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ANNUAL
REPORT

**Fiscal
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1989**

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

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OFFICE OF THE DIRECTOR

Report of the Director (DIR)	1
Report of the Deputy Director (DIR)	11
Assistant Director for International Health (DIR IH)	17
Planning and Evaluation Section (OPEC PES)	25
Public Inquiries and Reports Section (OPEC PIRS)	31
Research Data and Management Information Section (OPEC RDMIS)	37
Financial Management Section (OAM FMS)	41
Personnel Management Section (OAM PMS)	43
Equal Employment Opportunity Program (EEO)	45

INTRAMURAL RESEARCH PROGRAM

Bone Research Branch (BRB)	49
Cellular Development and Oncology, Laboratory of (LCDO)	73
Clinical Investigations and Patient Care Branch (CIPCB)	97
Diagnostic Systems Branch (DSB)	131
Developmental Biology and Anomalies, Laboratory of (LDBA)	145
Immunology, Laboratory of (LI)	191
Microbial Ecology, Laboratory of (LME)	215
Neurology and Anesthesiology Branch (NAB)	233
Oral Medicine, Laboratory of (LOM)	259

EXTRAMURAL PROGRAMS

Report of the Acting Director	273
Research Training and Manpower Development (RTMD)	279
Caries and Restorative Materials Research Branch (CRMR)	283
Periodontal and Soft Tissue Diseases Branch (PSTDB)	299
Craniofacial Anomalies, Pain Control and Behavioral Research Branch (CAPCBBR)	313

EPIDEMIOLOGY AND ORAL DISEASES PREVENTION PROGRAM

Report of the Office of the Director	327
Office of Program Support (OPS)	333
Microbial Systematics Section (MSS)	343
Epidemiology Branch (EB)	351
Analytical Studies Branch (ASB)	371
Disease Prevention and Health Promotion Branch (DPHPB)	385

INDEX BY PROJECT NUMBER

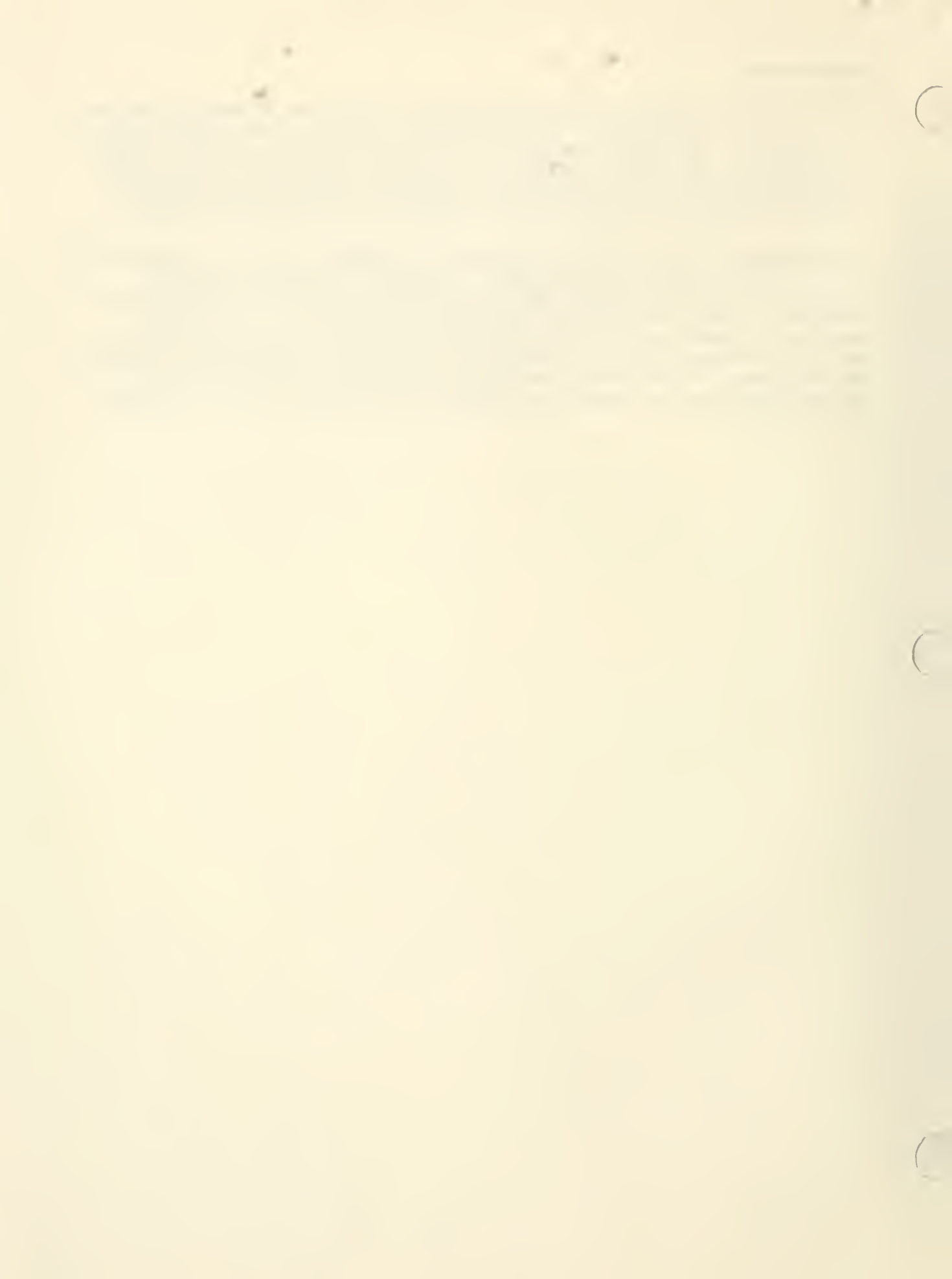
INDEX BY PRINCIPAL INVESTIGATOR

INDEX BY TITLE

INTRODUCTION

By the close of Fiscal Year 1989, the National Institute of Dental Research had completed the picture of the oral health of America from kindergartners to centenarians. The tangible benefits of research became clear as the decay rate among schoolchildren continued to decline dramatically, edentulousness among middle-aged adults approached elimination, and Americans in general enjoyed a high level of dental care. This was the good news of FY 1989.

Also emerging from the picture, however, are subpopulations at increased risk for a variety of oral health problems. The special needs of an aging population must be addressed. Finding solutions to the scientific enigma of AIDS and its oral complications must remain a priority in the research portfolio. These and other oral health problems that are as yet unsolved serve as reminders that in biomedical science, every accomplishment leads to a new challenge. As we enter a new decade, the NIDR brings with it both a renewed commitment to research excellence and anticipation of the good news for FY 1990.





OFFICE OF THE DIRECTOR

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REPORT OF THE DIRECTOR

Research Directions and Policy

Fiscal Year 1989 marked the end of NIDR's celebration of 40 years of research excellence and the beginning of the Institute's fifth decade of commitment to continued advances in the dental sciences. As NIDR leaves the 1980's, preparations are under way for the development of a long-range plan which will serve as a blueprint for research into the nineties. A sequel to "Challenges for the Eighties," the plan for the next decade will summarize the state-of-the-science in principal areas of oral health research, identify promising research avenues, enumerate impediments to progress, suggest specific research objectives, and propose strategies to help implement these objectives.

Also initiated in FY 1989, the "Research and Action Program for Improving the Oral Health of Adults and Older Americans" has as its goal the elimination of tooth loss in the U.S. population and the prevention of further deterioration of the oral status of individuals who have compromised dentition. The role of the NIDR, in collaboration with various public and private agencies, is to expand the knowledge base and to accelerate the transfer of laboratory findings to the dental profession and the public. These activities, while aimed at the adult and older segments of the population, will have far-reaching benefit for the oral health of all Americans.

The NIDR and the research community are forging deeper ties to meet the needs of those at special risk for oral disease and to maintain the momentum that has generated the many advances in the dental sciences. As this decade comes to a close, the Institute looks forward to a mixture of research challenge and scientific achievement in the years to come.

Program Management: Research and Training Support Activities

In FY 1989, the Director initiated and provided leadership in developing a comprehensive "Research and Action Plan to Improve the Oral Health of Adults and Older Americans." Collaborative implementation strategy was formalized with the Centers for Disease Control, and the program was subsequently elevated to a Public Health Service-wide initiative in the Office of the Assistant Secretary for Health. The Director oversaw the preparation and submission of a congressional report on efforts designed to improve the oral health of adults and older Americans.

The Director presented the NIDR annual research plan to the Director, NIH, in December 1988 and testified before the House and Senate Appropriations Subcommittees the following April. The testimony utilized the President's budget, the annual plan, the NIDR Long-Range Research Plan: FY 1985-1989, as well as recent research results from NIDR-supported projects.

The Director provided oversight for the development of the Long-Range Research Plan for the Nineties which involved six expert scientific panels, a workshop on international collaborative oral health research, a special meeting to enhance NIDR-industry collaboration, and a working group to discuss minorities in oral health research.



As Acting Associate Director of the Epidemiology and Oral Disease Prevention Program (EODPP), the Director continued to advance research targeted to at-risk populations of adults and older Americans. In this capacity, the Director oversaw the development of both the oral health component of the Walter Reed Study of the National History of HIV Infection and the oral examination component of NHANES III. In related activities, he also directed the meeting of dental public health constituencies designed to accelerate the implementation of preventive procedures, oversaw the further development of intraoral controlled release devices for the treatment of oral diseases, and directed the provision of scientific and technical assistance to the lay public and professionals regarding relationships between fluoride and health.

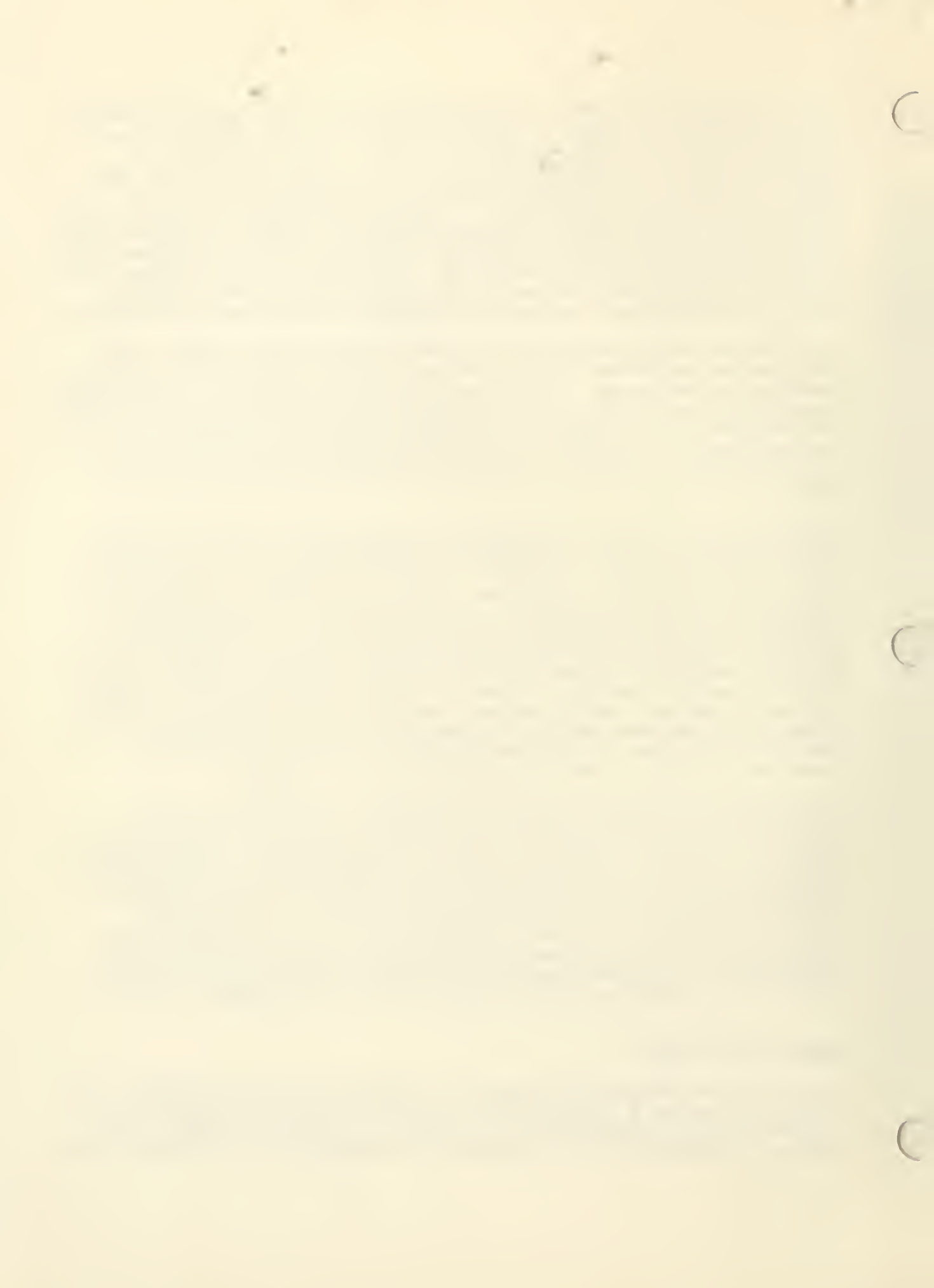
The Director, serving as Acting Chief, Periodontal Diseases Section, EODPP, presented the periodontal disease findings of NIDR national surveys at major meetings here and abroad. He continued as senior investigator in an ongoing longitudinal study of the natural history of periodontal diseases, and oversaw the development and calibration of periodontal disease measurements and training manuals in NHANES III, the Walter Reed Study of the Natural History of HIV Infection, and in major studies of periodontal disease among the elderly.

Extramurally, the Director oversaw the preparation and issuances of Requests for Applications (RFAs) on clinical core research centers, HIV inhibitory factors in human saliva, development and characterization of immortalized salivary gland epithelial cell lines, and on the evaluation of commonly used orthognathic treatment procedures. He also guided the development and issuance of program announcements on oral motor function, biology of tooth movements and eruption, effects of oral factors on taste and smell, and nutrition research. In other activities, the Director participated in Program Advisory Committee meetings on molecular biology research related to caries, periodontal and soft tissue diseases, and nutritional oral health research; promoted the consensus development conference on Oral Complications of Cancer Therapy; and utilized over 300 consultants in the review of 253 RFAs, conference proposals, research training issues and workshops.

In the Intramural Program, the Director selected a new chief for the Laboratory of Microbial Ecology, which focuses on research in oral bacterial toxins. A search is ongoing for a chief of the Laboratory of Developmental Biology and Anomalies. AIDS research was expanded to include studies of HIV detection in saliva, protective effects of saliva, and clinical studies of bacterial flora on HIV positive individuals. In FY 1989, the Board of Scientific Counselors positively reviewed the Clinical Investigation and Patient Care Branch as well as the Neurobiology and Anesthesiology Branch. Modernization of existing laboratory facilities continued this fiscal year, and plans are progressing for the tower addition to Building 30.

Employee Opportunity

The Director reaffirmed the Institute's commitment to equal employment opportunity through a variety of activities and programs throughout FY 1989. The Director kept informed of EEO, affirmative action, civil rights, and contract compliance issues through regular meetings with the Institute's EEO



Manager. He fully endorsed the activities of the EEO Advisory Committee such as forums in which speakers addressed a variety of career enhancement issues for NIDR employees. These presentations led to increased applications from NIDR employees to NIH training programs.

The Director's encouragement of supervisors and managers to recruit and employ minorities in the Institute's summer program resulted in an employment roster of 30 percent minorities and 61 percent women. In addition, NIDR supported five MARC (Minority Access to Research Careers) students for a summer research training experience. FY 1989 also saw a four percent increase in the minority work force profile of the Commission Corps, an eight percent increase in postdoctoral training programs, and nine percent in the GS and GM categories.

The Director encouraged funding support for and staff participation in conferences and symposia, including the annual MARC Scholars Conference, 16th NIH-MBRS (Minority Biomedical Research Support Program) Symposium, Symposium on Career Opportunities in Biomedical Sciences sponsored by AMPHS, and NIH and Indian Health Service Symposium on Biomedical Careers for American Indians and Alaskan Natives, which focused on the under-representation of minorities in the biomedical sciences. He also directed a Symposium on the Oral Health of Minorities presented as part of a Long-Range Plan for the Nineties meeting dealing the special oral health needs of minority subpopulations. The Director endorsed the participation of seven supervisors/managers in a special seminar on handicap awareness sponsored by the Division of Equal Opportunity.

Under the Director's leadership, the NIDR funded a variety of research and training grants pertaining to minorities totalling \$3,153,000. He also supported broader outreach activities in the minority community which included staff participation as special judges in the Washington, D.C. Annual Science Fair; expansion of NIDR Resource Collection to 27 minority colleges and universities and two high schools; and assisting science teachers at a local high school to boost awareness of research among minority science students.

Organizational Activities

In FY 1989, the Director continued to implement successive components of the multi-phased organizational development intervention program introduced in the previous fiscal year. This program is designed to identify organizational issues and problems, develop action goals, and assess the impact of the activities on issue resolution.

During this fiscal year, the Director orchestrated an organizational realignment within the Intramural Research Program. A Laboratory of Microbial Ecology was formed to study the adherence, physiology, biochemistry and genetics of oral microorganisms as well as oral manifestations of AIDS. Several other sections and units also were established within IRP in FY 1989.

The Director recruited and recommended for the Secretary's approval a new Director for the Extramural Program, while assuring continuity of EP operations during the lengthy personnel process.

Under the Director's leadership, an in-depth analysis of the budget activity and management accounts structure has resulted in a major overhaul of the



Institute's financial framework, enabling more efficient operations. In addition, several recent innovations in budget automation have further increased the accuracy and efficiency of both budget execution and formulation. Internal policies and procedures were also developed relating to overtime, utilization of patent royalties, the unconditional gift fund, controlled substances, ADP (automatic data processing) acquisition, and property management, among others.

The Director initiated a major reorganization and centralization of procurement activities which resulted in a reduction in the number of ordering officials from 63 to 8. In related activities, an assessment of administrative operations/services throughout the Institute was undertaken. Recommendations were developed and are being implemented, including staffing of the OD Administrative Office. A risk assessment for waste, fraud and abuse related to travel was also performed. As a result, internal controls and monitoring related to budget, positions, foreign travel and procurement activities were significantly enhanced.

The Director led Executive Staff in implementing the EPMS Performance Award System for FY 1988. An awareness of supervisory responsibility was maintained by continuing the mandatory inclusion of a supervisory element in all FY 1989 performance plans for SES/SSS/PMRS staff. The Director continued to encourage awards and honors to stimulate productivity and morale, and maintained an "open-door" policy for all employees.

The Director chaired regular meetings of the Executive Staff and Small Staff (immediate OD directors) and periodically attended program-level staff meetings to assure cognizance of management issues at all levels of the Institute and to be available to staff for open discussions.

Professional and Public Communications

The Director addressed numerous professional audiences at a variety of national and international dental meetings during FY 1989. He also stressed interagency cooperation among Federal organizations, including the establishment of an Interagency Coordinating Committee to implement an adult oral health research and action program involving the Indian Health Service, the National Center for Health Services Research and Health Care Technology, and the Health Resources and Services Administration. In related activities, the Director established more effective liaison with relevant components of the Food and Drug Administration including the Divisions of Obstetrics and Gynecology; Ear, Nose and Throat, and Dental Devices. The Director also explored collaboration with the Veterans Administration Dental Longitudinal Studies and the Normative Aging Studies, as well as the establishment of an NIDR Oral Health Research Facility at Walter Reed Army Medical Center.

During this fiscal year, the Director actively participated in the Central Services Review Committee, the NIH Task Force on Physician Scientist Training, the Next Generation of Biomedical Researchers Advisory Group, the search committee for the Director of the National Institute of Deafness and Other Communication Disorders, and the regular NIH BID Directors meetings.



The Director provided interviews and background material to print and broadcast media, including: The New York Times, Washington Post, Los Angeles Times, Self Magazine, Weight Watchers Magazine, Copley News Service, Asbury Park Press and the Ft. Worth Star-Telegram. He gave television interviews for Cable News Network; WGBH, Boston; and the Danish Broadcasting System in Copenhagen. Topics of discussion included the revolution in dentistry, periodontal disease, tooth decay, dental care from infancy, and others.

Honors and Awards

The Director was recognized for scientific achievement during this fiscal year by an honorary doctorate from the University of Toronto. He also was awarded the Swedish Dental Society's International Prize and the Surgeon General's Medallion for Exemplary Service.

Presentations

"The Impact of Advances in Research on Dental Education and Practice." American College of Dentists Annual Meeting, Washington, D.C., October 7, 1988.

"Dental Science--Dental Health: 40 Years of Progress." American Dental Association (ADA)/Federation Dentaire Internationale (FDI) Joint 1988 World Dental Congress, Washington, D.C., October 7-11, 1988.

Remarks: International AIDS Symposium. ADA/FDI Joint World Dental Congress, Washington, D.C., October 7-11, 1988.

"Re-Imaging Dentistry." Royal College of Dental Surgeons of Ontario/Toronto, Canada, October 15, 1988.

Remarks. NIDR Awards Ceremony, Bethesda, MD, October 21, 1988.

"Going for the Gold at the Silver Anniversary." Goldman School of Graduate Dentistry, Boston University, Boston, MA, November 4, 1988.

"NIDR Plans for the Future." Deans Meeting, Palm Springs, CA, November 28, 1988.

"Research and Action to Improve the Oral Health of All Americans." American Association for Dental Research (AADR), New Jersey Section, Newark, NJ, December 6, 1988.

"The Impact of Science on the Practice of Dentistry." Ohio State University College of Dentistry Research Day, Columbus, OH, March 3, 1989.

Opening Statement and Highlights. House and Senate Appropriations Committees, Washington, D.C., April 3, 1989.

Remarks. Workshop on International Collaboration for Oral Health Research, NIH, Bethesda, MD, April 6, 1989.



"A View of the Natural History of Periodontal Disease." British Society of Periodontology Spring Meeting, Liverpool, England, April 10, 1989.

"Periodontal Disease in Industrialized Countries." British Society of Periodontology Spring Meeting, Liverpool, England, April 11, 1989.

"The Changing Face of Dentistry." Turner and Newall Lecture, University of Manchester, Manchester, England, April 12, 1989.

Welcoming Remarks. NIH Consensus Development Conference on the Oral Complications of Cancer Therapies: Diagnosis, Prevention and Treatment, NIH, Bethesda, MD, April 17, 1989.

"Oral Disease Prevention in Research and Practice." Second World Congress on Preventive Dentistry, Beijing Medical University, Beijing, China, June 23, 1989.

"Future Directions in Dental Research at the NIDR." Faculty Seminar, University of Connecticut, Farmington, CT, June 1, 1989.

Commencement Address. University of Toronto Faculty of Dentistry, Toronto, Canada, June 9, 1989.

Remarks, Introduction and Presentation of Awards. 1989 Kreshover Lecture, NIH, Bethesda, MD, June 13, 1989.

"The Changing Face of Dentistry." Scandinavian Society of Periodontology, 25th Anniversary of the Jysk/Fysk Society of Periodontology, Copenhagen, Denmark, June 15, 1989.

"The Changing Face of Dentistry." Distinguished Speaker's Lecture, University of North Carolina, Chapel Hill, NC, August 26, 1989.

"Changing Disease Patterns: Their Impact on Dentistry." Dentistry in the 21st Century: A Global Perspective, September 10, 1989, Berlin, West Germany.

Keynote Address. Charlotte Area Health Education Center, 40th Anniversary of Water Fluoridation, Charlotte, NC, September 28, 1989.

Publications

Kingman, A., Morrison, E., Löe, H. & Smith, J. 1988. Systematic errors in estimating prevalence and severity of periodontal disease. *Journal of Periodontology* 59:707-713.

Löe, H. 1988. Research on oral diseases and its impact on dental education and practice. *Advances in Dental Research* 2(2):199-203.

Löe, H. 1988. Concluding Remarks. *Advances in Dental Research* 2(2):411.

Löe, H. 1988. Symposium Report: Molecular and Genetic Basis of Growth and Development: Introductory Remarks. NIH Consensus Development Conference. *Journal of Craniofacial Genetics and Developmental Biology* 8:279-280.



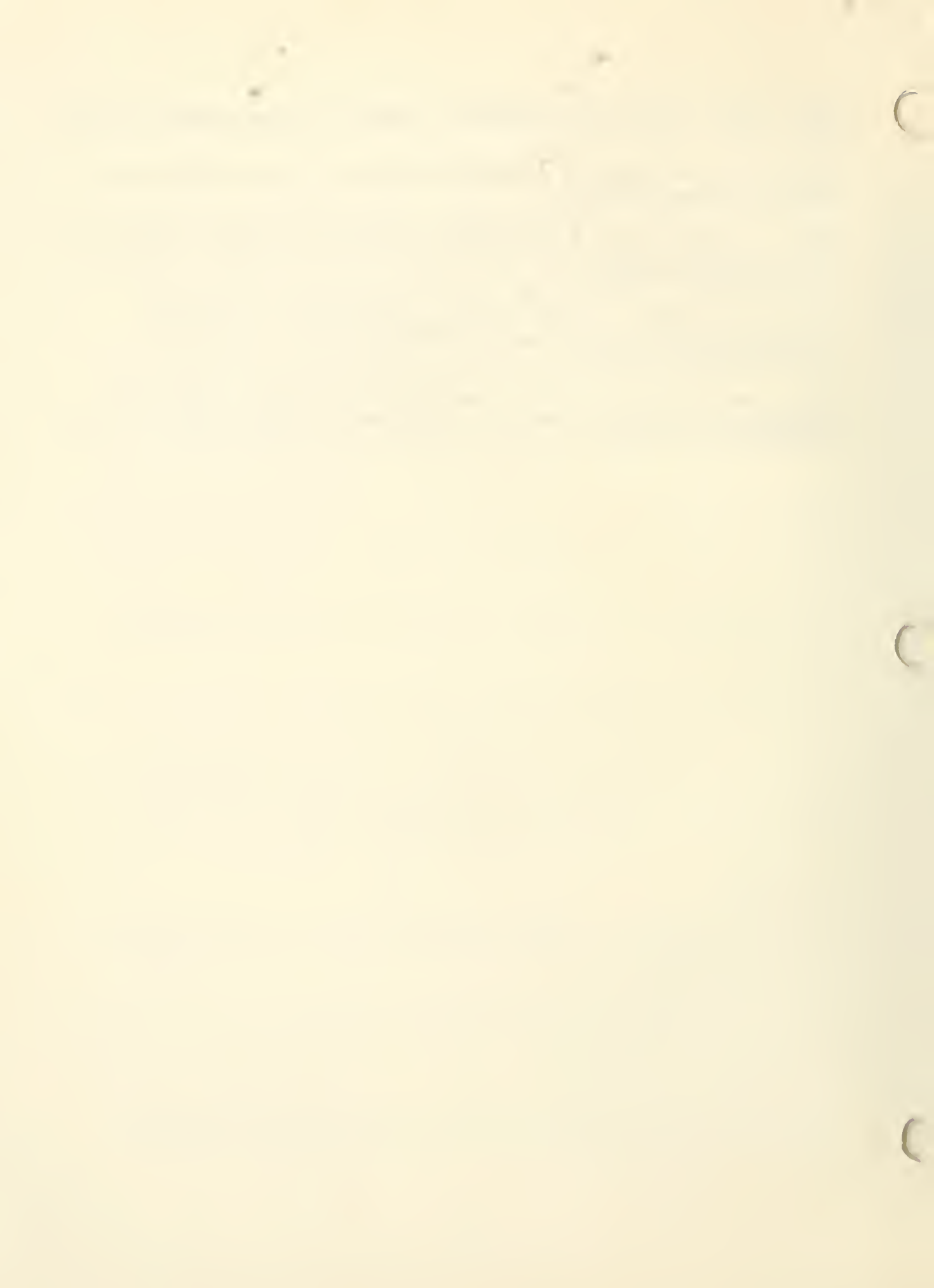
Löe, H. 1989. Forty years of progress. *Advances in Dental Research* 3(1):3-6, 1989.

Löe, H. 1989. The impact of advances in research. *Journal of the American College of Dentists* 56:33-41.

Brown, L.J., Oliver, R.C. & Löe, H. 1989. Periodontal diseases in the U.S. in 1981: prevalence, severity, extent, and role in tooth mortality. *Journal of Periodontology* 60:363-370.

Oliver, R.C., Brown, L.J. & Löe, H. 1989. An estimate of periodontal treatment needs in the U.S. based on epidemiologic data. *Journal of Periodontology* 60:371-380.

Löe, H. and Morrison, E., 1988. Epidemiology of periodontal disease. In Contemporary Periodontics, Genco, R., (Ed.) The C.V. Mosby Company, St. Louis (in press).



REPORT OF THE DEPUTY DIRECTOR, NIDR

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

Institute Management

The Deputy Director participated in the development of the FY 1991 budget proposal, and the preparation for and defense of the the President's 1990 budget, including participating with the NIDR Director at the House of Representatives Appropriations Subcommittee hearings and the development of responses to the questions of the House and Senate Appropriations Subcommittee members.

Initiated this year under the Deputy's leadership was an agreement with the National Institute on Aging to have our Equal Employment Manager serve in this capacity for both Institutes. The size of the staff of this office will be proportionately increased to better serve both Institutes but with an aggregate reduction of cost. This arrangement also increases the career opportunities for the incumbent. The Deputy Director serves as the supervisor and rating official of this individual.

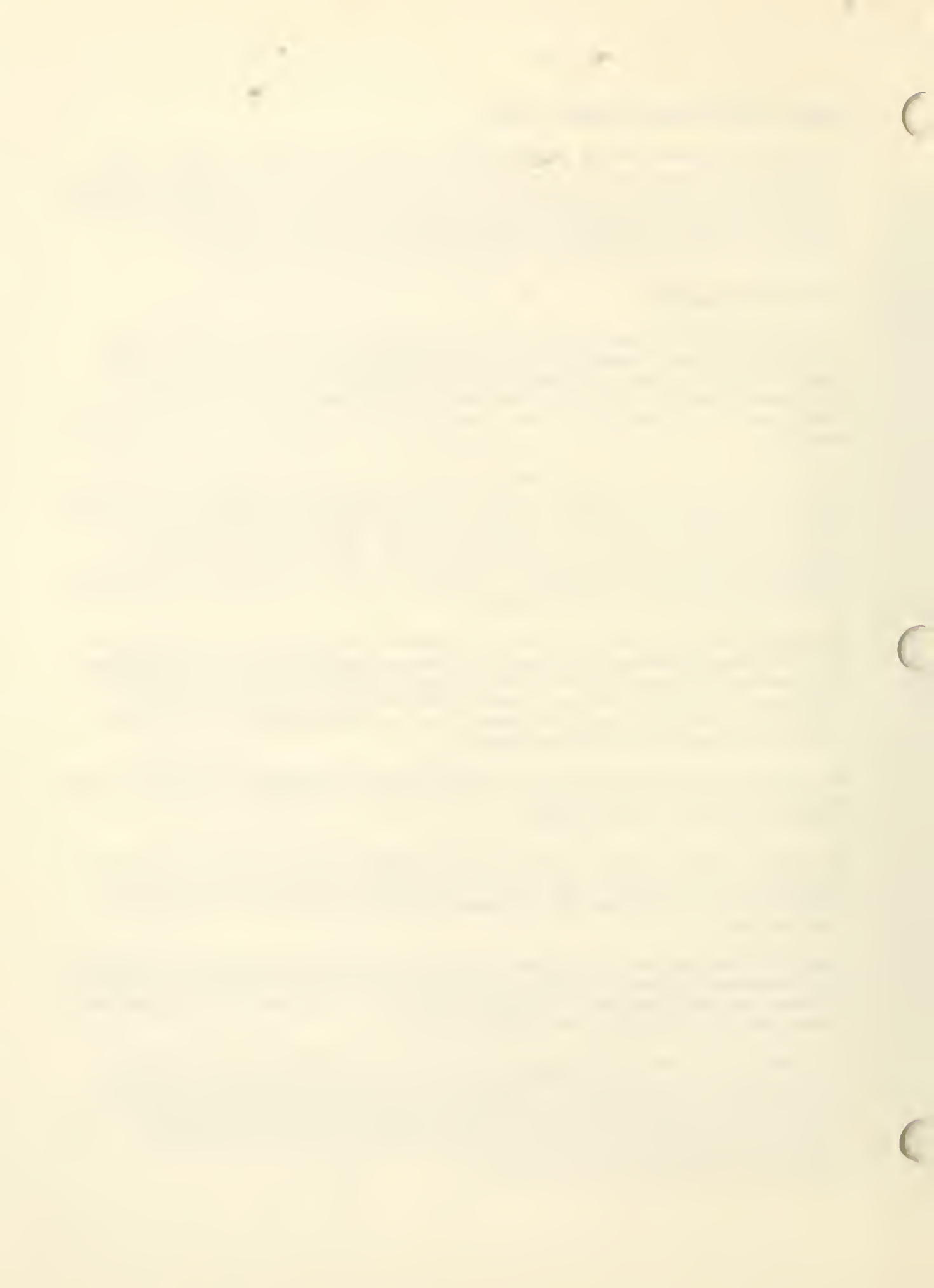
The Deputy Director serves as the Performance Recognition Group Manager for all Performance Management Recognition System employees and as the Employee Performance Management System Budget Manager for the Office of the Director employees. He also serves as a member of the review group for all NIDR quality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

The Deputy Director renegotiated a contract with a management consultant team designed to improve the managerial operations of the NIDR. He serves as the project officer of that contract.

He worked closely with the newly appointed NIDR Executive Officer and Budget Officer in their initial year with the Institute. FY 1989 was somewhat atypical due to stringent FTE personnel ceilings imposed, as well as fiscal constraints.

Special attention has been given this year to implementing program information and management systems to ensure that the Office of the Director has needed information for budgetary development and control purposes while attempting to reduce respondent burden upon program staff.

The Deputy Director participated in the final round of interviews of the finalists for the position of Director of the NIDR Extramural Program (EP). He has worked closely with the Acting Director of the Extramural Program pending the appointment by the Secretary, DHHS, of the new EP Director. The search for a permanent Director of the Epidemiology and Oral Disease Prevention Program has been initiated.



The Deputy Director designed and implemented a three-year, part-time managerial internship program that is linked to a NIDR-conducted residency program in oral medicine. This program is designed to acquaint promising PHS career dentists with the breadth of the NIDR activities, provide them with training leading to specialty board eligibility in oral medicine, and improve their managerial capabilities. The first candidate, Dr. William Kohn, an Indian Health Service Dental Officer, was selected and began the program in March 1989.

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

NIDR Advisory Council and Committee Activities

The Deputy Director oversees the committee management function of the NIDR. The NIDR has four formally chartered advisory committees, one of which is the National Advisory Dental Research Council. The Deputy Director serves as the Executive Secretary for this Council. He was responsible for the conduct of the January, June, and September meetings in FY 1989. A special initiative was implemented in FY 1989 to seek broadly qualified candidates to serve on the Council, and a system to manage the process is being developed. Nominations for replacement of Council members whose terms have expired have been forwarded to the Secretary, DHHS, in compliance with new requirements for both lay and scientific members and with requisite attention to both minority and women representation and geographic balance. The Deputy is responsible for conducting the orientation of new members of the Council and this year initiated the development of a comprehensive and up-to-date orientation manual.

NIDR and NIH Representation Activities

In the absence of the Director, the Deputy Director represented the NIDR at the weekly NIH Bureau/Institute/Division (BID) meetings. In addition, under his personal leadership, the Deputy convened and serves as a founding member of the NIH BID Deputy Director group which now meets on a monthly basis under a rotating chairmanship arrangement. This group has substantially increased communications among the respective BIDs and has facilitated both the resolution of some problems and cooperative involvement on special initiatives. The Deputy Director also served as a member of the NIH Task Force on Population-Based Research Training that was established by the Director, NIH, to guide future NIH activities in an area that has up to now received inadequate attention.

The Deputy Director served as the NIDR senior representative to the joint NIH-Indian Health Service "Symposium on Biomedical Careers for American Indians and Alaska Natives" held in April 1989 in Phoenix, AZ. This was a special initiative designed to increase the representation of minorities in research.

Special NIDR Initiatives

The Deputy Director served as the Institute's spokesperson for the FY 1989 dental sealant utilization initiative. He participated in both national and



state press conferences, as well as numerous radio, TV, and press interviews. This program was designed to better acquaint the public with a valuable preventive procedure.

The Research and Action Program to Improve the Oral Health of Adults and Older Americans remains a high priority for the NIDR, and the Deputy Director is actively involved. He has sought endorsement and input by professional organizations, such as the American Dental Association, the National Dental Association, and the American Association for Dental Research, and also the active participation of other Federal agencies. In concert with the Federal collaborating agency, the Centers for Disease Control, a decision was made to proceed with the establishment of a PHS coordinating committee and request that this be done so by the Assistant Secretary of Health. The Assistant Secretary of Health, DHHS, has agreed to this request and the appointment and charge to the coordinating committee is being formalized.

The development of the NIDR Long-Range Research Plan for the 1990s has been a prime NIDR OD priority in FY 1989. In addition to participating in numerous planning activities, the Deputy Director served as the Chairman for a special meeting of minority representatives held May 22. The purpose of this meeting was to seek input and review of the developing Long-Range Research Plan for the 1990s.

Liaison Activities

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

The Deputy Director obtained total Federal support for a special monograph being developed by the American Dental Association on the oral issues related to AIDS beyond infection control in order to ensure that it will be distributed to every dentist in the United States, dental libraries, and other health organizations. Through his efforts, support was obtained from the Office of the Assistant Secretary of Health, the Food and Drug Administration, the Indian Health Service, the Health Resources and Services Administration, the Centers for Disease Control, the Department of Veterans Affairs, and the National Institutes of Health.

PHS Dental Responsibilities and Activities

In addition to his NIDR responsibilities, Dr. Littleton serves as Deputy Chief Dental Officer, PHS. In this role, he assists the Chief Dental Officer in providing leadership and coordination of PHS national and international oral health activities, and dental professional affairs of the Office of the



Surgeon General; represents the Surgeon General to local, state, national and international groups and professional societies; provides advice and guidance on matters such as recruitment, retention, career development of PHS dental personnel; and performs other duties as assigned by the Surgeon General. He has spent considerable energy in assisting in the oversight of dental activities of the PHS and carrying out a broad range of assignments.

Particularly noteworthy in this assignment was the Deputy Chief Dental Officer's participation on the program of the October 1988 international meeting of Chief Dental Officers (CDO) that was held in Washington, D.C., as part of the World Dental Congress. He helped organize and co-host a formal reception held at the NIH Fogarty International Center (Stone House) for the international representatives. The next international meeting of Chief Dental Officers was also held during this fiscal year in Amsterdam, The Netherlands, in conjunction with the annual Federation Dentaire Internationale meeting. The Deputy Chief Dental Officer was selected to present the keynote address at the opening session of the CDO meeting.

Liasion with national dental organizations is an important activity. The Deputy Chief Dental Officer served as the alternate delegate to the House of Delegates of the American Dental Association and as delegate to the American Association of Dental Schools. The Deputy Chief Dental Officer joined the Chief Dental Officer in meeting with the president and executive director of the National Dental Association (NDA) about minority issues of concern to both the PHS and the NDA. In addition, serving in both his NIDR and Deputy Chief Dental Officer roles, he met with the Executive Committee of the NDA.

The Deputy Chief Dental Officer represented the USPHS by his participation and serving as a presenter at the Annual Meeting of the Association of Military Surgeons of the United States. Additionally, the Deputy Chief Dental Officer actively participated in the many activities related to the Centennial observation of the establishment of the USPHS Commissioned Corps and the 70th anniversary of the Dental Corps. The Deputy Chief Dental Officer represented the Chief Dental Officer at PHS meetings and meetings with the dental components of the Armed Forces.

In his role as Deputy Chief Dental Officer and as an ex-officio member of the Surgeon General's Dental Professional Advisory Committee, he was actively involved in a number of important PHS initiatives particularly involving revitalization of the Commissioned Corps. This includes recruitment, with emphasis on enhancing minority representation; standardization of billets (with the dental standardized billets ultimately becoming the model for the other commissioned corps specialty areas); specialty pay; licensure; awards; and promotions. He provided input regarding the Research Officer Group of the Public Health Service Commissioned Corps, which addresses some long-standing problems facing Commissioned Corps personnel in the research field.

The Deputy Chief Dental Officer led a major initiative carried out by the Chief Dental Officer on behalf of the Secretary, DHHS. In response to a request by the Senate and House Appropriations Committee, a comprehensive study was conducted on dental activities within the Department. It examined goals and priorities in dental health in the areas of research, education, prevention and service and formulated recommendations concerning needed organizational and administrative arrangements within the PHS. The Deputy



Chief Dental Officer was intimately involved in all aspects of this activity that resulted in a report submitted to Congress in May 1989. The Deputy Chief Dental Officer assisted the Chief Dental Officer in a broad range of other activities. At the request of the Surgeon General, the Deputy Chief Dental Officer served as the Chairman of the Selection Board reviewing candidates for the position of Chief Veterinary Officer.

Professional Activities

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

Honors and Awards

In this fiscal year, the Deputy Director/Deputy Chief Dental Officer was honored with the PHS Meritorious Service Medal, a PHS Unit Commendation Award, Georgetown University's Dental Alumni Distinguished Service Award, and was elected as a Fellow in the American College of Dentists.

Presentations

Keynote Address. The Dental Public Health Role in Infection Control: Infectious Diseases -- Effective Controls for Both Industrialized and Developing Countries. 6th Annual Conference of Chief Dental Officers, Federation Dentaire Internationale, Amsterdam, The Netherlands, September 3, 1989.

Opportunities in Dental Research. National Dental Association 76th Annual Convention, Washington, D.C., July 30, 1989.

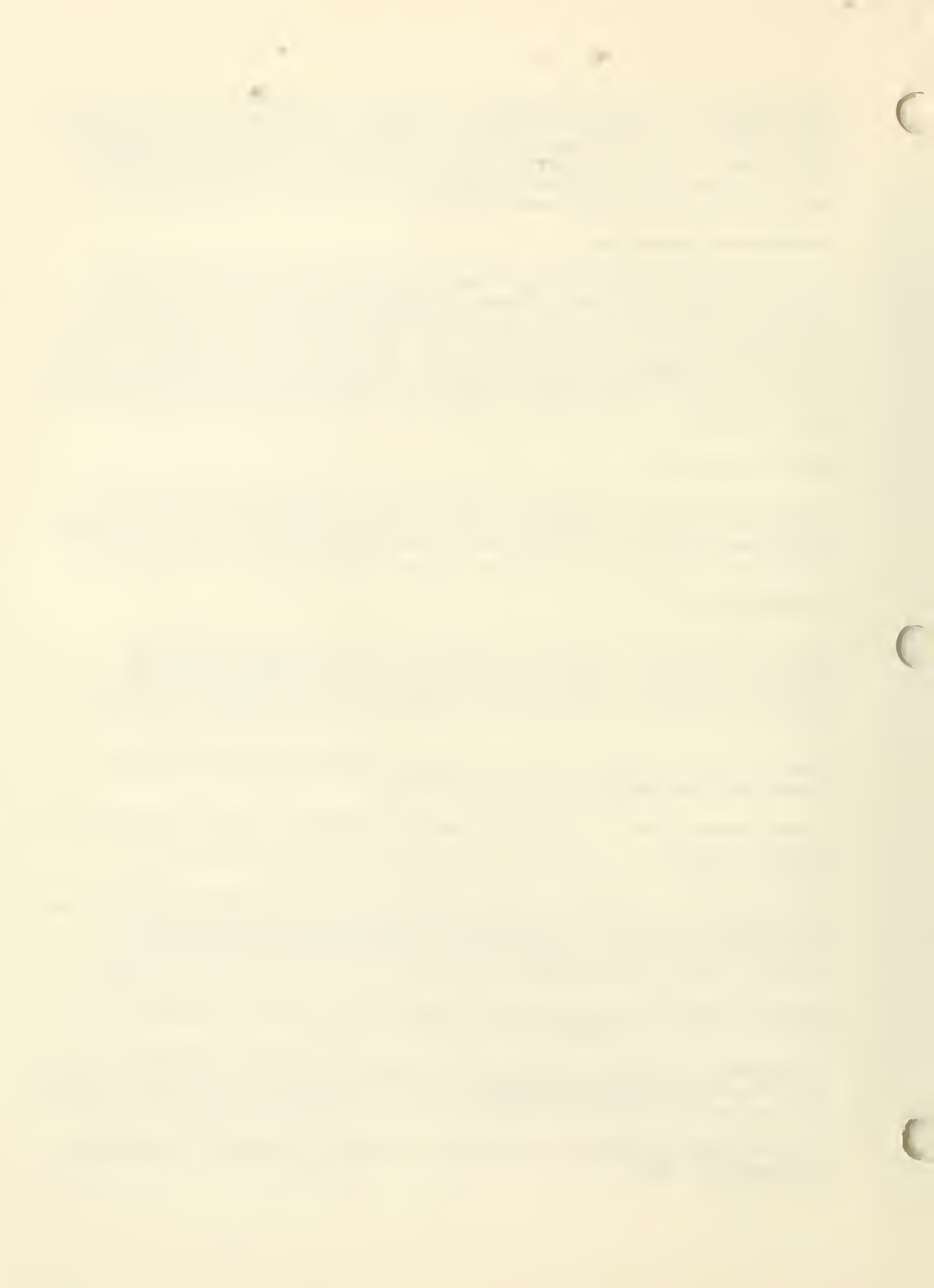
Dental Research Training Opportunities for Minorities. Indian Health Service-National Institutes of Health Minority Research Conference, Phoenix, AZ, April 24, 1989.

Keynote Address. Dentistry -- The Profession with the Brightest Future: Can You Believe What We Have Accomplished? Can You Imagine What We Can Accomplish? Homecoming 1989, School of Dentistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, April 1, 1989.

Panelist, NIDR Forum. 18th Annual Session of the American Association for Dental Research, San Francisco, CA, March 17, 1989

Challenges to Dentistry -- Challenges to Future Dentists. San Antonio Section of AADR and Dental School Research Committee Meeting (at AADR Annual Meeting), San Francisco, CA, March 16, 1989.

National Press Conference Panel Participant, Dental Sealants. San Francisco, CA, March 16, 1988.



Research and Action Program to Improve the Oral Health of Adults and Older Americans. Council of Deans Session, 66th Annual Session of the American Association of Dental Schools, San Francisco, CA, March 12, 1989.

Salute to USPHS Dental Corps -- Synopsis of 70 Years of Accomplishments. Annual Meeting of the Tri-Service Dental Society, Fort Meade, MD, March 2, 1989.

State Press Conference Panel Participant, Dental Sealants. Ohio State and Cincinnati Health Departments, Cincinnati, OH, February 22, 1989.

Initiatives in Dental Public Health Affecting the PHS Dental Corps. Annual Meeting of the Association of State and Territorial Dental Directors and National Oral Health Conference, Cincinnati, OH, February 20, 1989.

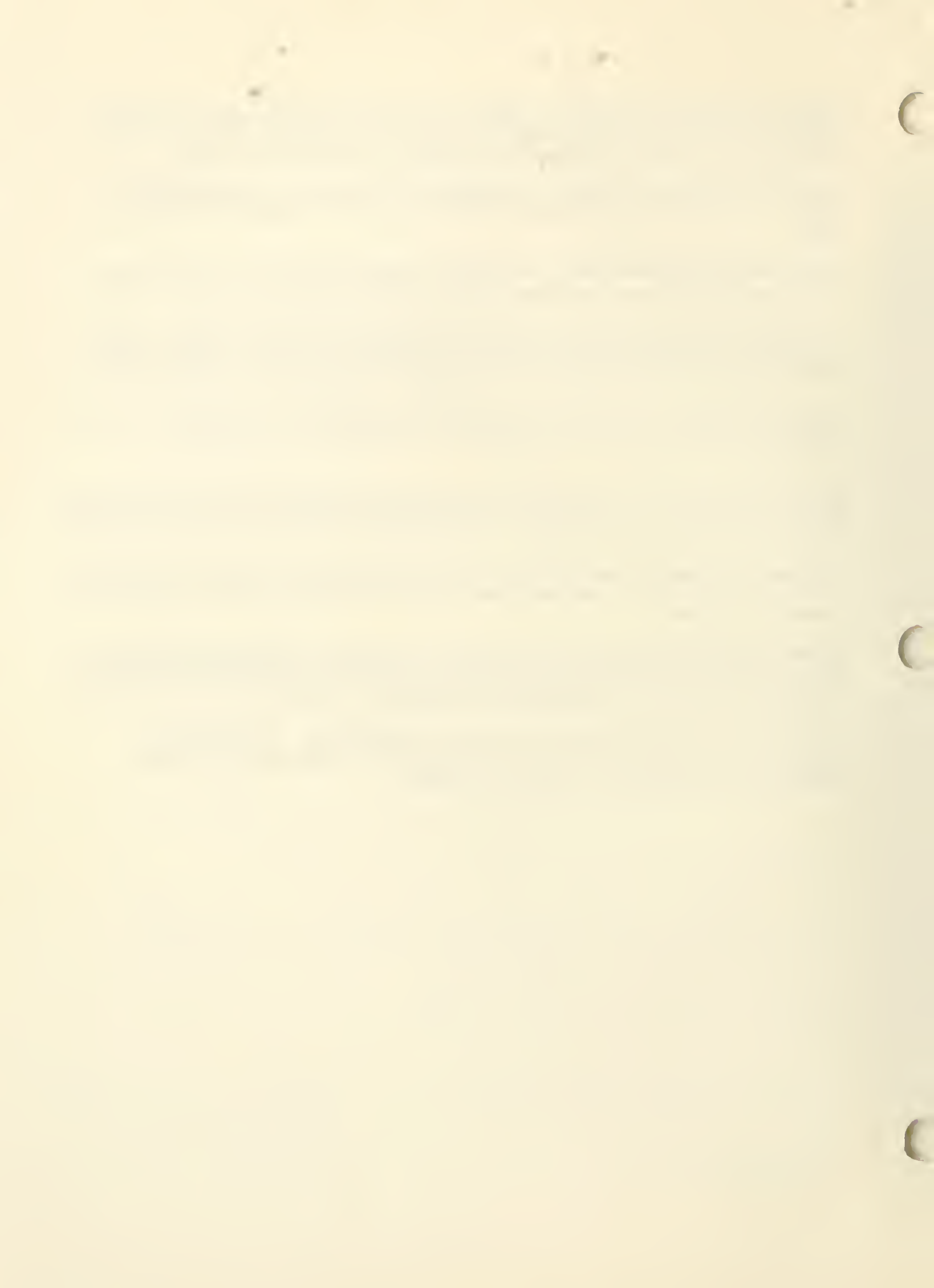
Keynote Address. Excitement, Expectations, and Ethics. 39th Annual Pinning Ceremony, Fones School of Dental Hygiene, University of Bridgeport, Bridgeport, CT, February 5, 1989.

Current Realities in the Support of Dental Research at U.S. Dental Schools and Research Institutions. 30th Annual Conference of Dental School Deans, Rancho Mirage, CA, November 28, 1988.

The Impact of Dental Research on Dental Public Health. Dental Health Section, 116th Annual Meeting of the American Public Health Association, Boston, MA, November 16, 1988.

Improving the Oral Health of the Nation: Transition from Knowledge Base to Action Research. 95th Annual Meeting of the American Association of Military Surgeons of the U.S., San Antonio, TX, November 1, 1988.

Oral Health Systems Research, Panelist and Rapporteur. Fifth Annual International Conference of Chief Dental Officers, FDI/ADA World Dental Congress, Washington, D.C. October 9, 1988.



ASSISTANT DIRECTOR FOR INTERNATIONAL HEALTH, NIDR

The Assistant Director for International Health is responsible for coordinating global oral health initiatives, ongoing programs and activities, and facilitating their progress as well as promoting communication among U.S. dental investigations and scientists abroad. During Fiscal Year 1989, major effort was expended on planning innovative new approaches toward international collaborative research.

INTERNATIONAL COLLABORATION FOR ORAL HEALTH RESEARCH (ICOHR)

The NIDR together with the Fogarty International Center (FIC) and the World Health Organization (WHO) completed a major initiative begun in July 1988 to enhance international collaboration for oral health research. With the assistance of consultant Professor Philip Holloway, University of Manchester, U.K., a report was completed of a study which he organized and implemented. The study focused on the development of a research agenda for questions which require international collaboration or which would be greatly enhanced by such collaboration. Research training needs as well as facilitating factors also were included in the inquiry. This effort involved a series of systematic interviews of NIDR and NIH staff, U.S. researchers involved in international collaborative research and selected foreign investigators and administrators also involved in such activities. A workshop was convened in April 1989 to specify priorities in a variety of dental science subject areas. Position papers were produced and refined and these were integrated into a final report which was presented to the National Advisory Dental Research Council, the Fogarty Advisory Board, as well as key staff in both organizations and the WHO. Discussions of the feasibility of implementation of both the short and longer-term recommendations are proceeding at the staff level. Progress reports of the initiative were given to the local Washington Section of the American Association of Dental Research and a variety of dental research institutions in the U.S. A successful exhibit featuring ICOHR was in use at the annual meeting of the International Association of Dental Research (IADR) held in Dublin in June 1989. Currently NIDR staff are exploring the potential utility of an international meeting of funders of dental research, to further the implementation of the report.

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

Continuing the implementation of the International Collaborative Study involving NIDR, the WHO, the National Center for Health Services Research (NCHSR), the Indian Health Service (IHS) and the Centers for Disease Control (CDS) and several other countries, the ICS-II is proceeding on schedule. Data collection is near completion in New Zealand and has begun in the USSR and Poland. Pending OMB approval, data collection is imminent in the Baltimore metropolitan and non-metropolitan areas and in comparable native-American study sites. A meeting of organizers and coordinators for the U.S.-based sites was hosted by the NIDR in April and the



Assistant Director for International Health delivered two papers on the progress of the study at the American Public Health Association in Boston, November 1988 and at the International Association for Dental Research in Dublin in June 1989.

Technical assistance to staff involved in the replication studies sited in India and in Israel was provided during this year and cooperation of the National Institute on Aging was explored to enhance the support of the research segment which focuses on older persons.

Organizational Liaison: World Health Organization (WHO)

NIDR continues to be active in its role as the WHO Collaborating Center for Epidemiology, Prevention and Treatment of Oral Diseases and Conditions. Aside from ICOHR and ICS-II programmatic activities, the Institute hosted two coordinating meetings on the subject of the oral manifestations of HIV infection and participated in a third meeting held in conjunction with the Annual World Dental Congress held in Amsterdam. The purpose of these meetings is to sustain essential communications among all WHO Centers working in this area and to further the collaborative activities among centers and the Federation Dentaire Internationale (FDI). A special workshop was organized in April by the NIDR staff member in the Epidemiology and Oral Disease Prevention Program on Minimal Essential Data needed for epidemiologic and surveillance purposes.

Other activities included: informal meetings with staff of the WHO Special Program on Research in Aging, discussions with the Deputy Director-General of WHO, Dr. M. Abdelmorimere on WHO's ability to facilitate international collaborative funding for oral health research and provision of partial conference proceedings support for The Second World Congress on Preventive Dentistry, May 1989 in Beijing.

Organizational Liaison: Federation Dentaire Internationale (FDI)

As the 1988 World Dental Congress of the FDI (and the American Dental Association), held in Washington, D.C. in October, the NIDR played a substantial role in both the planning and implementation of a number of programs and activities related to this major meeting. Not only was it the largest FDI Congress in their history but it was the largest meeting ever housed by the D.C. Convention Center, thus posing considerable strain on the resources of the local dental organizations, including the NIDR. The Assistant Director for International Health was the lead organizer for the following: reception at the Stone House for the Chief Dental Officers of the world; commemorative symposium in honor of the 40th anniversary of the NIDR; scientific session on improving access to oral health care; tours of NIDR, NIH museum exhibit and the NLM exhibit on dental research; lecture on dental science stamps; U.S. Postal Service commemorative NIDR 40th anniversary postal mark; Smithsonian Institution exhibit on dental research and opening gala



and poster associated with this exhibit. The Assistant Director also served as chairperson for Working Group 3 on Oral Health Promotion of the Commission on Oral Health, Research and Epidemiology and participated in the Executive Meetings of that Commission. She also is a member of the Scientific Programme Committee of the FDI and actively contributed to all four of the meetings held in D.C. and through correspondence during the year subsequent to the Congress. She represents the organization Behavioral Scientists in Dental Research to the FDI General Assembly.

During Fiscal Year 1989, she served on the jury for the Johnson and Johnson Preventive Dentistry Awards and at the 1989 Annual World Dental Congress in Amsterdam (September) completed her term of service on that jury as well as on the Working Group. The final report of the latter was submitted for publication by the FDI, Promoting Oral Health: Guidelines for Dental Associations. In her role on the Scientific Programme Committee she participated in a special meeting on revamping the structure of the scientific sessions. She also organized a session entitled "Getting the Message Through" and presented a paper on the Leadership Role of National Dental Associations. The FDI/WHO Joint Working Group on AIDS was facilitated by the Assistant Director and the Chief, Soft Tissue, Craniofacial Defects and Pain Section, Epidemiology and Oral Diseases Prevention Program, NIDR.

Organizational Liaison: International and American Associations for Dental Research (IADR/AADR)

During the annual IADR meeting in Dublin, a special exhibit on ICOHR was highlighted. It successfully conveyed the message of NIDR's interest in developing stronger partnerships with foreign investigators and to facilitate such relationships with U.S. investigators for the purpose of strengthening the science and pooling scarce resources. Relevant materials were distributed on NIDR extramural support mechanisms as well as those from the Fogarty International Center. The AADR has completed its project on New Frontiers in Dental Science which was initiated to complement the NIDR long-range research planning effort for the decade of the 90's. That report and the ICOHR report have been integrated into the draft report of the plan submitted for consideration in September by the National Advisory Dental Research Council and the Coordinating Panel for the plan.

Organizational Liaison: American Dental Association (ADA)

The Council on ADA Sessions and International Relations has utilized the Assistant Director as an active consultant in its liaison relationships to the FDI, with the National Council on International Health (NCIH) and Health Volunteers Overseas (HVO). She helped plan and moderate a program at the NCIH for health voluntary organizations which have dental programs or would wish to develop some. At that meeting, there was a unique opportunity for the ADA to acquire essential information on their own potential



role as a resource for these groups. The ADA Board of Trustees voted in June to affiliate with HVO to provide the programmatic direction and technical assistance needed for U.S. dentists wishing to volunteer their services in developing countries. Data obtained from the NCIH meeting will be discussed by ADA staff and officers to arrive at an appropriate and feasible role for the organization in their work with HVO, and in concert with the global role of the Oral Health Unit of the WHO (including PAHO). More recent liaison includes discussions of ADA and the International Executive Service Corps.

Organizational Liaison: Fogarty International Center (FIC)

The Assistant Director represented the NIDR at the BID International Representatives meetings held every six weeks and coordinated by the FIC; submitted the NIDR annual international report as a contribution toward the NIH annual report document; transmitted NIDR comments on a variety of circulated documents including WHO, PAHO and Western Pacific Regional Office plans and reports, provided information on proposed FIC international fellows; attended FIC Advisory Board meetings held three times annually; served on the advisory committee on the FIC contract dealing with international health research expenditures data comparability system and cooperatively planned, directed and funded the initiative, International Collaborations on Oral Health Research (ICOHR).

FIC staff have assisted NIDR extramural grantees' participation in the International Training Programs Related to Epidemiology of AIDS. They have continued to assist NIDR in stimulating involvement in the U.S.-Mexico agreements as well as the U.S.-India and U.S.-Italy agreements, and potentially the Memorandum of Understanding with Egypt. The India and Egypt mechanisms will be of use to facilitate the involvement of those two middle-income developing countries in ICS-II. The Mexico project continues to focus on neuroanatomy and the Italian agreement focuses on bone research.

NIDR international travel is coordinated with FIC staff and such travel is reviewed for appropriateness by the Assistant Director. On occasion justification for international travel is prepared by the Assistant Director for meetings at which several NIDR need to be in attendance (e.g., IADR in Dublin) or when several foreign scientists attend meetings in the U.S. at NIDR's expense (e.g., Hybridema Data Bank Coordinators Meeting in Rockville, Md.).

Other International Activities

During this fiscal year, the Assistant Director coordinated staff reviews on applicants seeking support from the U.S.-Israel Binational Science Foundation, provided orientations for visitors from a number of countries in attendance at the 1988 World Dental Congress held in Washington, D.C. and others from Australia, China, Israel, Japan, Poland, South Africa and the WHO. She also provided



written responses to inquiries from Australia, China, Colombia, Denmark, Federal Republic of Germany, German Democratic Republic, Great Britain, Hong Kong, India, Ireland, Nigeria, Scotland, South Africa, Sri Lanka, Sweden, The Netherlands, Yugoslavia and the WHO. Requests for information ranged from possible pre and post-doctoral research opportunities to commentary on ongoing or proposed research initiatives. A larger than usual proportion of inquiries came from China during the 3rd and 4th quarter of the fiscal year.

A new NIDR set-aside evaluation project to begin in late FY 1989 will initiate an ongoing monitoring system of international dental research performance by institutions and countries according to specific research areas from the NIDR Long-Range Research Plan for the 90s. The Evaluation Officer presented two papers on evaluating dental research internationally utilizing bibliometrics and citation analyses, one at the AADR meeting in San Francisco (March) and another at the Workshop on International Collaboration for Oral Health Research in Bethesda (April).

The Assistant Director also presented information on NIDR's international program during visits to Harvard School of Dental Medicine, Loma Linda School of Dentistry, California State University and the Sparkman International Center at the University of Alabama School of Public Health.

Presentations and Publications

Cohen, L.K. "Global Strategies for Oral Health: New Agenda Requires International Collaboration." International Health News, October 1988, Vol. 9, No. 8, pp. 5 and 8.

Cohen, L.K. "Impact of Oral Health Care Delivery Systems on Oral Health Status," Presented at the 116th Annual Meeting of the American Public Health Association, Boston, Massachusetts, November 16, 1988.

Cohen, L.K. "Facilitating International Collaborations for Oral Health Research," Presented at the Workshop on International Collaboration for Oral Health Research, NIH, Bethesda, Maryland, April 5-7, 1989.

Cohen, L.K. "Psychosocial and Cultural Factors in International Health." Seminar presented at Department of Health Sciences and Human Ecology, California State University, San Bernardino, California, April 19, 1989.

Burt, B.A., Albino, J.E., Carlos, J.P., Cohen, L.K., Dubner, R., Gershen, J.A., and Greene, J.C. "Advances in the Epidemiological Study of Oral-Facial Diseases," Advances in Dental Research, May 1989, Vol. 3, No. 1, pp. 30-41.

Cohen, L.K. "Research Planning for the 90's and Implications for Dental Education and Practice," Presented at faculty retreat, School of Dentistry, University of Washington, Rosario Resort,



Orcas Island, August 21-22, 1989.

Cohen, L.K. "Leadership Role of Dental Associations: Oral Health Promotion," Paper presented at 77th Annual World Dental Congress of the Federation Dentaire Internationale, Amsterdam, The Netherlands, September 5, 1989.



OFFICE OF PLANNING, EVALUATION AND COMMUNICATIONS (OPEC)

OPEC directs and coordinates all scientific program planning as well as all information necessary to support budget submissions, Congressional testimony and related documents which reflect NIDR's program goals and objectives. It is also responsible for coordinating and directing all program evaluation activities, assessing the impact of and return on NIDR's investments in research and training. OPEC prepares responses to inquiries from Congress, other Federal and nonfederal agencies, the professions and the lay public; and collects appropriate materials and data to respond to such inquiries and to disseminate the informational outcomes of research to potential user organizations and communities. This Office often provides orientation services to visitors, new staff and consultants; organizes NIDR seminars on issues related to science policy; and provides essential support services to the Director of the Institute in developing documentation for speeches, presentations and publications. OPEC includes: Branch Chief, Special Assistant for Special Projects, Planning and Evaluation Section, Research Data and Management Information Section, and Public Inquiries and Reports Section.





PLANNING AND EVALUATION SECTION (PES)

The Planning and Evaluation Section, OPEC, coordinates all planning and evaluation activities for the NIDR, originates special planning initiative projects, and responds to internal (NIDR) and external requests for information relevant to planning and evaluation. When appropriate, functions are coordinated with both budget and information systems in concert with operating programs of the Institute. PES also serves as NIDR contact with the Division of Legislative Analysis, NIH.

Planning and Evaluation Activities

Planning. The major planning activity involved the NIDR Long-Range Research Plan for the 1990s. Staff and contractor summarized an extensive amount of background material from responses to a notice placed in the Federal Register. Information consisted of accomplishments and discoveries in oral health and disease research over the past ten years, current state of the science in twenty research areas, key contributors to research advances, projected topics for research during the coming decade, and implications of potential research findings for dental practice and education. Staff developed letters of invitation to prospective panel members selected to assist the NIDR develop state-of-the-science reports and recommendations for six panels to be incorporated in the new Plan for the Nineties. These panels encompass all 14 subject areas of the previous Long-Range Research Plan, Challenges for the Eighties, as well as new areas. Staff, working with the Research Data and Management Information Section, OPEC, provided panel chairs with extensive historical data on research grants and training for specific subject areas, and arranged agenda for a special meeting of the panel chairs; attended and assisted at all panel meetings; worked with contractor in all follow-up activities, including a second meeting of panel chairs. Ongoing activities are the writing of the plan and editing panel reports.

Also in conjunction with the new plan, staff developed agenda/invitational letters for special meetings involving private industry and representatives of minority groups. The purpose of these meetings was to invite comment and participation from these individuals and organizations in the development of the new plan. Staff also assisted contractor prepare written summaries of both meetings. Staff worked with Chief, OPEC to present Long-Range Plan summaries to international representatives convened at a special 3-day meeting to discuss the potential for collaborative international research. At this meeting, staff also presented a summary of previous and ongoing projects to evaluate the status of oral research output of various countries and institutions.

Staff also developed and coordinated the annual research planning and briefing sessions with the Director, NIH; and prepared materials for the ad hoc Committee on Biomedical Research Funding.



Evaluation. Several evaluation research projects were completed by staff in FY 1989. The first was an assessment of relationships among the NIH, industry and academia in restorative dental materials research. The second developed a comprehensive data base of 1981-1984 research publications included in MEDLINE that acknowledged financial support from the NIDR or were performed by NIDR intramural staff, and analysed publications according to specific NIDR subject area, journal, and location of authors (i.e., extramural, intramural, or combination). The third project developed a CRISP keyword system to identify NIH behavioral and social science research projects; determined all such grants, by BID, that were funded by the NIH between 1980-1981; extracted all 1983-1984 papers in MEDLINE which acknowledged support from the NIH grants; and analysed the distribution of NIH-supported behavioral and social science publications by journal, BID, and both of these variables combined.

Two new NIDR set-aside evaluation projects were approved by the NIH and Assistant Secretary, Planning and Evaluation, DHHS, for start in late FY 1989 or early FY 1990. The first project will develop a data base of research and professional accomplishments of individuals supported by the NIDR during research training through the NRSA and Research Career Development mechanisms, as well as a sample of dental researchers who did not receive NIDR funding. This information will provide baseline data for future evaluations of the Dentist Scientist Award trainees. The second project will be used to initiate an ongoing monitoring system of international dental research performance by institutions and countries according to specific research areas from the NIDR Plan for the 90s.

Staff also developed the FY 1990/1991 NIDR Evaluation Plan.

Legislative Activities

Staff served as legislative contact for the Institute, attending meetings and coordinating NIDR responses to legislation and inquiries in areas that include AIDS, fetal tissue research, the use of animals in research, fraud and misconduct, executive salary increases, the human genome and NIH reauthorization legislation proposals.

Other Activities

Planning and Evaluation Staff (PES) worked with the Director, NIDR and the Budget Office in preparing the FY 1990 Congressional Justification narrative, opening statement and responses to Congressional questions for the record. Staff worked with the Director, NIDR in the preparation of oral presentations and articles before a variety of audiences. PES, working with intramural and extramural representatives, developed plans for and coordinated a workshop on "New Approaches to Differential Diagnosis of Chronic Orofacial Pain", held April 3-4, 1989 at the NIH. Proceedings will be published in 1990. PES reviewed documents for



"New Frontiers in Oral Health Research", prepared by the American Association for Dental Research under contract from the NIDR. Staff continued activities in association with staff of the Department of Veterans Affairs and the National Institute on Aging in developing plans for collaborative research and research training. PES also served as supervisor for four assignments by NIH Grants Associates in the areas of planning and evaluation; two presentations at the annual research session of the American Association for Dental Research resulted from these collaborations. Staff represented the Director, NIDR, at an invitational strategic planning conference on dental informatics, and served as leader of a workshop group at this conference on specific research issues in dental informatics. Staff successfully recruited a secretary during the year, and selected and supervised two dental students through the COSTEP (Commissioned Officer Student Training and Extern Program).

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

NIDR AIDS Task Force
NIH Evaluation Technical Review Committee
NIA/DVA/NIA Collaborative Project on Oral Health in Aging
NIH Animal Welfare Steering Committee
NIH Evaluation Course Planning Committee
National Cancer Institute Technical Review Committee

Presentations

Joan Wilentz

"Creating a National Research Agenda Addressing the Oral Health Needs of Special Care Patients," presented at the First Annual Conference of Special Care Issues in Dentistry, Chicago, May, 1989.

Summary and comments on "New Approaches to Differential Diagnosis of Chronic Orofacial Pain", a workshop sponsored by the NIDR which was held April 3-4, 1989 at the NIH.

James Lipton

"International Comparison of Biomedical Science Performance by Dental Researchers." Annual meeting of the American Association for Dental Research, San Francisco, California, March 1989.

"Identifying Outstanding Researchers in Dentistry." Annual meeting of the American Association for Dental Research, San Francisco, California, March 1989.

Chairperson and Discussion Leader, Behavioral Aspects Section, "New Approaches to Differential Diagnosis of Chronic Orofacial Pain", a workshop sponsored by the NIDR which was held April 3-4, 1989 at the NIH.

"Evaluating International Research in Oral Health and Diseases."



Workshop on International Collaboration for Oral Health Research,
Bethesda, Maryland, April 5-7, 1989.

"Evaluation Activities at the NIDR." Panel meeting organized by
the OD, NIH, concerning "Evaluation of the NIH-MEDLINE Bibliometric
Database," June 8, 1989.



Joan Wilentz

Special Assistant for Special Projects
Office of Planning and Evaluation/ OPEC

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

- o American College of Dentists Annual Meeting/Washington D.C.
The Impact of Advances in Research on Dental Education and Practice
- o ADA/FDI Joint 1988 World Dental Congress/Washington D.C.
Dental Science--Dental Health: 40 Years of Progress
- o ADA/FDI Joint 1988 World Dental Congress/Washington D.C.
Remarks: International AIDS Symposium
- o Royal College of Dental Surgeons of Ontario/Toronto
Re-Imaging Dentistry
- o NIDR Honor Awards Ceremony
Remarks
- o Goldman School of Graduate Dentistry, Boston University/Boston
Going for the Gold at the Silver Anniversary
- o Deans Meeting/Palm Springs, California
NIDR Plans for the Future
- o American Association for Dental research, New Jersey section/Newark
Research and Action to Improve the Oral Health of All Americans
- o Ohio State University College of Dentistry Research Day/Columbus
The Impact of Science on the Practice of Dentistry
- o House and Senate Appropriations Committees/Washington
Opening Statement and Highlights
- o Workshop on International Collaboration for Oral health Research/NIH
Remarks
- o Turner and Newall Lecture, University of Manchester/Manchester, England
The Changing Face of Dentistry
- o NIH Consensus Development Conference on the Oral Complications of Cancer Therapies: Diagnosis, Prevention and Treatment/NIH
Welcoming Remarks



- o Second World Congress on Preventive Dentistry/Beijing Medical University/
Beijing, China
Oral Disease Prevention in Research and Practice
- o University of Toronto Faculty of Dentistry/Toronto
Commencement Address
- o 1989 Kreshover Award Lecture
Remarks/Introduction and Presentation of Awards
- o Scandinavian Society of Periodontology/25th anniversary of the
Jysk/Fysk Society of Periodontology/Copenhagen
The Changing Face of Dentistry
- o Distinguished Speaker's Lecture/University of North
Carolina/Chapel Hill
The Changing Face of Dentistry
- o Dentistry in the 21st Century: A Global Perspective
Changing Disease Patterns: Their Impact on dentistry

Article and Brochures:

- o The Need for Technology Assessment in Oral Health Care-
Introduction to the oral health theme issue of the
International Journal of Technology Assessment in Health Care,
in press.
- o Provided design, text and captions for The NIDR: Exit the
Eighties; Enter the Nineties, distributed and discussed at the
NIDR forum at the 1989 AADR meeting in San Francisco.

Assistance to other Senior Staff and Consultants:

- o Preparation of Senate and House Reports on the Research and
Action Program for Improving the Oral Health of All Americans
(with Helen Gift)
- o Preparation of Report to the House of Representatives
Appropriations Committee on Oral Health Activities within the
Department of Health and Human Services (the Whiteside
Report).
- o Reviewed, with suggested edits, the manuscript for Dental
Science in a New Age by Ruth Harris, with particular emphasis
on chapters dealing with current oral health research.



PUBLIC INQUIRIES AND REPORTS SECTION (PIRS)

The Public Inquiries and Reports Section conducts a comprehensive information program using a variety of communications mechanisms. Research advances in the oral health sciences are shared with the public, the Congress and the dental profession through the development and distribution of patient and professional education materials, publications, exhibits, scientific reports, films, and extensive interaction with the trade and lay print and broadcast media.

Anniversary Projects

The Institute's 40th anniversary celebration came to an end during FY 1989. PIRS continued its role as principal planning and coordinating office for the commemorative functions. Staff successfully coordinated the completion of a major documentary film on dental science. Negotiations were initiated to secure broadcast of the film on NOVA. Entitled "The Changing Faces of Dentistry," the program is tentatively scheduled to air in late 1989 or early 1990.

Plans are under way in PIRS for disseminating the NOVA film to school systems, dental schools and dental societies, as well as for the production of a half-hour version of the program. Staff is currently planning the development of materials for schoolchildren which will be available in conjunction with the film.

PIRS organized an NIH Public Affairs Forum, "Behind the Scenes at NOVA," featuring NOVA executive editor William Grant. Staff also arranged meetings with Grant and the NIH Director and staff to explore possibilities at NIH for future scientific public programming.

Staff prepared a major commemorative Institute publication, "Dental Science--Dental Health: NIDR at 40," featuring articles on NIDR history and current research advances. The booklet was distributed at the World Dental Congress held in Washington, D.C. in October 1988 and has been used as model by other NIH Institutes.

PIRS planned and carried out a variety of projects in conjunction with the World Dental Congress. Staff, in cooperation with the U.S. Postal Service, arranged for a special postal booth and commemorative cancellation at the meeting site. PIRS coordinated a symposium, "Dental Science--Dental Health: 40 Years of Progress; arranged for a viewing of the dental research documentary; and conducted tours of the NIH facilities for approximately 100 meeting attendees. As part of their tour, participants heard a guest lecture on dentistry in stamps and visited a companion exhibit. The stamp exhibit, also coordinated by PIRS, was displayed through October in the NIH Clinical Center.

In conjunction with the Institute's 40th anniversary, staff researched, gathered historic photographs, wrote copy and designed an exhibit on "Dental Science for Dental Health." The historical exhibit, covering approximately 300 square feet, was displayed at the Smithsonian Institution's National Museum of American History from October through December 1988. The exhibit



highlighted 40 years of dental research that has significantly improved oral health or that holds future promise. Many of the attendees at the meeting of the World Dental Congress had an opportunity to view the display. Staff also participated in negotiations to secure museum facilities and to plan and conduct a reception marking the opening of the exhibit. The reception was attended by over 2,000 persons, including many foreign visitors. PIRS later arranged for this exhibit to be displayed on the NIH campus.

Following completion of the final draft, staff oversaw the publication and distribution of the NIDR history monograph as a hardback book. Dental Science in a New Age was written by a professional historian in conjunction with the Institute's fortieth anniversary.

New Initiatives

Other special projects carried out in FY 1989 included the planning and design of two new exhibits for display at professional meetings. An exhibit entitled "Research and Action Program--Initiative for the Nineties" was developed for use at the American Association of Dental Schools/American Association for Dental Research meetings held this year in San Francisco. The display was designed to familiarize dental scientists and practitioners with a new NIDR-initiated program aimed at improving the oral health of adults and older Americans.

A second exhibit, "Fostering International Collaboration for Oral Health Research," was prepared for the International Association for Dental Research (IADR) meeting in Dublin, Ireland. This display reflects the joint efforts of the NIDR and the World Health Organization in improving global oral health status.

A member of PIRS staff served on a steering committee to plan and organize a special 3-day conference in Phoenix, Arizona, entitled "Exploring Tomorrow's Frontiers". Targeted to native American students, the conference focused on career opportunities for minorities in biomedical research. Staff provided public information support, staffed an exhibit, and attended meeting sessions.

PIRS drafted copy for a brochure to serve as an introduction to the Institute's new "Teeth for Life" program. Aimed at adult and older Americans, this campaign will focus on maintaining the natural dentition for a lifetime.

Members of PIRS staff attended a joint symposium on fluoride sponsored by the IADR and the European Organization for Caries Research. The international meeting, held in Pine Mountain, Georgia, was the first of its kind in 13 years. Approximately 50 scientists presented lectures related to the mechanisms of action, effectiveness, and recommendations for use of fluorides.

Public and Professional Education

During this fiscal year, PIRS carried out the following activities in the areas of public and professional education: directed preparation of press summaries on NIDR-supported research advances for the AADR annual meeting in San Francisco and the IADR general session in Dublin; prepared bimonthly



issuances of the "NIDR Research Digest" for inclusion in the IADR newsletter; provided articles for publication in the NIH research advances section of the Journal of the American Medical Association; and contributed articles on recent research advances to the Journal of the American Dental Association for the continuing NIDR series.

Staff prepared background material on NIDR AIDS research activities for a special issue of the Journal of the American Dental Association, and developed an article for nursing oncology journals on the oral complications of cancer therapies.

PIRS staff served on the planning committee for the NIDR Consensus Development Conference on Oral Complications of Cancer Therapies: Diagnosis, Prevention, and Treatment, and arranged for all publicity for both the profession and the public. These activities included the development of posters, tent cards and registrations forms; preparation of "Federal Register" and "Calendar of Events" notices, and "NIH Record" story; notification of the media for the press conference, and facilitating media access to conference participants. In addition, staff assisted in editing the consensus statement and coordinated administrative aspects of publication of the proceedings. This included negotiating and gaining approval to produce the proceedings as a Journal of the National Cancer Institute monograph, and coordinating mailing of the proceedings to ensure that it reaches target professional audiences.

Staff planned, coordinated and directed all arrangements for the 1989 Seymour J. Kreshover Lecture, "Pathogens, Probes, and Perception: The Story of Multidisciplinary Oral AIDS Research," delivered by Drs. Deborah and John Greenspan of the University of California, San Francisco. The lecture also included a preview of the upcoming NOVA documentary, "The Changing Faces of Dentistry." Arrangements were made for the lecture to be videotaped and distributed to all dental schools in the United States.

PIRS also coordinated the program, publicity and reception for a symposium entitled "Mineralized Tissues: Through a Looking Glass" held at NIH in spring 1989.

A story on the availability of DENTALPROJ, a new database developed by the NIDR, was prepared by PIRS and published in the NLM Technical Bulletin. The article alerts readers to the fact that DENTALPROJ is now online and is accessible through MEDLINE.

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

Staff assisted in publicizing industry and minority meetings held at NIH as part of the planning process for NIDR's Long-Range Plan for the 1990s.

PIRS exhibited at the annual scientific sessions of the American Association of Dental Schools, American Association for Dental Research, International Association for Dental Research, National Dental Association, American Public Health Association and the American Dental Association.



During this fiscal year, staff prepared articles on dental health for publication in the syndicated "NIH Search for Health" column, contributed regularly to the "NIH Record" and "NIH News and Features," and continued periodic publication of "NIDR Research News."

In related activities, PIRS staff provided background material for articles on NIDR research efforts to professional journals and health-oriented publications including Medical World News, Health and You, Dental Management, American Health Magazine, Dental Health Advisor, ADA News, American Society of Microbiology News, Pharmacy Practice News, and Encyclopedia Britannica.

Staff provided assistance to the Centers for Disease Control in the final editing of "Complications of Diabetes Mellitus: Periodontal Disease," a chapter in a new primer for physicians about the prevention and management of the long-term consequences of diabetes.

Publications

PIRS developed a handbook for new members of the National Advisory Dental Research Council during this fiscal year. Also in progress are patient education pamphlets on temporomandibular joint dysfunction and the oral effects of smokeless tobacco.

The following publications were updated and reprinted in FY 1989:

"Graduate Training Supported by the National Institute of Dental Research,"
"National Institute of Dental Research: Research Training and Career
Opportunities in the Dental Sciences,"
NIDR, Extramural, and Intramural brochures.

Media

During FY 1989, PIRS arranged interviews and provided background information on dental research topics to numerous general circulation magazines such as Parents' Magazine, Washingtonian, Working Mother, Self, and Reader's Digest; to the trade press; and to a variety of major metropolitan newspapers, news magazines, and wire services, including the New York Times, Washington Post, Los Angeles Times, Baltimore Sun, USA Today, Cincinnati Inquirer, Parade, Associated Press and Science News Service. Staff also handled information requests from broadcast media, including WUSA's Health and Science Show, Medvision, Cable News Network and local network news.

Staff directed a major press conference on the subject of low sealant use in the United States at the San Francisco meeting of the American Association for Dental Research. PIRS prepared a press kit and a video press release which detailed the study findings and was broadcast nationally via satellite. Both print and broadcast media featured the story throughout the country.

PIRS prepared features for the press on NIDR-supported research which focused on dental implants, use of electroporation to genetically engineer bacteria, and the development of a transgenic mouse model for HIV infection, as well as a historical perspective on the work of NIDR's Laboratory of Developmental Biology and Anomalies with basement membranes and laminin.



Reports

PIRS contributed to four Special Reports to Congress on scientific activities carried out in FY 1988. These included research advances in the following areas: human immunodeficiency syndrome, diabetes, maternal and child health, and international activities. Staff also reviewed final galleys for the NIDR Director's Report for the NIH Biennial Report, 1987-1988.

General ~~Comm~~unications Activities and Services

In FY 1989, PIRS responded to approximately 8,000 requests for general information on a broad range of topics from the public, professionals, Congress and the media, and distributed over 400,000 publications.

PIRS handled a large volume of press inquiries on water fluoridation and controversies arising out of the 1986-87 children's oral health survey. This effort involved numerous telephone interviews and background information sessions with reporters from the New York Times, Associated Press, United Press International, Washington Post, Boston Globe, Atlanta Constitution, ABC Radio, Pittsburgh Press, the Today Show and others. PIRS prepared an Institute statement reaffirming the efficacy of this anti-caries measure.

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

PIRS continued to oversee a contract with a clipping service to monitor NIDR-related research activities reported in the press. Staff also initiated a contract with a video monitoring service to retrieve NIDR-related news items that appear on television across the country. Negotiations were successfully concluded which established an exhibit services contract for storage, shipping and handling, and related needs for meeting services.

In FY 1989, PIRS coordinated development of numerous responses to congressional and controlled correspondence for the Director and other DHHS officials.

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

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

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



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

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

Personnel Activities

Robert Kuska, a graduate of the Master's program at the Northwestern University School of Journalism, completed a two-year assignment as an information intern in the Public Inquiries and Reports Section.

Kristen Kennedy, a student at Bucknell University, performed a variety of assignments as an information intern during breaks in the school year.

Awards

Brent Jaquet, Chief, PIRS, received the NIH Merit Award in FY 1989 for exceptional leadership in developing a positive, assertive communications program for the NIDR.

Staff of the Public Inquiries and Reports Section was presented a Group Cash Award for an extraordinary team effort in developing, managing and successfully implementing a year-long program of activities in conjunction with the Institute's 40th anniversary.

Publications

Sheridan, P. NIDR--40 years of research advances in dental health. Public Health Rep 1988; Vol. 103, No. 5:493-499.

Sheridan, P. Dental caries continues downward trend in children. J AM Dent Assoc 1988;117:625.



The research and management information needs of the Institute continue to be the primary mission of the Research Data and Management Information Section. One of three sections in the Office of Planning, Evaluation, and Communications (OPEC) in the Office of the Director, NIDR, this section concentrates on the collection, retrieval, and reporting of data related to dental research and its management. Working closely with the Division of Research Grants, the Grants Management Section and the Contracts Management Section of the Extramural Program; intramural scientists in the Intramural Research Program and the Epidemiology and Oral Diseases Prevention Program, and the Financial Management Section, data are collected on all active supported projects, and reports are generated to reflect the substantive and fiscal posture of the Institute. Computers, computer programmers and information scientists facilitate the processing and delivery of this information to its many users for a variety of purposes, some of which are described in the following paragraphs.

Technical Reports

The recurring need for certain kinds of information and the need for an easily accessible archival record of such, prompted the development of several printed technical reports on a fiscal year basis. The *National Institute of Dental Research Programs* is a comprehensive collection of charts and tables which list and display, in a variety of formats, all of the projects supported by the Institute. The *NIDR Indexes* provide information about these projects by subject using the Computerized Retrieval of Information on Scientific Projects (CRISP) thesaurus of terms. Reports resulting from contracts are frequently not published in the scientific literature and may only be identified through our *Selected List of Technical Reports*. *Trainees and Fellows Supported by the National Institute of Dental Research* is extremely difficult to compile but provides the only hard-copy record of that very important area of support. And finally, the *Annual Report*, required by the National Institutes of Health, and its companion, the *NIDR Code Book*, provides us with not only a yearly record of activities but is the source of data elements on projects that are essential to the information needs of the Institute.

NIDR ONLINE

The NIDR ONLINE system, introduced in 1986 as an experiment to improve the transfer of information from the NIDR to its constituency, had grown to over 230 registered users. Changes at the Division of Computer Research and Technology (DCRT), which controls the mainframe computer used in this project, allowed us to convert the system to a public database making it easier for users to access and eliminating more of the administrative functions that accompanied the original system.



DENTALPROJ

After nearly two years of planning, programming and testing, the DENTALPROJ file is now available to the public through the National Library of Medicine's MEDLARS system. DENTALPROJ contains summaries of ongoing dental research projects supported by the National Institutes of Health. It is unique in that it allows the scientific community to access information while the research is in progress, before results are published and otherwise available. This system provides the user with a variety of information, including who is currently performing the research (principal investigators), where the research is being conducted (performing institutions), and what the research is actually about. Each research summary is indexed using MeSH (Medical Subject Headings) by specially trained scientific indexers. These indexers evaluate each project and assign terms which describe the nature of the research. This detailed level of coding is extremely beneficial to RDMIS, for it gives us the ability to perform in-depth subject searches in response to the many inquiries we receive. The next implementation phase of DENTALPROJ will include the addition of projects sponsored by the Veterans Administration and the Department of Defense.

FEDLINK

As a member of the Federal Library Information Network (FEDLINK) operated by the Library of Congress, we are able to utilize several commercial information vendors like BRS, DIALOG, and PAPERCHASE at reduced rates and by means of an interagency agreement. DIALOG is the more versatile of the three with access to nearly 300 databases in a broad scope of disciplines containing in excess of 152,000,000 records. PAPERCHASE, though limited to MEDLINE, is more popular at NIDR because it is menu driven and requires little or no training to use.

Slide Production

The addition of the PCR/Matrix slide production equipment has produced considerable saving this fiscal year. An average of about 150 slides per month at a cost of \$.32 cents per slide versus \$ 10.50 per slide using an outside vendor equates to a savings to the NIDR of about \$ 18,000. This amount more than covers the cost of the equipment in the first year.

Before purchasing our own slide production equipment, there were only two alternatives for slide production. One of those alternatives, the NIH Graphic Arts department, was very expensive, labor intensive and slow. The other alternative was producing the slide, via the Harvard Graphics software, on a personal computer and providing a diskette to a local vendor at a cost of \$ 10.50 per slide. Now, a relative novice can use the menu driven Harvard Graphics package to design a chart and save it on a diskette. After giving the diskette to a member of the RDMIS staff, slide production can take place in a matter of minutes.



Budget Reports

Due to extensive changes in the NIDR Common Accounts Structure (CAN) and reorganizations, the budget reports produced for management have undergone extensive computer code revisions. These reports, which employ the Division of Financial Management (DFM) Allotment Ledger Master File (ALMF) data, provide monthly status of funds information. These reports aid NIDR management in making prudent budgetary funding decisions. Other changes incorporated include the addition of a separate report to show all NIDR reimbursable activities and the exclusion of reimbursables from all of the other nine reports.

Improved Printing Capabilities

The use of a Hewlett Packard Laserjet series II printer coupled with WordPerfect Version 5.0 and Bitstream font generation software has added a new dimension to high quality printing within the RDMIS. The use of the Bitstream package to produce "soft" fonts can then be directly utilized by WordPerfect to produce print shop quality flyers, announcements, invitations etc. at substantial savings in time and money. Prior to development of these techniques, it took a considerable amount of planning to insure that document printing requests would be returned from the print shop in a timely manner. High quality originals can now be produced and copied on a standard copier in minutes.

Local Area Network (LAN) Activities

With the installation of the NIH OD LAN proceeding, RDMIS, along with other elements of the NIDR, are giving thought to possible installation of an Institute LAN. As a first step in this process, we will be developing forms for conducting an inventory of all personal computer hardware and software. This inventory will be collected and entered into a Dbase IV database which will be used to produce reports to aid in determining LAN requirements.

Personnel Changes and Employee Related Activities

At the end of August, Dr. Kenneth C. Lynn, Chief of the Section, was retired and Sheldon A. Fishman, who joined us in June was selected to be the new Chief. Mary Eileen Lukes, who had been detailed to the Section as a data transcriber, was officially transferred in January.

A member of the RDMIS staff, who also serves as an Information Resources Management (IRM) representative, has been appointed a member of a new committee organized by the Division of Research Grants (DRG). One of the first tasks of the group will be to consider how the DRG IMPAC system can be successfully migrated into a Database Management System (DBMS) environment.



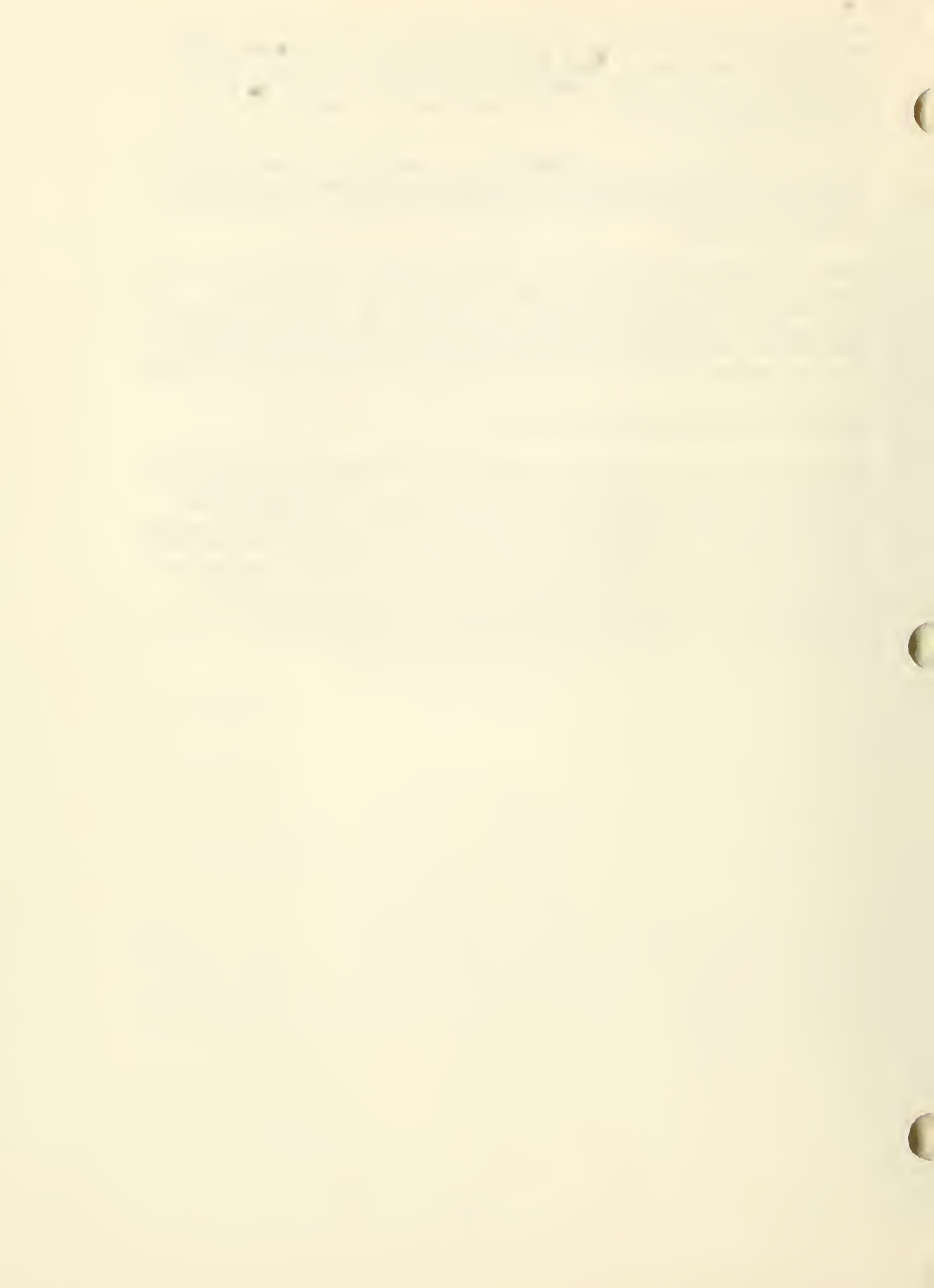
As a part of this effort the MITRE corporation has tentatively been awarded a \$ 99,996 contract to review available DBMS software packages and provide, among other objectives, a requirements analysis to DRG. The NIDR along with the other NIH B/I/Ds will be contacted by the contractor to assess their various requirements to insure continuity within a DBMS structure.

Section staff members represent NIDR on a number of NIH coordinating groups including the ADP Systems Planning, ADP Systems Security, ADP/ Extramural, Handicapped Employees, Office Technology, and Information Resources Management.

Special recognition was given to Carla Flora by the Division of Research Grants for her development of the S-CRISP program; to Mary Ann Williamson for assisting the panels developing new research initiatives for the NIDR Long Range Plan for the Nineties; and to Ronald Ruben for his part in an information systems study for the National Center for Nursing Research, and an Information Resources Strategic Plan for NIDR.

Freedom of Information Act/Privacy Act

The Section Chief serves as the Freedom of Information Act (FOIA) and Privacy Act (PA) coordinator for the Institute. All requests of this nature are routed through this office for purposes of tracking and distribution to the program areas responsible for the records requested. The NIDR FOIA/PA Coordinator is the official liaison with the NIH FOIA or PA officers who interpret the regulations and policies and are the only ones who have the authority to deny the release of sensitive information. The Institute receives about 30 such requests each year which can vary in size from a few to hundreds of pages, much correspondence, and many hours of program staff time.



FINANCIAL MANAGEMENT SECTION (FMS)

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

NIDR Budget

During FY 1989, the FMS assumed full responsibility for the formulation of the Institute's budget request for FY 1991 by developing the initial budgetary levels; preparing justifications and statistical materials that supported the request; and apprising the Institute Director of changes made through negotiations as the budget was transmitted through NIH, PHS, the Department, and OMB.

Because of the overlap in the budget cycles, the FY 1991 budget was negotiated as the FY 1990 budget request was reviewed by the Congress for legislative enactment. If approved, the FY 1990 request will be incorporated into an appropriation bill. As part of this budget process, FMS staff accompanied the Director to the formal congressional appropriations hearings and provided additional justification materials to the Committee as needed. After action on the request by both houses of Congress, the FMS prepared statements that summarized the effect of congressional action on the Institute's budgetary operations and program goals for FY 1990.

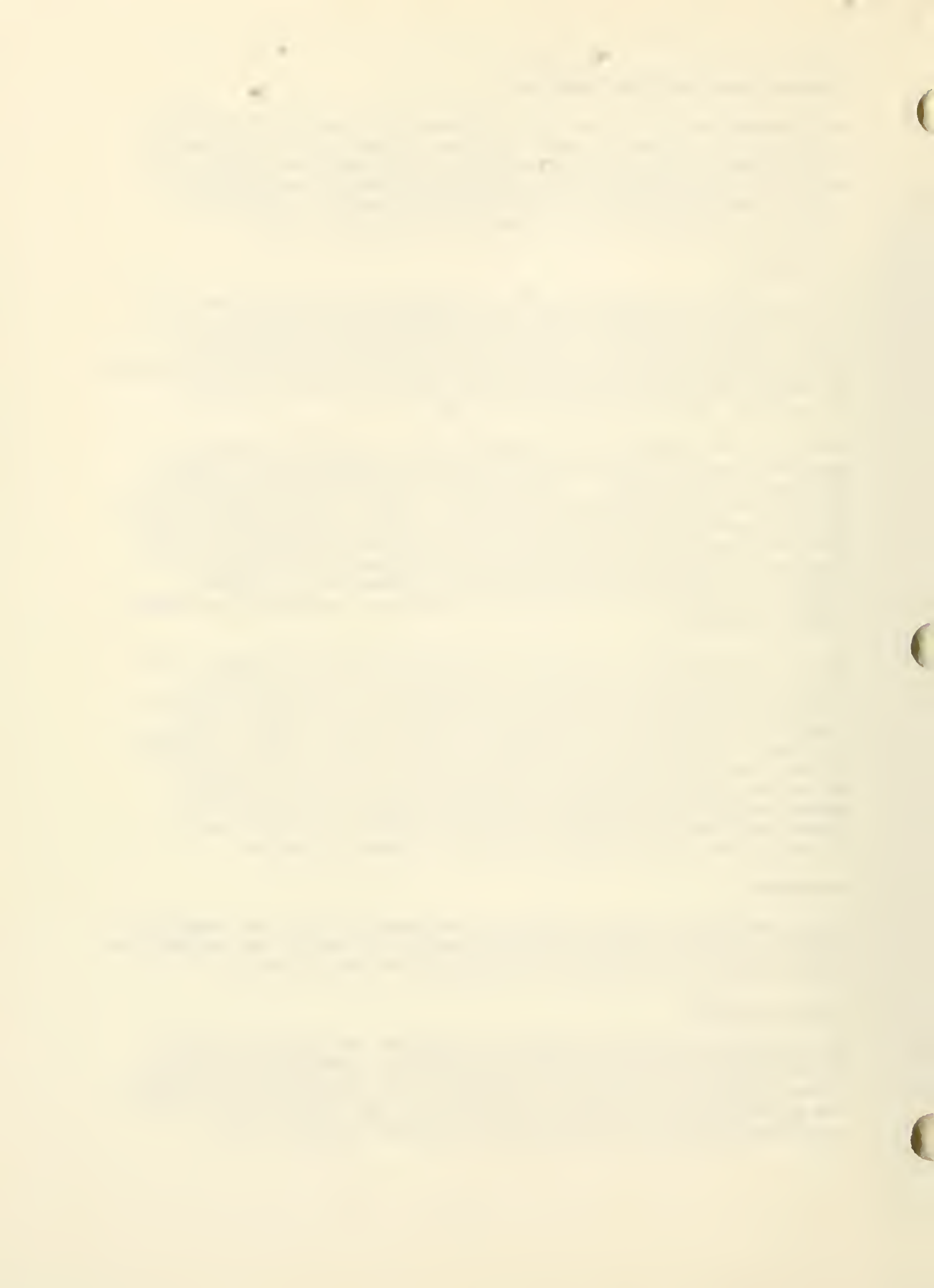
While the formulation and legislative processes were in progress, FMS staff monitored grant, contract, intramural, and direct operating expenditures for FY 1989 and provided managerial and financial advice regarding the Institute's extramural and intramural programs. When necessary, they worked with Institute administrative staff to determine if reprogramming actions would be necessary. To facilitate ease of operations, the FMS undertook a complete overhaul of the Institute's budget activity and management accounts structures. The FMS also apportioned funding by quarter to support planned activities and, at the end of the year, prepared the actual obligation reports.

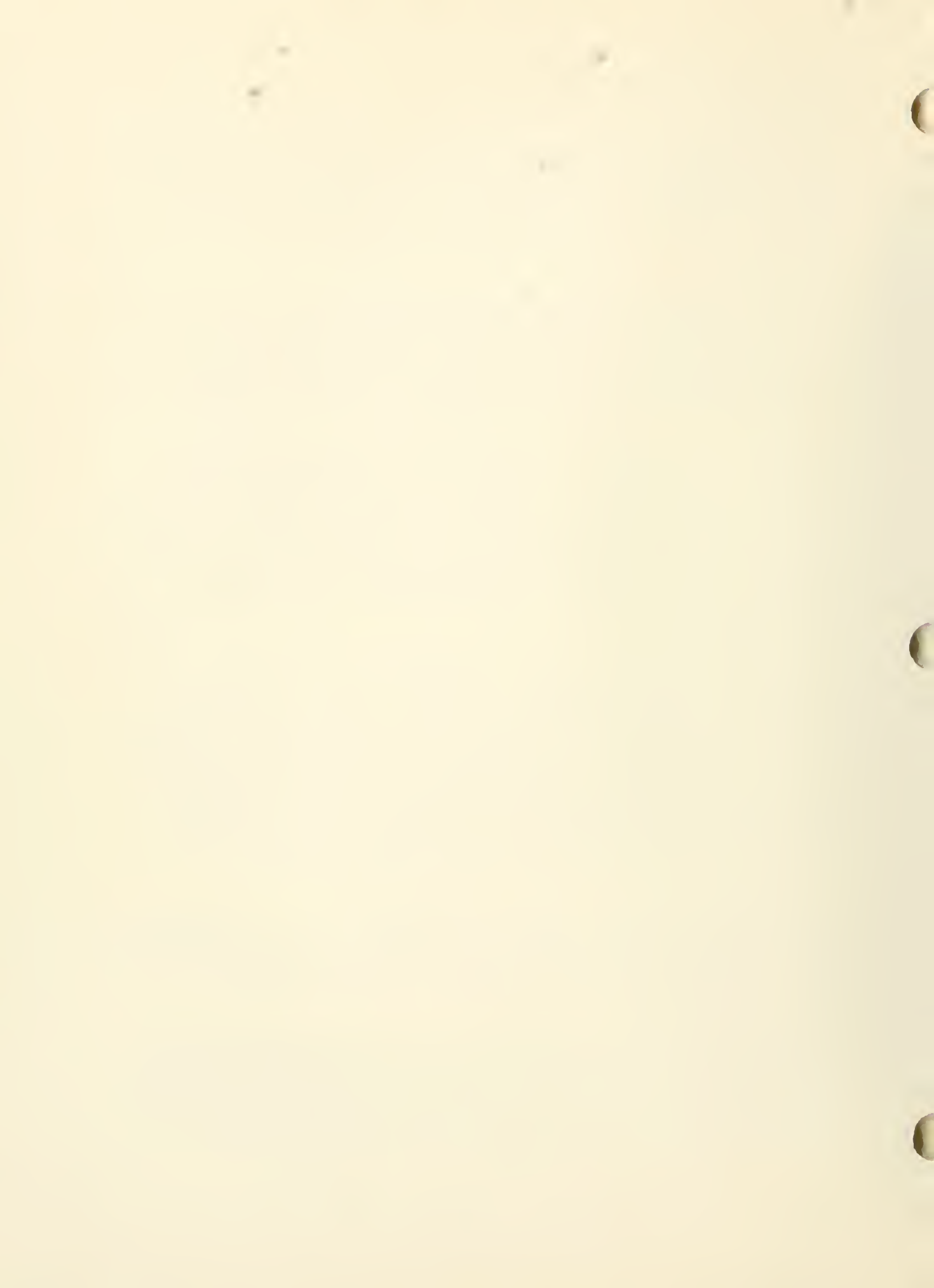
Automation

During this fiscal year, acquisition and installation of new computer work stations for all FMS staff were completed. Several new computerized programs have been developed to facilitate the budget process.

Other Activities

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





PERSONNEL MANAGEMENT SECTION (PMS)

The Personnel Management Section (PMS) is the focal point for Institute civilian and Commissioned Corps personnel management activities including staffing, placement (including merit promotion), classification, pay administration, employee relations, and employee development and training.

Reorganizations

Several minor reorganizations/realignments took place during FY 1989. In the Intramural Research Program, several units and sections were established, and in the Extramural Program, the Caries and Restorative Materials Research Branch was renamed to include salivary gland research in its title -- the new title is Caries, Restorative Materials and Salivary Research Branch.

Staffing and Recruitment

Staffing and recruitment activities received considerable attention again this year. The retirement of the Director, Extramural Program, necessitated a Senior Executive Search for a replacement, while in the Intramural Research Program, recruitment activities for several branch chief positions took place.

Other major staffing issues on which the PMS staff provided information, comments or guidance concerned the changing policies of the PHS Commissioned Corps, particularly those involving senior officers, research investigators and promotional opportunities; the increasing delegations of authority from the Office of Personnel Management (OPM) for the examination and direct hire for many civil service occupations, as well as the elimination of examinations for some positions; the direct hire authority of high quality college graduates into entry-level administrative positions; and establishment of several innovative programs for combined training/employment of pre- and postdoctoral fellows in intramural research.

Classification and Pay Administration

The PMS staff carried out its regular classification program throughout the year. Several special classification studies were undertaken and satisfactorily concluded. In the area of pay administration, the staff reported, once again, on the recruitment and retention of several groups of employees. Information was provided regarding general salary levels, salary levels for the special rate categories (clerical/technical positions where typing, stenographic or dictating machine transcribing skills are involved and some dental patient care positions), and pay rates for senior level scientific and managerial positions.



Employee Relations

With the change in administrations, much attention was focused on ethical conduct of federal employees and, in particular, on the Department's Standards of Conduct. The PMS staff worked closely with other NIH offices to gain a clearer understanding of the complex regulations, and was responsible for assuring that NIDR employees were made aware of the Standards and its requirements.

Awards

The Institute continues to have an active awards program, as its managers and supervisors nominate staff for recognition for both NIDR and other incentive and honor awards. As a result, some 70 staff members were recognized at the Annual NIDR Awards Ceremony in October. Staff were recognized for special acts or service, high quality work performance, and for their special or professional contribution to the mission of the NIDR, NIH, PHS or DHHS.

The PMS staff worked closely with NIDR Budget Managers to implement the new Departmental performance award system for EPMS staff. The new system was operational for the first time in 1989 for performance during 1988. The new system gave supervisors the opportunity to consider employees for two types of awards for performance--a quality increase or a performance award, with the quality increase considered NIDR's highest form of recognition for performance. The Budget Managers met in the Spring to consider Institute employees for both types of awards, and to authorize the EPMS performance awards, which were paid in July.

EEO Activities

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

Professional Activities

PMS staff continued their participation in trans-NIH personnel activities such as career and job fairs, personnel operations workshops, the qualifications review, interview and selection process for the NIH Career Curricula program, service on other qualifications review boards, participation on several workgroups to improve personnel management operations, as well as continuing membership and active participation in the International Personnel Management Association.



EQUAL EMPLOYMENT OPPORTUNITY PROGRAM

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

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

Discrimination Complaints

The Institute had two formal discrimination complaints outstanding in 1989. The EEO Manager and Counselor continue to provide, on an as needed basis, career counseling, guidance on job applications, training opportunities, and problem-solving in supervisor/employee relations.

EEO Advisory Committee

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

During 1989, the Committee invited guest speakers (three) to various meetings to discuss safety, animal care, and building maintenance (areas of major concern to NIDR staff). In addition, the Committee nominated to the Director one outstanding employee for the NIDR EEO Special Achievement Award.



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

Recruitment and Awareness

The EEO Manager continues to identify and communicate with minority, women, and handicapped organizations and associations concerning our mission and activities. This office, in conjunction with NIDR Handicapped Advisory Committee Representatives, participated in the National Symposium on Perspectives on Employment of Persons with Disabilities and the Annual Meeting of the President's Committee on Employment of the Handicapped for networking and for discussing dental research.

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

The EEO Manager, in an effort to stimulate interest in educational opportunities at the Office of Personnel Management (OPM) and the Department, conducted a mass mailing to NIDR employees in grades 11/12 on two excellent training programs. As a result of this effort, one employee submitted an application and was accepted to OPM's Women's Executive Leadership Program.

Staff also participated in two special workshops--Windmills and Perspectives: AIDS in the Workplace--on handicap awareness sponsored by the Division of Equal Opportunity.

Staff exhibited at the 46th Joint Annual Meeting of the National Institute of Science Beta Kappa Chi Scientific Honor Society and the Brookhaven Semester Program in Nashville, Tennessee. The meeting provided an opportunity for networking and discussing research training opportunities at NIDR.

The EEO Manager represented NIDR at a Symposium on Career Opportunities in Biomedical Sciences in Long Beach, California. The symposium was sponsored by Morehouse Medical College and the Association of Minority Health Professional Schools. These groups are attempting to help reverse the trends of underrepresentation of minorities in the biomedical sciences.

Staff participated in a national symposium sponsored by the Indian Health Service and NIH in Phoenix, Arizona. The purpose of the symposium was to familiarize high school, college and graduate students, particularly Native Americans and Alaska Natives, with the various career paths and programs of support in biomedical disciplines.



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

Minority Access to Research Careers Program Minority Biomedical Research Support Program

Through cooperative agreements with the National Institute of General Medical Sciences and the Division of Research Resources, the NIDR supports components of the Minority Access to Research Careers (MARC) and the Minority Biomedical Research Support (MBRS) Programs that relate to the overall mission of the Institute. Staff participated in the annual MBRS Symposium held in Los Angeles, California and the annual MARC Conference held in Washington, DC. The two events provided an opportunity for staff to discuss research training opportunities at NIDR with faculty and students. Moreover, the Institute sponsored five MARC students for a summer research training experience in NIDR laboratories.

Community Outreach

The EEO Office continues to provide certain minority institutions (9) with the Institute's surplus scientific materials. This service has been expanded to include a high school in Montgomery County and 27 minority colleges/universities.

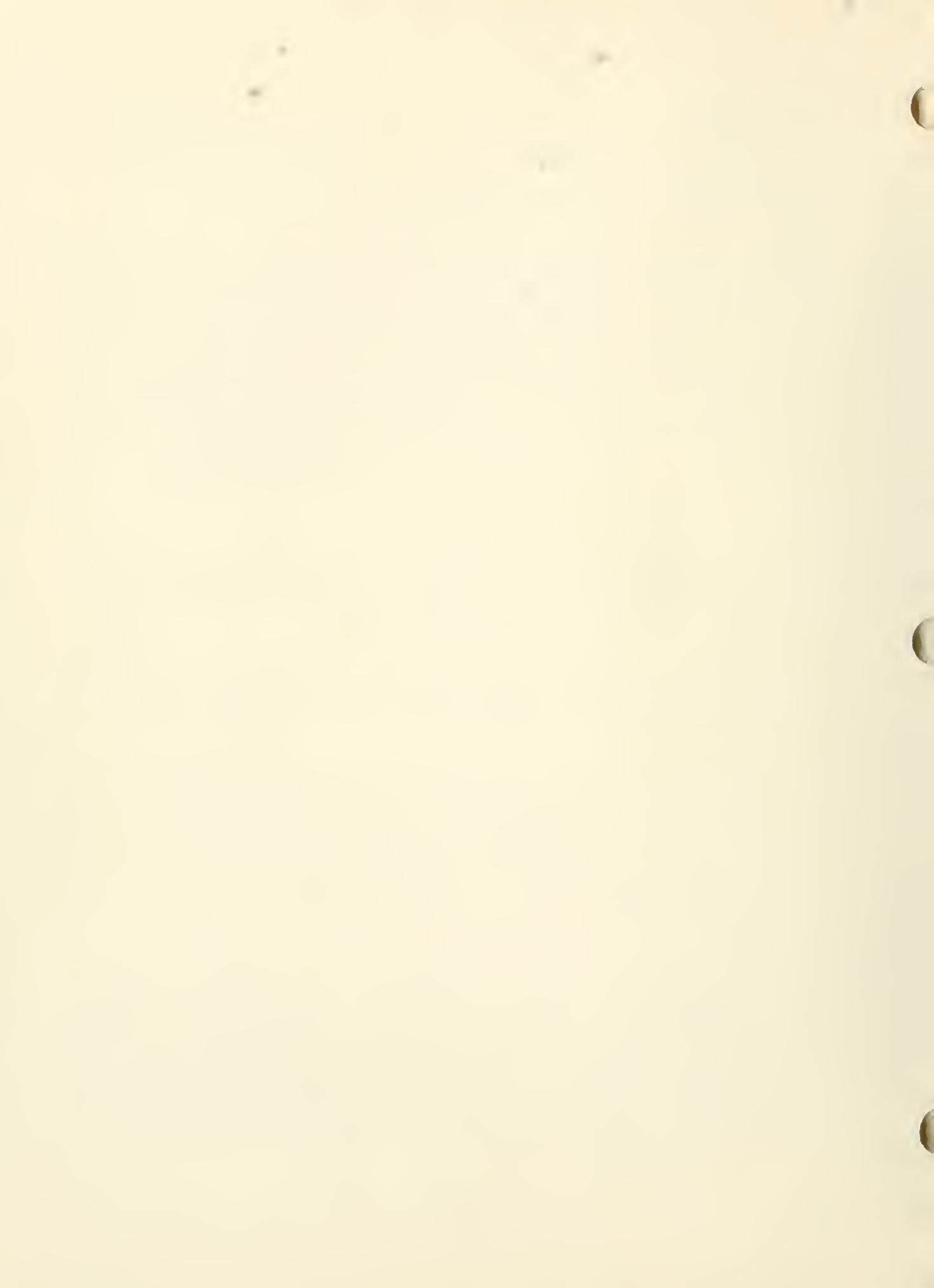
Two employees from the Intramural Program and the EEO Manager represented the NIDR as special judges in the District of Columbia Annual Science Fair. Four outstanding students were recognized for their excellent projects in the Annual Science Fair.

Staff have also interacted with science teachers at Montgomery Blair High School in Silver Spring, MD. The school has an excellent science program and numerous minority students interested in scientific research and the life sciences. To increase their awareness on biomedical research, particularly at NIH, a seminar was arranged with the students and a minority scientist from NIH.

Civil Rights

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





INTRAMURAL RESEARCH PROGRAM





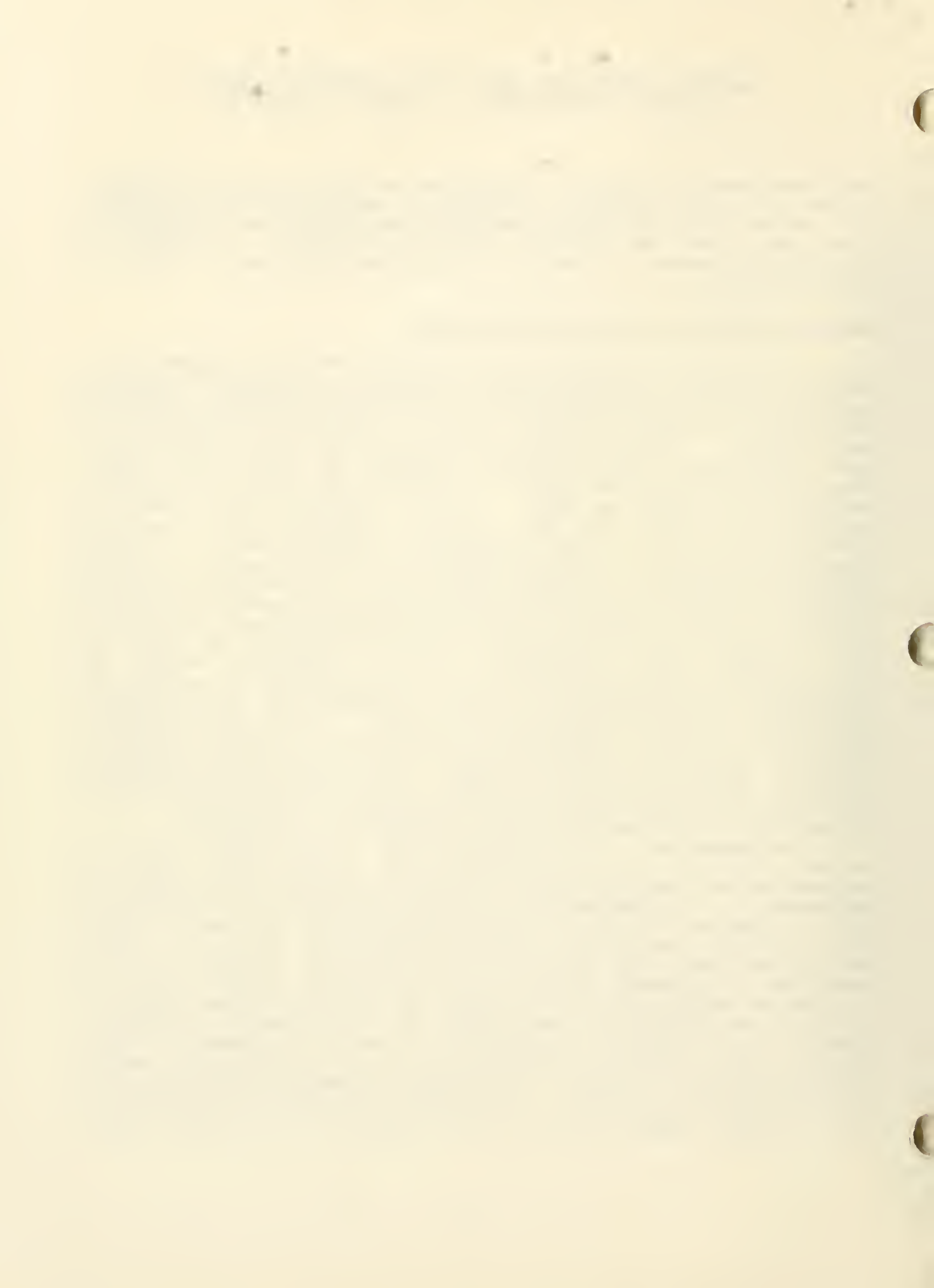
ANNUAL REPORT OF THE BONE RESEARCH BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

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

Mineral Chemistry and Structure Section

In the Mineral Chemistry and Structure Section, headed by Dr. Edward D. Eanes, artificial lipid vesicles (liposomes) continued to be used as a model system for investigating mineral deposition processes in matrix vesicles, nuclei for initial calcification in many tissues. Studies conducted during this fiscal year suggest that cholesterol, an essential component of vesicle membranes, could play an important role in the calcification of these extracellular structures in vivo. It was found that high levels of cholesterol (>35%) in the lipidic membrane of liposomes effectively blocked calcium phosphate precipitation in their aqueous interiors. This inhibition appears to result from the inflexible cholesterol molecule imposing a rigid ordering of other membrane constituents, thereby preventing transmembrane transport of the Ca ions necessary for intraliposomal precipitation to occur. This rigidity in structure may also account, in part, for our finding that cholesterol had the opposite effect on extraliposomal precipitation, i.e. it increased the propensity for liposomes to spontaneously initiate precipitation in surrounding metastable calcium phosphate solutions. The most probable explanation for this latter result is that by decreasing membrane fluidity, cholesterol helped stabilize potential seeding sites on external surfaces of the liposomes. These contrasting findings point up the complex manner by which membrane constituents such as cholesterol may influence in vivo calcification processes.

The expansion of the mineral front from a matrix vesicle milieu occurs in an extracellular environment rich in macromolecular complexes such as collagen fibers and proteoglycan aggregates. A collaborative study was undertaken this year with Dr. V.C. Hascall of the Proteoglycan Chemistry Section to assess the possible influence that proteoglycans (PG) and their constituent parts may have on this expansion process. It was found that PG monomers dissolved in the external solution phase of a liposome suspension had no effect on apatite crystal formation in the interior aqueous compartments of the liposomes themselves. However, the monomers significantly slowed the spread of these endogeneously produced crystals into the suspending medium surrounding the liposomes. Additional studies with chondroitin sulfate showed that the PG glycosaminoglycan (GAG) components were most probably responsible for this inhibition. The data suggest



further that the slowdown was not the result of GAG restricting diffusion of the reactant ions through the medium but instead suggest that the GAG molecules physically covered growth sites on the crystals, preventing reactant ions from incorporating into the crystal lattice at these sites. These results suggest that PGs may play a significant role in controlling extracellular mineral growth in vivo.

Collaborative studies with members of the American Dental Association and Dental/Medical Materials Groups at the National Institute of Standards and Technology continue to represent an important part of the Section's research effort. The collaborative study with M. Markovic and W.E. Brown of the ADA on the formation of octacalcium phosphate (OCP) - carboxylate double salts was extended during the fiscal year to include 17 mono- and polycarboxylates. Nine of these seventeen carboxylates formed double salts, eight did not. The data suggest that polycarboxylate incorporation in OCP depends upon the anions having the proper stereochemistry for replacing acid phosphate groups from the hydrous interlayer of the OCP structure. Another collaborative study (with J. Antonucci and S. Venz of the Dental/Medical Materials Group) focussed on the development and use of calcium metaphosphate ($\text{Ca}(\text{PO}_3)_2$) as a possible filler for composite structures used in dental and medical materials applications.

Protein Biophysics Section

Work during the past year in this Section, headed by Dr. Dennis A. Torchia, focused upon studies of the structure and dynamics of staphylococcal nuclease in the crystalline state and in solution, with the purpose of attaining a better understanding of the protein structure-function relationship.

The difficult problem of assigning the one thousand or more signals observed in the nmr (nuclear magnetic resonance) spectra of staphylococcal nuclease was overcome by combining novel two- and three-dimensional nmr experiments with appropriately labeled protein samples. In this way, we have assigned signals of wild type and E43D mutant S. nuclease complexed with thymidine-3', 5'-diphosphate and Ca^{2+} . In addition, we assigned the signals of the apo enzyme S. nuclease, the largest protein for which such extensive assignments have been obtained.

Combining the information obtained from NOESY spectra and the resonance assignments, we have shown that three alpha helical S. nuclease domains and a six-strand beta barrel domain are observed in solution. This result is in agreement with structural domains observed in the crystal. In addition, a comparison of solid state nmr and solution nmr spectra confirmed the close structural homology in these regions of the protein.

We have also derived information about the values of the NH-alpha-H dihedral angles. We find these are in general to be in good agreement with results obtained in the crystalline state, except for fine residues which are involved in intermolecular contacts in the crystal. These observations show that nmr is able



to discern regions in the crystal conformation that differ from the solution structure. NOESY spectra of S. nuclease have shown that the Lys 116-Pro 117 bond is predominately in the cis conformation in solution for the apo-I and for the liganded molecule. This observation explains the slow kinetics of protein refolding which requires trans-cis isomerization of the K116-P117 peptide bond.

Taken together, our results show that nmr spectroscopy is able to determine solution structure with an accuracy of a few tenths of an angstrom. With this point established, we are now comparing the solution of the wild-type enzyme with the structure of several site-directed mutants.

Our structural studies also indicate that residues 44-55 of S. nuclease do not adopt the crystalline conformation in solution. In fact, measurements of nmr relaxation times and linewidths show that residues in this region undergo a conformational fluctuation on the timescale of milliseconds. We hypothesize that flexibility in this region allows S. nuclease to accommodate a wide variety of substrates, thus explaining the broad specificity of the enzyme.

Proteoglycan Chemistry Section

The Proteoglycan Chemistry Section, headed by Dr. Vincent Hascall, continues to explore the biochemistry of proteoglycans in a wide variety of biological settings. These include: a) proteoglycans in intracellular storage granules of mouse (M1) and human (HL60) leukemic cells; b) proteoglycans on cell surfaces of rat ovarian granulosa cells and a calcium responsive rat parathyroid cell line; and c) proteoglycans and hyaluronic acid in extracellular matrices of mouse cumulus cell-oocyte complexes, embryonic chick cornea, a rat osteogenic tumor cell line (UMR-106), rat chondrosarcoma chondrocytes and bovine articular cartilage.

Most if not all hemopoietic cells contain storage granules which contain cationic, bio-active enzymes and other substances. The granules also contain characteristic proteoglycans which provide a highly anionic matrix for concentrating the cationic molecules. These proteoglycans have small core proteins with embedded serine-glycine repeat sequences that provide the initiation points for the glycosaminoglycan chains. We have explored the structure and metabolism of this class of proteoglycans in mouse (M1) and human (HL60) leukemic cell lines which undergo differentiation to granulocytes when exposed to appropriate cytokines. Both before and after differentiation, these cells synthesize almost exclusively, a proteoglycan with a small (~40 kDa) core protein that contains 3-5 chondroitin sulfate chains and 3-5 O-linked oligosaccharides attached. However, the newly synthesized proteoglycans are segregated, probably at the final stage of adding the glycosaminoglycan chains, into two compartments; one of which is secreted into the medium after ~1 h, and the other of which is retained in an intracellular compartment with a half-life of ~3.5 h before complete degradation in lysosomes. Differentiation is accompanied by a 4-5 fold increase



in proteoglycan synthesis with the majority entering the secretory compartment. Experiments are underway to determine if the core proteins of the proteoglycans in the two compartments are identical and to define where in the maturation pathway they are segregated.

Many cells contain a cell surface heparan sulfate proteoglycan with the following overall structure; a core protein of ~70 kDa with 3-5 heparan sulfate chains and a few O- and N-linked oligosaccharides attached. In some cells, such as the rat ovarian granulosa cell or the rat osteosarcoma UMR cell, up to 20% of these proteoglycans are anchored in the plasma membrane through phosphatidyl inositol linkages, whereas the remainder appear to be intercalated. In other cells, such as the rat parathyroid cell line, only the intercalated form is present. These proteoglycans are involved in a variety of cell membrane pathways, involving flow from the Golgi to the cell surface, reinternalization and ultimate degradation in lysosomal pathways. In the parathyroid cells, these proteoglycans appear to be intimately associated with their biological response to ionized calcium. At physiological Ca^{+2} levels, newly synthesized heparan sulfate proteoglycans do not appear on the cell surface, but rather remain in an intracellular compartment which, after ~2 h, begins to be eliminated through lysosomal degradation. In reduced calcium (~10% of physiological) more than 70% of the newly synthesized proteoglycans appear on the cell surface within ~30 min; further, they cycle rapidly between the cell surface and an intracellular compartment with a halftime of a few minutes. When cells at physiological levels are switched to low calcium conditions, the proteoglycans sequestered in the intracellular compartment are rapidly mobilized to the cell surface and initiate the cycling process. When cells in low calcium are switched to physiological levels, the proteoglycans are rapidly internalized and no longer cycle to the surface. The kinetics of the cycling process are similar to those for receptor-mediated uptake pathways and the results suggest that the proteoglycans are localized in specialized regions of the membrane which respond to the biological effector, namely reduced levels of calcium, to mobilize 'receptors' to the surface and initiate synthesis and secretion of parathyroid hormone active peptides.

In most mammalian ovaries, the cumulus cell-oocyte complex rapidly expands (within a few hours) at the time of ovulation by depositing an extracellular matrix between the cumulus cells. Expansion correlates with a programmed ~20 fold stimulation of synthesis of hyaluronic acid (HA) by the cumulus cells followed by its deposition and organization into a lattice-like array in the extracellular matrix. In vitro, we found that stimulation of HA synthesis requires hormone (follicle stimulating-hormone) or cAMP treatment and soluble factor(s) produced by the oocyte. Further, a factor(s) in fetal calf serum or follicular fluid is required to retain the newly synthesized hyaluronic acid in the extracellular matrix and achieve expansion. The nature of these factors and their effects on cumulus cells is under investigation.

The rat osteosarcoma cell line (UMR-106) synthesizes several proteoglycans which are secreted into the extracellular matrix or medium. The large proteoglycan produced by the cells probably



contains the same core protein as that for the cartilage proteoglycan synthesized by rat chondrosarcoma chondrocytes. However, the mature forms of these two proteoglycans are very different. That from the bone tumor cell contains far fewer chondroitin sulfate chains (~20 vs ~100) and far more O-linked oligosaccharides (~200 vs ~120) than that from the cartilage tumor cell. This suggests that the addition of the complex carbohydrate structures onto the core protein during the final stages of maturation is not determined directly by the core protein. The current hypothesis under investigation is that xylosylation of serine residues at a proportion of the serine-glycine sites in the core protein is the critical step and determines the number and location of the chondroitin sulfate chains in the final proteoglycan. O-glycosylation, then, is a subsequent, low specificity step in which most of the accessible threonines and the remaining accessible serines are then substituted. Work with the chondrosarcoma chondrocytes has shown that xylosylation occurs late in the intracellular half-life of the core protein precursor, but probably significantly earlier (a few minutes) than the addition of the rest of each chondroitin sulfate chain. Work is underway to identify the location in the Golgi where xylosylation occurs.

The rat bone tumor cells also synthesize and secrete large amounts of a sulfated sialoprotein. Amino acid sequence analysis of the N-terminus showed that the sialoprotein is identical to bone sialoprotein II (see Skeletal Biology Section report). Metabolic labeling studies showed that the sulfate resides both on tyrosine residues within the protein and on N- and O-linked oligosaccharides. Approximately 15% of the O-linked oligosaccharides are sulfated, with most of these being hexasaccharides. The structures of these oligosaccharides have been partially determined and found to be similar or identical to those previously described on the large proteoglycan isolated from the rat chondrosarcoma. Metabolic studies also revealed that a similar proportion of the O-linked oligosaccharides on the large proteoglycans synthesized by both the osteosarcoma and chondrosarcoma cells are sulfated. The sites of sulfation within the oligosaccharide structures is being determined by chemical and selective enzymatic degradation procedures.

Transforming growth factor β , at physiological concentrations, is sufficient as the sole medium supplement to maintain steady state metabolism of proteoglycans in articular cartilage organ culture. Northern analysis revealed significant levels of mRNA for this growth factor in chondrocytes from this tissue, and immunoprecipitation revealed that it is synthesized in appreciable levels by the cultures. These results suggest that this factor is at least partially responsible for autocrine regulation of proteoglycan metabolism in normal hyaline cartilage.

Skeletal Biology Section

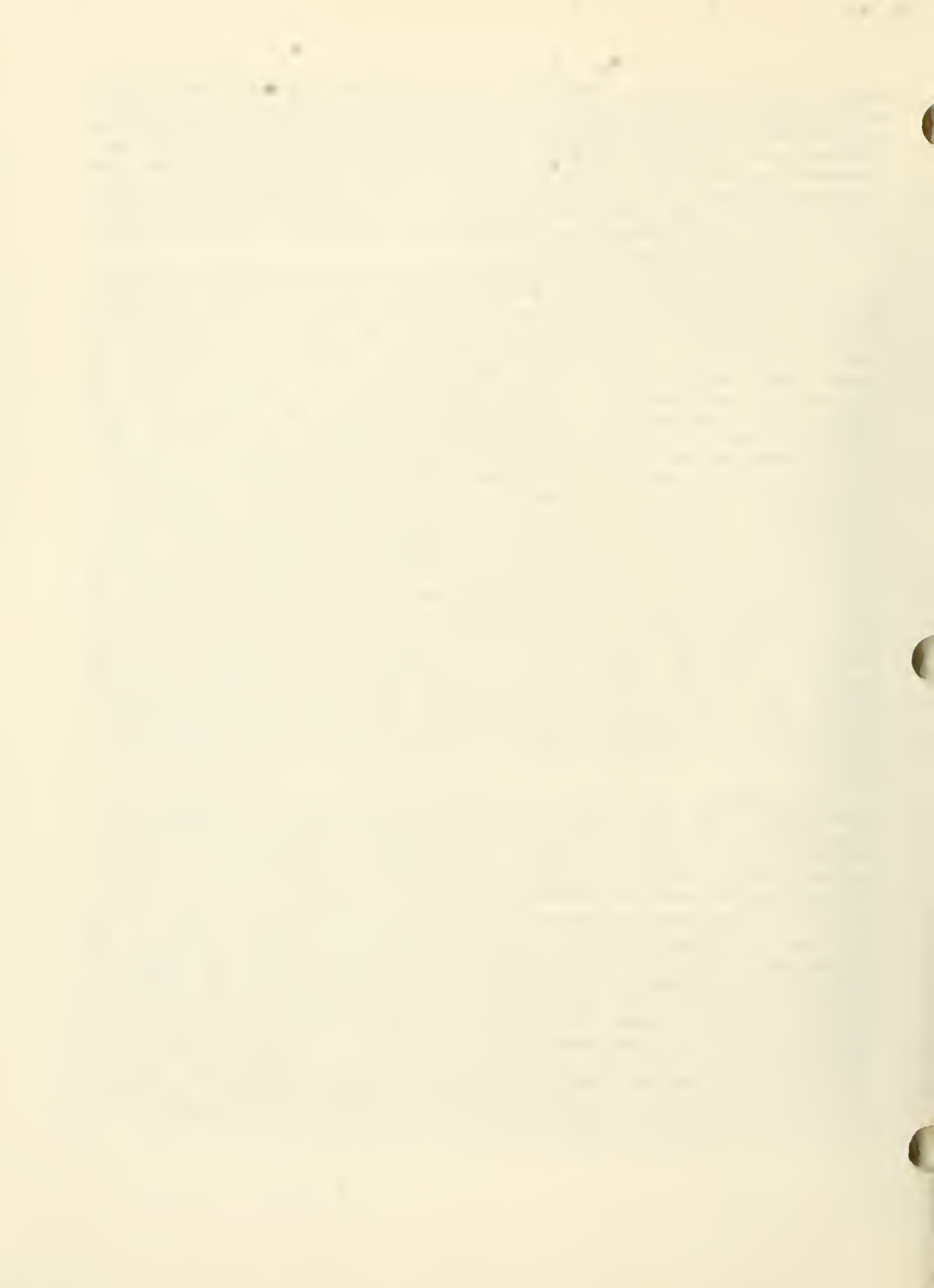
Work in this Section (Dr. John D. Termine, Chief) originates from three independent groups each under the leadership of a



Senior BRB scientist. The Bone Cell Biochemistry program, headed by Dr. Pamela Gehron Robey, is continuing to define osteoblastic metabolism by comparison of osteoblastic properties at both the tissue and cellular levels. Osteoblastic gene expression was assessed at the human tissue level via in situ hybridization and immunolocalization. At the cellular level, in vitro metabolism was followed by analysis of human osteoblast growth, gene expression (mRNA production and protein synthesis), and modulation by hormones and growth factors.

Continued study of the localization of osteonectin in adult tissues revealed that not only is it present in bone matrix, osteoblasts and osteocytes, but it is also found in cells actively involved in ion transport such as renal distal tubule epithelium and striated duct epithelium of salivary glands. In vitro, at least one renal epithelial cell line (LLCPK₁) was found to synthesize osteonectin. Cell types actively synthesizing and depositing the small proteoglycans I and II (biglycan and decorin), and the bone sialoproteins (osteopontin and BSP) were also investigated by in situ hybridization and immunolocalization in developing human fetal tissues. Although biglycan and decorin are highly homologous in amino acid sequence, they exhibited a remarkably different pattern of tissue expression, pointing to potentially different functions. Biglycan was localized to cell surface or pericellular areas of endothelial, epidermal, muscle and some renal tubular epithelial cells, whereas decorin was enriched in dermis, tendon, sclera and cornea. In cartilage and bone, biglycan was distributed in the territorial matrix of proliferating cartilage, in osteocytic lacunar walls and in bone matrix. Decorin was found in the inter-territorial matrix of cartilage and in bone matrix. Osteopontin was found in bone matrix and in only a few osteoblasts, but interestingly was also found in small marrow mononuclear cells and in occasional osteoclasts. The major human bone sialoprotein, BSP, was found in virtually all osteoblasts and in only a few non-bone mononuclear cells.

Continued analysis of osteoblastic cell cultures has identified the following secretory products: thrombospondin, plasminogen activator inhibitor, along with collagen, osteonectin, osteocalcin, proteoglycans (a large chondroitin sulfate proteoglycan, heparan sulfate proteoglycan, biglycan and decorin), sialoproteins, and fibronectin, as well as known growth factors such as transforming growth factor- β . Proteoglycan metabolism has been further analyzed with increasing temporal age of the osteoblast cultures. With time, the heparan sulfate proteoglycan and decorin increase at a linear rate, while biglycan, and to a lesser extent, the large chondroitin sulfate proteoglycan dramatically increase at longer time points which correlate with matrix deposition (as assessed by electron microscopy), perhaps indicative of function in this process. Using synchronized cultures, the effect of cell cycle on gene expression is also under investigation. Expression of alkaline phosphatase enzymatic activity is greatly reduced during G₂/M phase, for example. The

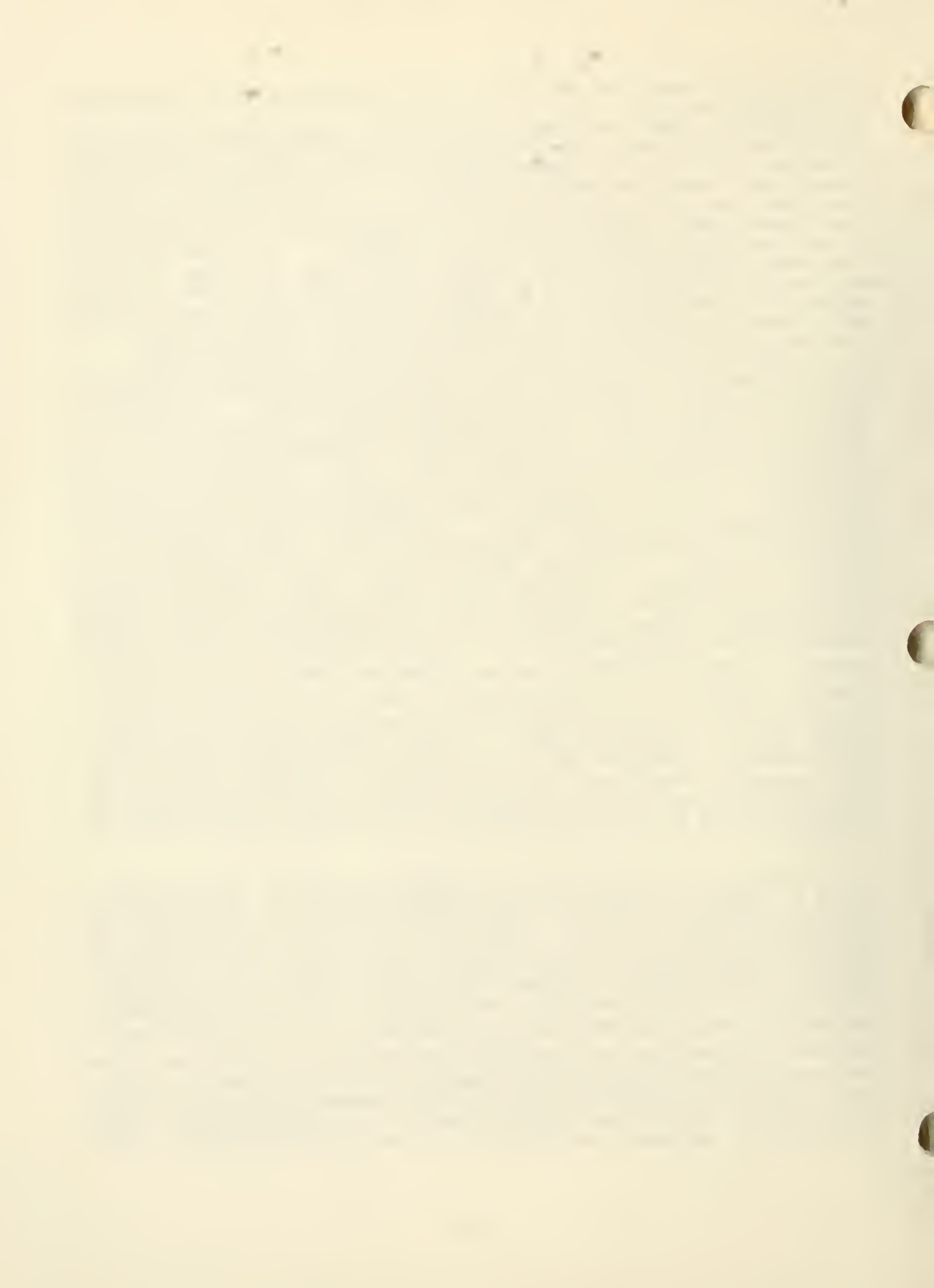


cell cycle seems to influence the expression of other bone proteins, as well.

Factors that modulate osteoblastic metabolism were also identified this year. Platelet-derived growth factor and insulin-like growth factor-1 were found to be mitogenic for human osteoblasts under serum-free conditions. Since fluoride is one of the few therapies found to increase bone mass in certain individuals with osteoporosis, its effects were investigated in fetal and adult human bone cell cultures. Fluoride did not increase cell proliferation rates, nor did it alter protein synthesis, indicating that it most likely does not have a direct effect on differentiated osteoblasts, and that the target cell may be either a precursor not present in our culture system, or another cell type which produces factors that influence bone.

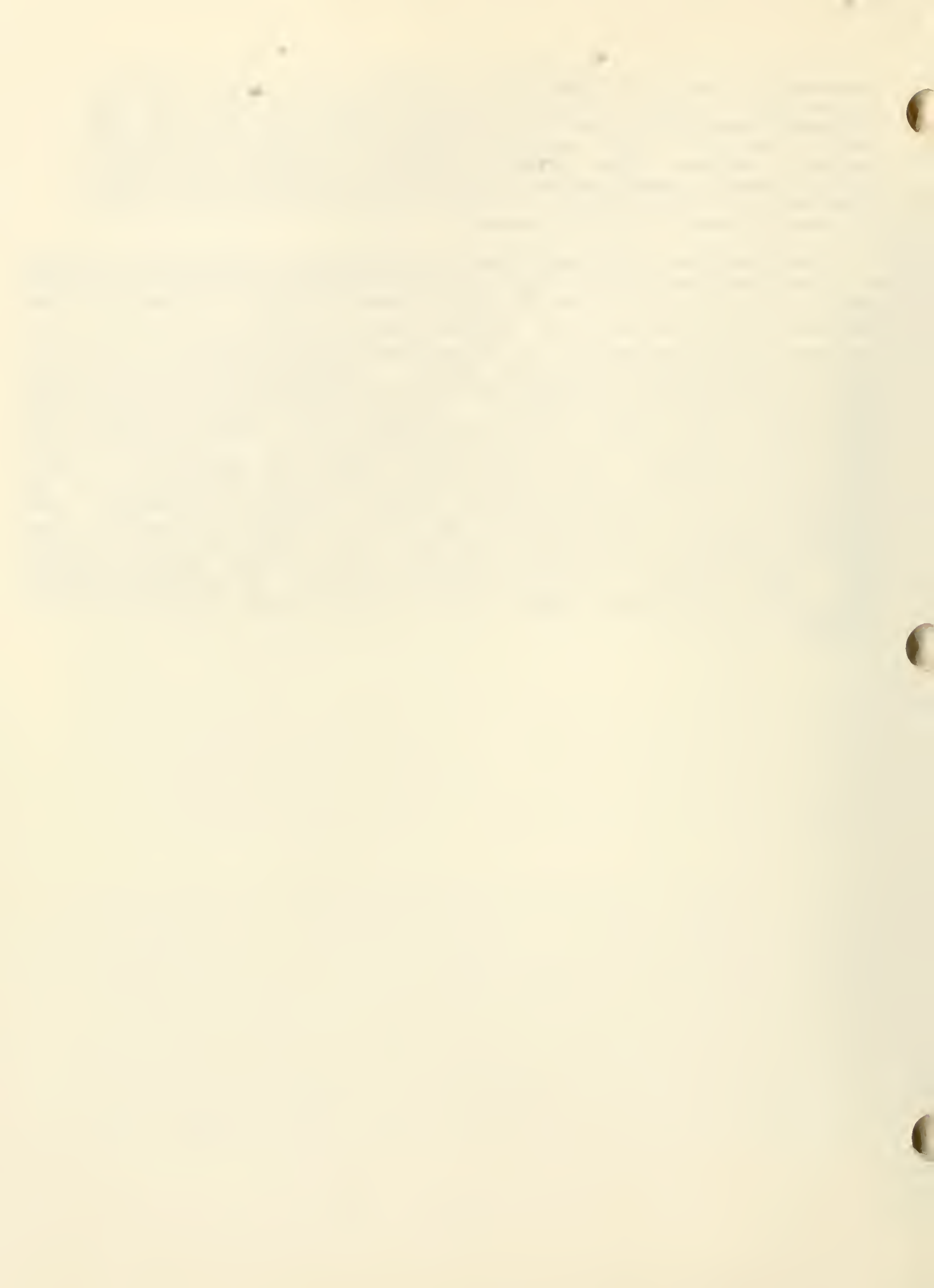
Utilizing the cells and their secreted products, functional assays have been developed to evaluate hydroxyapatite binding, cell attachment, spreading and cell proliferation. Under non-denaturing conditions, it was confirmed that osteonectin as well as other secreted cell products bind to hydroxyapatite, whereas in strongly dissociative conditions, only osteonectin and thrombospondin remain tightly associated, indicative of high affinity. In cell attachment assays, attachment only was mediated by thrombospondin, while both attachment and spreading was mediated by fibronectin, osteopontin and a bone sialoprotein-derived peptide. A newer approach was used to explore bone protein function, utilizing anti-sense DNA oligonucleotides (oligos) to inhibit synthesis of a particular protein. Using radiolabeled oligos, whole cell autoradiography demonstrated uptake of the antisense oligos into the cytoplasm. A transforming growth factor- β anti-sense oligo inhibited DNA synthesis by 75%, lending support to the hypothesis that TGF- β is an autocrine growth factor for osteoblasts. Interestingly, osteonectin anti-sense probes inhibited only a small proportion of the total osteonectin, a fraction only recognized by a monoclonal antibody. This suggests that there are two slightly different forms of the molecule synthesized by osteoblasts. The use of anti-sense DNA oligos then is a promising tool for elucidating protein function in vitro.

The Bone and Tooth Protein Chemistry program, headed by Dr. Larry W. Fisher, continued to have good success in the production of monospecific antisera to synthetic peptides derived from the sequences of bone proteins. This work was done in collaboration with Dr. Frank Robey, LCDO, NIDR. The current list includes: several animal species-derived osteonectins; human proteoglycan I (biglycan) and II (decorin); osteopontin; alkaline phosphatase; the N- and C-propeptides and the C-telopeptide of $\alpha 1(I)$ collagen; and the C-propeptide and N-telopeptide of $\alpha 1(III)$ collagen. The Protein Chemistry group, in collaboration with Dr. Marian Young, has cloned and sequenced cDNAs encoding human biglycan, decorin, osteopontin and bone sialoprotein. Interestingly, human osteopontin appears to have at least two different forms



presumably due to differential splicing. Dr. Paolo Bianco, currently visiting the Branch, has successfully used both the antisera and the cDNA to localize both protein and mRNA respectively for these molecular species in human tissues (see above). We have also recently produced monospecific antisera against dentin phosphophoryn and have isolated mRNA from bovine odontoblasts in preparation for the cloning and sequencing of this interesting dentin-specific protein.

The Bone and Tooth Molecular Biology program, headed by Dr. Marian F. Young, was the focus for the cloning of the above human bone proteins and for construction of the in situ probes for them. In addition, the effects of 17- β estradiol on cultured bone cells was studied and shown to have significant effects on collagen mRNA expression but no effect on cellular proliferation in this system. The genes for several of the major non-collagenous proteins of bone were mapped in the human genome using human-rodent cell hybrids. Biglycan is located on chromosome Xq13-qter, while decorin is on chromosome 12q. Both osteopontin and bone sialoprotein are located on chromosome 4. A high frequency restriction fragment length polymorphism (RFLP) was observed in or near the osteopontin gene due to the presence or absence of a BgII site. An analysis of the transcriptional control of the osteonectin gene indicates there are both positive and negative promoter elements. These elements have been localized to intron 1, exon 1 and 1kb of 5' flanking DNA. cDNA encoding the bovine enamel gene has been isolated and has been used to show that there are two species of related enamel message: a predominant mRNA of 1.4 kb and a minor one of 2.8 kb in length.



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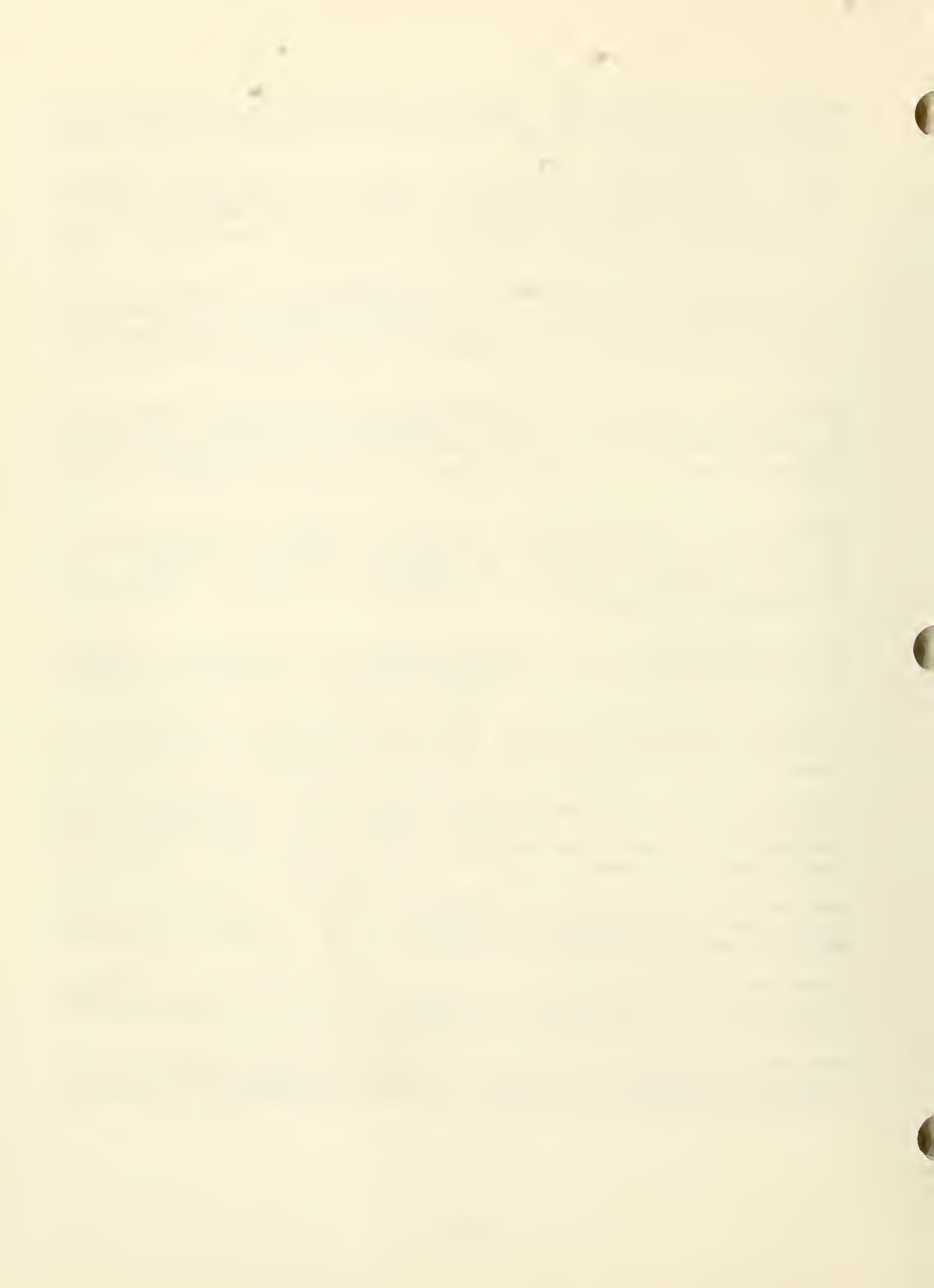
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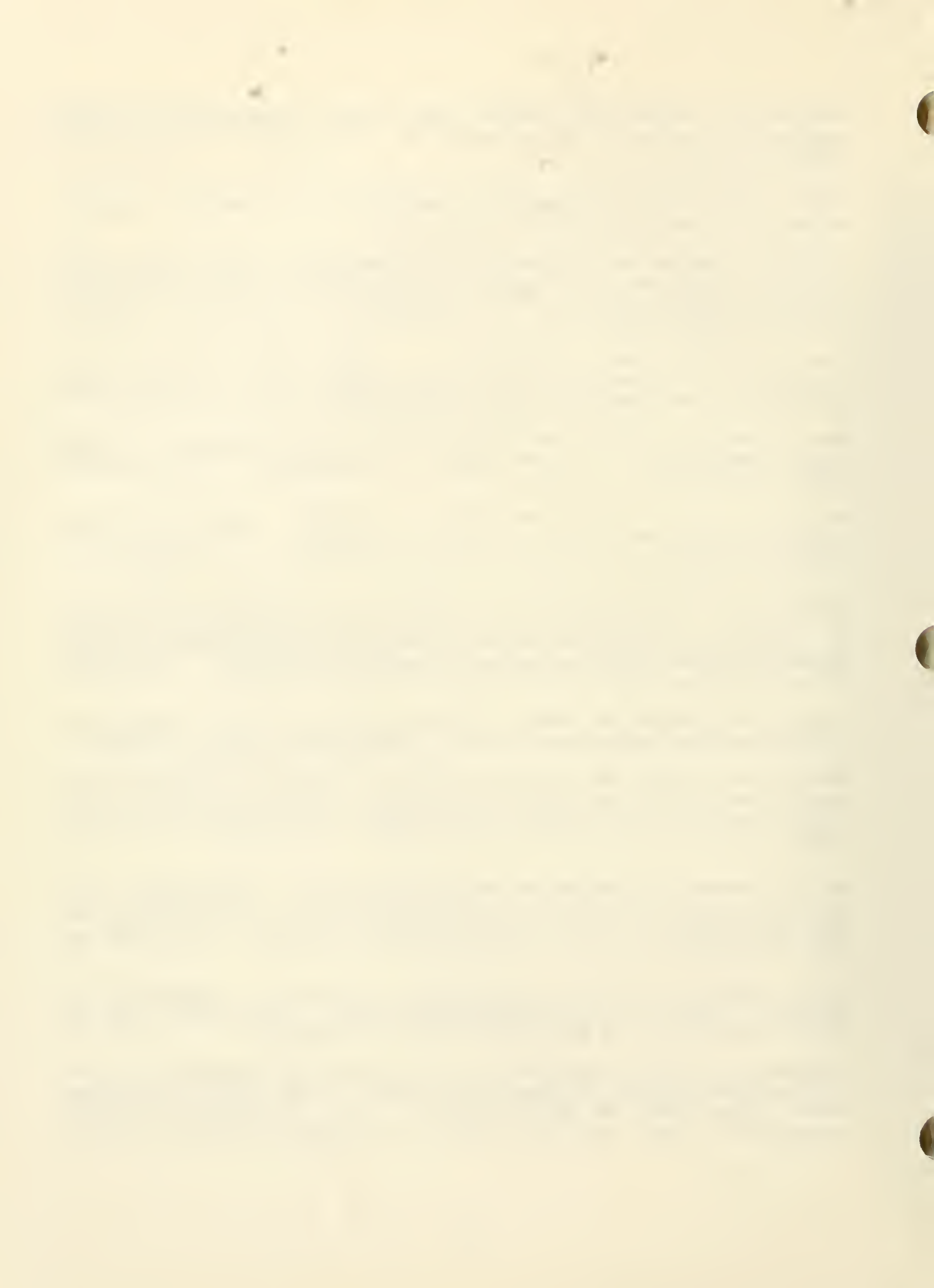
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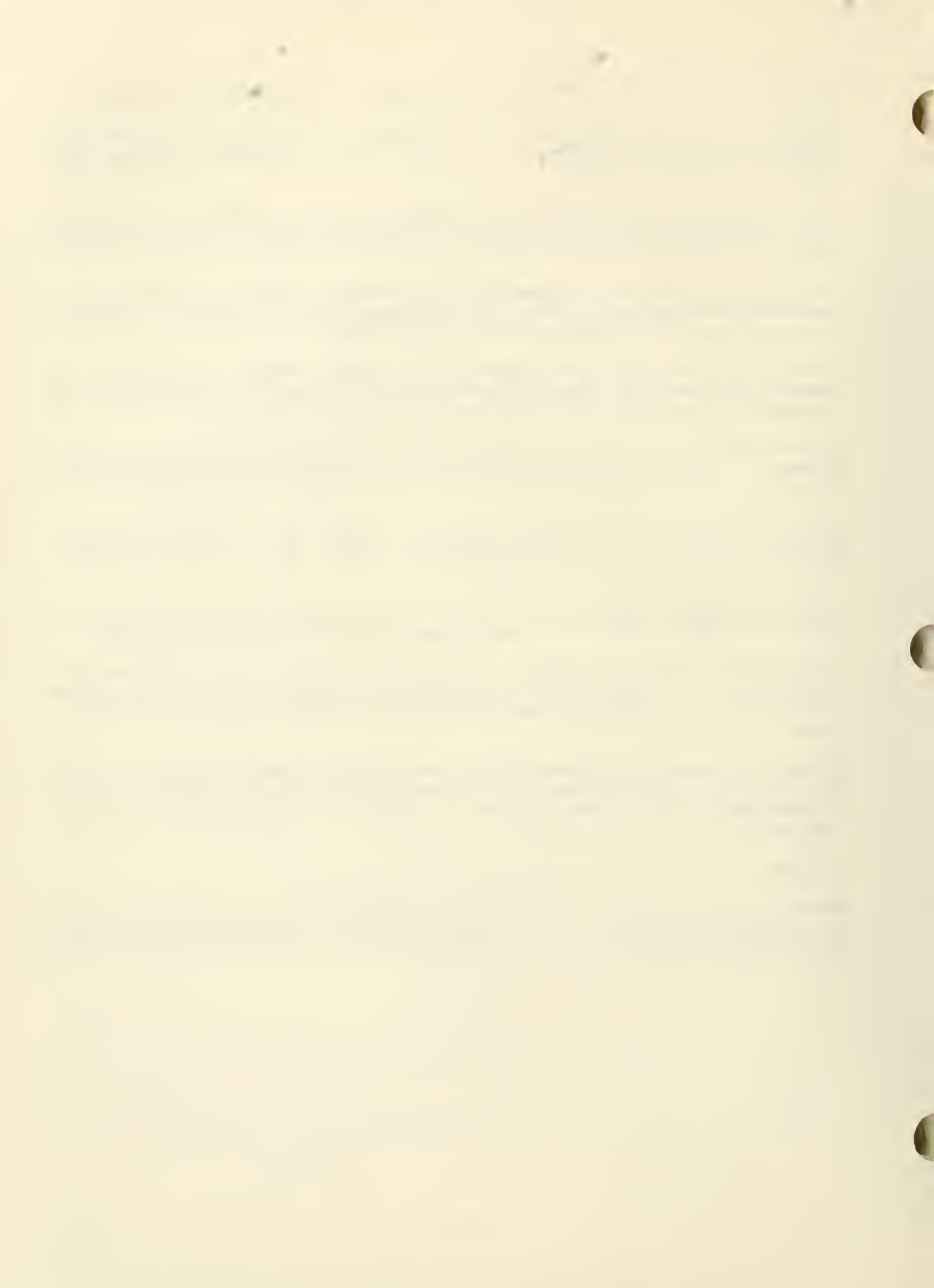
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Patents:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00012-27 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

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

B.O. Fowler Research Chemist BRB, NIDR

COOPERATING UNITS (if any)

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

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.

1.25

PROFESSIONAL:

1.00

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

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

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

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (e.g., hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral data (temperature dependence and polarization) are then utilized to establish composition and structural details of the apatites in question which include: the type and geometry of constituent ions; the size or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00074-17 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Bone and Tooth Matrix Biochemistry and Metabolism

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

L.W. Fisher	Senior Staff Fellow	BRB, NIDR
J.D. Termine	Chief	BRB, NIDR
N.C. Tuross	NIH Postdoctoral Fellow	BRB, NIDR

COOPERATING UNITS (if any)

SIU, School of Dentistry, Edwardsville, IL
 University of New Mexico, Albuquerque, NM
 Food and Drug Administration, Bethesda, MD

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.

3.20

PROFESSIONAL.

1.70

OTHER.

1.50

CHECK APPROPRIATE BOX(ES)

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

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

The extracellular matrix proteins of the bones and teeth are key elements in the structure and metabolism of these tissues. The goal of this project is to study matrix proteins specific to each mineralizing skeletal tissue in order to understand their molecular structure and biological function.

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binding, RIA, ELISA, etc.) have been developed to quantitate the levels of noncollagenous proteins in bone including osteonectin, bone sialoproteins I and II, bone proteoglycans I and II, dentin phosphophasyn and the N-propeptide and C-propeptide of type I(I) collagen in (a) surgical specimens of bony tissue and (b) serum (osteonectin). Changes in the noncollagenous protein profile with age and variety of bone (and tooth) diseases have been observed in man and several animal models. We have been highly successful at producing antisera against synthetic peptides for all of the human bone noncollagenous proteins. These antisera have proven useful in immuno precipitation studies, immunolocalization, immunodetection and on Western blots. We have successfully cloned and sequenced human bone proteoglycans I (biglycan) and II (decozin), sialoprotein (BSP) and osteopontin.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00088-16 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Chemical, Structural and Morphological Studies on Calcium Phosphates

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

E.D. Eanes	Chief, Mineral Chemistry and Structure Section	BRB, NIDR
D. Skrtic	Visiting Fellow	BRB, NIDR
V.C. Hascall	Chief, Proteoglycan Chemistry Section	BRB, NIDR

COOPERATING UNITS (if any)

American Dental Association Health Foundation, Paffenbarger Research Center, National Bureau of Standards, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.

3.00

PROFESSIONAL:

2.00

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

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

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

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, healthy bone and teeth. Developmental defects in the deposition of these salts or their destruction and loss by disease can severely impair the function of these skeletal tissues. The purpose of this project is to study the physical, chemical, and ultrastructural properties of these salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable these salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued is the use of artificial lipid vesicles (i.e., liposomes) as in vitro models for investigating the physico-chemical aspects of calcium phosphate precipitate formation in matrix vesicles. The liposome experiments are being conducted with the goal of better understanding how matrix vesicles, the loci for early mineralization in many vertebrate hard tissues, can initiate precipitation in their membrane-bound interior spaces and control the expansion of this initial precipitate into the surrounding extracellular space. Present finds show that membrane components such as cholesterol can affect both precipitate initiation and expansion whereas extravesicular factors such as proteoglycans affect mainly the latter event.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00134-15 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Structure and Biosynthesis of Proteoglycans

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

V.C. Hascall	Chief, Proteoglycan Chemistry Section	BRB, NIDR
T.I. Morales	Senior Staff Fellow	BRB, NIDR
R. Midura	Staff Fellow	BRB, NIDR
A. Calabro	Guest Worker	BRB, NIDR
S. Shibata	Guest Worker	BRB, NIDR

COOPERATING UNITS (if any)

Rush-Presbyterian-St. Luke's Medical Center; Univ. of Lund, Sweden;
University of West Virginia; University of Michigan

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.67

PROFESSIONAL:

4.67

OTHER:

0

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of the project is to study the chemical and physical properties and metabolism of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the core protein of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of core protein precursors and processing to mature proteoglycans; 3) Effects of growth factors (insulin-like growth factor 1 and transforming growth factor beta) on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilages; 4) sulfate-proteoglycan and dermatan sulfate-proteoglycan in chick, cat and human cornea; 5) Analyses of sulfated oligosaccharides on proteoglycans and sialoproteins in bone and cartilage tumor cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00157-14 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Biophysical Studies on the Structure of Connective Tissue

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

D.A. Torchia	Chief, Protein Biophysics Unit	BRB, NIDR
S.W. Sparks	Staff Fellow	BRB, NIDR
H.B.R. Cole	Staff Fellow	BRB, NIDR
D.M. Baldisseri	IRTA Fellow	BRB, NIDR

COOPERATING UNITS (if any)

York College, SUNY, Jamaica NY; LCP, NIDDK, NIH; University of Maryland; LB, NCI, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Unit

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.

3.75

PROFESSIONAL:

3.00

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The purpose of this project is to investigate the molecular structure and dynamics of proteins and model compounds. The structural and dynamical information obtained will be correlated with function. Areas of present interest are 1) Calcium binding proteins and staphylococcal nuclease. We are using multinuclear nmr to study (a) the molecular dynamics; (b) the structure; and (c) the interactions of staphylococcal nuclease with calcium, and with inhibitors and model substrates. 2) Effects of site-directed mutation on enzyme function and structure. We are comparing the solution structure of s. nuclease E43D with wild type nuclease in order to better understand sequence-structure-function relationship in the protein.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00379-06 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Structure and Bone Matrix Gene Expression

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

M.F. Young	Senior Staff Fellow	BRB, NIDR
J.D. Termine	Chief	BRB, NIDR
P. Dominguez	Visiting Fellow	BRB, NIDR
D.I. Deutsch	Visiting Fellow	BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS.

4.85

PROFESSIONAL:

3.60

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

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

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

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective in this investigation is to study the biosynthesis of these macromolecules and to understand the regulation of their expression.

The expression of bone matrix proteins have been studied by constructing recombinant cDNA libraries from bone cell mRNA. cDNA clones encoding several bone and tooth matrix proteins have been isolated using expression DNA vectors and polyclonal antisera directed against individual bone matrix and ameloblast proteins. The clones were used to study the primary structure and regulation of expression of these genes in cultured bone cells.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00380-06 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Metabolism of Bone Cells in Vitro

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

P. Gehron Robey	Biologist	BRB, NIDR
J.D. Termine	Chief	BRB, NIDR
N.S. Fedarko	IRTA	BRB, NIDR
J.B. Kopp	Staff Fellow	BRB, NIDR
T.E. Hefferan	Biol. Lab Technician	BRB, NIDR
P. Bianco	Visiting Associate	BRB, NIDR
U.K. Vetter	Visiting Associate	BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.45

PROFESSIONAL

3.95

OTHER

1.50

CHECK APPROPRIATE BOX(ES)

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

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

Bone cells derived from a variety of animal species including human, bovine, ovine and rodent, and of varying developmental ages, have been utilized to: 1) study the biosynthesis and deposition of extracellular matrix proteins such as collagen, osteonectin, bone proteoglycan and other bone proteins, and alterations of matrix production in the disease Osteogenesis Imperfecta; 2) study the responsiveness of the cells to a variety of hormonal and pharmacological factors (such as 1,25-dihydroxy vitamin D3 and fluoride); 3) elucidate the production and interaction of growth factors (such as TGF- β , insulin-like growth factors and platelet-derived growth factors); 4) study the potential function of bone matrix proteins through the use of functional assays and the use of anti-sense DNA to inhibit specific protein synthesis; and 5) serve as a source of mRNA and DNA for studies of the proteins at the genomic level.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00431-03 BRB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Metabolism of Proteoglycans

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

M. Yanagishita	Visiting Scientist	BRB, NIDR
V.C. Hascall	Chief, Proteoglycan Chemistry Sect.	BRB, NIDR
Y. Takeuchi	Visiting Fellow	BRB, NIDR

COOPERATING UNITS (if any)

University of Rome, Italy; University of Lund, Sweden; Mateus Bickel, FDA; HGB, NICHD, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS

2.33

PROFESSIONAL:

2.33

OTHER:

0

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of the project is to study biochemical and physical properties, biological function and metabolism of proteoglycan under physiological and various pathological conditions using a number of tissues and cell systems. Topics of present interest include: (1) analysis of proteoglycan structure in a osteoblast-like cell line; (2) analysis of proteoglycans in a parathyroid cell line; (3) analysis of proteoglycans and hyaluronic acid in mouse cumulus cell-oocyte complex; and (4) analysis of proteoglycans in mouse and human leukemic cell lines.



ANNUAL REPORT OF THE LABORATORY OF CELLULAR DEVELOPMENT AND ONCOLOGY

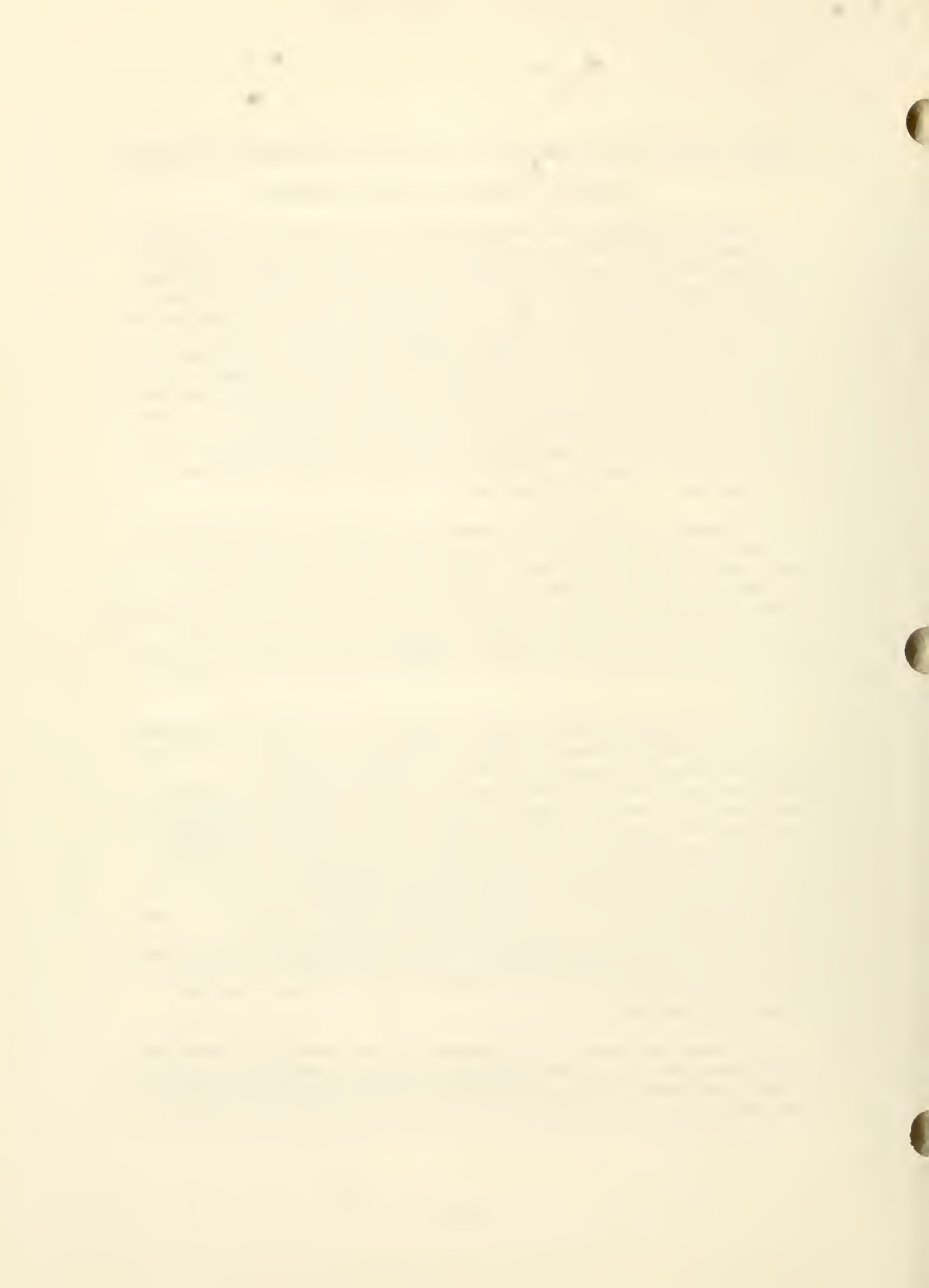
NATIONAL INSTITUTE OF DENTAL RESEARCH

The restructuring of this laboratory from what formerly was the Laboratory of Oral Biology and Physiology was essentially completed this year with the official transfer of Dr. Keith Robbins from the National Cancer Institute to serve as chief of the Molecular and Cellular Biology Section. The only remaining step in reorganization is the selection of a chief for the Laboratory. In the interim Dr. John Folk, Chief of the Enzyme Chemistry Section, continues to serve as the acting chief. The laboratory of about 25 members now contains three Sections and one Unit. All are of similar size; only the Section on Molecular and Cellular Biology at present has support staff. Within the research staff the ratio of temporary (post-doctoral fellows and visitors) to tenure staff is about 4:1. One-half of the temporary staff are supported by plans that do not count against our position ceiling.

Although the size of the Laboratory programs is theoretically limited by this ceiling and our ability to recruit through other mechanisms, program size must actually be measured to include collaborations with scientists outside of this laboratory. All of the scientific staff collaborate closely with other scientists within and outside of NIDR and NIH. In this way productivity is mutually increased. This becomes even more necessary since space within NIDR has become more limited each year, without much hope of any major relief.

The principle product of this laboratory is original biological research in several areas related to normal function and to disease. Unity is achieved through a search for structure-function relationships. The Laboratory meets or exceeds whatever standards could be set in terms of number and quality of publications which are the accepted measure of productivity. In addition to laboratory research, the Peptide and Immunochemistry Unit provides a service in custom peptide synthesis for all intramural programs. Furthermore, senior scientific personnel function in a variety of other professional activities of importance to biological science. These include attendance at, and participation in, national and international scientific meetings, review of manuscripts for journals, evaluation of applications for grants and fellowships, lecturing before groups within and outside NIH, serving on committees and organizing meetings.

The numerous research achievements of the Laboratory summarized below according to section were made possible by the diverse expertise of the staff members, many collaborations with scientists outside of the Laboratory, the development of new and powerful technologies, and



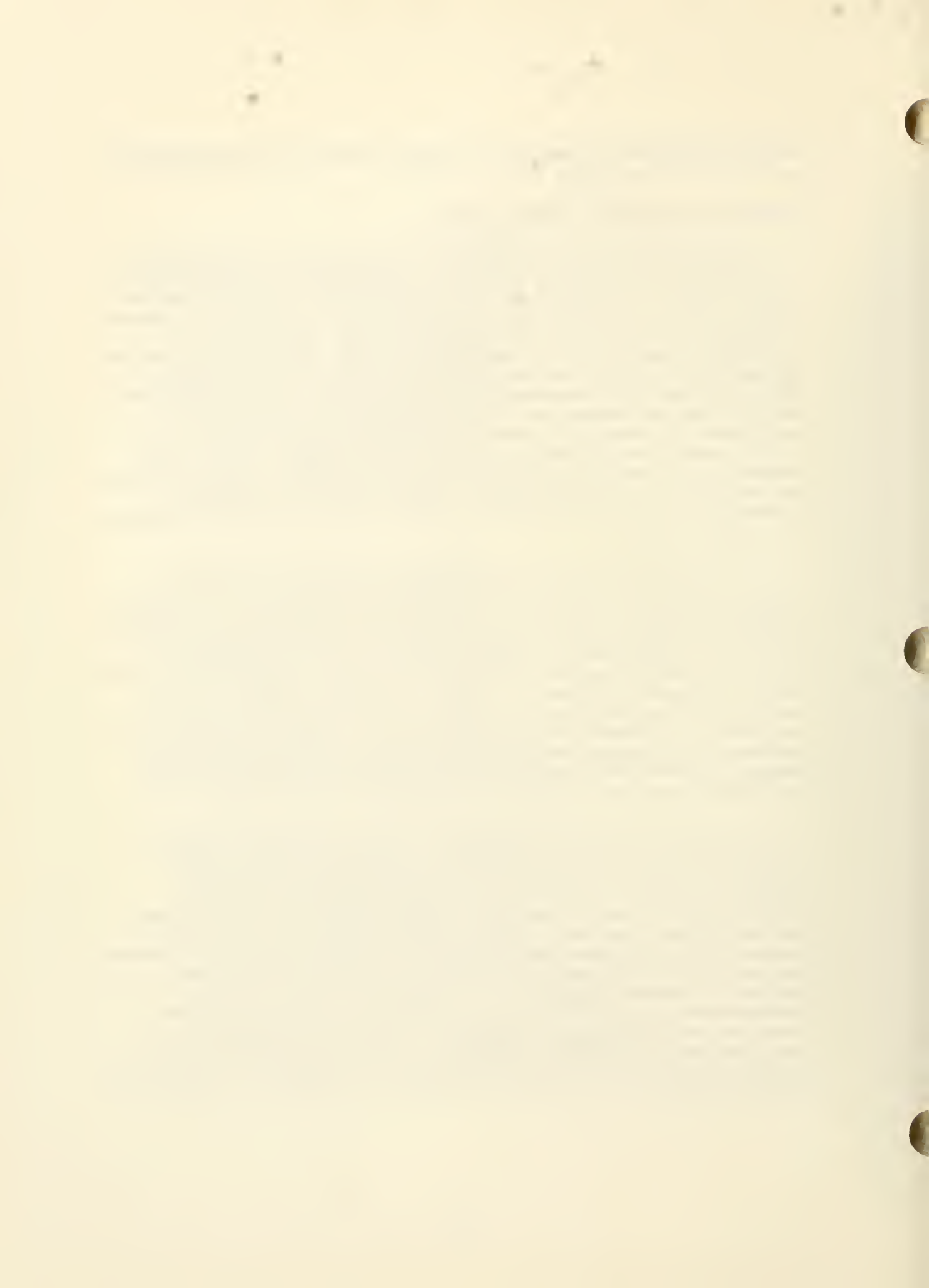
their applications to the various programs, and the interdisciplinary nature of the Laboratory.

Molecular and Cellular Biology Section

The long term goal of the Molecular and Cellular Biology Section is elucidation of molecular mechanisms responsible for conversion of normal cells to a malignant state. The approach to this problem has been to focus on the oncogenic sequences within the genomes of certain tumorigenic retroviruses. The fgr oncogene was identified as the transforming component of Gardner-Rasheed feline sarcoma virus and the fyn gene was isolated from normal human fibroblasts. In addition, cDNA molecules which represent fgr and fyn proto-oncogene transcripts were isolated and sequenced. This work provided the basis for development of immunologic reagents necessary for the identification of the translational products of these proto-oncogenes. Both gene products are protein-tyrosine kinases with conserved catalytic domains and unique amino terminal regions. Current efforts are aimed at determining their normal physiologic roles as well as their mechanism as oncogenic agents.

In a search for sites of c-fgr proto-oncogene action, it was found that expression of its mRNA is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50 to 100 copies of c-fgr mRNA per cell. The expression of p55^{c-fgr} in normal human neutrophils (PMN) was also investigated. Peptide antibodies against the amino - or carboxyl terminal regions of p55^{c-fgr} detected a protein of 55 kDa in lysates of PMN but not in control cells establishing the presence of this protein in normal neutrophils. Neutrophil-derived p55^{c-fgr} was enzymatically active as demonstrated by immune complex kinase assays. Phosphoamino acid analysis of p55^{c-fgr} revealed only phosphotyrosine. These findings established neutrophil p55^{c-fgr} as a protein-tyrosine kinase.

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



The translational product (P70gag-actin-fgr) of Gardner-Rasheed feline sarcoma virus (GR-FeSV) is a protein-tyrosine kinase structurally unique among retrovirus transforming proteins in that it contains components derived from at least two different cellular genes, gamma actin and c-fgr. Based on the knowledge of this composition and a finding that certain human cells express an aberrant form of actin, an investigation was commenced to determine whether the actin domain contributes to the oncogenic activity of P70gag-actin-fgr, either by interfering with normal cytoskeletal organization or by directing the fgr kinase to substrates not normally exposed to tyrosine phosphorylation. By comparing the biologic and biochemical properties of cells expressing wild-type or deletion mutant genes, it was established that the actin domain inhibits focus formation and that its presence impairs the protein-tyrosine kinase activity of P70gag-actin-fgr.

To examine the possible role of human fyn as an oncogene, the gene was cloned into retrovirus expression vectors and transfected into NIH/3T3 cells. It was observed that, under conditions of high expression, the fyn proto-oncogene induces morphologic transformation and anchorage-independent growth. Moreover, within the population of cells overexpressing fyn, genetically altered fyn genes were detected. Such mutants were capable of converting NIH/3T3 cells to a fully malignant state. Isolation and nucleotide sequence analysis of three such oncogenic fyn genes revealed that they are mutated in vivo in such a way that their translational products are truncated at their carboxyl termini. On the basis of these findings, a search is underway in human tumor cells for overexpression of normal and truncated versions of the fyn gene product. Included in this survey are lymphatic tumor cells with chromosomal aberrations at 6q21, the site of the fyn locus within the human genome.

Peptide and Immunochemistry Unit

The serum amyloid P component (SAP) is closely related in structure to another protein in human serum, C-reactive protein (CRP), peptide portions of which can stimulate B cell proliferation. It was found that a peptide modeled on a 13 member sequence of SAP displays strong cell attachment activity. Native CRP and SAP, on the other hand, do not possess these biological activities. It was suggested that these two proteins may function as storage sources of small biologically active peptides. A search is underway to reveal the mechanism of release of the peptides in vivo.

HIV-1 is the causative agent of AIDs. Efforts are underway to develop means of introducing antiviral drugs specifically into cells through CD-4, the HIV-1 receptor. To this end monoclonal antibodies have been prepared to a 22-member peptide segment of the CD-4 sequence. An in vitro assay developed to measure binding of HIV-1 envelope to CD-4 receptor indicates that binding may depend upon the



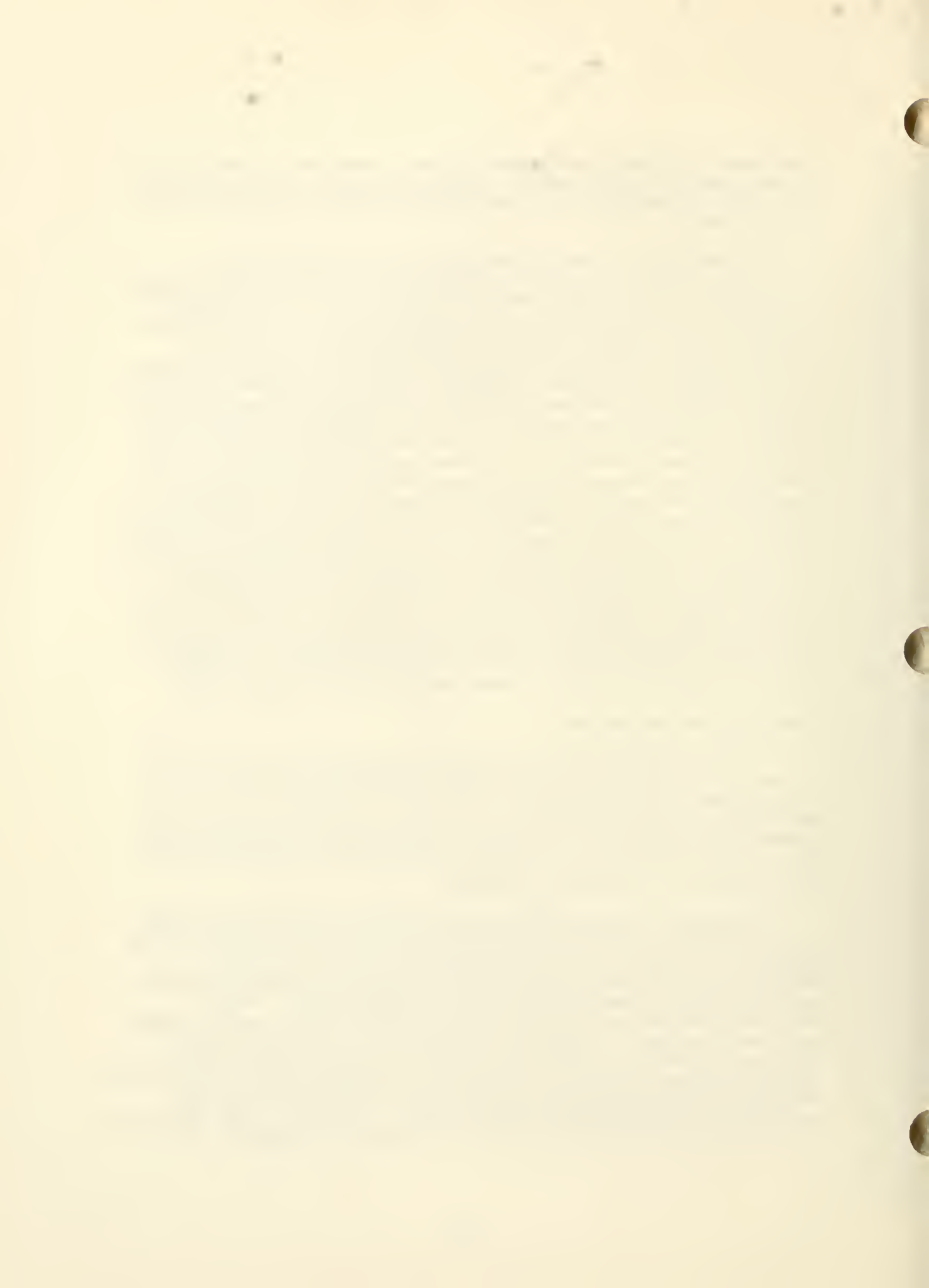
conformation of the CD-4-derived peptide. Attempts are underway to relate peptide modification to peptide conformation with the aim of using this information to obtain antibody specific enough for use in drug delivery.

In general, in order for antibodies to be formed against synthetic peptides it is necessary for the peptides to be conjugated to proteins. The reasons for this are not known. It is believed, however, that low molecular weight peptides are cleared through the kidney before an immune response can be developed. It is also believed that the longer an immunogen spends in the host the better are the chances of antibody formation. For these reasons, there is a need to improve the current strategies for forming antibodies against peptides. The obvious danger in the use of proteins as carriers is the antigenicity of the proteins. The use of high molecular weight polymers of small peptides as vaccines is being investigated. The method used for polymerization of peptides is to allow spontaneous reaction of -SH groups and haloacetyl groups located, respectively, at the carboxyl-terminal and amino-terminal positions of the synthetic peptide. This chemistry results in end-to-end straight chain polymers or in cyclic structures with peptides of less than 10 amino acids. The problem to date has been that the polymers formed reach only a molecular weight maximum of approximately 20,000. The goal is to prepare peptide polymers with molecular weights in excess of 50,000, the putative cutoff for the kidney clearance mechanism. Attempts to solve this problem involve cross-linking the polymers into higher molecular weight structures through an additional chemical step.

Bone Cell Biology Section

A challenging problem in bone cell biology is the local control of bone cell differentiation by growth and differentiation factors. It is common knowledge that bone cells are in intimate contact with extracellular matrix components. Current research is on the role of growth and differentiation factors in bone formation and repair; the goal of this research is to define the interaction among the various growth factors in bone and cartilage.

Osteogenin, a protein that initiates bone differentiation, was purified from bovine bone matrix and its activity monitored by an in vivo bone induction assay. The purification method utilized extraction by 6 M Urea, affinity chromatography on heparin Sepharose, and hydroxyapatite and molecular sieve chromatography. Active fractions were further purified by preparative sodium dodecyl sulfate gel electrophoresis. The amino acid sequence of several tryptic peptides of the gel-eluted protein were determined. These tryptic peptide sequences were used to synthesize oligonucleotide probes. The bovine genomic library was screened and a recombinant clone was selected that hybridized to the two probes. The bovine gene probe was used to determine a cell source for the mRNA for osteogenin. DNA



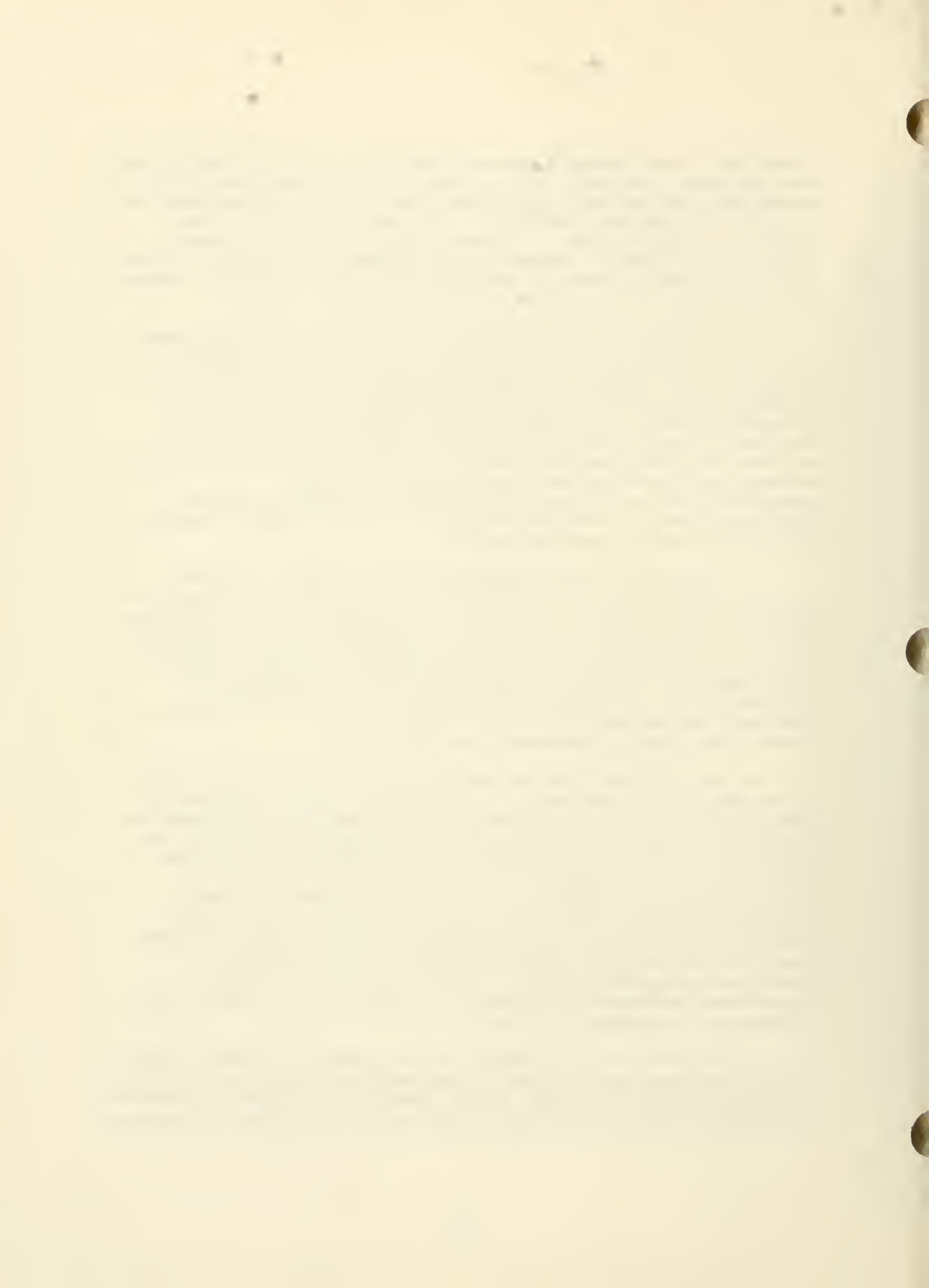
libraries of human placenta, monocyte and small cell carcinoma of lung were screened. The small cell carcinoma cell line expressed the osteogenin mRNA and full-length clones were selected and sequenced and the amino acid sequence predicted. The sequence of this 22 kDa protein is similar to BMP-3, a member of the transforming growth factor- β super family. Current work is focused on expression of this osteogenin cDNA in transient and stable eukaryotic expression systems with the goal of assessing the biological activity in vivo.

Past attempts to elicit antibodies to purified osteogenin were uniformly unsuccessful. In view of this and with the availability of the amino acid sequence of osteogenin, approaches to antibody production were made using synthetic peptides. Synthetic peptides representing amino- and carboxy-terminal sequences and internal domains were synthesized by solid phase methods. Antisera were elicited in rabbits and goats and were examined for reactivity with an enzyme-linked immunosorbant assay, protein blots and immunolocalization. The results indicate a usefulness of these antibodies, once purified, in careful studies of osteogenin mechanism and in histological localization work.

It is noteworthy that demineralized tooth matrix, like bone matrix, has the potential to induce cartilage and bone. Although the osteoinductive activity is similar it is unclear whether osteogenins from bone and tooth are identical. Osteogenin from tooth matrix was dissociatively extracted and partially purified by heparin affinity chromatography. The heparin binding fraction initiated the bone differentiation cascade when implanted with guanidine-extracted inactive bone and tooth matrices. Interestingly, the tooth matrix was found to be a better substratum than bone matrix.

As the first step towards understanding the mechanism of action of osteogenin, its influence on rat periosteal cells, osteoblasts, fibroblasts, chondrocytes and bone marrow stromal cells was examined in vitro. Osteogenin stimulates alkaline phosphatase activity and cAMP response to parathyroid hormone in periosteal cells and bone marrow stromal cell clones. In calvarial osteoblasts osteogenin stimulates alkaline phosphatase activity, cyclic AMP response to parathyroid hormone and the synthesis of collagen; however, cell proliferation is not affected. Osteogenin profoundly increases the production of sulfated proteoglycans in rat chondroblasts and in rabbit articular chondrocytes. These observations show the significant influence of osteogenin in stimulation of osteogenic and chondrogenic phenotypes in vitro.

On the basis of his leadership in the field of bone cell biology and his sustained contributions to the understanding of the role of bone growth factors, Dr. A.H. Reddi, chief of the section, was awarded the Bernard Sarnat Lectureship in Bone Biology at Tel-Aviv in January.



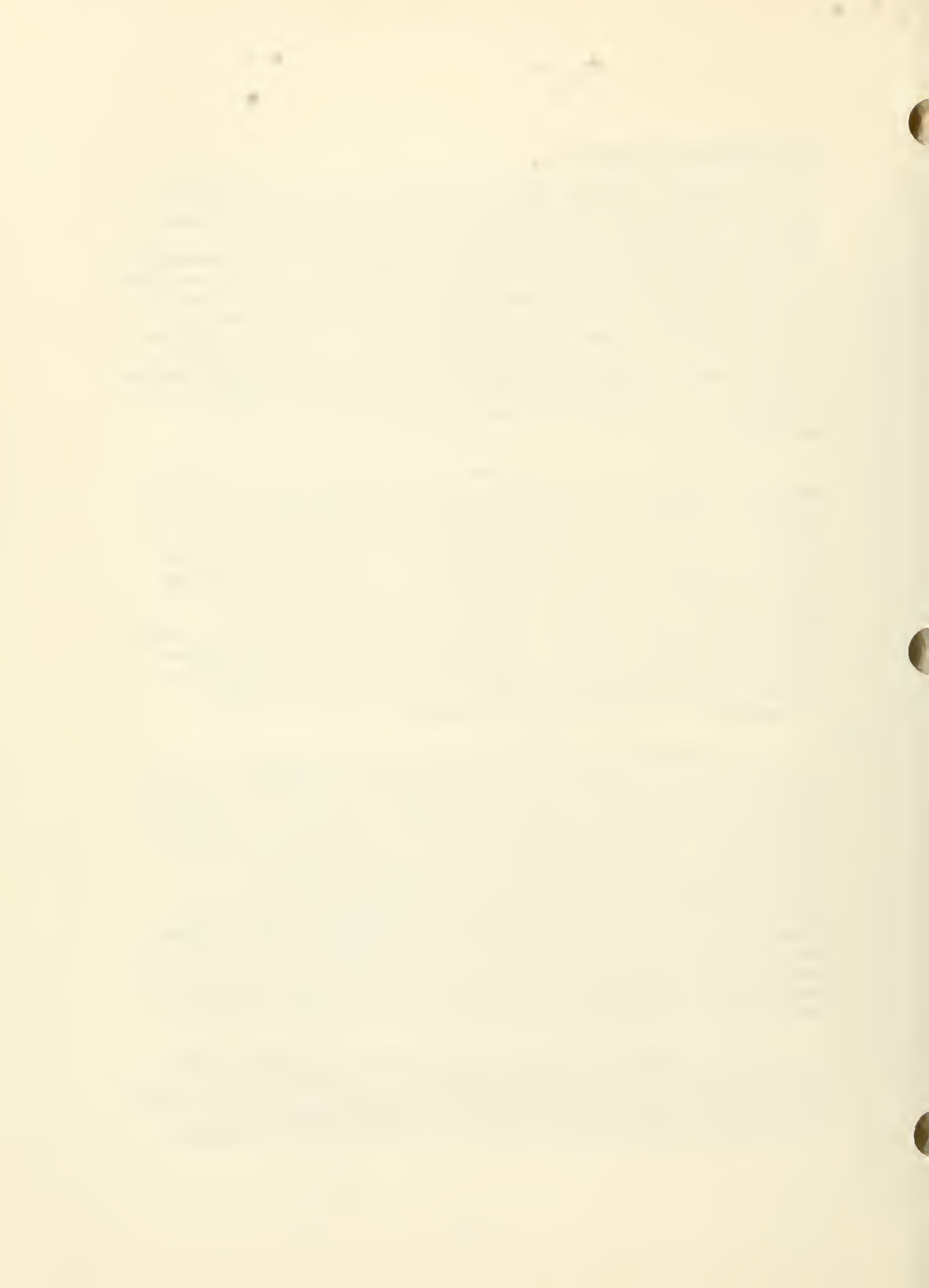
Enzyme Chemistry Section

The highly conserved eukaryotic protein synthesis initiation factor 4D (eIF-4D) is the only known cellular protein that contains the amino acid hypusine, [N^ϵ -(4-amino-2-hydroxybutyl)lysine]. Hypusine occurs at a single position in this protein. Two precursors of eIF-4D that contain lysine in place of hypusine were isolated from spermidine-deficient cells. A comparison of the stimulatory activity of these precursors and mature eIF-4D in the methionyl-puromycin synthesis assay was made. The finding that eIF-4D caused significant stimulation in this model for protein synthesis, whereas neither precursor had any effect, provides the first concrete evidence for an essential role of hypusine in eIF-4D and suggests that hypusine plays a vital part in eukaryotic protein synthesis and in cellular proliferation.

In the first step in the formation of hypusine the butylamine moiety of spermidine is transferred to the ϵ -amino group of a lysine residue to form the intermediate deoxyhypusine [N^ϵ -(4-aminobutyl)-lysine]. From double-labeling experiments it was shown that one of the protons on carbon 1 of the butylamine moiety is abstracted and replaced during this step in biosynthesis. Deoxyhypusine synthase, the enzyme that catalyzes this reaction, cleaves spermidine in the absence of eIF-4D precursors to form 1,3-diaminopropane and Δ^1 -pyrroline. Concurrently, a proton is transferred to NAD^+ . These findings form the basis for a suggested mechanism of deoxyhypusine production in which there is initial dehydrogenation of spermidine and transfer of the 4-aminobutyl group from an enzyme-bound imine intermediate to lysine in eIF-4D precursor.

Transglutaminases are enzymes that occur ubiquitously in eukaryotic cells, as well as in many extracellular regions. Although they vary significantly in molecular form, they catalyze a single covalent modification reaction, the outcome of which is the permanent attachment of certain protein molecules to one another subsequent to the assembly of their polypeptide chains. The importance of this post-translational event, which occurs through so called ϵ -(γ -glutamyl)lysine or bis-(γ -glutamyl)polyamine cross-links is evident in fibrin clot stabilization in hemostasis, vaginal plug formation as a result of postejaculatory clotting of seminal plasma, and production of the cell envelope during terminal differentiation of keratinocytes in the stratum corneum. Each of these reactions is catalyzed by a different transglutaminase and the characteristics of each reflects the individual specificity of the enzyme involved.

Several glutamine peptides of 8-12 amino acid residues were prepared by solid phase peptide synthesis. These peptides were modeled after the sequence surrounding glutamine 167 of β -casein and are excellent substrates for all of the transglutaminases, and especially for factor XIIIa. They were radiolabeled by reductive



amination using ^{14}C -labeled formaldehyde. Tests are underway using these labeled peptides to identify ϵ -amino groups in proteins that function as amine substrates for the transglutaminases. Particular attention is directed toward using those proteins known to participate in cross-linking reactions in order to verify the usefulness of the peptide method and to determine if there is enzyme specificity toward protein ϵ -amino groups. Preliminary findings with fibrin(ogen), which show reaction of one of the labeled peptides exclusively with fibrin α -chains, are encouraging and indicate a wide usefulness for this method.

Differentiating keratinocytes of the stratified squamous epithelia synthesize a cornified envelope consisting of cross-linked protein. The cross-linking process is induced by influx of calcium ions, which activate the intracellular transglutaminase(s). Several molecular forms of transglutaminase have been isolated from human skin, as well as from the epidermis of other animals. The disposition of the various forms of transglutaminase may be regulated in such a manner as to catalyze a proper and orderly production of cell envelope. Recent isolation and characterization of an inactive 77 kDa precursor of the well characterized 50 kDa epidermal transglutaminase led to the realization that the majority of attainable transglutaminase activity in epidermis is provided by activation of this proenzyme. Immunohistochemical staining of human tissues with monospecific antibody to the proenzyme showed that the expression of proenzyme is confined to suprabasal cell layers of epidermis and a few outer layers of squamous cells of non-keratinizing epithelia (buccal epithelium, cervix, etc.). The proenzyme is not expressed in cultured human foreskin keratinocytes. However, it is expressed when the cells terminally differentiate in the liquid-air raft culture. Activation of epidermal protransglutaminase was found to be induced by a number of factors, e.g., proteases, high temperature, organic solvents, increased Ca^{2+} ion, and reducing agents. The activation response to a wide variety of activators suggests that production of cell envelope as a protection against the environment may occur as a result of proenzyme activation by environmental insults.

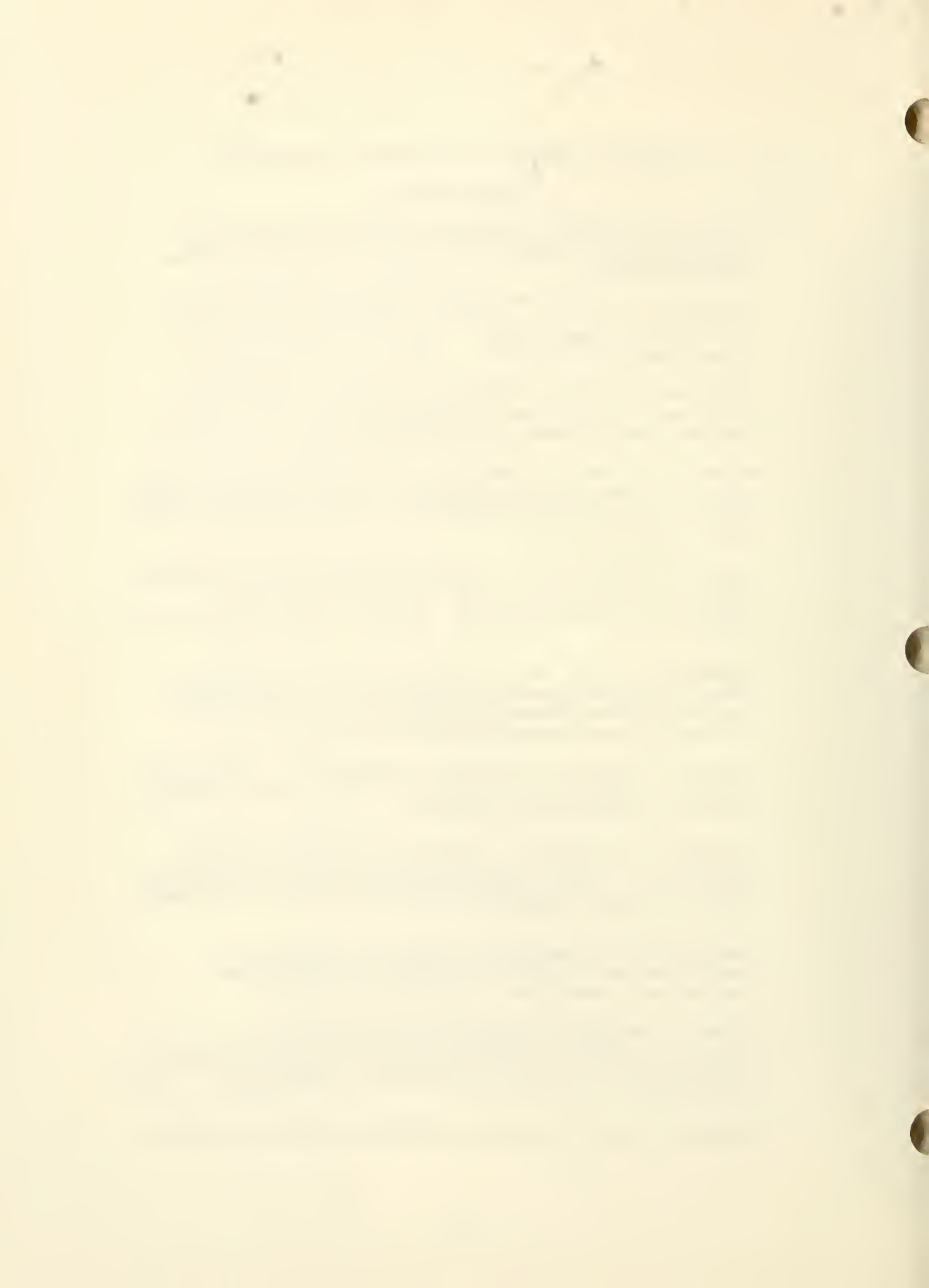
Protein cross-linking in the cell envelope of human epidermis occurs through the polyamine spermidine, as well as through $\epsilon(\gamma\text{-glutamyl})\text{lysine}$ bonds. The polyamine cross-link, bis($\gamma\text{-glutamyl}$)-spermidine, was observed at a level of about 1.5 cross-links per 1,000 amino acid residues in envelopes from normal skin, and much higher, at approximately 10 cross-links per 1,000 amino acid residues, in envelopes from psoriatic lesions. Despite the high level of another polyamine, spermine, in skin, and particularly in the involved areas of psoriatic skin, no cross-linking through this polyamine was seen. In an effort to determine the basis for specificity in polyamine cross-linking, the effects of liver polyamine oxidase on $\gamma\text{-glutamylpolyamines}$ and derivatives was examined. It was found that the size of the acylating group on the second primary amino group of

the polyamine has a pronounced influence on the activity of the polyamine oxidase. Thus, whereas both mono and bis acetyl spermine are substrates, only the mono γ -glutamyl compound is a substrate. In addition to providing further knowledge of polyamine oxidase specificity, this result suggests a mechanism by which spermine is diverted from participating in transglutaminase catalyzed envelope cross-linking.

Laboratory of Cellular Development and Oncology

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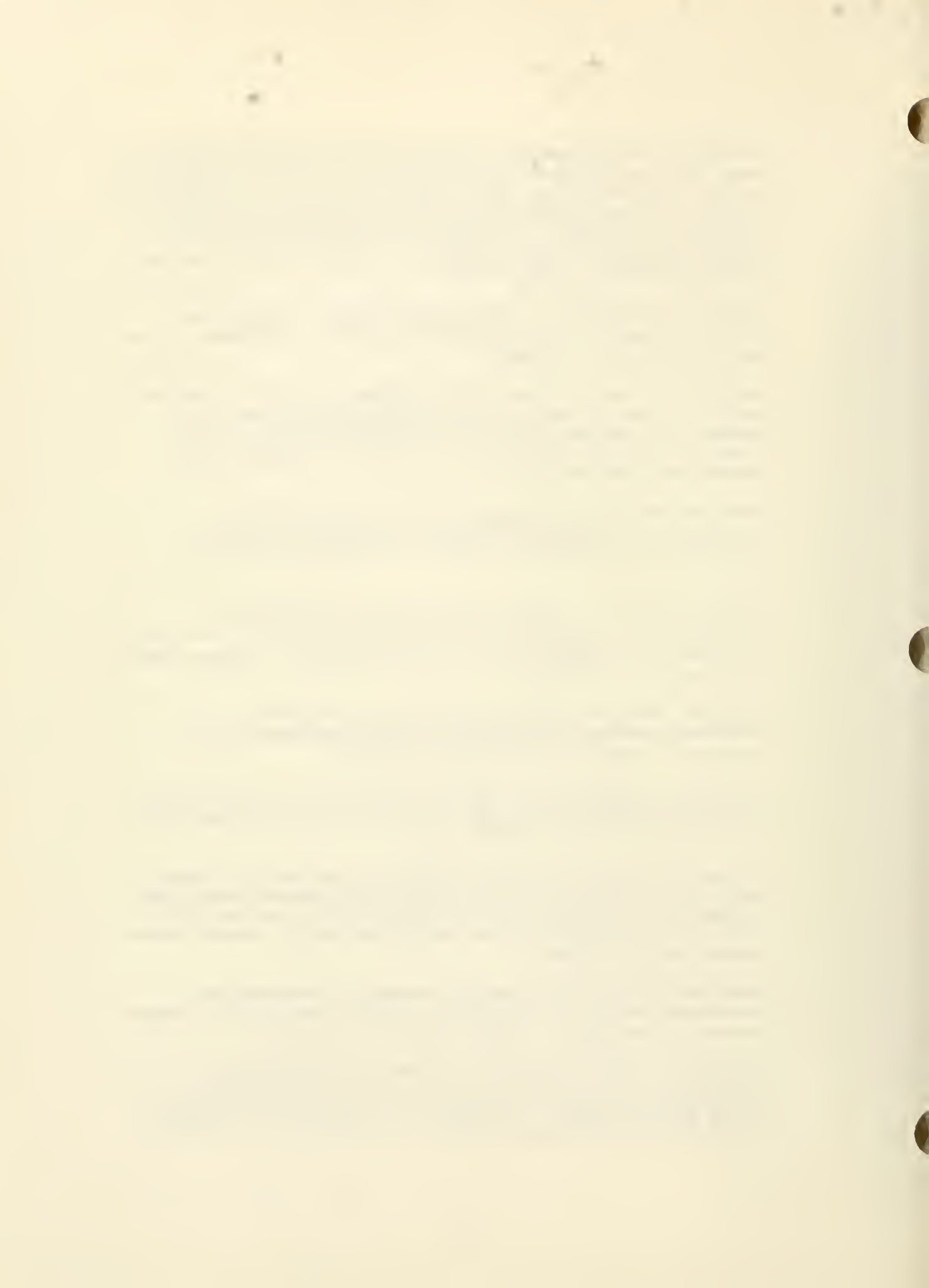
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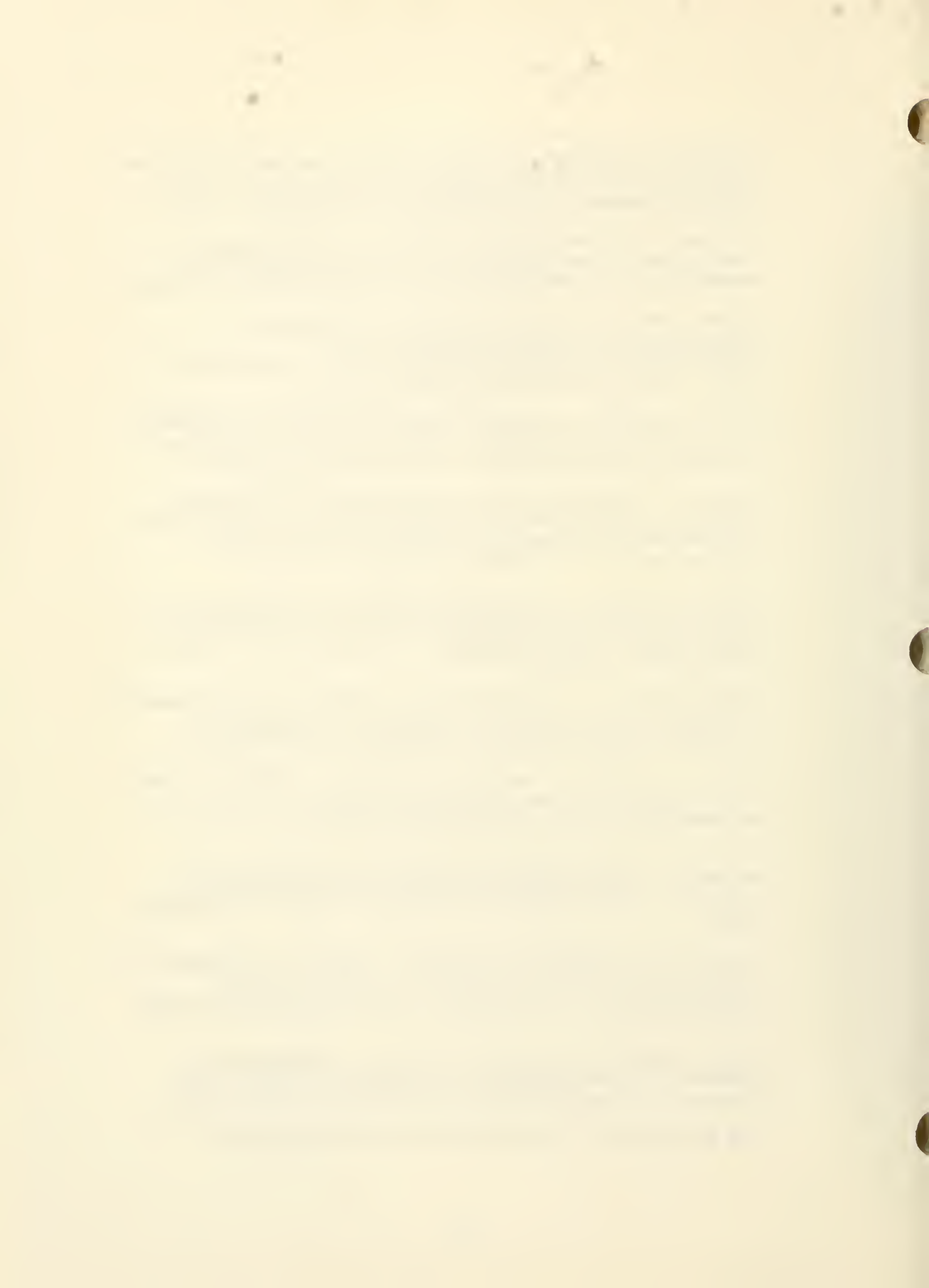
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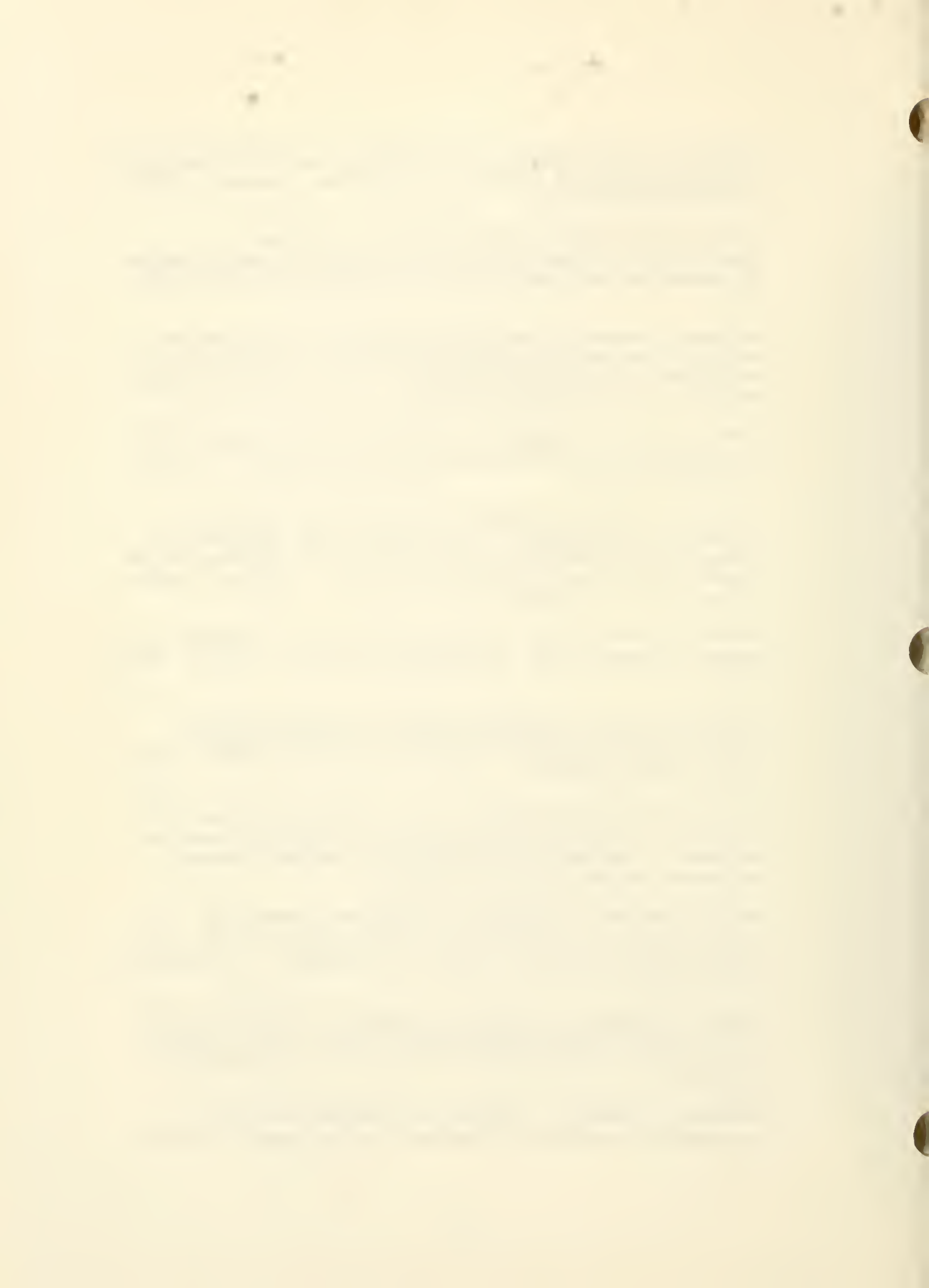
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00001-37 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Transglutaminases: Functions, Control and Biological Roles of Products.

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

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

COOPERATING UNITS (if any)

Dr. J. Gorman, CSIRO, Australian National Health Laboratory, Geelong, Australia;
 Dr. M. Fink, Baylor University, Waco, Texas; Dr. L. Fesus, University School of
 Medicine, Debrecen, Hungary.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

2.70

PROFESSIONAL.

2.50

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

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

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

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



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00049-18 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Physiological Function of Transglutaminases

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

PI:	Chung, S.I.	Research Chemist	LCDO NIDR
OTHERS:	Cardinali, M.	Visiting Fellow	LCDO NIDR
	Kanemitsu, K.	Visiting Fellow	LCDO NIDR
	Kim, H.C.	Guest Researcher	LCDO NIDR

COOPERATING UNITS (if any)

Dr. Sang Chul Park, Seoul National University, Seoul, Korea

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.85

PROFESSIONAL:

3.75

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

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

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

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of "provisional stromata" (fibrin or fibrin-connective tissue), during tissue or bone fracture repair, and in the modulation of specific cellular processes. The coagulant layer formed at injury sites is one of the vital elements of hemostasis and diathesis. The major constituent of this coagulant gel is fibrin. Fibrin stability appears to dictate overall healing and restoration processes and is modulated by factor XIIIa-catalyzed cross-linking of fibrin subunits and of α_2 -antiplasmin to α -chain of fibrin. A number of other plasma proteins are also known to cross-link to fibrin, e.g., fibronectin, thrombospondin and von Wilerbrand Factor. Osteonectin, a non-collagenous bone glycoprotein which exhibits high affinity for type I collagen, thrombospondin and hydroxyapatite was shown to cross-link to fibrin and also found to be a substrate for cellular transglutaminase. Osteonectin was found in plasma and bone as a tight complex with albumin. Fibrin clots provide matrices for the initial phase of cell migration and anchorage, and have been found to be covalently cross-linked to cell membrane (i.e., in B16 melanoma cell). The fibrin cross-linked to fibrin provides a protective shield for melanoma cells against LAK cell-induced cell-lysis. Cellular transglutaminases in terminally differentiated epidermis catalyze the cross-linking of cellular proteins to form stabilized cornified envelope. The main cytosol transglutaminase was shown to occur in an inactive form in the differentiated cells. It was activated by either high calcium concentration or by proteases that are known to be active participants in the terminal differentiation process.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00204-13 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Growth and Differentiation Factors in Bone and Cartilage

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

PI:	Reddi, A.H.	Chief, BCBS	LCDO NIDR
OTHERS:	Carrington, J.	IRTA Fellow	LCDO NIDR
	Cunningham, N	Research Chemist	LCDO NIDR
	Harrison, E.	Research Biochemist	LCDO NIDR
	Luyten, F.	Visiting Associate	LCDO NIDR
	Ma, S.	Visiting Fellow	LCDO NIDR
	Muthukumaran, N.	Guest Researcher	LCDO NIDR
	Vukicevic, S.	Guest Researcher	LCDO NIDR

COOPERATING UNITS (if any)

Dr. J. Hollinger, U.S. Army Institute of Dental Research, Washington, DC;
 Dr. William I. Wood, Genentech, South San Francisco, CA.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Bone Cell Biology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

7.60

PROFESSIONAL

7.50

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The objective of the project is to investigate the role of growth and differentiation factors in bone and cartilage. The projects currently under investigation with the salient findings are: 1) Isolation and purification of osteogenins from bone matrix. Osteogenins have been isolated and purified from bovine, porcine and human bone matrix. Osteogenin was purified by affinity chromatography on heparin Sepharose, hydroxyapatite and Sephacryl S-200. Bovine osteogenin was further purified by preparative SDS-gel electrophoresis. Osteogenin activity was localized in a zone between 30 and 40 k Da. Amino acid sequence of a number of tryptic peptides were determined. Current work is directed towards assessing structure-function relationships; 2) Production and characterization of antibodies to synthetic peptides. Amino and carboxy terminal and internal peptides have been synthesized and antisera elicited. These antisera are being systematically scrutinized by a battery of techniques; 3) Cloning and expression of osteogenin by recombinant DNA methods. Osteogenin has been successfully cloned and currently attempts are being made to express in transient and stable expression systems; 4) Affinity of osteogenin, an extracellular bone matrix associated protein initiating bone differentiation, for lectins. Partially purified osteogenin binds to concanavalin A but not to wheat germ agglutinins or soybean lectin. Osteogenin is a glycoprotein and concanavalin A Sepharose chromatography is a useful technique; 5) Purification of osteogenin from rat tooth matrix by heparin affinity chromatography; 6) Stimulation of the expression of osteogenic and chondrogenic phenotypes *in vitro* by osteogenin, and 7) Response of chick limb bud mesodermal cells to transforming growth factor β -type 1.

The significance of this investigation is in the realms of correction of cranio-facial anomalies, oral surgery and fracture repair in orthopaedic surgery.



OTHERS:

Katz, R.
Robey, F.

Dental Staff Fellow
Chief, PIU

CIPCB NIDR
LCDO NIDR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DE 00311-09 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Protein Translation Initiation Factor 4D; Structure, Biosynthesis and Control.

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

PI: Park, M.H. Research Chemist LCDO NIDR

OTHERS: Folk, J.E. Acting Chief LCDO NIDR

Wolff, E.C. Chemist LCDO NIDR

COOPERATING UNITS (if any)

Dr. Hanauske-Abel, Harvard Medical School, Boston, MA;

Dr. A. Abbruzzese, 1st Medical School, University of Naples, Naples, Italy.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

2.60

PROFESSIONAL

2.50

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

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



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00433-03 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Functional Aspects of C-Reactive Protein

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

PI: Robey, F.A. Research Chemist LCDO NIDR

COOPERATING UNITS (if any)

Tosato, G., CBER, FDA; Dhawan, S., LOM, NIDR

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

.55

PROFESSIONAL:

.45

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Two serum proteins, C-reactive protein (CRP) and serum amyloid P component (SAP), are closely related in their primary structures. Perhaps the key difference between the two is their mode of biosynthesis; whereas SAP occurs in normal serum at constant levels regardless of the disease state, the serum level of CRP raises rapidly with the onset of inflammation. CRP is believed to mediate the removal of chromatin present in the cellular debris following cell death. The function of SAP is unknown.

We found in the primary structure of SAP an amino acid sequence, YIGR, which resembles that of the cell attachment peptide. YIGR tested negative for cell attachment activity. In contrast to our expectations, a control peptide of 13 amino acids from the primary structure of SAP was found to have strong cell attachment activity. This SAP peptide was found to bind to fibronectin. Thus, we have identified a unique fibronectin-binding peptide that may be useful as a substrate for attachment of cells to insoluble matrices.

This novel cell attachment peptide may be formed by proteolysis of native SAP in vivo. This may be a first step in the repair of damaged tissue.

We have also found small peptides in the CRP structure that resembles tuftsin, Thr-Lys-Pro-Arg, and that have the ability to stimulate human B cell proliferation. Maintaining a supply of these biologically active peptides may be an important role for CRP.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00434-03 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Synthesis and Use of Peptides from HIV-1 Having Homology to Host Proteins

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

PI: Robey, F.A. Research Chemist LCDO NIDR
OTHERS: Nahor, O. Visiting Fellow LCDO NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.81

PROFESSIONAL

1.50

OTHER:

.31

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

HIV-1 is the causative agent of AIDS. To date there are no promising vaccines or drugs to combat HIV-1. Our approach to the AIDS problem is to use peptide chemistry to develop potential vaccine candidates and new drugs. The work is based on the fact that CD-4 is the cellular receptor for the virus and that the amino acid sequence of CD-4 is well-known. In addition, we are relying on published data to guide us in determining the ligand for CD-4 on the virus. Our goal is to design and synthesize reagents that are capable of blocking the binding of the virus to cells. Anti-viral drugs will be conjugated to these reagents and the conjugates will be tested to evaluate their in vitro efficacy.

The primary focus of our work is to develop a "guided missile" anti HIV-1 reagent. Such a reagent should be able to recognize the receptor for the virus, bind to the receptor and enter the cell through the receptor. In addition, our goal is to not damage the cell but to kill the virus. We are currently developing monoclonal antibodies directed against CD-4, the HIV-1 receptor. Antibodies are against peptides derived from the receptor and should react with the native receptor protein. This makes them unique from what is commercially available. Studies are currently underway to evaluate which anti CD-4 monoclonals are taken up by CD-4 expressing cells.

The techniques involved include peptide synthesis, receptor isolation and characterization, amino acid analyses, high performance liquid chromatography, affinity chromatography, new methods of conjugating nucleic acids to antibodies, antibody development and all the associative methods involved in production of specific monoclonals. Cell culture is used to produce large amounts of cells bearing the CD-4 receptor for use in our studies.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00437-03 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Peptide Polymers as Vaccine Candidates

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

PI: Robey, F.A. Research Chemist LCDO NIDR

OTHERS: Haven, Nelly Visiting Associate LCDO NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.31

PROFESSIONAL:

.31

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

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

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

In a few isolated instances, researchers have found that synthetic peptides act as suitable immunogens to provide protection against viruses. In order to function in this manner, a peptide must be coupled to a carrier protein in order that it remain in the host long enough for an immune response to develop. We are trying to improve the current peptide-carrier protein conjugation strategy by developing vaccines composed of peptide polymers. Theoretically, a high molecular weight polymer of a peptide should be immunogenic whereas the peptide monomer may or may not. Such vaccines of highly defined chemical composition may be specific for a certain pathogen, without the side effects that often occur upon using the peptide-carrier protein approach.

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



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00479-01 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Molecular Mechanisms Responsible for Oncogenesis.

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

PI: Robbins, K.C. Chief, MCBS LCDO NIDR

OTHERS; Gutkind, J.S. Visiting Fellow LCDO NIDR
 Moriuchi, R. Visiting Fellow LCDO NIDR
 Sartor, O. Senior Staff Fellow LCDO NIDR

COOPERATING UNITS (if any)

Joseph B. Bolen, LTVB, NCI

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

4.10

PROFESSIONAL:

3.00

OTHER:

1.10

CHECK APPROPRIATE BOX(ES)

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

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

The translational product (P70gag-actin-fgr) of Gardner-Rasheed feline sarcoma virus (GR-FeSV) is a protein-tyrosine kinase structurally unique among retrovirus transforming proteins in that it contains components derived from at least two different cellular genes, gamma actin and c-fgr. On the basis of this composition and findings that certain human cells express an aberrant form of actin, we investigated whether the actin domain might contribute to the oncogenic activity of P70gag-actin-fgr either by interfering with normal cytoskeletal organization or by directing the fgr kinase to substrates not normally exposed to tyrosine phosphorylation. By comparing biologic and biochemical properties of cells expressing wild-type or mutant genes, we have established that the actin domain inhibits focus formation and that its presence impairs the protein-tyrosine kinase activity of P70gag-actin-fgr.

Our laboratory has isolated and sequenced cDNA molecules which defined the fyn proto-oncogene transcript, and on the basis of this work, has developed immunologic reagents which make it possible to identify its translational product. fyn is a nonreceptor protein-tyrosine kinase with a conserved catalytic domain and a unique amino terminal region. We have shown that under conditions of high expression the fyn proto-oncogene induces morphologic transformation and anchorage-independent growth of NIH/3T3 cells. Moreover, within the population of cells overexpressing fyn we have identified genetically altered fyn genes which are capable of converting NIH/3T3 cells to a fully malignant state. Isolation and nucleotide sequence analysis of three such oncogenic fyn genes revealed that they are mutated in vivo such that their translational products are truncated at the carboxyl terminus. On the basis of these findings, we are searching in human tumor cells for overexpression of normal and truncated versions of the fyn gene product. Included in this survey are lymphatic tumor cells with chromosomal aberrations at 6q21, the site of the fyn locus within the human genome.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00480-01 LCDO

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

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

PI: Keith C. Robbins Chief, MCBS LCDO NIDR

OTHERS: J. Silvio Gutkind Visiting Fellow LCDO NIDR
Pedro Lacal Guest Researcher LCDO NIDR

COOPERATING UNITS (if any)

Timothy J. Ley, Washington University, St. Louis, Missouri

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

3.10

PROFESSIONAL.

2.00

OTHER:

1.10

CHECK APPROPRIATE BOX(ES)

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

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

We have shown that *c-fgr* proto-oncogene expression is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50 to 100 copies of *c-fgr* mRNA per cell. We have also investigated $p55^{c-fgr}$ expression in normal human neutrophils (PMN). Peptide antibodies against amino or carboxyl terminal regions of $p55^{c-fgr}$ detected the protein of 55 kd in lysates of PMN but not control cells. Neutrophil-derived $p55^{c-fgr}$ was enzymatically active as demonstrated by immune complex kinase assays, and phosphoaminoacid analysis of $p55^{c-fgr}$ revealed only phosphotyrosine. These findings established neutrophil $p55^{c-fgr}$ as a protein-tyrosine kinase.

In an effort to define possible cellular locations where the tyrosine kinase activity of $p55^{c-fgr}$ might be exerted, we fractionated human PMN into cytosol and particulate membrane compartments including primary, secondary and tertiary granules as well as plasma membrane. Abundant $p55^{c-fgr}$ was detected in the plasma membrane enriched fractions as well as fractions containing secondary and tertiary but not primary granules. When secondary granule secretion was induced with the chemoattractant peptide, fMLP, a marked decrease in $p55^{c-fgr}$ and *c-fgr* kinase was observed in fractions depleted of secondary granules. Concomitantly, the concentration of $p55^{c-fgr}$ and its enzymatic activity were increased in fractions containing plasma membrane. From these findings, we conclude that $p55^{c-fgr}$ is associated with functional secretory granules and is redistributed within normal neutrophils in response to their activation.





ANNUAL REPORT OF THE CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Clinical Investigations and Patient Care Branch (CIPCB) functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinical and basic research programs; (2) to offer consultation on oral and dental problems to other institutes and render clinical care to specified patients; (3) to encourage and facilitate clinical research activities of other branches and laboratories within the Institute; and (4) to sponsor an oral medicine training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research-oriented dental clinicians.

This past year has seen continued and significant progress in both our basic laboratory and clinical research programs. The basic laboratory research effort remains directed at studying the regulation of glandular epithelial cell secretory processes (i.e. ion fluxes and fluid movement, gene expression, protein release). Likewise our strong clinical research efforts remain focused in four areas; salivary gland hypofunction, diagnostic and management problems posed by specific compromised patient groups (oncology, congenital and acquired dental/skeletal disorders), aging and oral physiology, and oral manifestations of AIDS. The research programs of the Branch were reviewed during this year by the NIDR Board of Scientific Counselors and received an extremely high and very enthusiastic endorsement. Our success is due to the continued, exceedingly high level of effort, cooperation, flexibility and understanding by Branch personnel.

Indeed, organizational progress has been so positive that we have been able to restructure our administrative framework from the previously established two sections/two units scheme to a present four section organization. The four sections are as follows: Patient Care and Clinical Studies Section (M.W. Roberts, Chief; formerly Patient Care Section); Clinical Investigations Section (P.C. Fox, Chief; formerly Clinical Studies Unit); Membrane Biology Section (R.J. Turner, Chief; formerly Membrane Transport Unit); and Secretory Physiology Section (B.J. Baum, Chief; formerly Clinical Investigations Section). We expect that the Branch will continue to make great strides towards reaching our goals.

Patient Care and Clinical Studies Section

The Patient Care and Clinical Studies Section (PCCSS) conducts the daily operation of the NIDR Dental Clinic and is the focus of clinical oral and dental health concerns at NIH. The Section provides a wide range of diagnostic consultative services to NIH clinical care and research programs. Staff dentists and dental hygienists routinely participate in medical rounds and patient care conferences integrating oral health care concerns to total patient management.

The Section's staff continue their deep commitment to the Dental Staff Fellow program. They are primarily responsible for the clinical training, and introduction to clinical research, of the Dental Staff Fellows. Scheduled rounds, a Fellowship lecture series, oral medicine seminar series and a journal review are conducted weekly throughout the academic year. The lecture and seminar series brought in speakers from outside the NIH as well as from laboratories and branches of the



various institutes of NIH including the Intramural Research Program, NIDR.

The PCCSS has continued to expand its research programs this past year. This effort continues to be tangibly aided by a close working relationship with the other sections of the Branch as well as by collaborations with other programs at NIH. Several major protocol based study areas continue to be central to this Section. One addresses management problems of compromised patients undergoing cytotoxic chemotherapy and therapeutic radiation. Because of studies in the NCI, this group forms a large percentage of Section patients. Thus, we are in a unique position to be able to evaluate the efficacy of different regimens of oral health care in a rigorous, controlled fashion and to assess the influence of such regimens on the general health status of these compromised patients. Another general area involves patients with congenital disorders; particular attention being directed towards the characterization and management of dental-craniofacial manifestations. For example, PCCSS staff are involved in efforts to better diagnose patients with ectodermal dysplasia through roentgencephalometric analyses. Expanded management research is also directed at standardizing fixation methods following orthognathic surgery and at rigorously evaluating endosseous titanium implants in edentulous persons including persons with congenital dental agenesis such as ectodermal dysplasia. In particular, we are making use of advanced diagnostic imaging technologies (ultrasound, 3-dimensional infared image analysis) to evaluate quantitatively normal oral functions (lip and jaw movements during speech and food consumption; the oropharyngeal phases of swallowing) following orthognathic surgery and endosseous implant insertion. These procedures allow for "active" assessment of treatment modalities and patient progress, and represents a considerable advance in assessment capabilities than presently available with the traditional use of "static" head films alone.

PCCSS staff have continued to play an important role in the Branch's efforts to study oral manifestations of AIDS through studies directed at understanding the development of oral candidiasis in HIV-1 infected patients. Studies during this past year have demonstrated that AIDS patients, with oral candidiasis, have high titers of antibody (secretory IgA) in their saliva which is directed at candida. The antibody titers are significantly elevated over values seen with control subjects but patients are still unable to control oral candidal growth.

Another major area of emphasis within the Section involves studies of oral physiological status during normal aging. We now have seen ~500 normal volunteers from the National Institute on Aging's (NIA) Baltimore Longitudinal Study of Aging (BLSA) since we initiated an oral physiological component. Each volunteer is subjected to a detailed evaluation of salivary gland, oral motor and oral sensory performance, and oral mucosa status. In addition, we have extended our aging studies using a cohort of healthy, non-medicated men and women seen in a cross-sectional aging program operated by the NIA at the NIH/CC. These individuals represent a control group for a NIA study on dementia of the Alzheimer type (DAT). Accordingly, we have also begun to study oral function in patients with DAT. During this past year we have extended our normative studies which show that among healthy, non-medicated persons there is no diminution in major salivary gland fluid secretion across the life span. Age-related declines in oral sensory function have been specifically noted with regard to the magnitude of response and the quality of performance when judging pressure applied to the dorsum of the tongue. Assessments of intensity perception for temperature, texture and taste (sweet, salty) did not show such age-related declines. The initial studies of pressure-sensitivity changes with age have expanded to include 4 intra- and extra-oral sites for pressure



evaluation: namely, the tongue tip, the right dorsum of the tongue (the site used in the initial test), and the upper and lower lips. These sites were selected to allow correlation of changes in sensory function with changes in motor function (related to speech) as judged by diadochokinetic rates, which require use of the muscles of the tongue and lips. These data, as well as findings from other speech testing, will answer questions concerning changes in articulatory mechanisms (compensation) with age that continue to allow non-pathologic aging subjects to produce normal-sounding speech. Our initial analysis of olfactory data confirms earlier reports in the literature that olfactory function declines in many individuals over age 65.

Age-related changes in the clinical appearance of oral mucosa, including buccal and labial mucosa, floor of the mouth, and hard and soft palate were evaluated in a cross-sectional study combining data from the BLSA and the normative aging study at the NIH/CC. Data were obtained from 182 healthy different aged persons, using both subjective complaints and a semi-quantitative clinical rating scale. No changes in either criterion were detected with increased age, suggesting that aging, per se, does not lead to changes in the appearance of the oral mucosa. A separate evaluation of tongue mucosal appearance was conducted with BLSA subjects. In this study, twenty dentists were asked to evaluate photographs of the tongue of 60 healthy BLSA subjects ranging in age from 21 to 95. Analysis suggests the lack of correlation of clinical appearance of the dorsum of the tongue with chronological age.

Initial studies of patients with DAT have focused on evaluating salivary gland fluid output in a population with early stage disease. We observed that submandibular flow rates were significantly lower among patients with DAT compared to controls. Importantly, these patients showed no such differences in parotid flow rates. Our findings suggest a selective impairment in submandibular gland function occurs in essentially healthy patients with DAT.

PCCSS staff are also continuing participation in a multi-center study evaluating the effects of the non-steroidal anti-inflammatory drug flurbiprofen on periodontitis in adult subjects. The study is sponsored by the Upjohn Company and is being conducted at four other research centers besides NIDR; Emory University, Harvard University, University of Michigan and University of Texas-San Antonio. This study should be completed during the next fiscal year.

The Section recently has also developed an interest in oral sequela associated with the eating disorders anorexia and bulimia nervosa. In cooperation with the National Institute of Mental Health, patients are referred to our clinic for oral soft and hard tissue evaluation, oral motor examination and glandular saliva collection. Initial studies have demonstrated a high incidence of salivary gland enlargement associated with bulimia and a reduction in unstimulated parotid saliva flow but not in stimulated rates. A significant loss of both pharyngeal and velar gag reflexes has also been documented along with two highly unusual oral swallowing patterns.

The PCCSS has also continued its academic affiliations with the Baltimore College of Dental Surgery of the University of Maryland, Georgetown University School of Dentistry, and Montgomery College. These arrangements provide graduate dental students, senior dental hygiene, dental assistant, and dental laboratory students an opportunity to experience alternative practice settings beyond those offered in the school core curriculum, as well as provide our staff with academic clinical dental teaching responsibilities. The Section also participates in the NIH Clinical Electives Program, which emphasizes providing dental care to medically compromised



patients in a hospital environment and an introduction to clinical research.

Clinical Investigations Section

The new Clinical Investigations Section (CIS) has evolved from the old Clinical Studies Unit. This principle focus of the CIS is understanding the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders. In addition, the CIS has been central to Branch studies on oral manifestations of HIV-1 infection. The central CIS activity remains the dry mouth (xerostomia) clinic, in which we have now involved more than 650 patients. Considerable progress continues in our efforts to develop more specific diagnostic and treatment approaches for patients with dysfunctional salivary glands. About 40 new individuals per year are now admitted as inpatients for intensive study under the protocol "Evaluation and treatment of salivary gland dysfunction". Approximately 60 persons are studied as outpatients. We have continued two treatment protocols for salivary dysfunction which were begun previously. In one protocol, we utilize a steroid (prednisone), and a non-steroidal anti-inflammatory drug (peroxicam), to control the autoimmune damage to salivary glands in primary Sjogren's syndrome. There are approximately 1-2 million Americans with primary Sjogren's syndrome and as yet no specific therapy exists for the condition. Patients with early and late stage disease are studied. Treatment is designed both to arrest progressive gland dysfunction and improve existing function. Early results (~20 patients) suggest a clear beneficial effect of prednisone on salivary secretion, but peroxicam was without effect. Patients continue to be enrolled in this study. In the other protocol, we employ the parasympathomimetic drug pilocarpine to preserve salivary function during head and neck radiation. Approximately 50,000 Americans each year are diagnosed with a head and neck cancer. Most are treated, at least in part, with radiation. The single greatest area of post-treatment complaint in surviving patients is related to oral damage reflective of salivary dysfunction. Thus far six patients have completed this protocol. The initial results suggest that pilocarpine may have beneficial effects on parotid (but not submandibular) fluid secretion, and on chemosensory perception. Both of these protocols address significant clinical management problems. In addition, we have completed the third trial study of pilocarpine, reported last year. In this study, patients with gland hypofunction, but with evidence of residual gland parenchyma present, receive pilocarpine three times per day over a 6 month period (one month is a double-blind placebo period). Thirty-one individuals completed this study. Patients experienced no significant side effects due to prolonged treatment with pilocarpine. Side effects, while common, were mild, transient and well-tolerated. Sustained subjective relief was observed by 87% of the study patients and two-thirds (20) of the patients manifested increased salivary output after drug therapy. All study subjects clearly stated that if given a choice, they would prefer to remain using pilocarpine.

The major grouping of salivary gland dysfunction patients which we see remains individuals with primary Sjogren's syndrome (SS). We continue to examine intensely the status and function of oral tissues in these patients. Recently, we completed a study of salivary function in 69 SS patients. Our findings demonstrate that submandibular gland function is much more severely impaired in these patients, than parotid gland function and that, indeed, stimulated parotid gland secretion (an often used clinical test) is relatively well-preserved. We also have examined oral sensory function in SS patients. These patients show a specific defect in oral temperature perception, but essentially normal judgements of intensity for taste, tactile and textural stimuli. This pattern of alteration is distinct from that

generally observed by us to occur in older persons (i.e. altered perception of oral pressure). Whether such a perceptual change may be associated with the occurrence of oral mucosal burning sensations, which have often been reported in post-menopausal women with salivary dysfunctions, is at present not clear.

The CIS has continued to study the relationship between salivary secretion and deglutition. Previously, we observed that patients with salivary hypofunction display a dysphagia of oro-pharyngeal etiology; the time of the oral phase of swallowing is increased ~ 2 fold. In addition, recently, we observed that a significant subgroup of studied SS patients ($\sim 40\%$) show an unusual pattern of oral swallow; their oral phase of swallow requires more time to complete in the presence (versus the absence) of water. Although the presence of saliva appears critical to successful completion of an oral swallow by a patient with salivary hypofunction, in healthy individuals who are free of xerostomic complaints there is no relationship between the performance of an oral swallow (time; number per unit time) and salivary flow rate. That is, healthy persons with salivary flow rates which differ by 10-50 fold show comparable oro-pharyngeal swallows.

During this past year the CIS has also made two important technical contributions to the clinical evaluation of salivary secretions. For many years it has been recognized that minor salivary glands, of which there are hundreds scattered around the mouth, are very important to the basal protection of oral tissues. However, no simple, reproducible, quantitative means have been developed to measure minor gland fluid output. Section investigators have used a device (gingival fluid flowmeter), which operates by measuring the dielectric constant of paper, to measure minor gland secretory rates. Secretions are adsorbed to a standardized filter paper strip, the dielectric constant is recorded and compared to a standard curve prepared by measuring known volumes of adsorbed fluid. Although there is considerable variability between patients, individual reproducibility is extremely high. This is a rapid and reliable method which should prove widely useful. CIS scientists, in collaboration with the NIH Biological Engineering and Instrumentation Branch, have also developed a simple, universal submandibular salivary collector. Although satisfactory collectors exist at present, they are (i) generally bulky (and therefore would provide considerable tactile stimulation; not ideal for basal measurements), (ii) not useful for patients with salivary hypofunction (it is hard to measure small fluid volumes) and (iii) awkward to use with patients who have oral mucosal lesions (e.g. patients receiving chemotherapy). The universal collector is based on the use of a micropipet to collect saliva directly from the orifice of Warton's duct. The micropipet is attached, via a small trap, to a suction device. It is simple to use, yields reproducible data and also should prove widely applicable in salivary physiological studies.

As noted above, the CIS has played a major role in Branch efforts to understand the oral manifestations of HIV-1 infection. We previously have reported, based on observations in 3 healthy men, that saliva contains a factor which inhibits the ability of HIV-1 to infect lymphocytes. Recently, we extended this study to 34 persons, including healthy women, children (plus additional healthy men) and HIV-1 positive men. All were found to contain the inhibitory activity in their saliva, suggesting its ubiquitous existence. Efforts (involving CIPCB and other NIDR staff) are now underway to determine the mechanism by which this inhibitory activity occurs. These observations may, in part, help to explain the apparently extremely low risk of oral transmission of HIV-1 infection. We also have reported that relatively soon after HIV-1 infection (i.e. in asymptomatic, generally-healthy individuals who are HIV-1 antibody positive), alterations in salivary gland



performance can be detected. As saliva forms a key component of the oral host defense mechanism, this change could have negative consequences to the patient by changing host resistance and increasing risks of infection via an oral route. We have extended these studies through the careful examination of the status of salivary anti-microbial proteins in AIDS patients. We measured the concentrations of lactoferrin, lysozyme, secretory IgA and histatins (the latter are anti-candidal proteins) in AIDS patients and controls. We observed a general increase in the levels of these proteins in patients and, in particular, saw the greatest elevations of lysozyme and histatins in patients with oral candidiasis. These changes may indicate a response by the host to the HIV-1 infection and associated opportunistic microbial threats. However, despite the increased levels of these anti-microbial proteins, oral infections (i.e. candidiasis) still proceed (see above related studies by the PCCSS). A final major area of our AIDS studies involves a collaboration with the NIDR Epidemiology and Oral Disease Prevention Program (EODPP). The EODPP has established a longitudinal epidemiology study, at the Walter Reed Army Medical Center, to determine (and to follow) the oral manifestations of AIDS. Branch staff are collaborating in several aspects of these studies including work on salivary gland functional assessments and on oral candidiasis.

A major strength of our Branch is the linkage of our laboratory and clinical components. Because of (i) our location, within the NIH Clinical Center, (ii) our mixture of basic scientists and clinicians, and (iii) the close physical approximation between our various groups, we are able to enhance each other's research efforts and help to fulfill our mission. This is especially obvious in the many interactions which occur between Branch basic scientists and CIS investigators. For example, as noted above, the CIS has devoted considerable effort to the study of the autoimmune exocrinopathy, primary SS. Using an established human submandibular ductal cell line, cloned by scientists in the Secretory Physiology Section (SPS), CIS staff have begun to study possible antigenic targets in the salivary glands of SS patients. Sera from SS patients show potent reactivity with this cell line, in a nuclear staining pattern, which is absent from both healthy persons and non-SS patient controls. Although the reactive cellular component (s) has not yet been identified, it appears not to be either the SS-A or SS-B antigens. Another area of strong collaboration involves the CIS and Membrane Biology Section (MBS). At present, we (health practitioners) do not have the clinical (i.e. in vivo) diagnostic tools to delineate specific alterations in the neurotransmitter signal transduction pathway of salivary glands which can lead to secretory hypofunction. In order to accomplish a molecular diagnosis of salivary gland dysfunctions MBS scientists have established a videofluorescence imaging system which will allow CIS (or other Branch) investigators to monitor signal transduction events on a single cell level. We can use clinical samples (e.g. needle biopsies of major glands; minor gland biopsies) attached to coverslips and monitor, by appropriate fluorescent probes, neurotransmitter regulation of Ca^{2+} mobilization, pH and membrane potential changes. All of these are critical to normal salivary cell function (see below). A final (but not the final) example of the excellent clinical-laboratory collaboration which occurs in our Branch is related to studies reported last year on anti-microbial peptides found in minor salivary glands. These peptides are antigenically related (perhaps identical) to the frog skin magainins. MBS scientists have worked with CIS staff to demonstrate the presence of these peptides in human minor salivary glands, while similar collaborative efforts with SPS staff have permitted demonstration of magainin immunoreactivity in saliva samples. Further, CIS and PCCSS scientists have teamed together to develop a sensitive, reproducible and functional assay for salivary anti-microbial activity using the E. Coli strain utilized in magainin studies. Such

cooperative efforts have allowed the Branch to continue to develop a strong, comprehensive program to evaluate all components (saliva, commensal flora, mucosal barrier) of oral defense.

Membrane Biology Section

The Membrane Biology Section (MBS) was established this year in response to the excellent scientific and organizational performance by the old Membrane Transport Unit. The major focus of the MBS is directed at understanding biochemical steps involved in the formation of saliva. It is well accepted that saliva has a critical role in the defense, and functional maintenance, of all oral tissues. Saliva contains water and electrolytes, derived from serum, and specific exocrine proteins synthesized by glandular epithelial cells. Salivary glands are useful models of secretory processes and studies with these glands have proved important to our understanding of basic concepts of secretion and to appreciating pathogenesis in conditions such as cystic fibrosis.

Recent studies have indicated that fluid secretion in many exocrine glands is related to transepithelial anion movements. It has been suggested that a model first proposed by Silva et al. for the shark rectal gland applies to a number of these tissues. In this model, four plasma membrane ion transport systems act in concert to move Cl^- , and ultimately fluid from the interstitial space to the luminal space in response to secretory stimuli. These transport systems are (i) an electroneutral, loop diuretic-sensitive Na/K/Cl cotransporter located in the basolateral membrane of the secretory cell, (ii) a basolateral K^+ channel, (iii) an apical Cl^- channel, and (iv) the Na/K ATPase. According to this model the electrochemical gradient for Na^+ generated by the Na/K ATPase causes Cl^- to be driven into the secretory cell against its electrochemical gradient via the basolateral cotransporter. Stimulation of the gland results in the opening of the basolateral K^+ channel and the apical Cl^- channel. These increases in basolateral K^+ conductance and apical Cl^- conductance allow K^+ and Cl^- to flow out of the cell down their electrochemical gradients resulting in an accumulation of Cl^- ions in the lumen. Na^+ is thought to follow Cl^- by leaking through the tight junctions between the cells in order to preserve electroneutrality. The resulting osmotic gradient for NaCl causes a net transepithelial movement of water from interstitium to lumen.

Previous studies by MBS scientists have demonstrated the presence of the Na/K/Cl cotransporter in parotid basolateral membrane vesicles and shown that this was the predominant mechanism by which Cl^- can enter the resting and stimulated parotid acinar cell. We also have shown the existence of a muscarinic-agonist stimulated Cl^- exit pathway in these cells, likely the hypothesized apical membrane Cl^- channel, and demonstrated the presence of another possible means for Cl^- entry, coupled Cl/HCO_3 and Na/H exchange in the basolateral membrane. We, in fact, showed that in rat parotid acinar cells ~25% of the transepithelial Cl^- flux was due to Cl/HCO_3 exchange. Our past studies on the regulation of intracellular pH by muscarinic receptors in acinar cells, also gave strong support for the important role of Cl/HCO_3 and Na/H exchange, and of a HCO_3 dependent component of secretion, in the elaboration of salivary fluid.

This past year we have continued studies aimed at understanding the regulation of the Na/H exchanger by physiological stimuli. Using the fluorescent probe BCECF we showed that within 30 sec of stimulation by the muscarinic agonist carbachol, rat parotid acini respond to an acute acid load (decreased intracellular pH) by an activation of the Na/H exchanger. This activation was not mimicked by phorbol



esters, which can activate protein kinase C, nor is it prevented by agents which block stimulation of this kinase. Also, Na/H exchanger activation was unaffected by chelation of intra- and extra-cellular Ca^{2+} . These results strongly suggest the possibility that the agonist-occupied muscarinic receptor can more directly (i.e. not through a second or third messenger) activate the Na/H exchanger. This might be via direct coupling to a G protein or even involve the possibility that the muscarinic receptor subtype involved possesses Na/H exchange activity. It thus appears that in rat parotid acinar cells Na/H exchanger activity can be regulated at two levels. The above described Ca^{2+} independent control is associated with the initial phase of secretion, while a Ca^{2+} dependent mechanism (reported last year) is associated with sustained secretory responses. Physiologically, a stimulation of the Na/H exchanger would be expected to increase intracellular HCO_3^- levels (by increasing pH_{in}) and thus increase HCO_3^- dependent fluid secretion. Increased pH_{in} may also have other less direct effects on the fluid secretory process, e.g., by changing the rates of certain intracellular enzymatic and other processes.

These results have given impetus to related studies aimed at providing a more detailed characterization of the anion (Cl^-) channel proposed to exist in the apical membrane of acinar cells. One line of experimentation which we have used employs BCECF fluorescence to follow the secretion of various weak acids loaded into acinar cells, and thus characterize the anion specificity presumed to be found in the apical anion channel. We observed that rat parotid acini can secrete many anions but with the following order of effectiveness: $\text{HCO}_3^- \gg$ acetate $>$ propionate $>$ formate $>$ n-butyrate \gg lactate. We also have used another, quite different, approach to study the HCO_3^- exit pathway. Anion loss from acinar cells must be accompanied by K^+ loss according to the above described model. We use $^{86}\text{Rb}^+$ as a marker for K^+ and have shown that the marked $^{86}\text{Rb}^+$ efflux observed from parotid acini after stimulation by a muscarinic agonist in a "physiologically complete" medium is severely blunted when both Cl^- and HCO_3^- were replaced by the relatively impermeant anion gluconate. This observation strongly supports our model for salivary fluid secretion involving the coupled loss of K^+ and an anion (Cl^- , HCO_3^-) from the acinar cell by showing that there is no significant anion-independent K^+ efflux pathways in these cells. $^{86}\text{Rb}^+$ Loss from agonist stimulated cells can, however, occur both in a HCO_3^- free medium (with Cl^- present) or a Cl^- free medium (with HCO_3^- present) with the following rate constants; 0.209 and 0.162, respectively. This too strongly supports the notion that rat parotid acini can display significant levels of HCO_3^- dependent fluid secretion. Considerable other data also are consistent with the idea that Cl^- and HCO_3^- share the same exit pathway across the apical membrane in these cells. Recently, we have extended these studies to the bovine parotid acinar cell. This cell seems to be much more dependent on HCO_3^- for secretion than its rat counterpart. It also has a more cuboidal shape than rat or human cells and therefore may prove to be an excellent model with which to study the apical anion exit pathway.

MBS scientists have also continued their studies on the Na/K/Cl cotransporter, the predominant mechanism for Cl^- entry into acinar cells (and thus the primary driving force for fluid secretion). These efforts are directed at understanding the molecular and biophysical processes involved in the operation of this key component in the above described model. We have made considerable progress, in particular, in the purification of this cotransporter. Based on our previous work showing that [^3H] - bumetanide binds directly to the Na/K/Cl cotransporter, we have used [^3H] - bumetanide binding to follow the solubilization and partial purification of this protein. We have used low concentrations of the non-ionic detergent Triton x-100 (<0.3%) for solubilization. Binding activity is stabilized by the addition of



Na⁺, K⁺ and Cl⁻ salts and by exogenous lipids. Indeed, in the absence of lipids all diuretic binding activity is lost. Addition of specific lipids (~0.15%, potency order as follows: phosphatidyl inositol > phosphatidyl glycerol = phosphatidyl serine > phosphatidic acid = cardiolipin) allow recovery of binding. Many lipids (e.g. phosphatidyl choline, cholesterol, etc.) were without effect. The effect of lipids on the Na/K/Cl bumetanide binding site appears to be a specific interaction. These findings should greatly help in our efforts to purify the Na/K/Cl cotransporter and understand its mechanism of operation.

Other efforts by MBS staff have been directed at understanding the programmed appearance of marker exocrine proteins during rat submandibular gland development and following growth stimuli. Several salivary proteins are followed including several neonatal (protein B₁, protein C, protein D) and adult (mucin; glutamine/glutamate-rich protein, GRP) species. Previously we showed that in fetal glands protein B₁ and C are found in separate cells (type III, type I, respectively). The adult secretory proteins, mucin and GRP, first appear in some newborn type III cells. These newborn cells appear to be transitional cells between the fetal type III cells and adult mucous acinar cells. GRP and mucin are exclusively found in the latter cell type in adult glands. Recently MBS investigators have followed the localization of protein D during development. Protein D is found in both types I and III cells of fetal animals. In the adult protein D is found in both intercalated duct cells and mucous acinar cells. We have also examined secretory protein localization in adult rat parotid glands following chronic stimulation with the β -adrenergic agonist isoproterenol (this treatment causes hyperplastic and hypertrophic growth of the gland). The proteins followed include amylase, proline-rich proteins (PRP) and a leucine-rich protein (PSP). Following isoproterenol treatment, detection of PRPs is increased while diminished immunoreactivity for both amylase (~50%) and PSP (~90%) are observed. Parallel studies (see below under SPS) are examining the regulation of mRNA transcription in isoproterenol treated parotid glands, in an effort to better understand the molecular switches necessary to control the growth and differentiation of these cell types.

Secretory Physiology Section

This section represents most of the CIPCB effort housed in what was formerly termed the Clinical Investigations Section. The investigations of the Secretory Physiology Section (SPS) are primarily involved in efforts to understand the events which underlie neurotransmitter regulation of exocrine epithelial cells. Specifically these efforts are directed (i) at controlling steps in second messenger formation (cAMP, inositol trisphosphate, IP₃, and Ca²⁺) and (ii) understanding the post-receptor "switches" which account for the growth control and differentiation (i.e. tissue specific gene expression) of salivary cells. These mechanistic questions are not only central to understanding neurotransmitter regulation of salivary secretion but, indeed, are pivotal questions central to all hormone regulated physiological processes.

Previously we have shown that β -adrenergic stimuli, via a cAMP second messenger, were capable of activating PIP₂-specific phospholipase C and mobilizing Ca²⁺ in rat parotid acinar cells. This is an excellent example of receptor crosstalk between different signalling systems. During this past year we have concentrated considerable effort on studying interactions between similar (both ~IP₃; e.g. via α_1 -adrenergic and muscarinic) and different (such as cAMP and IP₃; e.g. via β -adrenergic and muscarinic) signal transduction mechanisms. To further study the latter phenomena, we have utilized the B82 cell which is a mouse "L" cell possessing



native PGE₁ and transfected human β_2 -adrenergic receptors. This cell provides a more simple signalling system than rat parotid acinar cells, in that it possesses no native "Ca²⁺-mobilizing" receptors (therefore there exists no possibility of non-specific activation). We observed that both PGE₁ and isoproterenol increased cAMP in B82 cells as well as increased cytosolic Ca²⁺ levels ([Ca²⁺]_i). Both isoproterenol and 8 Br-cAMP rapidly increased IP₃ levels, indicating both agents were capable of activating a PIP₂-specific phospholipase C. These results are similar to what we have observed in parotid acinar cells and demonstrate a direct stimulatory effect of cAMP on the IP₃/Ca²⁺ mobilizing system. The mechanism underlying this interaction is not clear but efforts by SPS staff have led to suggestions that this interaction may occur at the level of a transducing G protein. Strong, though indirect, evidence comes from experiments using the muscarinic antagonist atropine and the non-receptor activator of G proteins, AlF₄⁻, in both parotid acinar and B82 cells. In parotid cells, 10⁻⁵M atropine has no effect on isoproterenol-stimulated amylase release but blocks isoproterenol-stimulated changes in [Ca²⁺]_i. AlF₄⁻ can elevate parotid [Ca²⁺]_i and this response is also blocked by atropine. However, atropine has no effect on either the isoproterenol- or AlF₄⁻-elicited Ca²⁺ mobilization observed in B82 cells. Importantly, parotid acinar cells, but not B82 cells, possess muscarinic receptors. When yet another cell type, M1 cells (a CHO cell with a transfected muscarinic receptor), is used, the AlF₄⁻-induced increase in [Ca²⁺]_i is blocked by atropine. Thus, it appears that a muscarinic antagonist occupying a muscarinic receptor can prevent IP₃ formation and Ca²⁺ mobilization induced by a β -adrenergic receptor (via isoproterenol) or via a non-receptor means of activating a G protein (AlF₄⁻). The common link in these crosstalk experiments may be the transducing G protein which activates PIP₂-specific phospholipase C (Gp).

Parallel experiments have looked at possible signaling interactions between receptors which purportedly transduce their signals via the same mechanism (α_1 -adrenergic and muscarinic; via IP₃ formation and Ca²⁺ mobilization). Simultaneous addition of agonists for these two receptor types (epinephrine and carbachol, respectively) do not lead to additive responses (either IP₃ or changes in [Ca²⁺]_i). This is observed at maximal and submaximal concentrations of the agonists. The latter (ie. submaximal) experimental results occur despite the presence of ample levels of PIP₂ in the cell membranes to allow for additive responses. Thus, some cross inhibition (heterologous inactivation) must be taking place. Interestingly, parotid cells are unable to mobilize Ca²⁺ in response to addition of AlF₄⁻ after simultaneous exposure to submaximal concentrations of epinephrine and carbachol. These experiments also suggest that receptor crosstalk may occur at the G protein level. We recognize that this conclusion is based on indirect data and accordingly we have begun experiments (by measuring [³⁵S] GTP γ S binding and GTPase activity) to study directly agonist and antagonist effects on the G protein activation involved in IP₃ formation and elevations in [Ca²⁺]_i. It is important to emphasize that our efforts directed at understanding crosstalk mechanisms in parotid cells are not only addressing key mechanisms of basic signal transduction, but also realistically examining a situation which parallels physiology, where multiple receptors are simultaneously activated by neurotransmitters.

While the above-described studies focus on the immediate post-receptor signals involved in neurotransmitter activation, in the case of physiological salivary fluid secretion cellular responsiveness is sustained (i.e. stimulated salivary fluid can readily be elicited for 20-40min). This sustained secretion of saliva is also dependent on Ca²⁺ (entry of extracellular Ca²⁺). Therefore, we have also focused

substantial effort into understanding receptor regulation of Ca^{2+} entry into the cell and the refilling of the intracellular target " Ca^{2+} pool". The initial phase of parotid acinar cell Ca^{2+} mobilization after addition of the muscarinic agonist carbachol is rapid (peak 3-4 fold basal reached within 5s) and transient (completed within 300-400s). Thereafter, the small (~ 1.5 -2 fold basal) sustained phase of elevated $[\text{Ca}^{2+}]_i$ occurs. The latter is dependent on the extracellular $[\text{Ca}^{2+}]$ and can be mimicked by AlF_4^- . This response (agonist or AlF_4^-) proceeds for >20 min and therefore likely provides the means for continuous, Ca^{2+} -dependent, stimulated salivary secretion which occurs during physiological events such as alimentation. The specific mechanisms by which the intracellular agonist sensitive Ca^{2+} pool (ASCaP) is refilled are not known, either in general or for exocrine cells specifically. We have examined this important mechanism by studying $[\text{Ca}^{2+}]_i$ mobilization in parotid cells in response to sequential addition of carbachol and epinephrine. Our data support the notion that refilling of this ASCaP can occur at least by two ways; one involving extracellular Ca^{2+} entry, the other involving a redistribution of intracellular Ca^{2+} previously not found in the ASCaP (i.e., it occurs with EGTA or La^{3+} in the incubation medium). Furthermore, our results indicate that the gradient of Ca^{2+} across the target intracellular IP_3 -sensitive membrane (ASCaP) to the cytosol can modulate the initial $[\text{Ca}^{2+}]_i$ response by an agonist, and that Ca^{2+} entry mechanisms (depending on the extracellular $[\text{Ca}^{2+}]$) can alter the steady-state level of Ca^{2+} within this compartment. Antagonists may alter this ASCaP to cytosol gradient by terminating the agonist-induced release of Ca^{2+} from this compartment.

As noted last year, we now have in routine use several established salivary cell lines which have shown considerable promise for characterizing neurotransmitter responses. Established cell lines offer several advantages over acute *in vitro* preparations including (i) the ability to carry out long term studies, (ii) the convenience and reproducibility which comes with cloned cell lines, and (iii) the general applicability of cell cultures to somatic cell genetic and molecular biological techniques. The following five cell lines have been used by us: A253, isolated from an adenocarcinoma of a human submandibular gland; A5, a cloned rat submandibular ductal cell line derived from RSMT cells which we have previously reported; HSG-PA, a cell line derived from the intercalated duct region of a human submandibular gland; HSY, a cell line derived from the human parotid gland; and HSG-MY, derived from HSG-PA following chronic exposure to sodium butyrate and purported to be a myoepithelial cell.

We have devoted substantial effort over the past year to studying receptor-operated Ca^{2+} mobilization mechanisms, and receptor-induced trans-epithelial cell K^+ fluxes, in these cells (particularly HSG-PA). For example, the HSG-PA cell has proven to be an excellent model with which to study the two distinct phases of cytosolic Ca^{2+} mobilization; intracellular Ca^{2+} release and extracellular Ca^{2+} entry. At maximal concentrations of carbachol ($100\mu\text{M}$), peak values of $[\text{Ca}^{2+}]_i$ due to ASCaP release increase from $104\pm 11\text{nM}$ to $329\pm 38\text{nM}$, while that due to Ca^{2+} entry increases from $84\pm 6\text{nM}$ to $244\pm 17\text{nM}$. At lower [carbachol], $\sim 2.5\text{nM}$, Ca^{2+} release is diminished more than Ca^{2+} entry. Kinetic experiments show that ASCaP release appears to be mediated via a single, relatively low affinity muscarinic receptor site, while Ca^{2+} entry appears mediated by two muscarinic receptor sites; one of low affinity similar to that involved in ASCaP release and one of higher affinity. The latter appears modulated by changes in membrane potential; depolarization with either high K^+ or gramicidin can block Ca^{2+} entry. The muscarinic antagonist oxotremorine-M, which purportedly recognizes high affinity receptors, primarily induces a Ca^{2+} entry response, which can be blocked by membrane depolarization. Our studies have



suggested that these salivary epithelial cells may exhibit two modes of agonist-induced Ca^{2+} entry. One is associated with ASCaP release (and therefore IP_3 formation) and is independent of membrane potential, while the other is less dependent on ASCaP release and IP_3 formation but is modulated by membrane potential (and, thus, may exhibit voltage-dependent gating).

We also have studied HSG-PA cell muscarinic receptor-induced Ca^{2+} mobilization using the calmodulin (CaM) antagonist W-7 as a probe. W-7 preferentially inhibited ASCaP release induced by carbachol (vs Ca^{2+} entry). The residual Ca^{2+} entry response was blocked by membrane depolarization. W-7 also inhibited IP_3 formation substantially. Thus, these experiments are also consistent with the model proposed from the above studies of HSG-PA cell Ca^{2+} entry. W-5, a weaker CaM antagonist, had much smaller effects on carbachol-induced Ca^{2+} and IP_3 responses. However, W-7 and W-5 were equivalent in their ability to displace radioligand binding to HSG-PA cell muscarinic receptors. The latter findings suggest that while W-7's effects on Ca^{2+} mobilization in HSG-PA cells may in part involve muscarinic receptor blockade, W-7 also likely antagonizes a CaM dependent regulatory step.

Other cell culture studies have utilized the HSG-MY cell. This cell line is purported to be a model for salivary myoepithelial cells, a cell type which normally represents ~2-3% of total salivary gland cells and therefore is extremely difficult to study. Previous in vivo studies by others have suggested that myoepithelial cell function may be regulated by muscarinic receptors in a Ca^{2+} dependent manner. We have shown that HSG-MY cells have high affinity muscarinic receptors which are coupled to Ca^{2+} mobilizing responses (both ASCaP release and Ca^{2+} entry). Further, we have shown that the characteristics of HSG-MY cell muscarinic receptors, and Ca^{2+} entry, are markedly different from those of their parent cell line (HSG-PA).

As noted above, considerable effort has been directed in parallel studies of K^+ fluxes in HSG-PA cells. Carbachol markedly affects K^+ (measured by ^{86}Rb) influx and efflux in HSG-PA cells. Agonist-elicited ^{86}Rb fluxes are Ca^{2+} dependent and partially blunted by activators of protein kinase C. Both quinine and charybdotoxin effectively inhibit ^{86}Rb fluxes induced by both carbachol and A23187 (a Ca^{2+} ionophore), suggesting that one or more types of Ca^{2+} -regulated K^+ channels are involved in the muscarinic responses. Interestingly W-7 treatment causes a large net decrease in ^{86}Rb equilibrium levels in these cells. This effect, however, is not blocked by atropine and is concentration dependent. W-5 does not mimic these responses suggesting that W-7 may be affecting a CaM dependent process and not directly increasing $[\text{Ca}^{2+}]_i$. Activators of protein kinase C do not alter these W-7 induced changes. It is possible that W-7 may have effects on HSG-PA cells by a direct (or indirect) interaction with membrane K^+ channels in a manner quite distinct from that observed following muscarinic stimulation.

We have also begun studies this year with the HSY cell, which is a cell line derived from a human parotid gland and is the first established cell line reported to synthesize and secrete salivary amylase. HSY cells, however, apparently do not have functionally coupled β -adrenergic receptors as treatment with isoproterenol does not lead to activation of adenylate cyclase or stimulated amylase release. HSY cells do have functional muscarinic receptors which when activated result in increased $[\text{Ca}^{2+}]_i$.

As mentioned above, SPS investigators are also examining the neurotransmitter control steps which are involved in regulating growth and differentiation in salivary cells. Past studies by us have shown that stimulation of the β -adrenergic



receptor (β -AR) induced an increase in steady state levels of c-fos proto-oncogene mRNA in rat parotid acinar cells. We have recently shown that β -AR stimulation induces similar changes (time, extent) in expression of the c-jun proto-oncogene in parotid cells. Both Northern blot analyses and in situ hybridization experiments demonstrate this coordinate expression after a single exposure of cells to isoproterenol. Similar acute β -AR stimulation had no effect on the levels of amylase or PRP in RNA transcripts. Chronic (9 days) administration of isoproterenol to rats (as noted above) results in hyperplasia and hypertrophy of parotid glands. While there is no correlation of c-fos or c-jun expression with these isoproterenol-induced growth responses, we did observe that chronic isoproterenol treatment resulted in high levels of the expression of the proto-oncogene c-abl. Interestingly, c-abl mRNA isolated from parotid glands (but not submandibular glands or heart) of rats treated chronically with isoproterenol displayed highly unusual transcripts (1.5 and 1.3kb) versus the typical 6.5 and 5.3 kb transcripts found in the other tissues. By using various specific cDNA probes of the c-abl gene we determined that the unusual parotid transcripts likely result from sequences at the carboxy-terminus of the c-abl gene. By S1 nuclease protection experiments, we demonstrated the presence in these transcripts of a 1.6kb EcoRI-Xho I fragment. These and other studies indicated the fragments mapped the region of the c-abl DNA around 360 nucleotides upstream from the Xho I site. These experiments show that c-abl gene expression is specifically and uniquely, modulated during growth stimulation of rat parotid acinar cells and as such may provide an important clue as to the operative molecular control mechanisms.

We have also studied β AR regulation of proto-oncogene expression in the A5 rat submandibular cell line. Both isoproterenol and 8BrcAMP can induce the transient expression of c-fos and c-jun in A5 cells in a pattern similar to that seen with rat parotid acinar cells. Also, c-fos and c-jun protein can be detected in nuclei of A5 cells by immunochemical staining. Expression of these proto-oncogenes was not correlated with DNA synthesis in synchronized A5 cells and β AR stimulation could induce c-fos and c-jun mRNA levels similarly in either G₁, S or G₂ phases of the cell cycle. Because of the advantages of using cloned cell lines, we expect such studies will greatly, facilitate our understanding of the roles of c-fos and c-jun in epithelial cell physiology.

Summation

The CIPCB has an unique mission in the NIDR. Our success in achieving this mission is made possible by the careful blending of a staff of clinical problem-oriented (yet basic science appreciating) individuals and basic science-oriented (yet clinical problem appreciating) individuals. We try to apply basic science knowledge and methodologies to address significant clinical concerns. We continue to make substantial progress in these goals since the reorganization of the Branch in 1982. Our considerable success thus far, acknowledged during our recent review by the Board of Scientific Counselors, is due to the high level of idealism, the strong sense of mission and the capacity for hard work generally manifested by Branch staff. We believe that we have an important responsibility to NIDR, and to dentistry, as the institute's major research group functioning within the NIH Clinical Center. We have the opportunity to make many contributions to clinical dentistry, to oral science as well as to fundamental biology. Our Branch recognizes this and works creatively and enthusiastically toward meeting this goal. We anticipate continued forward movement in our efforts to address questions of importance to the understanding and management of oral disease, thus contributing to the future development and direction of the dental profession.

CLINICAL INVESTIGATION AND
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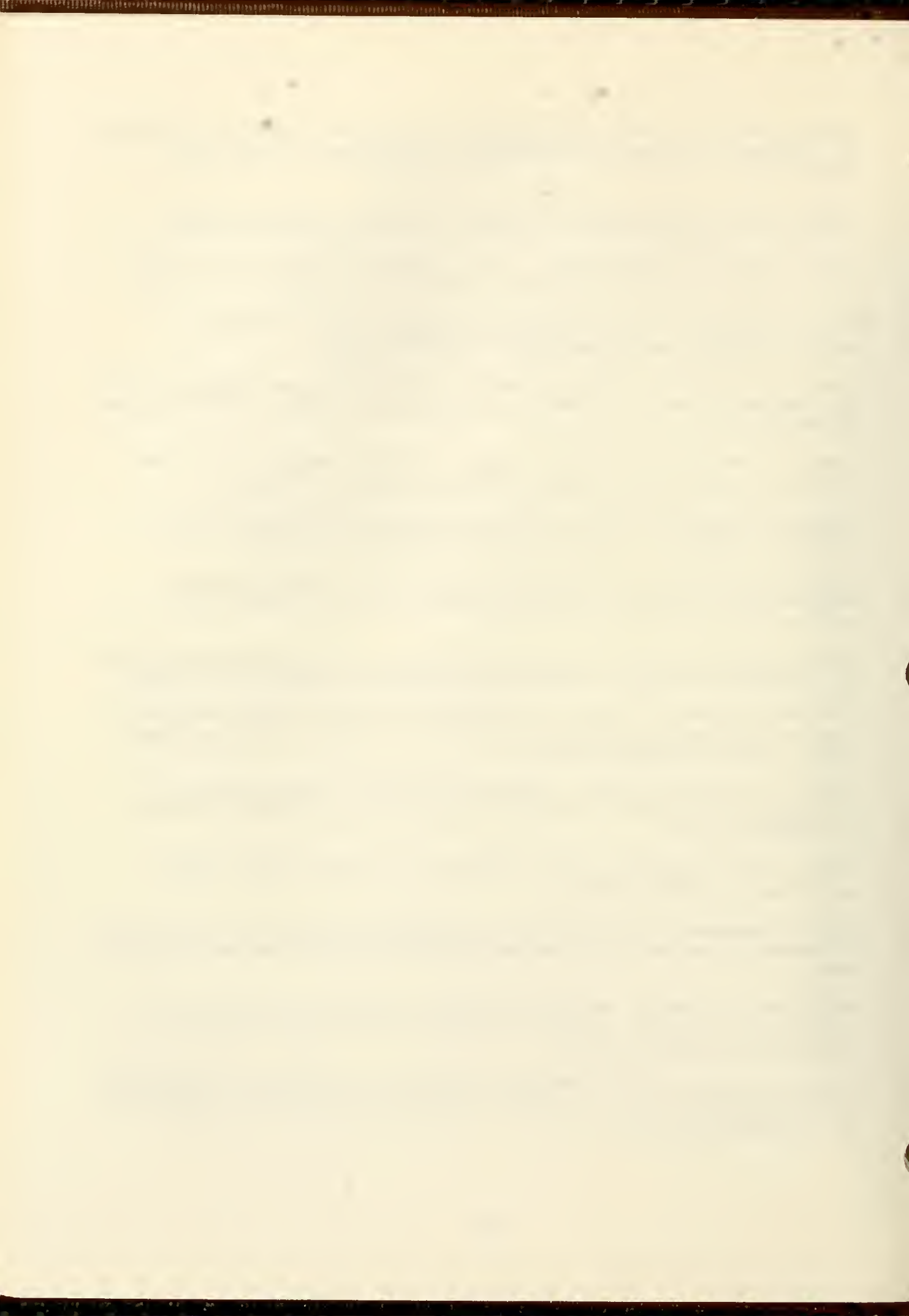
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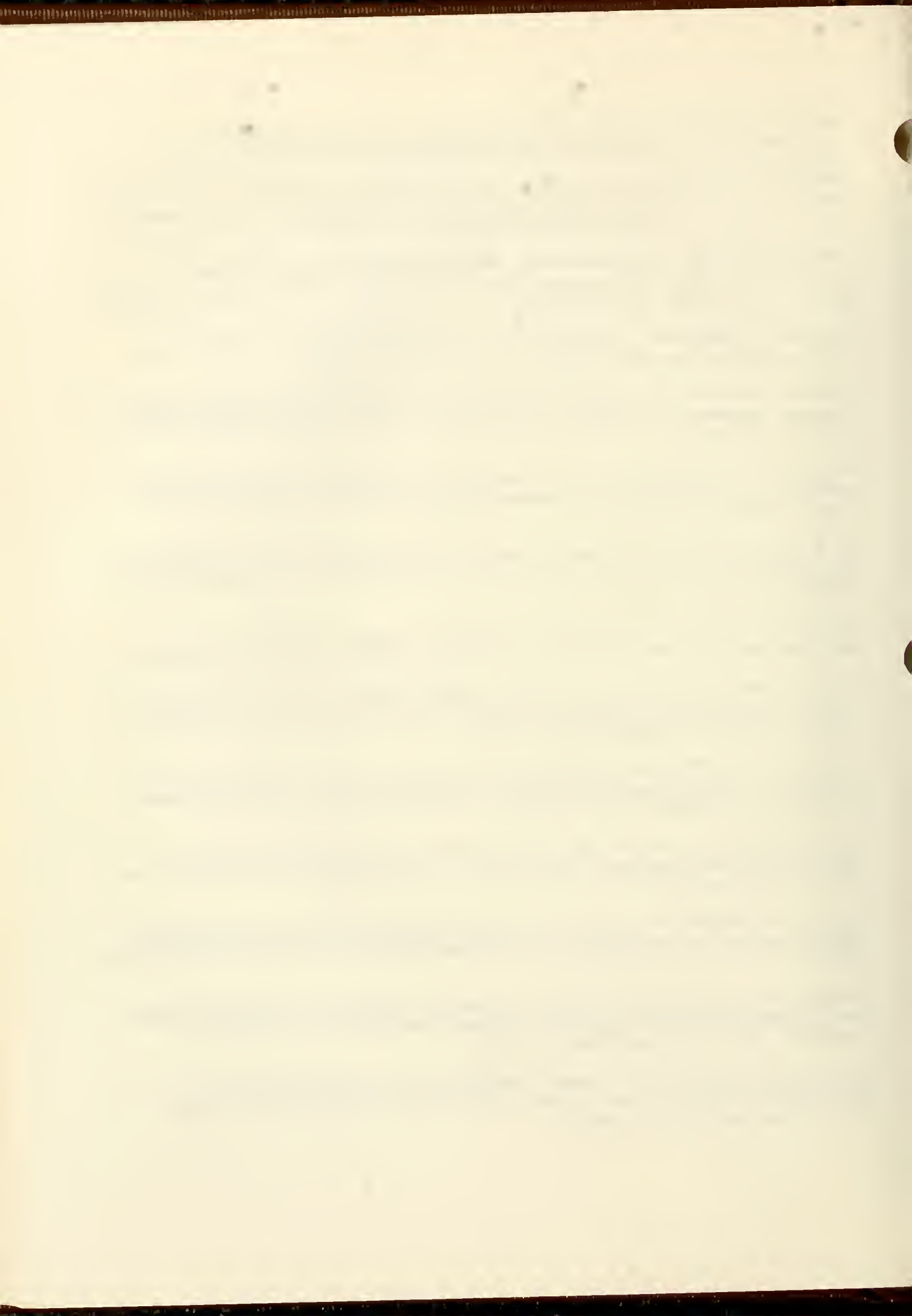
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00028-22 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Untrastructure and Cytochemistry of Secretory Cells

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

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COOPERATING UNITS (if any)

See next page

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

4.50

PROFESSIONAL

3.25

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

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

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

Basic mechanism of the secretory process are studied in animal and human salivary gland cells. Techniques utilized include light and electron microscopy, enzyme - and immunocytochemistry, radioautography, and biochemistry. Major areas of investigations are: (1) localization of secretory and cellular proteins in developing and adult salivary glands using fluorescent and colloidal gold immunolabeling procedures; (2) quantitative immunogold labeling of secretory proteins in salivary glands of rats chronically treated with isoproterenol; and (3) localization of magainin and related peptides in frog skin and mammalian tissues.



Cooperating Units

Tabak, L.A., Dept. of Dent. Res., Univ. of Rochester
Malamud, D., Dept. of Biochemistry, Univ. of Pennsylvania
Ball, W.D., Dept. of Anatomy, Howard University
Jungmann, R.A., Cancer Res. Ctr. Northwestern Univ.
Bevins, C., Division of Human Genetics and Molecular Biology,
Children's Hospital of Philadelphia
Eizirik, D.L., and Sandler, S., Dept. of Medical Cell Biology,
Uppsala University, Sweden
Takano, K., Dept. of Oral Histology, Nagasaki University, Japan
Mednieks, M.I., Dept. of Pediatrics, Univ. of Chicago
Bennick, A., Dept. of Biochemistry, Univ. of Toronto



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00212-13 CIPC

PERIOD COVERED
 October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Taste and Its Disorders

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

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Tylenda, Carolyn A.	Senior Staff Dentist	CIPC	NIDR

COOPERATING UNITS (if any)
 LSB, NIA; BPB, NIMH

LAB/BRANCH
 Clinical Investigations and Patient Care Branch

SECTION
 Clinical Investigations Section

INSTITUTE AND LOCATION
 NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

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

(a1) Minors

(a2) Interviews

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

This project seeks to elucidate the mechanisms by which oral sensory and perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. These methods, applied to the study of age-associated changes have provided insights into basic mechanisms of normal chemosensory perception. Functional variation under pathologic circumstances is assessed through objective evaluations of oral sensory disturbances occurring in association with systemic disease, salivary gland dysfunction, therapeutic x-irradiation, eating disorders or as an isolated complaint. Assessments of olfactory identification as well as sensitivity to local pressure on the tongue and to variation in the temperature or the viscosity, of an oral bolus are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DE 00332-08 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Clinical Investigations and Case Reports

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

Roberts, Michael W.	Dep Clin Dir NIDR/C	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Folio, John	Senior Staff Dentis	CIPC	NIDR
Shern, Roald J.	Senior Staff Dentis	CIPC	NIDR
Tylenda, Carolyn A.	Clinical Staff Dent	CIPC	NIDR
Wright, William E.	Senior Staff Dentis	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Kobel, Mary L.	Dental Hygienist	CIPC	NIDR

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Pediatric Branch NCI; Inter-Institute Genetics Program, CC; Diagnostic Systems Branch, NIDR

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

8.94

PROFESSIONAL

3.34

OTHER

5.60

CHECK APPROPRIATE BOX(ES)

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

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

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews, evaluation of various therapeutic regimens and roentgencephalometric analysis.



OTHER PROFESSIONAL PERSONNEL

Lyons, Janet L.	Dental Hygienist	CIPC	NIDR
Marini, Joan	Geneticist	CE	NCI
Mulvihill, John J.	Chief, Clinical Genetics Sect.	CE	NCI
Guckes, Albert D.	Chief, CODC	CODC	CC
Elin, Ronald J.	Chief, Clinical Pathology	OD	CC
Brandt Harry A.	Medical Officer	LCS	NIMH
Dubbert, Bellinda K.	Clinical Psychiatric Nurse	CC	NIMH
Pizzo, Philip A.	Chief, Pediatric Branch	PB	NCI
Termine, John J.	Chief, Bone Research Branch	BR	NIDR
Loe, Harald	Director NIDR	CIPC	NIDR
Cain, Janet L.	Volunteer	CIPC	NIDR
Royce, Leah S.	Dental Staff Fellow	CIPCB	NIDR
Valdez, Ingrid H.	Dental Staff Fellow	CIPC	NIDR
Katz, Ronald W.	Dental Staff Fellow	CIPC	NIDR
Patton, Lauren L.	Dental Staff Fellow	CIPC	NIDR
Ship, Jonathan A.	Dental Staff Fellow	CIPC	NIDR
Li, Shou-Hua	Statistician (Health)	EB	NIDR
Rudy, Susan F.	Clinical Nurse (General)	CC	NIDR
Webber, Richard L.	Chief Diagnostic Sys	DS	NIDR
Moffa, Joseph P.	Consultant	CIPC	NIDR
Teaford, Mark F.	Assistant Professor	The Johns Hopkins School of Medicine	

The professional staff of the Patient Care and Clinical Studies Section, CIPCB, NIDR, are encouraged to become involved in clinically related research investigations and documentation of unusual cases. Interesting types of oral pathology with or without other medical complications are often seen in the NIDR Dental Clinic. Publication of these cases with a multi-disciplinary review of the disorder can provide valuable information for the dental clinician who may be required to treat similar conditions in the future.

1. Plan and participate in clinical research projects
2. Recognize and document interesting and unusual cases
3. Devise innovative patient care techniques to solve unusual or difficult clinical problems.

METHODS EMPLOYED

Recognition of pathology or unusual cases which warrant investigation and documentation as well as independent clinical research is encouraged. A review of the pertinent literature is completed and a comprehensive evaluation of all dental and medical considerations is conducted with collaboration of experts in other laboratories, branches, or Institutes. Specific laboratory methodology is developed, or adapted, as required by projects.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00336-08 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Salivary gland secretion mechanisms during normal and altered functional states

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

Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Ambudkar, Indu S.	Visiting Associate	CIPC	NIDR
He, Xinjun	Visiting Fellow	CIPC	NIDR
Hiramatsu, Yukiharu	Visiting Fellow	CIPC	NIDR
Horn, Valerie J.	NRSA Fellow	CIPC	NIDR
Wu, Xiaozai	Guest Researcher	CIPC	NIDR

COOPERATING UNITS (if any)

LCMB, NIA

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD

TOTAL MAN-YEARS

3.65

PROFESSIONAL:

2.40

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

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

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

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize in vitro dispersed cells, and cultured epithelial cells of salivary glands, to understand mechanisms controlling saliva formation. We have focused these studies on autonomic neurotransmitter regulation of secretory events and associated signalling mechanisms. The aging rat parotid gland continues to be employed as a useful model to study autonomic receptor control of calcium handling in exocrine acinar cells. During this reporting period the primary focus of study has been directed at understanding the regulation of cytosolic calcium 2++ levels following muscarinic-cholinergic and α 1-adrenergic receptor stimulation. In particular, we have continued to examine mechanisms of calcium 2++ release from intracellular stores and extracellular entry of calcium 2++ via receptor- or second messenger-operated calcium 2++ channels. We have found evidence for at least two distinct modes of calcium 2++ entry in salivary epithelial cells. One is associated with inositol trisphosphate formation, intracellular calcium 2++ release and is independent of membrane potential, while the other is less dependent on inositol trisphosphate formation and intracellular calcium 2++ release and is modulated by membrane potential.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00337-08 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Oral Physiological Processes: Normal Function and Disease Perturbation

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

Fox, Philip C.	Dental Officer	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf CIPCB	CIPC	NIDR
Caruso, Anthony	Staff Fellow	CIPC	NIDR
Macynski, Alice A.	Research Nurse	CIPC	NIDR
Moreira, Jorge E.	Visiting Associate	CIPC	NIDR

*See Additional Investigators

COOPERATING UNITS (if any)

RM, CC; DR, CC; HGB, NICHD; LIR, NIAID; MD, NIDDK; LNS, NIA; LSB, NIA; Columbia University; Boston University; SUNY, Stony Brook; University of Rochester; University of California, San Francisco.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

8.25

PROFESSIONAL:

2.75

OTHER:

5.5

CHECK APPROPRIATE BOX(ES)

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

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

This project examines the function of various oral tissues during physiologic aging and in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness) utilizing the inpatient and outpatient services of the Dry Mouth Evaluation Clinic. Specific diagnostic approaches have been developed to aid in establishing the etiology of salivary gland dysfunction and defining criteria necessary for management decisions. A treatment protocol for selected patients continues, employing a regimen of oral administration of the parasympathomimetic drug, pilocarpine. Clinical and laboratory studies focusing on the etiology and character of the salivary gland component of Sjogren's syndrome, an autoimmune exocrinopathy, have advanced. An initial treatment protocol for primary Sjogren's syndrome is continuing. In addition, detailed studies of salivary-associated oral complaints (eg. taste and oro-pharyngeal swallowing disorders) have continued. Such studies include evaluation of oral sensorimotor performance across the adult life-span in order to better understand dysfunctional states. Recent efforts have been directed to studies of oral mucosa; in identification of potential endogenous anti-microbial factors and alterations associated with salivary gland dysfunctions. A major effort has been instituted to characterize oral alterations associated with HIV-1 infection and the acquired immune deficiency syndrome (AIDS). Studies focus on soft tissue changes, salivary gland function, and salivary-fungal interactions. We have identified a salivary factor which is capable of inhibiting the infectivity of HIV-1 for human peripheral blood lymphocytes.



Additional Investigators

Begleiter, Alfred	Guest Worker	CIPC	NIDR
Bevins, Charles	Medical Officer	HGB	NICHD
Delapenha, Robert	Medical Staff Fellow	CIPC	NIDR
Lane, H. Clifford	Medical Officer	LIR	NIAID
Pillemer, Stanley	Senior Staff Fellow	MD	NIDDK
Ship, Jonathan	Dental Staff Fellow	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Tylenda, Carolyn	Dental Officer	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wolff, Andy	Visiting Fellow	CIPC	NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC	NIDR



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00411-04 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Oral Health of Head and Neck Radiation Patients

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

Wright, William E.	Senior Staff Dentist	CIPC	NIDR
Kobel, Mary L.	Dental Hygienist	CIPC	NIDR
Lyons, Janet L.	Dental Hygienist	CIPC	NIDR

COOPERATING UNITS (if any)

Pediatric Branch, NCI, and Radiation Oncology Branch, NCI

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

1.55

PROFESSIONAL:

0.45

OTHER:

1.10

CHECK APPROPRIATE BOX(ES)

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

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

These studies are evaluating the effectiveness of a formal orientation program designed to inform and motivate patients receiving head and neck radiation treatments using specialized oral health care regimens, and comparing preventive effectiveness of three topically applied fluoride regimens on the overall oral health status in the same population.

The subjects are divided such that a control group, orientated to the potential harmful oral side effects of radiation therapy by conventional verbal means, can be compared with a study group, oriented by a formal color slide-narration program developed at the NIDR dental clinic. In addition, individuals from each of the groups are randomly assigned in equal numbers to one of three oral fluoride regimens. A series of questionnaires and clinical diagnostic parameters are used to evaluate differences in patient compliance and the effectiveness of the therapeutic regimens as related to dental caries incidence and periodontal health status.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01 DE 00412-04 CIPC
PERIOD COVERED October 1, 1988- September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Maxillofacial Surgery and Implant-Prosthetic Reconstruction		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Brahim, Jaime S.	Senior Staff Fellow	CIPC NIDR
Folio, John	Senior Staff Dentist	CIPC NIDR
Roberts, Michael W.	Dep Clin Dir/NIDR/C	CIPC NIDR
Wright, William E.	Senior Staff Dentist	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR
Guckes, Albert D.	Chief, CODC	CODC CC
Caruso, Anthony J.	Staff Fellow	CIPC NIDR
Gracely, Richard H.	Research Psychologist	NAB NIDR
COOPERATING UNITS (if any) Rehabilitation Medicine Department CC; Nutrition Department, CC; Surgical Services Department, CC; Diagnostic Systems Branch, NIDR; Commissioned Officers Dental Clinic, CC		
LAB/BRANCH Clinical Investigations and Patient Care Branch		
SECTION Patient Care and Clinical Studies Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland		
TOTAL MAN-YEARS 7.25	PROFESSIONAL 2.65	OTHER 4.60
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 (Use standard unreduced type Do not exceed the space provided.) <u>Evaluation of Rigid Versus Nonrigid Fixation Following Orthognathic Surgery</u> The purpose of this study is to determine the preferred method of fixation to avoid relapse following maxillary and mandibular osteotomy to correct facial developmental deformities. Correlations will be established, between rigid and nonrigid fixation techniques, and the degree of relapse as determined by radiographic cephalometric and clinical assessment. Any changes in the height of the gingiva or the width of the attached gingiva will be recorded. Pre and post-operative changes in facial contours and occlusion will be recorded. In addition, speech, swallowing and orofacial movements will be assessed. <u>Clinical Study of Oral Endosseous Titanium Implants in Edentulous Subjects</u> The endosseous implant system consists of titanium root analogues with a threaded surface designed to be surgically embedded in the anterior third of the mandible. The root analogues are covered with a mucoperiosteal flap and the surgical site closed and allowed to heal. After healing, the root analogues are uncovered and coronal segments are attached to each root analogue. A complete denture is constructed to restore the mandibular dentition. Cephalometric radiographs, the Cornell Medical Index, the Minnesota Multi-phasic Personality Inventory, the Denture Satisfaction Questionnaire, a body focus questionnaire, a three day diet record and a rating of foods with respect to difficulty of chewing are used to obtain data. The information obtained is utilized to determine if implant supported mandibular dentures significantly effect loss of verticle dimension of occlusion, satisfaction with dentures, food choices and nutrition, perception of difficulty of chewing selected foods, and body focus, when compared to treatment with conventional dentures.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00415-04 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Ion Transport and Fluid Secretion in Salivary Glands

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

Turner, Roy James	Visiting Scientist	CIPC	- NIDR
Manganel, Michel	Visiting Fellow	CIPC	NIDR
George, Janet N.	Chemist	CIPC	NIDR
Lee, Syng Ill	Guest Worker	CIPC	NIDR
Busch, Kathryn	Guest Worker	CIPC	NIDR
Dehaye, Jean-Paul	Guest Worker	CIPC	NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Sections

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

4.3

PROFESSIONAL

3.2

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

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

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

Saliva is the principle protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations in the salivary secretory mechanism can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of primary fluid secretion by salivary acinar cells and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following.

(1) The transport of ions (Na^+ , K^+ , Cl^- , HCO_3^-), whose transmembrane and transepithelial movements are thought to be related to the process of primary salivary fluid secretion, was studied in vitro, in intact rat and bovine parotid acini and/or in isolated rat parotid basolateral membrane vesicles.

(2) The regulation of the rat parotid Na/H exchanger by muscarinic agonists was studied in order to clarify the role of this transporter in the fluid secretory process.

(3) The stability and lipid specificity of the rabbit parotid Na/K/Cl cotransporter was investigated in preparation for further protein purification studies.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00438-03 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Molecular Mechanisms Regulating Calcium Flux in Salivary Glands

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

Ambudkar, Indu S.	Visiting Associate	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Horn, Valerie J.	NRC Fellow	CIPC	NIDR
Mertz, Lawrence M.	IRTA Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Department of Physiology, Johns Hopkins University, School of Medicine
 LN, NINCDS, NIH

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

2.45

PROFESSIONAL:

2.45

OTHER:

0

CHECK APPROPRIATE BOX(ES)

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

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

Neurotransmitter stimulation of salivary glands results in the flow of fluid and electrolytes. The cascade of events, initiated by the binding of neurotransmitter to the cell and leading to secretion, is presently not completely understood. We and others have established that the level of cytosolic Ca^{2+} plays a key role in this process. We have directed our research efforts towards understanding the molecular mechanisms which are involved in cellular Ca^{2+} regulation during the stimulus-secretion process. We are addressing three major areas: (i) regulation of signal generation, i.e. PIP_2 hydrolysis, a major event stimulating calcium mobilization; (ii) regulation of intracellular Ca^{2+} mobilization, and (iii) regulation of Ca^{2+} flux in the cellular membranes. We had shown earlier that Ca^{2+} mobilization responses in the rat parotid acinar cells are limited by the size of the agonist-sensitive intracellular Ca^{2+} pool and that Ca^{2+} influx mechanisms are involved in the maintenance of this pool, while the IP_3 generating system itself is subject to "cross-regulation" by other intracellular messengers, e.g. cAMP. Additionally, the activity of ATP-dependent Ca^{2+} pumps in the basolateral and endoplasmic reticulum membrane may play a role in the regulation of cytosolic calcium. In the present reporting period we show that cross-regulatory mechanisms between calcium-mobilizing receptors likely determine the final cellular secretory response. We have further assessed the activation of IP_3 generation by cAMP using a mouse fibroblast cell line (B82 L cells) and show that this process does not involve calcium mobilizing receptors. With regard to possible post-receptor effects of atropine, we show that it may involve the G-protein associated with the calcium mobilizing signal system. Our studies also demonstrate a sustained Ca^{2+} entry mechanism, which is induced via G protein activation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00455-02 CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Oral Candidiasis in AIDS

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

Tylenda, Carolyn A.	Clinical Staff Dentist	CIPC	NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC	NIDR
Handelman, Beverly	Biologist	CIPC	NIDR
Kovacs, Joseph A.	Senior Investigator	CCMD	CC
Lane, H. Clifford	Senior Investigator	LIR	NIAID

COOPERATING UNITS (if any)

Laboratory of Immunoregulation, NIAID
 Critical Care Medicine Department, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

2.2

PROFESSIONAL:

0.8

OTHER.

1.4

CHECK APPROPRIATE BOX(ES)

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

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

Oral candidiasis is one of the opportunistic infections commonly associated with AIDS. The overall objective of this work is to understand the relationship between the development of oral candidiasis and pathogenesis of AIDS. The initial study examined by culture the level of oral yeast in a population of HIV-1 antibody positive AIDS patients participating in NIH outpatient protocols with no history of opportunistic infections. Both the patient group and the normal control group consisted of non-smoking, non-denture-wearing males taking no medication. The mean level of yeast in the whole saliva of the AIDS patients was 13,000 colony-forming-units (cfu) per milliliter compared to a mean of less than 1.0 cfu/ml in the control group. Identification of the yeasts showed that Candida albicans was the predominant yeast in both groups.

These results indicate that 1) high oral yeast concentration in whole saliva may be an early sequela of HIV-1 infection, 2) high levels of oral yeast precede overt clinical signs of candidiasis, and 3) the oral yeast in these patients results from proliferation of the normal yeast oral flora rather than colonization by unusual species.

A second study is underway as part of the protocol entitled "Natural History of Oral Manifestations of HIV-Infection in a United States Military Population." The study population, which may eventually reach 1000 subjects, consists of HIV-1 serum positive army personnel who receive an initial comprehensive examination followed by reevaluations at 6 month intervals. Whole saliva is examined for the presence and level of yeast and total salivary bacterial count. In addition, the presence of Pneumocystis carinii will be ascertained using indirect immunofluorescence techniques. The appearance of oral pathogens will be followed in relation to both the stage of HIV infection and the appearance and progression of other clinical symptoms.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00457-02
CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Neurotransmitter Regulated Functions of Salivary Epithelial Cells in Culture

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

Wellner, Robert B.	Senior Staff Fellow	CIPC	NIDR
Turner, Roy James	Visiting Scientist	CIPC	NIDR
Ship, Jonathan A.	Clinical Staff Fellow	CIPC	NIDR
Patton, Lauren	Clinical Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD.

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.8

OTHER

0.7

CHECK APPROPRIATE BOX(ES)

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

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

Saliva formation is essential for the maintenance of oral health. Control of salivary formation is mediated by the autonomic nervous system, which influences the ion, water and protein transport properties of salivary cells. In order to better understand the mechanisms involved in the regulation of salivary gland secretion, we have sought to study clonal lines of salivary cells which possess neurotransmitter signal transduction mechanisms coupled to functional cellular responses. The use of established cell lines offers several advantages over acute in vitro preparations: (1) long term studies can be carried out, (2) studies can be carried out on specific types of cloned cell lines which possess the same genetic background, and (3) somatic cell genetic and molecular biological techniques can be utilized to investigate various regulatory mechanisms. During this reporting period we have characterized several neurotransmitter responses in established human salivary epithelial cell lines in culture, and we have cloned several mutant cell lines which might be defective in processes subject to neurotransmitter regulation.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00458-02
CIPC

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

β-Adrenoreceptors and Gene Regulation

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

Kousvelari, Eleni	Senior Staff Fellow	CIPC NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC NIDR
Mertz, Prema	Staff Fellow	CIPC NIDR
Chinchetru, Miguel	Visiting Fellow	CIPC NIDR

COOPERATING UNITS (if any)

Roche Inst. of Molecular Biology; Department of Pharmacology, University of Minnesota; MGH Cancer Center; Squibb Institute of Medical Research.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

3.05

PROFESSIONAL:

3.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

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

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

Specific stimulation of β-adrenoreceptors in rat parotid acinar cells (RPAC) in vitro; increases levels of cAMP, protein production, phosphorylation and secretion. In vivo stimulation of rats by the β-adrenergic agonist isoproterenol results in hyperplastic and hypertrophic enlargement of the parotid and submandibular gland. Our studies are designed to understand the mechanisms by which information from β-adrenergic receptors is transmitted to the nucleus and thus regulates processes such as protein synthesis and gland hyperplasia in the rat parotid glands. We chose to investigate the role(s) of the proto-oncogenes c-fos, c-jun and c-abl, in eliciting these β-adrenergic receptor responses. We have shown that c-fos expression is highly induced in RPAC and in a submandibular cellline RSMT-A5 by stimulation of β-adrenoreceptors or addition of 8-BrcAMP. The fos protein is increased in a fashion similar to that of c-fos gene expression and associates with the nucleus of the acinar cell. In addition stimulation of muscarinic receptors can induce c-fos expression in RPAC. Changes in c-fos gene expression in RPAC and in RSMT-A5 cells are not coincident with DNA synthesis. Finally, two aberrant c-abl mRNAs (1.5 and 1.3 kb) are seen in rat parotid glands after isoproterenol administration and are not due to gene rearrangement. During this reporting period we have; 1) demonstrated that c-jun gene expression in RPAC and RSMT-A5 cells after β-adrenoreceptor stimulation or addition of 8-BrcAMP is regulated in a fashion similar to that of the c-fos gene; 2) observed that the induction of c-fos and c-jun in RPAC and RSMT-A5 cells is transcriptionally controlled; 3) proved that c-fos and c-jun gene expression is not a requirement for RSMT-A5 cells to progress into the cell cycle; 4) shown the parallel expression of c-fos, c-jun, amylase and PRP genes in RPAC with in situ hybridization; 5) identified a 300b, S1 nuclease c-abl protected fragment in parotid gland after isoproterenol treatment.



ANNUAL REPORT OF THE DIAGNOSTIC SYSTEMS BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Diagnostic Systems Branch (DSB) is concerned with the identification and understanding of factors limiting the diagnostic performance, and the development of alternative methods designed to overcome these limitations. Particular emphasis is directed toward development of noninvasive image-based systems designed primarily for dentistry, but the scope is broad enough to include research applicable to a variety of biomedical tasks.

All continuing research efforts are encompassed by three broad project designations: 1) The enhancement of diagnostic images, 2) The development and evaluation of improved diagnostic systems, and 3) The exploration and assessment of new diagnostic modalities. The first designation deals primarily with systems where the performance is limited by the ability of the human observer to recognize the displayed information, and uses methods for manipulating diagnostic data in specific, task-dependent ways. The second project is more global in scope, addressing all aspects of data acquisition, possible noise limitations, as well as interpretation problems in the diagnostic process. The third is relatively explicit, involving the study of novel or unusual kinds of diagnostic techniques.

This year's emphasis of the research program was oriented more towards the implementation of methods developed earlier by DSB into specific clinical tools. Although the program was severely restricted by the departure of the Branch Chief, Dr. Richard Webber, and the loss of further positions, significant progress in that direction has been achieved. In the light of the planned closing of the DSB, no new collaborations were established and an attempt was made to bring some of the ongoing research activities to a proper conclusion.

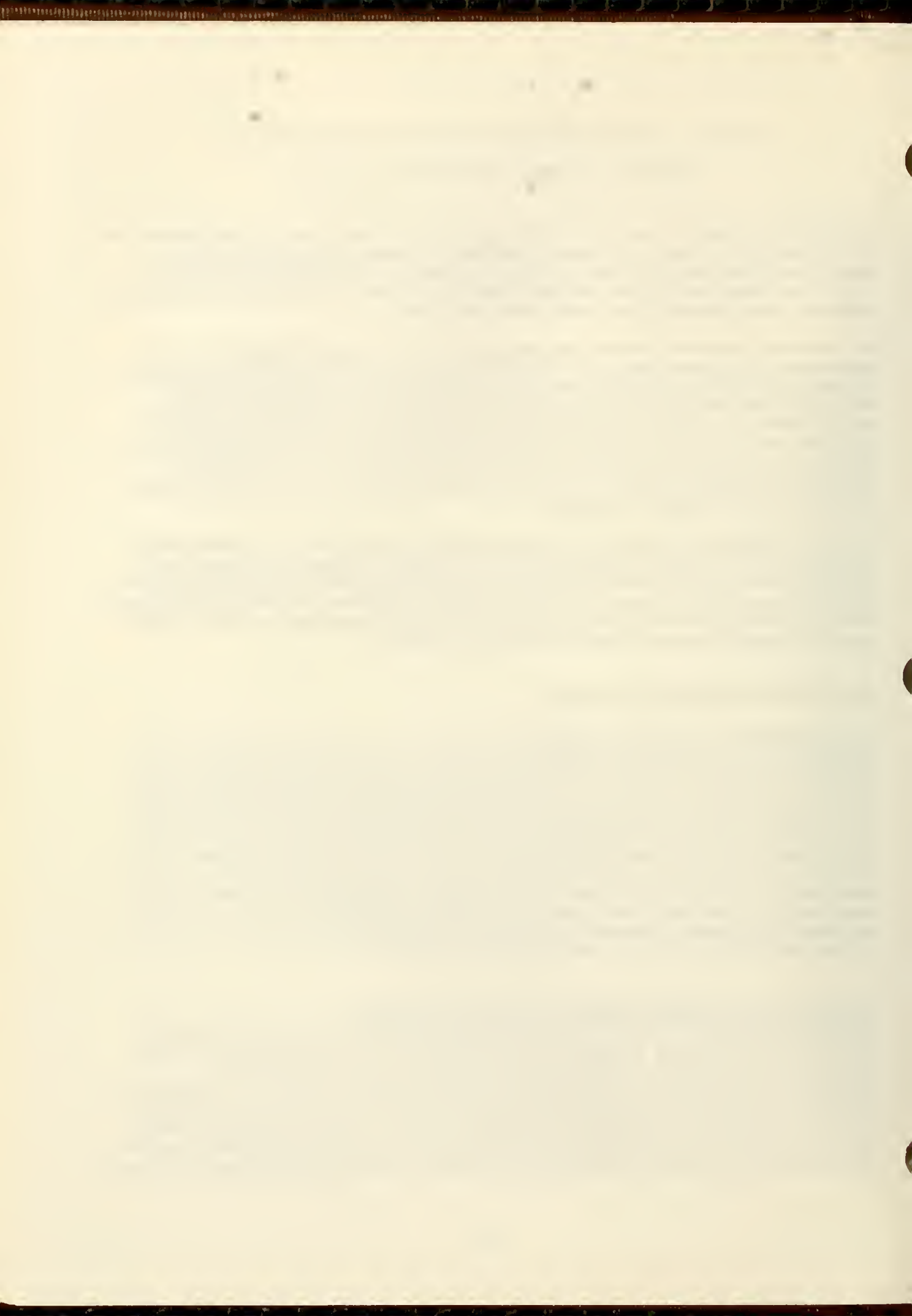
IMAGE ENHANCEMENT METHODS

Clinical monitoring of periodontal disease

In keeping with this year's emphasis on clinical applications, enhancement methods of imaging, in particular subtraction radiography, are being applied to a clinical study in collaboration with CIPCB, investigating the efficacy of a new anti-inflammatory drug in retarding loss of alveolar bone in patients with periodontal disease. This project conceptually parallels an ongoing multicenter field trial of this same anti-inflammatory drug which purportedly retards loss of alveolar bone in patients suffering from periodontal disease. The new investigation is designed to detect the effects of the drug much earlier than is likely to be possible in the larger multicenter study by using carefully controlled digital subtraction radiography. Additionally, periodontal pockets corresponding to regions demonstrating most severe bone loss are being surveyed for seven different potential pathogens to determine whether there is any longitudinal correlation of the presence of these specific bacteria with observed patterns of bone loss.

Follow-up of hydroxylapatite implants by subtraction radiography

Another clinical problem for which subtraction radiography may provide some solutions is the follow-up of bone implants. During the last decade, the use of hydroxylapatite (HA) has been gaining increased popularity in dental surgery. Although histologic analysis of the osteodynamics in and around HA implants can be a highly accurate method, it is of limited use in patients. Digital subtraction radiography may be a non-invasive diagnostic alternative for the follow-up of the status of these implants. Therefore, a preliminary study was made in collaboration with the Department of Oral and Maxillofacial Surgery at the University of Zürich, to evaluate the potential of subtraction radiography as a diagnostic method in the assessment of the status



of granular HA implants.

Patients with bony lesions were operated on at the University of Zürich by Dr. Werner Engelke, who is currently a Visiting Associate with DSB. Afterwards, the iatrogenic defects were reconstructed with porous HA granules (Calciite), and one lesion was left unfilled serving as a control. Standardized radiography over a time interval of 4 to 6 months postoperatively was made. None of the radiographic subtraction images indicated bone loss in the area of interest. In 50% of the patients, including the control patient, significant bone regeneration was detected, which was in 30% of all patients combined with volume loss of the implant material. In 20% only volume loss of implant material occurred, and in the remaining 30% no changes could be detected over time. Hence, subtraction radiography proved to be a useful means for the follow-up of HA implants. Improvements of the technique used to acquire the radiographs are required to yield quantitative data. In particular, the incorporation of a calibration step wedge made of bone equivalent material will permit absolute determination of bone mass gain or loss. Furthermore, extraoral head-stabilization by the cephalostat technique appears to have the best potential for improving the standardization of radiographic projection geometry because it is not affected by tooth movements.

Edge preserving image filtering

Intrabony lesions show up in subtraction radiography as dark regions, embedded within a flat background of uniform noise. Noise reduction by filtering is usually a first step that facilitates subsequent lesion mensuration. While linear filters reduce noise only at the expense of some blurring of the image edge contrast, which is detrimental, nonlinear filters such as the median filter can preserve edges better with, however, less noise reduction. The goal was, therefore, to design a nonlinear filter that achieves substantial noise smoothing without introducing edge blurring. As a model for this filter served the well-known nonlinear property of structures located in the visual cortex that display directional sensitivity. This property was used to detect the presence of edges within the moving filter window and avoid indiscriminate smoothing. This novel filter structure was termed directional filter.

In order to investigate the relative edge distortions produced by noise smoothing, linear filters, median filters, and directional filters were each applied to synthetic images simulating the appearance of lesions in noisy subtraction images. The residual noise variance in the subtraction images after filtering was least in all cases for the directional filter, whether measured over the the total region of interest (ROI), over a mask mapping the exact lesion extent, or the complementary mask within the ROI. On the other hand, the relative rankings between the linear and median filters were not uniform. Hence, as desired, the directional filter, when matched for equal noise smoothing, outperformed the linear and median filters with respect to preservation of contrast edges. This research in the area of neural networks has been terminated.

Film contrast correction

The correction of film-contrast mismatches arising from various technical inadequacies is an important step in any work in quantitative radiography, and digital subtraction radiography in particular. A digital contrast matching procedure has been developed previously by the DSB and proved to be very reliable and robust, and is now in use by several research groups. It is based on a general algorithm which generates a monotonic mapping of the gray levels derived from the histograms of common regions of interest in the two radiographs to be subtracted. Because radiographs are in practice always misregistered to a certain extent, it has been suggested by other researchers that the gray-level mapping should be derived from the co-occurrence matrix of the gray levels in pixels at identical coordinates of the two images. The rationale is that image misregistrations may produce outliers in that matrix which, by some curve fitting method, could be ignored in the derivation of the gray-level mapping function. In order to investigate the possibilities of this approach, a systematic study was initiated where both the histogram-based and the co-occurrence-based methods were applied separately to identical images with computer-



controlled amounts of contrast mismatch and misregistration. The results indicated that the co-occurrence method using the originally proposed curve fitting procedure was definitely inferior. Improvements of the curve fitting to the co-occurrence matrix were then developed which produced statistically significantly better results. However, the histogram matching method still achieved better contrast correction than any of the co-occurrence matrix methods. This led to the conclusions that until better ways are found to utilize the information inherent in the co-occurrence matrix, the histogram matching method is preferred because of its better performance and its vastly simpler and faster implementation by computer.

Bone characterization by fractal geometry

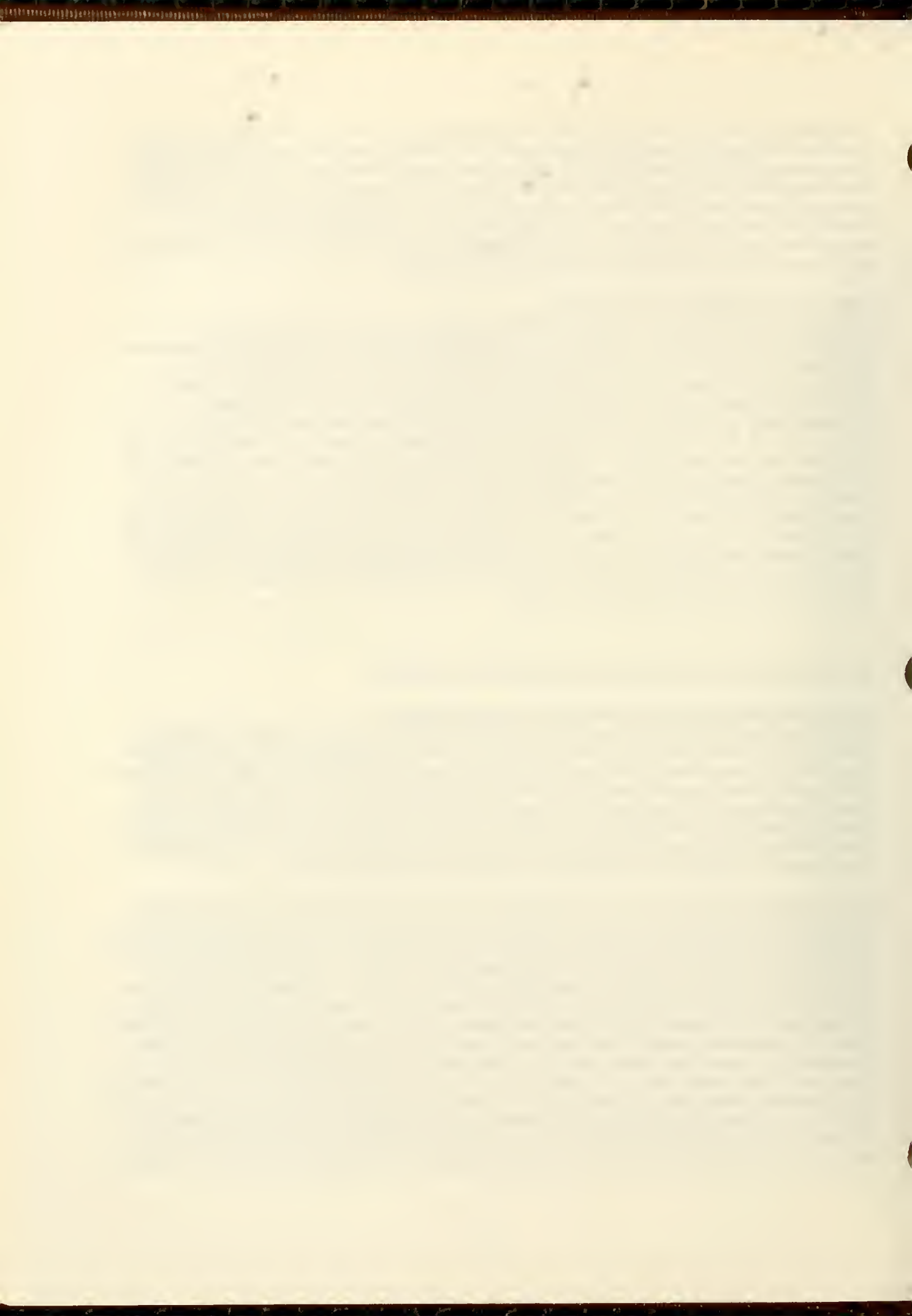
Automated recognition and delineation of certain regions of diagnostic interest within a radiographic image would be an important step towards the goal of a computerized mensuration and diagnosis system. The problem is complicated by the fact that trabecular patterns vary greatly among anatomic locations and patients, precluding the direct application of simple methods developed in texture analysis. Therefore, in order to obtain scale-invariant characterization of the trabecular patterns, the use of fractal geometry was investigated in collaboration with Dr. Walter Kuklinski, Department of Electrical Engineering, University of Lowell. Our initial studies have indicated that a texture model based on fractional Brownian motion provided an adequate descriptor for radiographs of bone. The implications of the validity of this model go far beyond this simple application to image partitioning. The fractal dimension of the bone surface, which may vary between 2 for a smooth and 3 for a maximally corrugated surface, may in itself be an important parameter that can be used to measure pathological differences of bone structure, particularly in characterizing structural changes due to osteoporosis. However, due to loss of positions in the DSB and the decision to discontinue its operations, planned research efforts in this area have been canceled.

DEVELOPMENT OF IMPROVED DIAGNOSTIC SYSTEMS

Optimal radiographic projection directions for caries detection

Bitewing radiographs have been commonly accepted as one of the most important diagnostic aids in detecting carious lesions in the interproximal surfaces of posterior teeth. However, precious little is known about the influence of changes in angulation of the x-ray beam with respect to the tissue of interest on the correct detection and interpretation of those small defects from bitewing radiographs. Therefore, a study was initiated in collaboration with Dr. Paul van der Stelt, Department of Oral Radiology, Academic Center for Dentistry, University of Amsterdam, to establish and elucidate the influence of variations in horizontal and vertical angulation of the x-ray beam on the detection performance of interproximal carious lesions.

Four hundred-and-twenty bitewing radiographs were made at different horizontal and vertical angulations from 10 sites of dry human mandibles before and after artificial, nonpenetrating lesions of various depths were induced by a burr. The radiographs were presented in random order to 25 dentists which were asked to give their assurance (5 categories) whether a lesion was present, and if present, rank the estimated lesion depth (< halfway, > halfway through the enamel, penetrating the DEJ). ROC analysis was performed with the assurance scores, and loglinear analysis was used to quantify the interactions between angles, lesion depths, observers and lesion sites. The association strength between actual lesion depths and assigned depth rankings was quantitated by ordinal categorical analysis. These analyses indicated that each lesion site had its own specific best projection angle centered within a tolerance range of about 7°. On the average, best detection performance was achieved with a horizontal angulation perpendicular to the tooth surfaces, but in the vertical direction, this reference position yielded the lowest performance. In that direction a bimodal characteristic was observed with performance maxima at either positive or negative tilts of 12 to 15° relative to the perpendicular. This bimodality was shown to be due



to the anisotropic shape of the interdental contact area, which is wider in the horizontal than in the vertical direction, causing the radiographic contrast resulting from a lesion in that region to increase as the vertical beam angulation departs from the perpendicular reference position. Deviations from the best horizontal angle resulted in an increase in the number of missed lesions, i.e. loss of sensitivity, while vertical misangulations caused mainly an increase in false positive readings, i.e. a drop of the specificity.

Development of a photo-electronic dental radiography system

Work has been continuing on the development of a prototype miniaturized tomosynthetic x-ray system, in collaboration with the Radiation Physics Group at the National Institute of Standards and Technology, and the United States Army Institute of Dental Research. This system will consist of a multi-focal spot x-ray tube with computer-controlled electronic hardware for tube activation, and an electronic intraoral x-ray image detector, also under computer control, for image acquisition and storage.

The prototype x-ray tube has been built by the Kevex Corporation and recently delivered to the Radiation Physics Group for initial testing and checking of radiation leaks. Its specifications are 70 kV continuous potential, maximum current 3 mA, and the position of the focal spot (1mm Ø) is electronically movable on the extended transmission target. This will permit the generation of any number of focal spots over a range of solid angles as required for tomosynthesis.

The image detector will consist of custom-designed solid state chip made available from a contract development for the U. S. Army, which is also supervised by the Radiation Physics Group. This chip will have the dimensions of a standard intraoral film with a resolution of 375 x 256 pixels, and is expected to have a detection sensitivity 10 to 20 times higher than the currently used film. The latter property would result in a substantial reduction of the patient's x-ray dose per image. Delivery of this new chip to the Radiation Physics Group is expected by Fall 1989, upon which it will be integrated into the complete system. Considering this schedule it is unlikely that the completed system will be available before the closing of DSB.

Control of the radiographic projection geometry by cephalostat and video feedback

The diagnostic value of subtraction radiography is critically dependent on the ability to reproduce reliably the radiographic projection geometry over a series of follow-up examinations. Consequently, the difficulty to attain proper control of the projection geometry has been the main practical limitation of the subtraction technique. Therefore, work continues on the implementation of a method for eliminating the present need of a stent to stabilize projection geometry when generating radiographs intended for subsequent subtraction. The use of a cephalostat to fix the head position with respect to the focal spot is a promising technique to control projection misangulation. With this fixation, head rotation is largely eliminated by the ear bars, but head nodding, i.e. rotation in the sagittal plane is still possible. Because the frontal teeth require imaging in the anterior-posterior direction, head rotation parallel to the sagittal plane results in a variable tilt of the tooth axes with respect to the central beam. Therefore, some additional control of the head position appeared to be of importance. The basic idea of this control was to allow the patient to reproducibly position himself into the cephalostat with the aid of visual feedback. For that purpose, a closed-circuit video system has been specially designed. The patient is facing a video camera placed next to a monitor and sees his face displayed in real time on the monitor. At the time the radiograph is made the corresponding video frame is digitized and stored in a computer disk file. At subsequent radiographic examinations, the patient views on the monitor a rapidly alternating display of the previously stored image and the live image originating from the camera, and repositions himself until the two images coincide. Because the x-ray tube, the cephalostat, the video camera and the monitor are all mounted on a rigid metal U-frame, coincidence of the video images also signifies coincidence of the x-ray



projection geometry. The U-frame, the video equipment, and the necessary mirrors were custom-built by a collaborative agreement with RTS Laboratories, Inc. Alachua, FL, who also provided hardware to enable the rapid alternations of the video image, as well as software to be run on an IBM PC serving as the control and image storage unit.

The ability to reposition patients using this video feedback system was evaluated using a laser pointer as an optical lever to measure the angular disparity arising from multiple positionings of human volunteers. In the posterior dental configuration, the device yielded angular disparities with a standard deviation of 0.6° in the horizontal plane and 0.8° in the vertical plane. Anterior projections using the video system produced horizontal and vertical disparities with SDs of 0.7° and 1.1° , respectively. However, uncontrolled rotation of the head about the ear rods rendered the cephalostat alone unusable for anterior registration applications, giving rise to SDs in the vertical direction larger than 6° . These data demonstrated that it was possible to reposition patients within acceptable tolerances necessary for digital subtraction radiography irrespective of the tooth position in the dental arch, in particular the video feedback system permitted to obtain anterior projections under sufficient control.

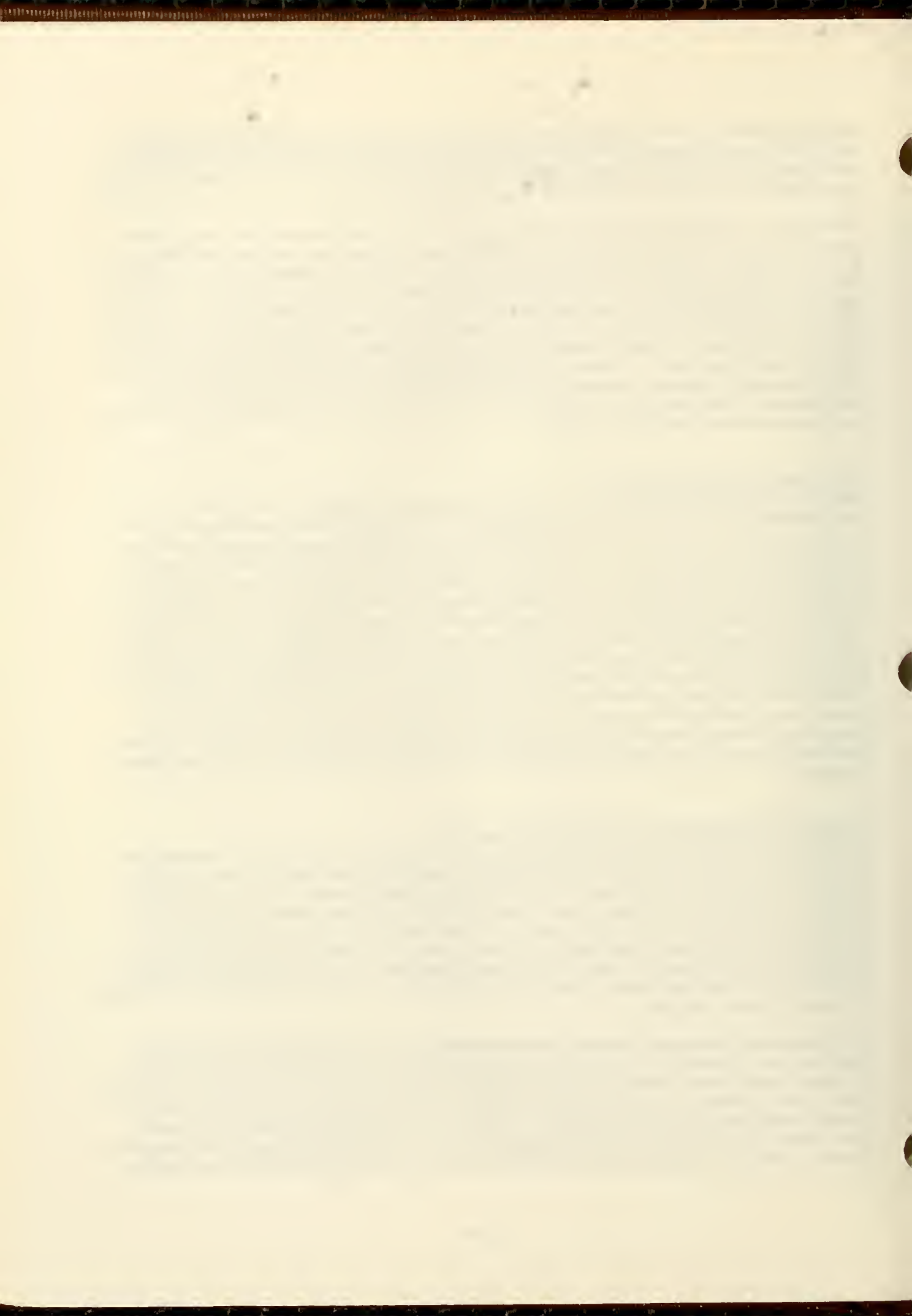
Dual energy subtraction methods

Methods developed by DSB have been steadily improving the precision of bone loss measurements derived by subtraction radiography. As a result, errors due to differences in the amount of soft tissues that may overlay the bone at the time the radiographs are produced have now become relevant. In theory, it is possible to eliminate these soft tissue effects by recording two radiographic projections with x-ray beams of different (low and high) photon energies. Research efforts were undertaken to determine whether passive filtration of the x-ray beam between simultaneously exposed films having different speeds could be used to achieve this goal. The theoretical and practical results indicated that passive filtration of the x-ray beam was not sufficient for reliable soft tissue elimination by energy subtraction radiography using films as image detectors. The use of films as radiation detection devices, because of their restricted latitudes, limits significant improvements of methods relying on passive x-ray beam filtration. Hence, it was concluded that under the constraints of currently available dental films, passive filtration of the x-ray beam does not provide sufficient energy separation to enable the elimination of soft tissue effects. Further investigations must await the availability of solid state image detectors.

Calibration of radiographic bone density measurements

Quantification of digital subtraction radiography commonly involves the use of a stepwedge or ramp of known material which serves as a reference standard against which relative changes in unknown exposure can be calibrated. A potentially important calibration error source is due to the effects of inevitable changes in spectral energy of the x-ray beam, called beam hardening, as it passes through various tissue combinations. This requires that the attenuation and scatter characteristics of all tissues penetrated by the x-ray beam as it passes through the region of diagnostic interest be identical to those of the calibration standard. These requirements are difficult to achieve in practical applications, and thus new calibration standards were developed to control for some of the error sources.

In one approach, a prototype intraoral cassette containing two periapical films was fabricated. The films were separated by a slab of bone-equivalent plastic with multiple wedges overlaying the entire field of view. Subtraction of the two films yields the image of the calibration standard alone, which, however, is now modified by the beam hardening and scattering effected by the dental tissues lying in front of the cassette. Comparison of this calibration method versus the conventional intraocclusal calibration wedge in an in-vitro study showed that the errors using the cassette method were significantly less than when using the conventional wedge-based calibra-



tion technique. The cassette system also permitted to correct for image distortions caused by changes in the position of the film plane relative to the teeth.

This cassette is currently being used in conjunction with the cephalostat head stabilization system and the closed-circuit video feedback for registration control in the ongoing clinical trial of a new anti-inflammatory drug. While theoretically the best approach, the intraoral cassette tends to be rather bulky and, particularly for maxillary teeth, may thus often preclude proper imaging of the root apices. Therefore, the concept of an intraocclusal calibration reference was further developed to include some means to correct for the beam hardening effect. Detailed studies with x-ray phantoms indicated that the impacts of differential beam hardening and contrast variations on bone mass calibration could be well characterized by a second order polynomial. Therefore, a calibration standard consisting of three different bone thickness levels that were superimposed over a continuous bone wedge was fabricated, enabling to estimate that polynomial function for each pair of radiographs to be subtracted. Knowledge of this calibration curve for each subtraction image will then permit to correct for beam hardening and film nonlinearity artifacts. This new calibration standard is incorporated into a newly designed film holder device. The performance of this new calibration standard and film holder is currently under evaluation.

Models for longitudinal data analysis

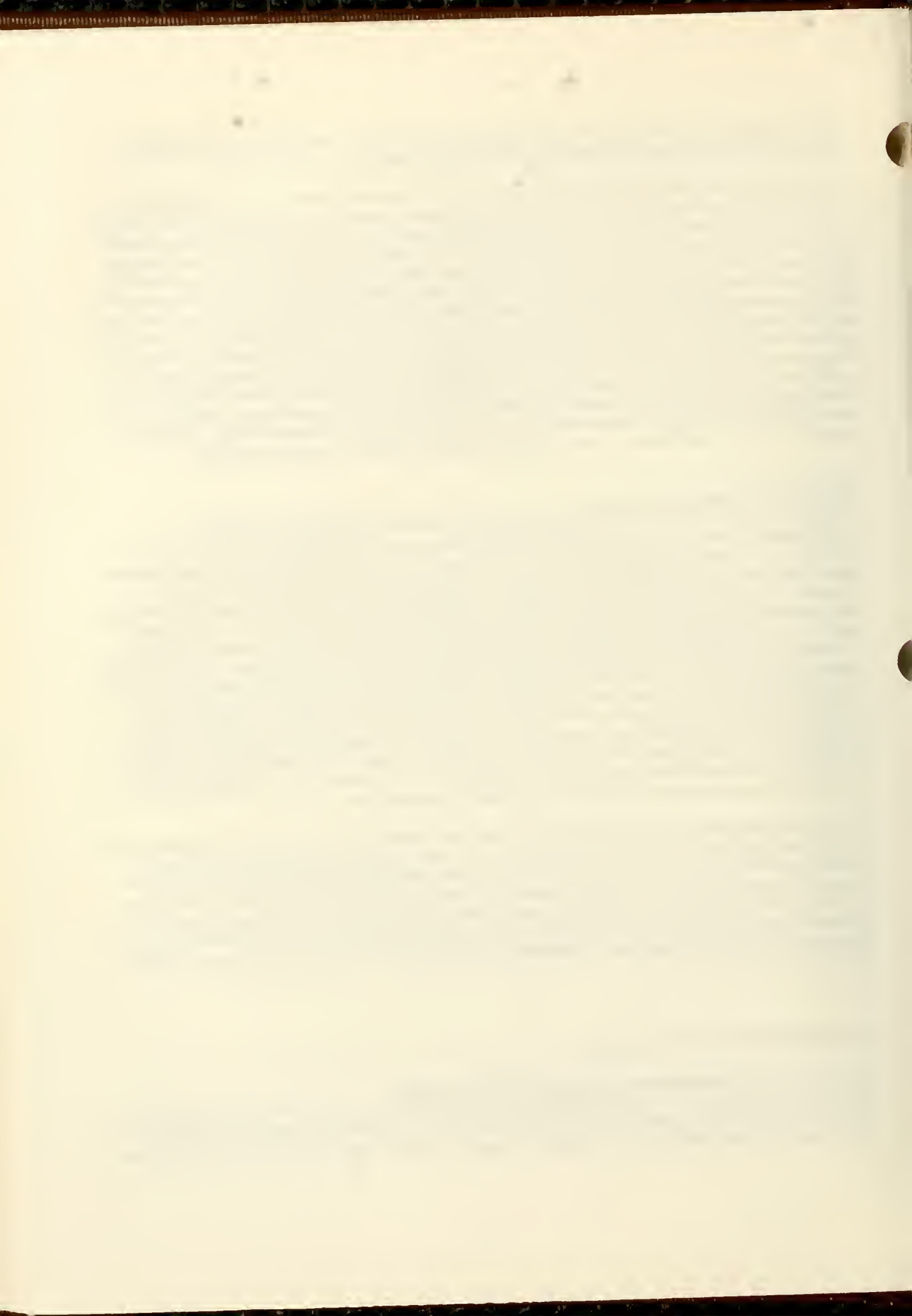
Objective prognosis of outcome for single patients based on longitudinally observed measurements is still in its infancy because either conceptual mathematical models are not available or not familiar, proper data are not collected, or the possible stages of outcome have not been objectively defined. DSB has continued the collaboration with Dr. Murray Pollack, Associate Director of the ICU in the Children's Hospital National Medical Center, in developing longitudinally oriented prognostic methods because in this environment all three of the above factors can be satisfactorily controlled. Outcome prediction has become increasingly important in studies concerned with issues such as quality of care, and resource utilization. However, most mortality risk predictors make use of patient data collected only over a single, short time period (e.g. admission day) and, thus, are not able to take advantage of the patient's changing status to improve predictor performance or to expand the relevance of the predictor. Therefore, using daily obtained physiologic data, a study was conducted to evaluate a) the importance of previous observations in making short-term outcome predictions, b) which time periods are most important for outcome predictions, and c) the best combination of observations from different time periods for short-term outcome prediction in pediatric intensive care.

The results indicated that only the most recent and the admission day data (with a weighting ratio of 3:1) were statistically relevant for prediction. The performance of the dynamic predictor in predicting final outcome of each patient's ICU episode was significantly improved over that obtained by a static predictor developed earlier, which was based on the admission day data alone. The ability of this predictor to update mortality risks permits objective charting of a patient's clinical course based on the latest available data, and may become an important tool for cost containment by identifying patients at a sufficiently low mortality risk for early discharge from the ICU.

NEW DIAGNOSTIC MODALITIES

Nuclear activity measurements during alveolar bone healing

Particularly promising in terms of modality-specific applications is research based on the use of radiopharmaceuticals to predict loss of periodontal bone. Specifically, it was investigated whether the healing of surgically induced alveolar lesions in beagle dogs could be followed-up by mea-



asuring the nuclear activity of ^{99m}Tc -MDP externally over the lesion sites with the aid of a commercially available miniature cadmium-telluride probe. Last year's results indicated that no statistically significant difference in nuclide uptake between the operated and unoperated side of the mandible could be demonstrated. Investigation of the properties of the miniature probe revealed lack of collimation, causing radiation across the dental arch to reach the detector. Thus, in collaboration with Dr. Fasle Hosain, Professor of Nuclear Medicine at the University of Connecticut, who spent part of his sabbatical leave with DSB, a removable probe collimator was designed and fabricated and a new series of in vivo measurements in dogs initiated.

Measurements made with the added collimator permitted reliable detection of all lesions and a tooth extraction by an increased ratio of the counts obtained over the operated versus the contralateral control site. Comparable data produced without collimation yielded only a small increase of the ratio for the extraction site. Hence, the sensitivity of this monitoring method critically depends on proper collimation of the detection probe. Because it is desirable for eventual clinical applications to minimize the radiation dose necessary to perform these measurements, the possibility of using more efficient detector elements than the currently used cadmium-telluride probe is investigated, in particular the suitability of sodium iodide crystals is considered.

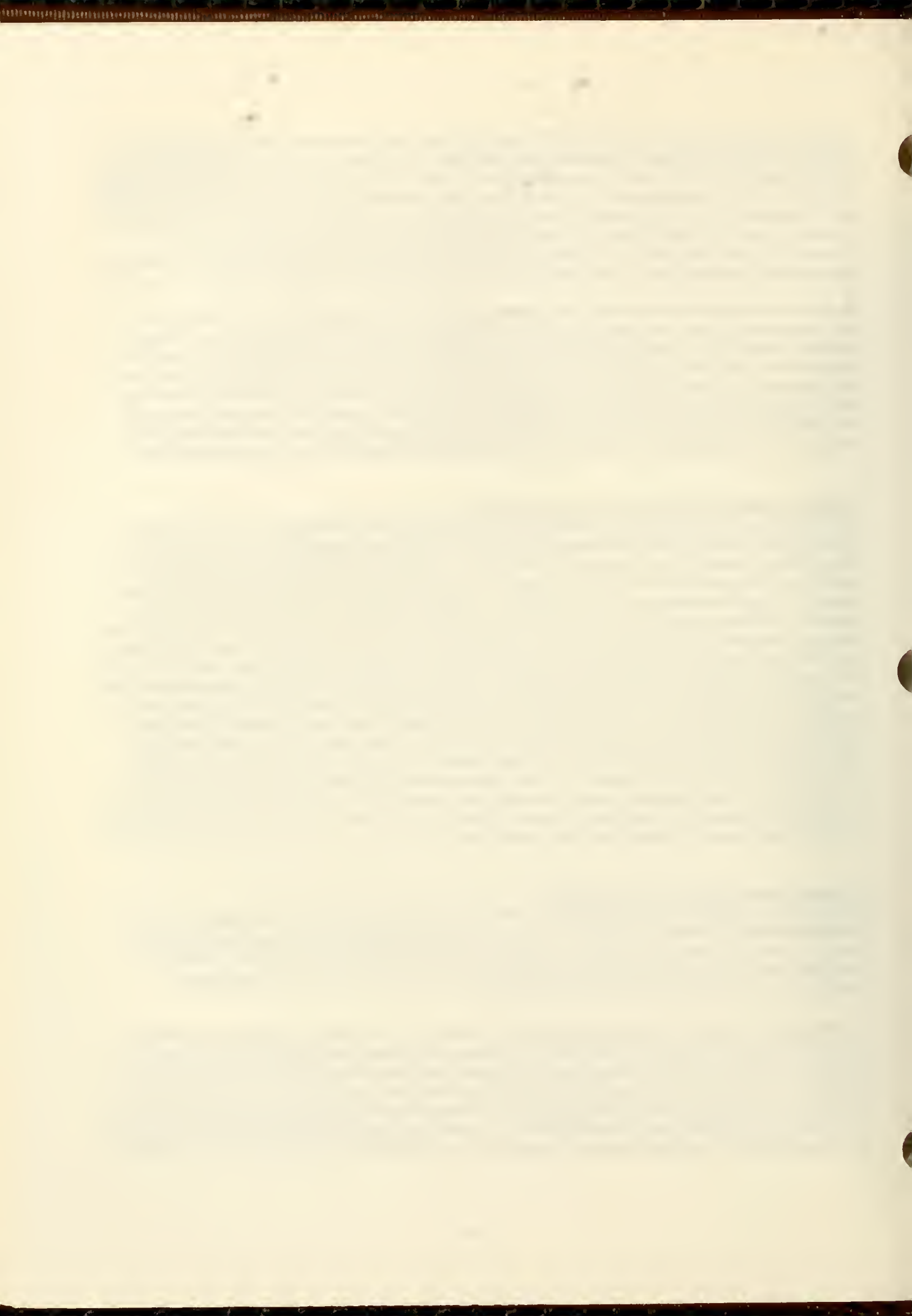
Magnetic resonance imaging of the salivary glands

The evaluation of salivary gland function has become important for diagnosis and prognosis in patients who with complaints of xerostomia such as in Sjogren's syndrome, after radiation or chemotherapy treatment, or in geriatric conditions. A currently available imaging technique involves the retrograde infusion of radio-opaque contrast material and subsequent projection radiography. The shortcomings of this technique are that it is painful, there is little information gained as to the extent of remaining functional parenchymal tissues, and, in the case of total duct blockage, the body of the gland can not be visualized at all. Because magnetic resonance imaging (MRI) reflects the water content in tissues, it was postulated that it might be possible to evaluate salivary function quantitatively using this modality. MRI would have the advantages of not using ionizing radiation, avoiding the injection of contrast material, and providing anatomical information in multiple planes. Therefore, a study with beagle dogs was initiated to determine whether ductal stenosis can be detected reliably from T1, T2 and inversion-recovery weighted (IR) images after salivary stimulation with pilocarpine. The results indicated that the signal strength observed in the T2 protocol, and to a lesser extent in IR images, may be used to assess water content in the glandular tissues, providing the potential for evaluating salivary gland function and duct patency by quantitative means. However, due to the personnel restrictions imposed on DSB this avenue of research has been terminated.

Photoplethysmography of the dental pulp

Collaboration with Dr. Joseph Schmitt from BEIB continued toward the development of a non-invasive method to detect the vitality of a tooth. This development arose from the desire to design a clinically useful and simple, yet objective test to determine pulp vitality. Presently available tests suffer from lack of reproducibility, may produce pain or other unpleasant sensation, and heavily rely on patient cooperation.

A prototype instrument was built by BEIB that is based on the analysis of light scattered from the deep structures of a tooth which has been flooded with broadband light through a fiber-optic bundle. The scattered light was collected by a prism attached to the tip of a fiber bundle on the opposite side of the tooth. Display of the signal derived from the scattered and subsequently filtered light intensity revealed a cardiac pulse, which was demonstrated by its synchrony with the simultaneously recorded electrocardiogram. In accordance with theoretical predictions, the best wavelength band for pulse detection was found to be within 530 to 590 nm, where the extinction



coefficient of whole blood reaches the highest values. These studies indicated that the demonstration of pulp vitality by showing the cardiac pulse appears possible and may be a potentially valuable aid to endodontic diagnosis, but improved techniques need to be developed to measure oxygen saturation in the pulpal tissues with sufficient accuracy. Further development of this methodology will be in the hands of the collaborators from BEIB.

Evaluation of diagnostic modalities suitable for monitoring the temporomandibular joint

In an effort to develop new diagnostic methods for possible applications in maxillo-facial surgery, a comparative evaluation of several different modalities was initiated. The particular aim was to assess whether subtraction radiography was feasible, and to compare its results with those obtained by tomosynthesis, nuclear medicine assays, and TMJ arthroscopy during the follow-up of bone lesions surgically induced into the condyle of beagle dogs. While the study is still in progress and not all results have been analyzed, some tentative conclusions can already be drawn. Arthroscopy furnishes detailed qualitative information about intracapsular soft tissues and particularly the status of the cartilage surface covering the joint which cannot be obtained by any other method. This modality may thus have a high diagnostic utility in case of restricted articulation, inflammation or other complications. Radionuclide activity measurements were clearly associated with local bone formation and may have applications for research purposes, however, at the current development stage, the technique is not sufficiently sensitive to warrant its use in individual patients with TMJ problems. The radiographic methods have the definitive advantages of their noninvasiveness and relatively low cost. Subtraction radiography was preferred over tomosynthesis because it provided in addition to about equally good qualitative information on bone remodeling, quantitative data regarding bone mass changes, and it required a smaller total radiation dose. The successful results obtained with subtraction radiography definitely merit the development of improved techniques enabling accurate and expedient control of the radiographic projection geometry in patients.

Measurement of arthritic lesions in the hand by subtraction radiography

The subtraction radiographic methods developed for measuring focal bone loss in the jaw bones were being tested for their applicability to quantitate arthritic lesions in the hand. This work was pursued in close collaboration with Dr. Mark Bolander, Chief of the Orthopedic Research Unit, NIAMS. While initial efforts focussed on the detection of arthritic lesions in the hand, the long-term goal was to develop techniques permitting quantitative imaging of the hip joint. Experiments were performed on cadaver hands where lesions were created surgically, simulating rheumatoid arthritis. Linear regression of lesion mass estimated by subtraction radiography versus the gravimetrically determined bone loss yielded correlation coefficients of 0.99 and a measurement precision of about 1 mg. Lesions as small as 2 mg could be detected. This sensitivity and precision for measuring focal bone loss is better than what could be achieved with computed tomography or photon absorptiometric methods. The primary problem in this application of subtraction radiographic methods is again proper control of the imaging geometry. Initial attempts to reposition hands of human volunteers under the x-ray machine yielded satisfactory subtraction images. Thus, the problems of attaining proper control of the projection geometry for standardized radiographic imaging of the hand may not be unsurmountable. Further development of this application has been discontinued upon the decision to terminate the operations of DSB.

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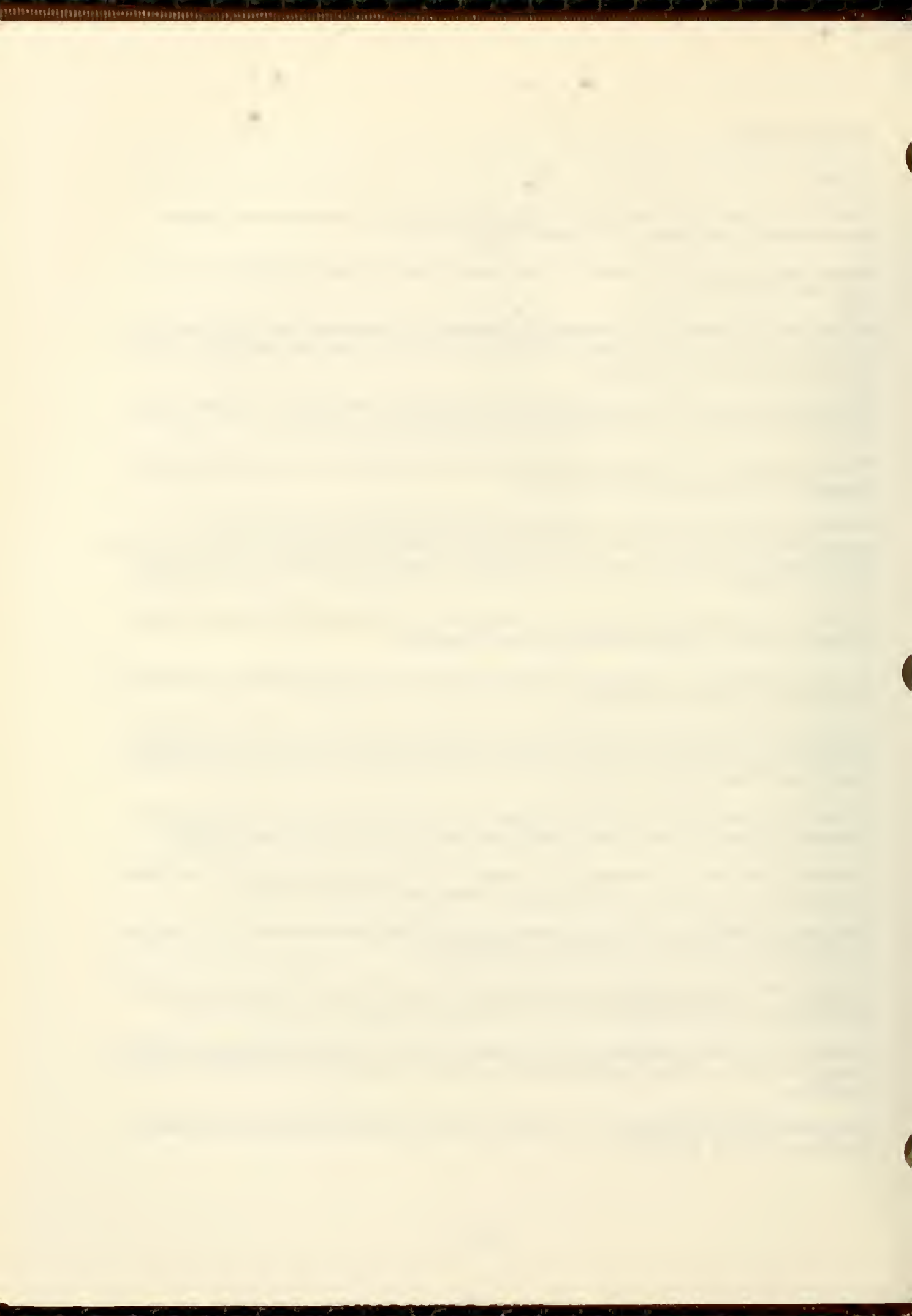
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Patents

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00065-18 DS

PERIOD COVERED
 October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Development and Evaluation of Improved Diagnostic Systems

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

Ruttimann, Urs E.	Acting Chief, Diagnostic Systems	DS	NIDR
Webber, Richard L.	Former Chief, Diagnostic Systems	DS	NIDR
Horvath, Gabriella	Summer Research Fellow	DS	NIDR
de Valk, Serge A.	Visiting Fellow	DS	NIDR
Tsuchimochi, Makoto	Visiting Associate	DS	NIDR

COOPERATING UNITS (if any)

Radiation Physics Group, National Institute of Standards and Technology
 Department of Oral Radiology, Academic Center for Dentistry, University of
 Children's Hospital National Medical Center Amsterdam

LAB/BRANCH
 Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS 1.58	PROFESSIONAL: 1.15	OTHER: .43
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CHECK APPROPRIATE BOX(ES)

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

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

This project is an extension of previous work directed toward the study of noninvasive methods to determine spatial and temporal relationships existing between tissues of clinical interest. The approach involves analytic formulation of the diagnostic task, computer simulations, in vitro measurements, and the development of prototypes suitable for clinical evaluation. Recent work has focussed mostly on studies directed toward development of a versatile computerized dental radiographic system to produce images which can be subtracted to show small changes in tissue occurring over long intervals of time, and which can be combined in ways permitting synthesis of desired projections or tomosynthetic display of specific slices of the teeth and jaws. The main difficulty with subtraction radiography is to attain proper control of the x-ray projection geometry. Therefore, work continued on the design of a head holder system using a cephalostat and closed-circuit video feedback to stabilize the projection geometry in film-based radiography. Measurements on a proto-type design demonstrated that it was possible to reposition patients within acceptable tolerances necessary for subtraction radiography with sagittal as well as frontal projections.

Bitewing radiographs are commonly used for detecting interproximal carious lesions, with little firm knowledge about the best projection directions. A controlled psychophysical study revealed best detection performance with angulation in the horizontal plane perpendicular to the tooth surfaces, and positive or negative tilts of 12 to 15° from the perpendicular in the vertical direction. Deviation from the optimal horizontal angle resulted in an increased number of missed lesions, i.e. loss of sensitivity, whole vertical deviations caused mainly an increase in false positive diagnoses, i.e. a drop of specificity.

New calibration methods are developed to control for nonlinear effects caused by differential x-ray beam hardening in the tissues of interest in order to achieve a more precise quantitation of bone mass loss by subtraction radiography.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00211-13 DS

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Enhancement and Processing of Diagnostic Images

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

Ruttimann, Urs E.	Acting Chief, Diagnostic Systems	DS	NIDR
Engelke, Werner	Visiting Associate	DS	NIDR
de Valk, Serge A.	Visiting Fellow	DS	NIDR
Horvath, Gabriella	Summer Research Fellow	DS	NIDR
Qi, Xiang-lin	Visiting Associate	DS	NIDR

COOPERATING UNITS (if any)

CIPCB, NIDR, NIH
 Department of Electrical Engineering, University of Lowell
 Department of Oral and Maxillofacial Surgery, University of Zurich

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

PROFESSIONAL.

OTHER

1.96

1.33

.63

CHECK APPROPRIATE BOX(ES)

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

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

This project investigates interpretation tasks in which the diagnostic performance is limited by the ability of the human observer to recognize or understand the displayed information. Of interest is the development of specific image processing techniques enhancing the visual data presentation or performing automated quantitation of images. Current work has centered on methods relevant to digital subtraction radiography that could be integrated into a computerized dental x-ray system. One specific goal was the automated recognition and delineation of areas in radiographs showing trabecular bone. Corresponding results indicate that fractal geometry provides a realistic mathematical model to recognize and characterize trabecular bone. Furthermore, the fractal dimension of the bone surface derived from the model identification process is a global image descriptor that may be developed into a measure enabling the quantitation of bone structure changes due to osteoporosis.

Noise reduction in subtraction images is a processing step that precedes lesion detection and measurement. However, this digital filtering step necessarily also incurs undesirable image blurring and thus, a filter is desirable that achieves substantial noise smoothing without causing undue image blurring. A filter, modeled after neural nets in the visual cortex that display directional sensitivity, was designed that recognizes contrast edges and performs smoothing only in directions that do not cross edges. This novel filter structure was shown to preserve image sharpness substantially better than known linear or median filters when matched to attain identical noise suppression.

Particular emphasis has been placed on clinical applications of the image subtraction method. One study investigated its use for following-up patients in which bony lesions were filled with hydroxyapatite implants. The method successfully demonstrated bone regeneration or loss of implant material over 4 to 6 months postoperatively. In another clinical study the efficacy of a new anti-inflammatory drug in retarding alveolar bone loss in periodontal patients is investigated by monitoring bone mass changes over a 12 months period and determining any correlations with the presence of specific pathogens sampled from gingival pockets.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00373-07 DS

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Exploration and Assessment of New Diagnostic Modalities

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

Ruttimann, Urs E.	Acting Chief, Diagnostic Systems	DS	NIDR
Tsuchimochi, Makoto	Visiting Associate	DS	NIDR
Engelke, Werner	Visiting Associate	DS	NIDR

COOPERATING UNITS (if any)

Biological Engineering and Instrumentation Branch, NIH
 Veterinary Resources Branch, NIH
 Diagnostic Radiology Department, CC, NIH

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

2.37

PROFESSIONAL:

2.03

OTHER

.34

CHECK APPROPRIATE BOX(ES)

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

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

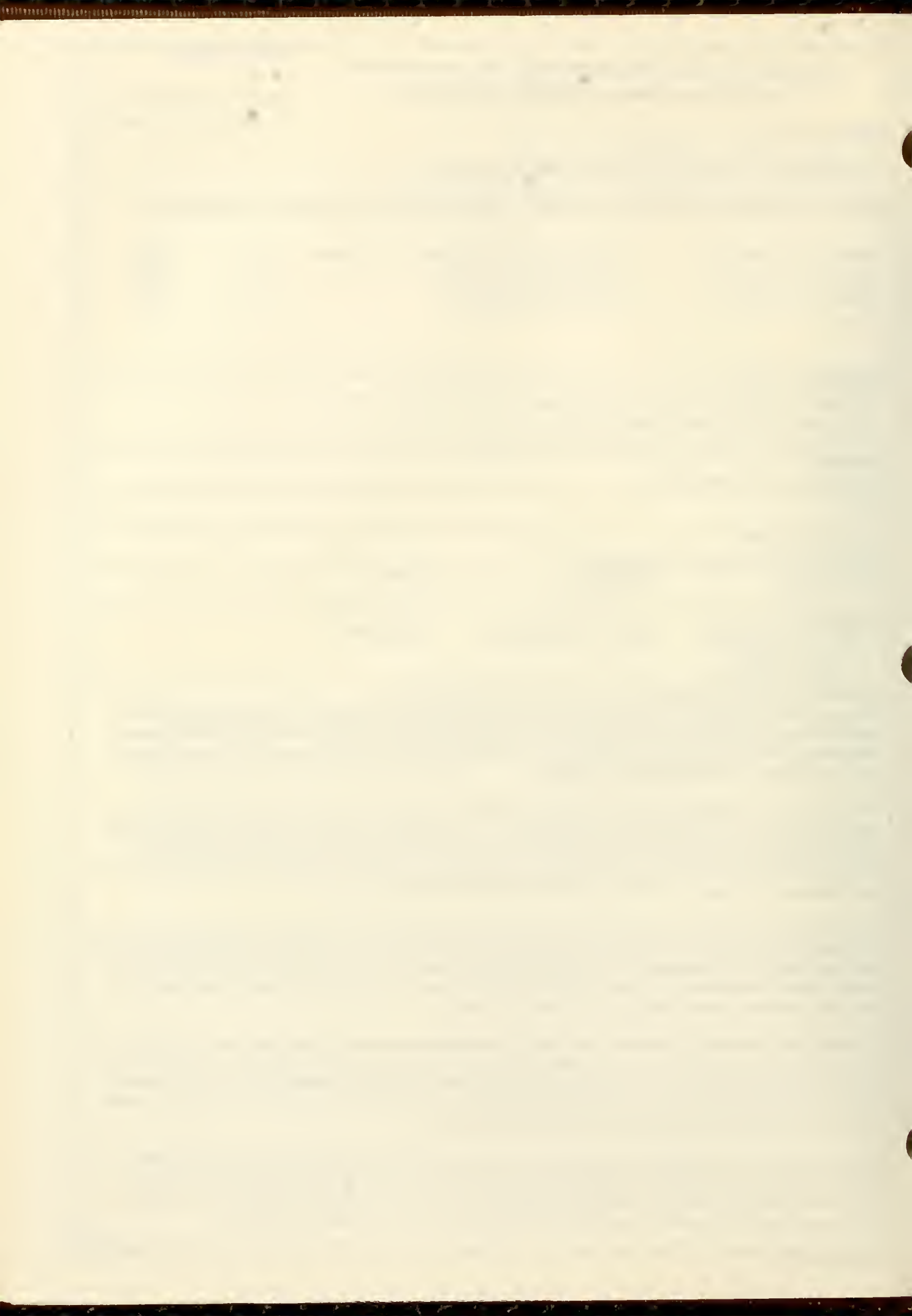
This project is concerned with the evaluation of variety of new diagnostic techniques which have yet to be applied in dentistry. Modalities studied include nuclear medicine (^{99m}Tc-MDP) assays, arthroscopy, fiber-optic systems making use of visible light (hemoglobin-specific spectrum analysis), and magnetic resonance (MR) imaging.

Measurements of differential uptake of ^{99m}Tc-MDP in beagle dogs following induction of tiny alveolar lesions indicated the need for redesign of a commercially available miniature nuclear probe to increase spatial specificity. The addition of a properly designed collimator to the hand-held probe permitted reliable detection of the bone regeneration process in induced lesions in the alveolar bone and the condylar head in the temporomandibular joint (TMJ).

In an effort to develop new diagnostic tools for TMJ evaluations, the healing of induced lesions in the condyle was also monitored by arthroscopy and subtraction radiography. Arthroscopy furnished detailed qualitative information on intracapsular soft tissues and the status of the cartilage surface which cannot be obtained by other methods, while subtraction radiography enabled quantitation of the bone mass and shape changes of the condylar head.

A prototype fiber-optic instrument was built to determine tooth vitality from the analysis of light scattered from the pulpal tissues. Display of the intensity ratio at two wavelengths within the 530 to 590 nm range demonstrated a cardiac pulse in dogs, which could be eliminated by root-tip transection. Similar pulsative signals could be derived from vital molars, bicuspid, and incisors in human volunteers, demonstrating potential utility for endodontics.

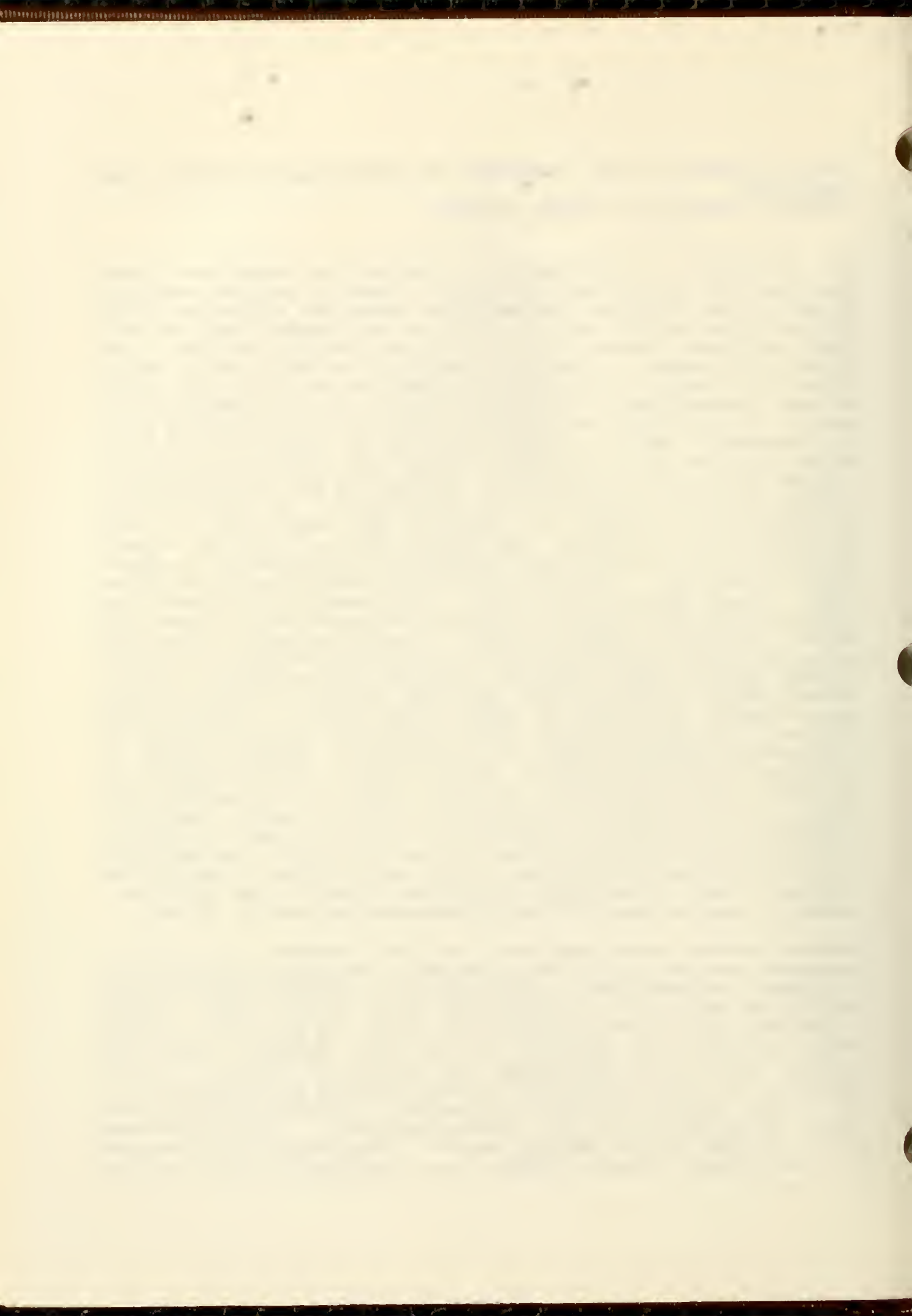
MR imaging was investigated to determine whether surgically induced stenosis of the salivary gland ducts can be detected reliably. The results indicate that T2-weighted images show significant differences between occluded and nonoccluded glands due to interacinar edema, and an excellent representation of the anatomy, possibly obviating the need for contrast sialography.



**ANNUAL REPORT OF THE LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES
NATIONAL INSTITUTE OF DENTAL RESEARCH**

The Laboratory of Developmental Biology and Anomalies (LDBA) continues an innovative and dynamic research program employing molecular and cellular approaches to study the structure of the extracellular matrix and its role in development and disease. There have been a number of changes in staff during this year. The Chief of 15 years, Dr. George R. Martin, has left LDBA to become Scientific Director of The National Institute on Aging. Dr. Martin is an internationally recognized expert on the extracellular matrix. His guidance and advice will be greatly missed by LDBA. Dr. Kenneth S. Brown, a geneticist who is known for his mouse mutant studies and for setting up the LDBA transgenic facility, retired after many years at NIDR. Several new people selected from a large pool of applicants, have joined LDBA. Dr. Paul Klotman, an Associate Professor of Medicine and Nephrology at Duke University Medical Center, has joined LDBA as a special expert. His studies will focus on gene expression and regulation in repair and in diseases. Several scientists on sabbatical have joined LDBA. These are Dr. Martin Dym, a professor and chairman from Georgetown Medical School, studying gene expression by developing Sertoli cells; Dr. Jeffrey Liebman, a senior scientist from Ciba-Geigy, studying osteoarthritis; Dr. Hari Reddi, chief of Bone Cell Biology Section, NIDR, studying cartilage differentiation; and Dr. Teruaki Mori, a neurosurgeon from Oita Medical School, studying brain tumors and repair of spinal cord injury. Several young fellows have also joined LDBA including Benjamin Weeks (a toxicologist from University of Connecticut), Yolanda Sanchez (a cartilage expert from Spain), Thomas Sweeney (an M.D.-Ph.D. from the University of Virginia), Katsunori Fukuda (an otolaryngologist from the University of Kagoshima, Japan), Radim Becvar (a rheumatologist from Czechoslovakia), Norio Shiraishi (a surgeon from Oita Medical School, Japan), Claudio Polistina (an internist from the University of Naples, Italy), Sergio Line (a dentist and collagen expert from Ludwig Institute, Brazil), and Dr. Makoto Sawada (a neurobiologist from Fujitagakuen University, Japan).

Several awards have been made to LDBA members. Dr. Leslie Bruggeman received an Arthritis Foundation Postdoctoral Fellowship. A patent for "Matrigel - Reconstituted Basement Membrane with Biological Activity" was awarded and issued in May, 1989. Royalty funds from this patent have been used to pay for speakers, for LDBA staff to travel to meetings, and for cash awards. Four other patents were filed this year, including: (1) laminin A chain peptide, (2) role of minoxidil in wound healing (with Upjohn Co.), (3) & (4) lipoxygenase and cyclooxygenase inhibitors which block tumor metastases (with G.D. Searle/Monsanto). Including these four, the lab has six patents pending. Two Cooperative Research and Development Awards (CRADAs) were approved by NIH and the



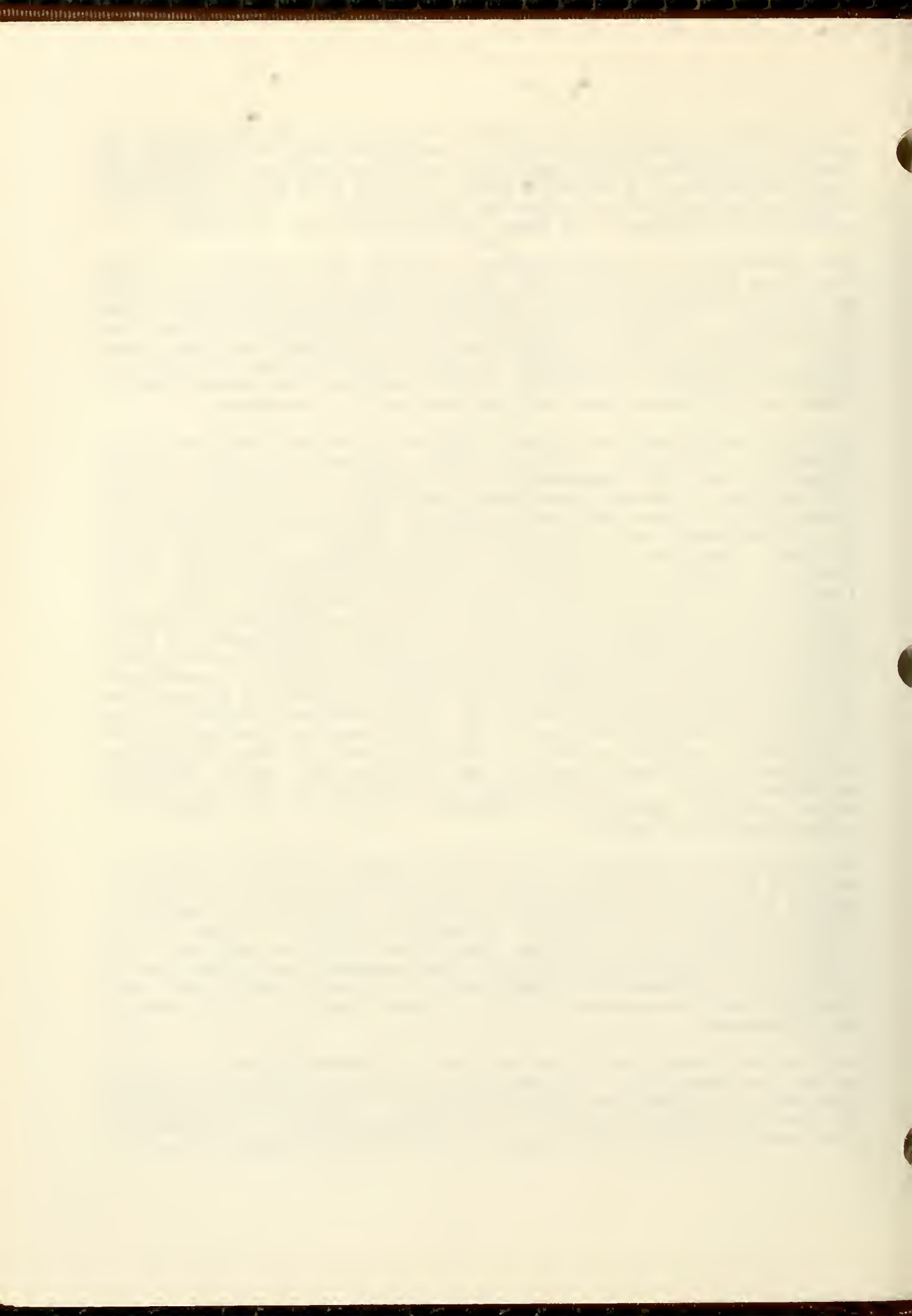
collaborative projects have begun with Eli Lilly to develop animal models for arthritis and with G.D. Searle/Monsanto to continue the laminin peptide-and lipoxxygenase inhibitor-mediated blockage of tumor metastases. A research grant has also been funded from Kyowa Hakko to study active peptides from laminin. We welcome the support and interest from these companies for our research.

The Laboratory of Developmental Biology and Anomalies (LDBA) continues to make significant advances in understanding the role of the extracellular matrix and its receptors in development and in disease. Using both molecular and cellular approaches, new directions have been developed and much progress has been made. This is particularly evident in our studies on gene regulation, basement membrane, cartilage, wound healing, the mechanisms of tumor cell invasion, and cell surface matrix receptors.

Basement membranes have been a major focus of LDBA where the structure, function, active sites, expression, and cellular receptors for the components are studied. Laminin, a major glycoprotein of basement membranes, was initially described by LDBA researchers 10 years ago, and last year, the cloning and sequencing of this molecule was completed also in LDBA. Previously, using synthetic peptides we identified a biologically active site, YIGSR (Table). During the last year, a 19 mer synthetic peptide on the laminin A chain containing the active sequence, IKVAV, was identified as active for promoting cell attachment, neurite outgrowth, collagenase IV activity, and tumor metastases in vivo. These activities are unique to this peptide. Non malignant cells when treated with this peptide become more invasive. In addition, the RGD containing sequence (PA21) from the laminin A chain has also been identified as active for cell adhesion, migration, and in promoting neurite outgrowth. With the characterization of these biologically active sites on laminin, most of the biological responses of laminin can now be mimicked by small synthetic peptides (Table). Such peptides may be used in vivo to promote nerve repair, improve the adhesion of cells to grafts, and accelerate wound repair.

The sequence of nidogen, an ubiquitous matrix protein in basement membrane has been completed as a result of a joint effort with Dr. Rupert Timpl, Max-Planck-Institut and Dr. Man-Li Chu, Jefferson Medical College. The deduced sequence revealed a number of EGF-like repeats and two globules, and agreed with the dumb-bell like structure predicted by EM-study. The sequence data also made it clear that nidogen was identical to entactin which had been characterized independently by Dr. Albert Chung at the University of Pittsburgh.

We have continued cloning and sequencing basement membrane heparan sulfate proteoglycan. At present, 80% of the core protein sequence (400 KD, 13 kb mRNA) has been determined. The sequence including the most N-terminus revealed three major domains: LDL receptor-like domain, laminin-like domain, and N-CAM-like domain (Fig. 1).



Table

Biologically Active Synthetic Peptides from Laminin

<u>Activity</u>	<u>B1 chain</u>	<u>A chain</u>	
	<u>YIGSR</u>	<u>PA21(RGD)</u>	<u>PA22-2(IKVAV)</u>
Adhesion	+	+	+
Migration	+	+	+
Spreading	0	+	+
Growth	0	0	+
Neurite outgrowth	0	0	+
Collagenase IV activity	0	0	+
Tumor metastases	↑↑	↓	↑↑
Heparin binding	0	0	+

+ denotes activity

0 denotes no activity

↑ or ↓ increases or decreases

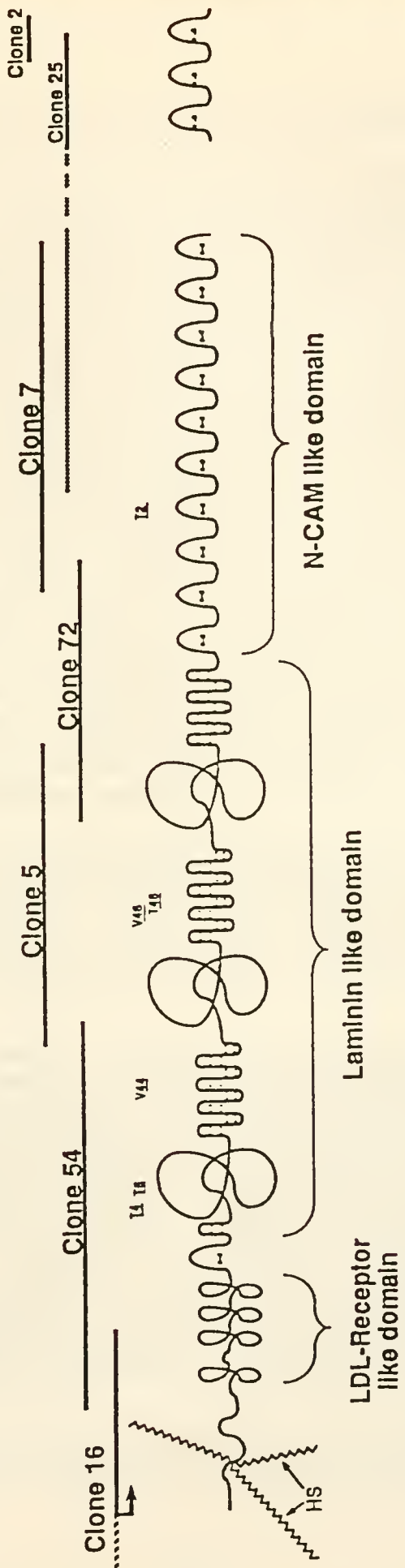
Three potential attachment sites for heparan sulfate have been found at the N-terminal part of the protein. The presence of a laminin-like domain prompted us to investigate the ability of the heparan sulfate proteoglycan to promote cell adhesion. Indeed, we have found that the protein core can promote attachment of a variety of cells. This attachment is mediated through a specific cell surface receptor of 38KD.

Cartilage has been another major focus of LDBA because of its importance in development and in human disease. DNA transfection analysis reveals that the level of collagen II gene expression is determined by both positive and negative regulatory DNA elements. In addition, a common nuclear factor(s) appears to interact with both the promoter element and the enhancer within the first intron of the gene. The link protein gene also has an enhancer in the first intron. The presence of the enhancer within the first intron of the link protein gene and in the collagen II gene raises the interesting possibility that the two genes, because they are co-expressed in chondrocytes were related as they evolved. The large cartilage proteoglycan forms an aggregate with both hyaluronic acid and link protein which functions to resist compression. This proteoglycan is the most extensively studied proteoglycan molecule but has not been named for more than 20 years, partly because of the heterogeneity of many preparations from different tissues. Now that we have completed sequencing of the rat proteoglycan core protein and its gene, we have submitted that this molecule be called aggrecan, to evoke its functional properties. Because it is a useful tool to study human skeletal dysplasia, we have isolated cDNA and the gene for the human aggrecan and completed its primary structure by DNA sequencing. The human aggrecan shows some



STRUCTURAL MODEL OF THE BASEMENT MEMBRANE PROTEOGLYCAN

Fig 1



structural differences from the rat. Part of this difference is due to an alternative exon splicing in the human gene.

The transfer of genes into the germ line of mammals is one of the major breakthroughs in biology. Creation of transgenic mice offers enormous potential to study mechanisms of development and protein function. We have employed this technique to explore gene regulation and function of connective tissue proteins. We have tried to create models in transgenic mice for hereditary and acquired human connective tissue disorders. We have shown that the collagen II promoter and enhancer can direct expression of a foreign gene, specifically in cartilage. We have also studied developmental regulation of the collagen II gene by introducing the diphtheria toxin A chain gene under the control of the tissue-specific collagen II promoter and enhancer sequences into mouse embryos. When the toxin is expressed, it kills the cells and eliminates any tissues that would have been derived from progenitor cells. Although no line of offspring have been generated in this experiment, we have found a series of abnormally developed embryos around day 12 gestation. These animals had underdeveloped limbs, no eyes, and kinked tails, and resembled the phenotype of chondrodystrophic mice strains. The expression of the toxin appeared to occur in the correct tissues, and at the right time frame of development. An expression vector with the collagen II promoter and enhancer could be useful to direct expression of any foreign genes such as cytokines and collagenase in transgenic mice. These transgenic mice can be used as models of human diseases of inflammation and weakened cartilage.

We previously reported that the basement membrane collagen IV genes are located on the same chromosome separated by only 130 bp in a head to head arrangement and are regulated by a common enhancer and a bidirectional promoter. Some of the regulatory sequences within the promoter and enhancer have now been identified and progress has been made in characterizing nuclear factors which bind these elements.

We have begun to study connective tissue gene expression in diseases. For example, we have demonstrated that the potent vasoconstrictor and platelet aggregatory product of arachidonic acid metabolism, thromboxane A₂, is markedly increased in kidneys from diabetic animals and further stimulated by protein feeding. This change in prostaglandin metabolism is associated with increased production of matrix proteins in the glomerulus, thus effacing the filtering surface. Recently, we have demonstrated that thromboxane directly stimulates laminin and collagen production by kidney cells in part by an increase in transcription of these genes. Studies are currently in progress to define the transcriptional regulatory proteins in diabetic kidneys and those induced by inflammatory mediators, cyclosporine toxicity, and renal allograft rejection.

Studies on the invasive, metastatic, and growth activity of malignant cells have continued as a major focus of LDBA. It has



been established that basement membranes are a barrier to the spread of tumor cells. Tumor cells attach, degrade and migrate through basement membranes. We have recently found that tumor cells which are hard to grow can be successfully grown in vivo in a gel of basement membrane. Even small numbers of cells and non malignant cells can be grown in vivo. Such approaches allow models for study of tumor growth. Collagenase IV activity appears responsible for much of the invasive and metastatic activity. We showed previously that direct inhibition of the activity of this enzyme blocked invasiveness in vitro and metastases in vivo. We now find that the synthesis of this enzyme is regulated by arachidonic acid metabolites. For example, inhibitors of either cyclooxygenase or lipoxygenase reduce invasiveness, metastases, and the synthesis of collagenase IV. A lipogenase inhibitor also increases the survival of nude mice bearing human ovarian carcinomas. Such novel drugs may be useful adjuvants to standard approaches (anti-mitotics) used to treat cancers.

We have also initiated studies on Kaposi's sarcoma, a tumor usually associated with skin that is a common manifestation of AIDs. We have focused much of our attention on the transactivator of transcription of HIV1, TAT. We have transfected a construct with a strong heterologous promoter (CMV) linked to TAT into a line of human T lymphocytes (H9, CD4+). These lymphocytes normally grow in suspension but when transfected with TAT and plated on laminin, they develop marked cytoplasmic outgrowths and demonstrate increased attachment. These data suggest that TAT may transactivate the laminin receptor on CD4+ T helper lymphocytes and, as a result, may markedly influence cellular immune responses and localization. Studies are currently in progress to evaluate whether laminin receptors are increased by TAT and, if so, the consequent functional implications of changes in laminin receptor number on immune function.

Wound healing has continued to be an important emphasis in the laboratory. We have described two models of impaired wound healing in the guinea pig, one caused by calcium-stimulated collagenase production and the second by an inhibitor of platelet derived growth factor. In both models, expression of collagen I mRNA is an important index of wound healing. We have applied these findings to small patient samples using the polymerase chain reaction. In patients with keloids or hypertrophic wounds, we have found that mRNA for collagen I was markedly increased and collagenase I was markedly decreased, suggesting that over-expression of collagen I and/or reduced production of collagenase contributes to the pathogenesis of these conditions.

Objectives and Approaches

Cell-matrix interactions play an important role in development. Components of the extracellular matrix promote cell adhesion, growth, migration, and differentiation. Our goals are to identify the molecular mechanisms which regulate the synthesis of extracellular matrix components and to understand the cellular



response to matrix components. Such studies should lead to an understanding of how the extracellular matrix functions in normal and abnormal development and should suggest strategies for therapeutic approaches. We focus on (1) cartilage, (2) basement membrane, (3) wound healing, (4) tumor growth and metastases, (5) diseases of connective tissue, and (6) matrix receptors. Our approaches include molecular biology, cell biology, transgenic mice, protein chemistry, and morphology.

Identification of Biologically Active Sites in Laminin

With the completion of the A chain sequence, the final structure of laminin could be determined and it became possible to prepare synthetic peptides and site-specific antibodies to laminin. Using such approaches, four active sites on laminin have been identified, including YIGSR and PDSGR on the B1 chain, and RGD and IKVAV on the A chain. Both YIGSR and PDSGR are unique to laminin and promote cell adhesion, migration and block in vivo lung colonization of melanoma cells. PDSGR is somewhat less active than YIGSR in these activities. YIGSR has also been found to reduce the size of small cell lung carcinoma derived tumors in nude mice.

RGD (Arg-Gyl-Asp) is a sequence present in the laminin A chain and in various other adhesive macromolecules including fibronectin, vitronectin, fibrinogen, thrombospondin, etc. Since there appears to be a common family of receptors (integrins) recognizing some RGD sequences of these adhesive proteins, we determined whether the RGD sequence functioned in laminin. The RGD-containing peptide (PA21, CQAGTFALRGDNPQG) from laminin is active for cell adhesion, spreading, and migration. This peptide can also compete for laminin-mediated adhesion with some but not all cells. By itself, it cannot promote neurite outgrowth. When coupled to keyhole limpet hemocyanin, it can promote neurite outgrowth for some but not all neuronal cells.

A 19 mer peptide (PA22-2) located near the end of the long arm of the A chain has been found to be an important and unique biologically active site. This peptide was very active for cell adhesion, migration, and neurite outgrowth when directly coated on the dish. Subsequent analysis revealed that an IKVAV sequence within PA22-2 was an active site for this biological function. Most neuronal cells tested respond to this peptide, including PC-12 cells, Type I astrocytes, cerebellar neurons, septal neurons, etc. This peptide competes with laminin for neurite outgrowth. Antibodies prepared to the polymerized form of this peptide block peptide-mediated outgrowth by 100% and laminin-mediate neurite outgrowth by 50%. We conclude that this site is a major active site in laminin for neurite outgrowth but other sites also likely exist. This peptide also increases the number of melanoma cells colonizing the lungs of mice. The increase in tumors is dose-dependent and observable when the peptide is coinjected with the tumor cells or when it is injected intraperitoneally 45 minutes after tumor cells. The peptide elevates collagenase IV activity to the same extent as laminin and likely promotes metastases due



to an increasing ability of the cells to cross the basement membrane of the blood vessel wall.

Ken-Ichiro Tashiro, Gregory Sephel, Yoshi Yamada, Hynda Kleinman, Reuven Reich

Matrix Receptors

Since laminin has at least four distinct active sites, it is expected that multiple cell surface receptors will interact with these sites. One receptor ($M_r=67,000$) has been cloned and sequenced. The clone coded for a much smaller protein $M_r=32,000$, than the expected $M_r=67,000$. Antibody raised to fusion proteins recognizes both molecular forms, but antibody to a synthetic peptide from the N-terminus reacts only with a protein of 32kD, suggesting that the two proteins share a common epitope. These antibodies localize to the cell surface in vivo, and in vitro. These antibodies also block laminin-mediated cell attachment. No effect on fibronectin-mediated cell attachment or laminin-mediated neurite outgrowth is observed. Using laminin affinity columns, and peptide binding assays (Western blots), it appears that the $M_r=67,000$ form recognizes the YIGSR sequence. Hepatocytes contain both the $M_r=32,000$ and $M_r=67,000$ forms and these increase when hepatocytes are placed in cell culture.

Tomeu Segui-Real, Bruno Clement, Yoshi Yamada

The basement membrane heparan sulfate proteoglycan has a large ($M_r=400,000$) core protein containing a domain whose sequences are 40% homologous to the short arm of the laminin A chain. A variety of cells attach well to this proteoglycan core and specific cellular receptors have been identified using affinity (core protein) chromatography. Bound protein ($M_r=38,000$) was eluted and found to be specific. Such data suggest that cells also recognized the proteoglycans by interacting with a specific cell surface receptor in the extracellular matrix.

Bruno Clement, Tomeu Segui-Real, Yoshi Yamada

Using peptide affinity columns, chemical crosslinking, and peptide (Western) blotting, receptors involved in the cellular response to the A chain sequence IKVAV are being identified. Both radiolabeled and unlabeled cultured cells as well as tissue extracts, are being examined. Studies are carried out with tumor cells, neuronal cells, and brain and kidney tissue. Such studies show $M_r=55,000$ and $M_r=110,000$ proteins are potential receptors. Antibody preparation and microsequencing are currently in progress to further characterize these receptors. Antiidiotype antibodies to the synthetic peptide IKVAV are also being prepared.

Hynda Kleinman, Beth Burrous, Norio Shiraishi, Benjamin Weeks, Yoshi Yamada



Studies on Blood Vessel Formation

Endothelial cells, both microvascular as well as large vessel, undergo differentiation slowly in culture under most conditions. When endothelial cells are cultured on Matrigel, a solid gel of basement membrane proteins, they rapidly align and form hollow tube-like structures. Using a quantitative in vitro assay, tube formation appears to be a multi-step process induced by laminin (a major basement membrane component). An RGD containing sequence in the A chain of laminin through an integrin receptor on the endothelial cell induces their attachment to the matrix while a YIGSR site in the B1 chain induces cell-cell interactions and the resulting tube formation. We also show that the laminin-derived synthetic peptide YIGSR contains sufficient information to induce single endothelial cells to form ring-like capillaries. In related studies, the YIGSR peptide blocks new blood vessel formation in the chick chorioallantoic membrane and in the rabbit eye when placed in slow release pellets. A third site at the end of the A chain on the laminin molecule containing the amino acid sequence, SIKVAV, was found to bind strongly to endothelial cells and potentiated an invasive/degradative behavior involving the production of type IV collagenase. The process of differentiation on Matrigel requires many intracellular changes involving de novo protein synthesis (particularly on collagen IV), the redistribution of laminin receptors on the cell surface, and cytoskeletal changes.

Derrick Grant, Hynda Kleinman, George Martin

Studies on Sertoli Cells

Primary cultures of Sertoli cells and cultures of TM4 cells, a Sertoli cell line, are being grown on various extracellular matrix molecules, including synthetic peptides to the laminin molecule to study the effects on gene expression, protein secretion, and signal transduction. We have recently shown that YIGSR may be important for tubule formation during early Sertoli cell differentiation. Furthermore, Sertoli cells appear to differentiate when grown on the laminin A chain IKVAV containing peptide, as compared to cells grown on plastic. Using ligand blots, the binding proteins to both YIGSR and IKVAV are present in Sertoli cells from young rats. We are currently carrying out a developmental study to determine whether there are any age-related changes in the binding proteins.

Martin Dym, Benjamin Weeks, Hynda Kleinman

Intracellular Events in Neuronal Cells Exposed to Laminin

Laminin has potent effects on neuronal cells. Within 1-4 hours, cells rapidly attach and extend long processes. Little is known about the intracellular events involved in the cellular response. The role of phosphorylation-dephosphorylation events in the transduction of the laminin signal is being investigated by following free phosphate in ^{32}P , as well as by the use of drugs which either stimulate or inhibit kinases and phosphatases.



Current results suggest that laminin alters the phosphorylated state of a specific protein in neuronal cells. The possibility that this protein is a cytoskeletal protein or a laminin receptor is being investigated. In the future, the amino acid residue being affected will also be determined. Also, kinase inhibitors (K252A) and stimulators (TPA), as well as phosphatase inhibitors (okadaic acid, vanadate, and isoproterenol) have been observed to inhibit the laminin signal. In the future, GTPase activity will also be measured in response to laminin. Thus, our data suggest that intracellular phosphorylation events are involved in mediating the laminin signal. A subtraction study will be performed using cDNA libraries prepared from laminin stimulated and non stimulated neuronal cells to further characterize new genes that are regulated by laminin.

Benjamin Weeks, Hynda Kleinman

Alzheimer's Disease

Alzheimer's disease involves degeneration of the central nervous system. The possible involvement of the extracellular matrix in this process is currently being studied in our laboratory, in collaboration with Dr. Smita Kitur from the National Institute on Aging. The effect of cerebral spinal fluid, brain tissue homogenate and sera from Alzheimer's patients on laminin-induced neurite outgrowth in cell cultures is being investigated. Preliminary studies demonstrate that there is laminin-like activity in the Alzheimer's tissue homogenates. Immunofluorescence and Western blot studies confirm that the Alzheimer's tissue is enriched in laminin deposits. Northern blot analysis of RNA from these tissues will also be studied.

Benjamin Weeks, Hynda Kleinman, George Martin

Expression of mRNA for Basement Membrane Components and a Laminin Receptor

The appearance of basement membrane molecules and their receptors is a key event in the differentiation of cells of the kidney. Steady state mRNA levels for the laminin A, B1 and B2 chains, the $\alpha 1$ (IV) collagen chain and a laminin receptor (LBP32) were examined in mouse kidneys at 16 days' gestation and birth, when cell differentiation is active, and at 1-3 weeks after birth when this activity has subsided. Both northern analysis and in situ hybridization revealed that mRNA expression of laminin receptor precedes the $\alpha 1$ (IV) and laminin B chains whereas laminin A chain mRNA was low. At 16 days' gestation, laminin receptor mRNA was elevated in cells of newly forming glomeruli and in the proximal and distal tubules of the nephrogenic zone located in the kidney cortex. These cells also expressed mRNA for $\alpha 1$ (IV) and laminin chains. At birth, mRNA expression of the receptor and all chains remained high in glomeruli but was reduced in proximal and distal tubules. At 1 week after the birth, expression was located in the medulla over collecting ducts and loops of Henle. Little



expression was detectable by 3 weeks. These results suggest that cellular expression of mRNA for these proteins is temporally linked, with the laminin receptor proceeding first and thereafter subsiding.

We have also studied mRNA levels for these proteins in developing brain since the developing brain has been reported to contain subtle patches of laminin which is the most potent extracellular matrix protein active for neurite outgrowth. Elevated mRNA levels of laminin receptor were found at and before birth when neurons are actively migrating from the proliferative ventricular zone to layers in the cerebral cortex. In situ hybridization localized the newborn brain expression to the ventricular zone and cerebral cortex. A similar pattern was found for laminin B2 chain whereas laminin A and B1 chains and collagen IV mRNAs were very low. The results suggest that neuronal migration and sorting may be a laminin receptor-mediated phenomenon.

Gordon Laurie, Norio Shiraishi, Satoshi Horikoshi, Yoshi Yamada.

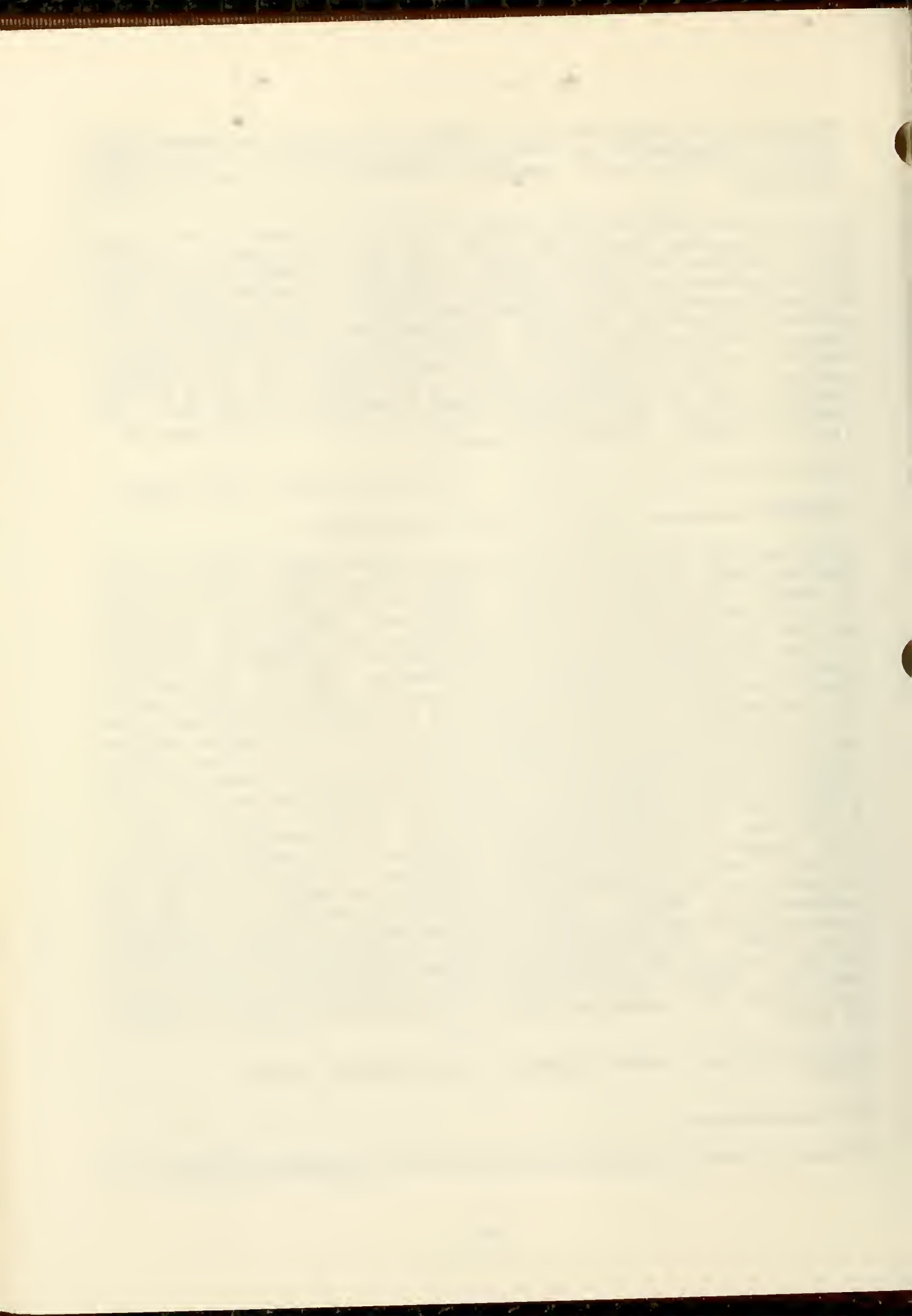
Basement Membrane Heparan Sulfate Proteoglycan

Previously we isolated and sequenced partial cDNA clones for the basement membrane proteoglycan. These clones hybridized to a mRNA of about 13kb which is enough to code for the 400kD core protein. We have now isolated several additional cDNAs which together account for about 80% of the proteoglycan core sequence including the most N-terminal portion of the molecule. The deduced sequences predict a protein with three large domains (Fig 1). The most 5' domain consists of cysteine repeats very similar to those found in the LDL receptor. The next domain is homologous to the short arm of the laminin A chain. These consist of three globular regions separated by three cysteine-rich regions. All of the laminin chains contain similar cysteine-rich repeats as those in this domain of the proteoglycan. The globular regions are also most similar to the analogous regions in the laminin A chain. The 3' domain has 10 repeats which are similar to repeats in the neural cell adhesion molecule, N-CAM. There are 14 of these repeats; 10 at the 3' end of the contiguous sequence, 3 in a non-overlapping sequence and 1 separating the laminin-like domain from the LDL receptor-like domain. This domain also contains a conserved sequence which may represent a potential heparan sulfate attachment site, DSGEY. The presence of similar domains in the proteoglycan and in laminin could indicate common functions including cell adhesion. Indeed, we have found that the proteoglycan core protein can promote cell adhesion through a specific cell surface receptor of 38kD.

Douglas Noonan, Bruno Clement, Beth Horigan, Yoshi Yamada

Nidogen/Entactin

Nidogen is one of the ubiquitous proteins in basement membrane and



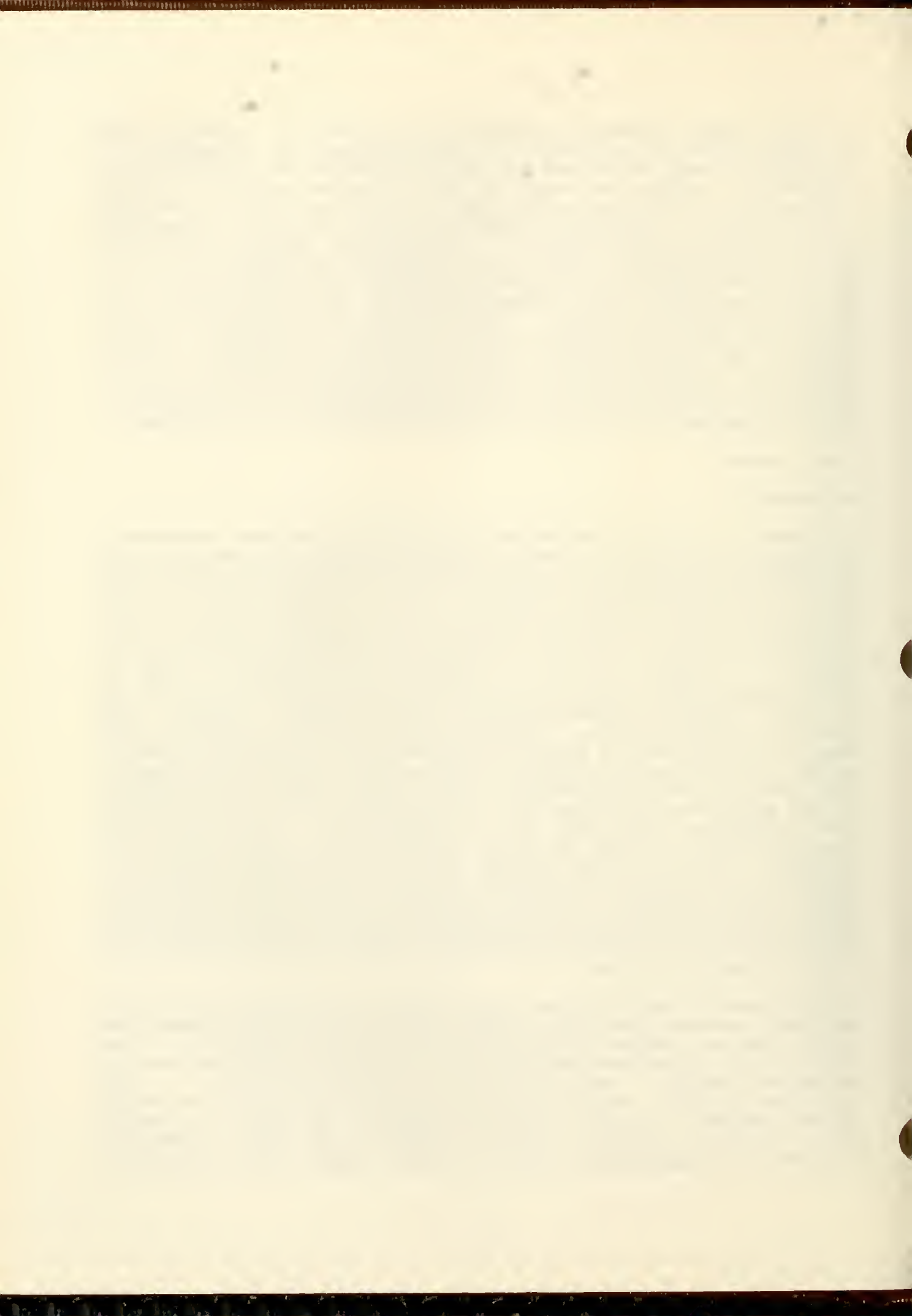
is often found in close association with laminin. Nidogen binds to laminin and to collagen IV, and promotes cell adhesion. A complete primary structure has been determined by cDNA cloning. Originally, nidogen and entactin were isolated separately by two laboratories. DNA sequencing made it clear that the two proteins were identical. The protein consists of some 1217 amino acids plus a 28 amino acid-signal peptide. The sequence analysis supports a previously proposed dumb-bell like structure of nidogen (Fig. 2). The N- and C- terminus consists of a globule-like structure. There are five EGF-like repeats constituting the rod-like domain and a smaller C- terminus globule. Two more EGF-like repeats interrupt the N-terminal and terminate the C- terminal sequences. Nidogen contains two consensus sequences for tyrosine sulfation and for asparagine β -hydroxylation, two N-linked carbohydrate acceptor sites and, within one of the EGF-like repeats an RGD sequence. This RGD sequence was shown to be functional for cell attachment.

Yoshi Yamada

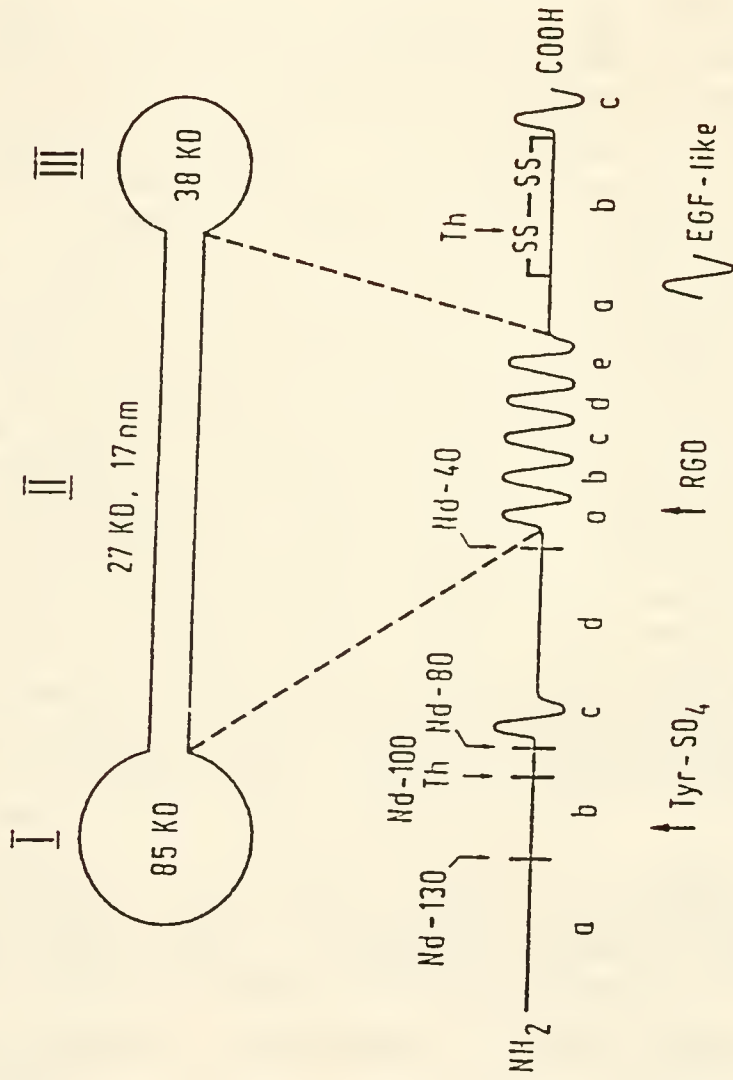
Collagen IV

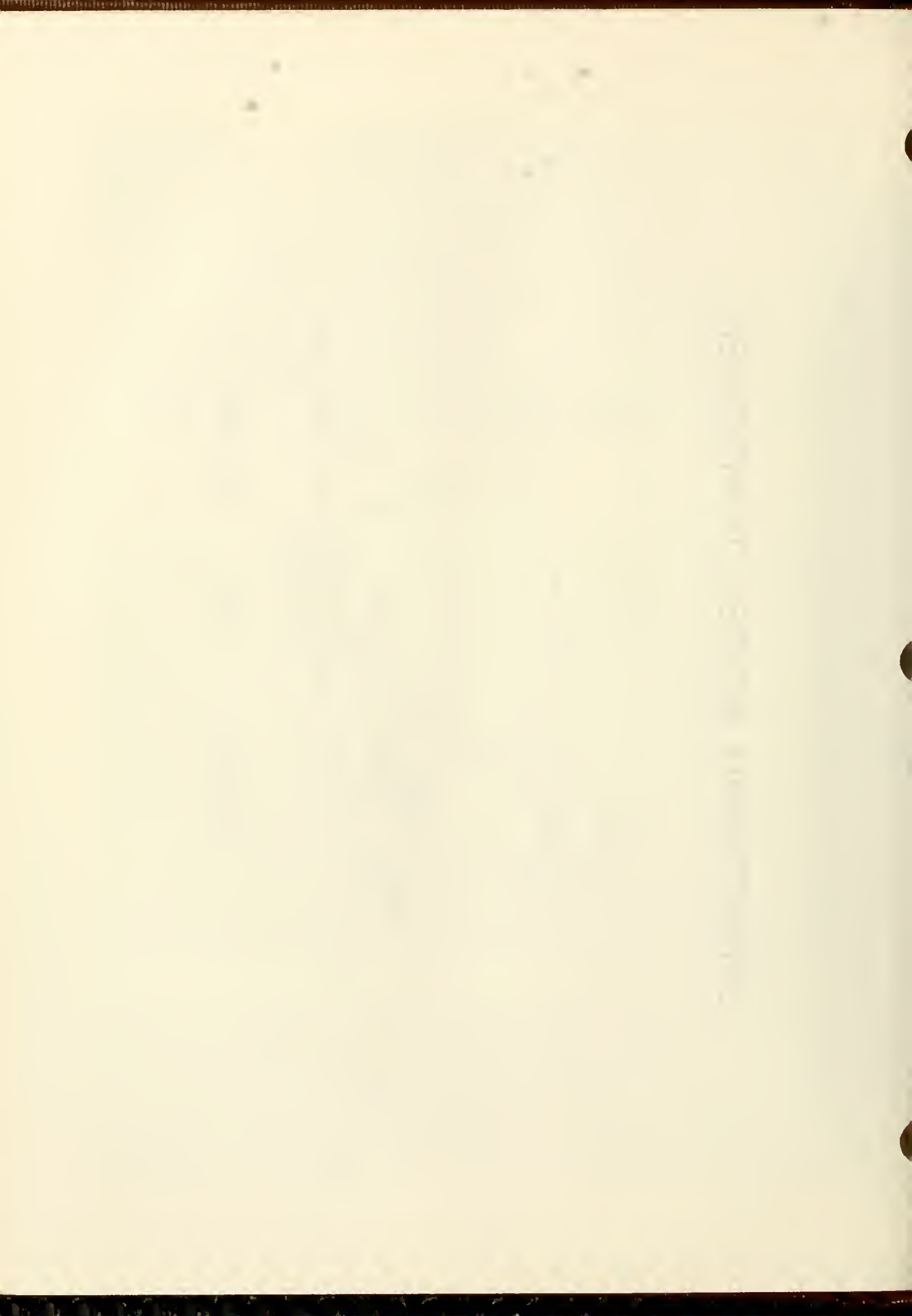
Collagen IV, the major structural component of basement membranes, is composed of two $\alpha 1$ (IV) and one $\alpha 2$ (IV) chains. The genes for the two chains are mapped in a head to head arrangement, separated by 130 bp in both the murine and human. We have also shown that both genes share bidirectional promoters and a common enhancer within the first intron of the $\alpha 1$ (IV) gene. The enhancer functions in a type-specific manner except in undifferentiated F9 teratocarcinoma cells. In spite of the low levels of collagen IV mRNA in undifferentiated F9 cells, transient transfection experiments using an $\alpha 1$ (IV) collagen promoter-enhanced-CAT construct showed a high level of transcriptional activity in these cells. We find that when this construct is stably integrated into undifferentiated F9 cells, CAT expression does not occur unless the cells are treated with retinoic acid and dibutyryl cAMP which induce the synthesis of the endogenous collagen IV. Treatment of the cells containing the integrated construct with 5-azacytidine, an agent which demethylates DNA, also activates transcription of the CAT gene. Analysis of DNA isolated from F9 cells revealed that there was a specific demethylation of the DNA at the 5' and of the collagen IV genes after treatment with both retinoic acid and dibutyryl cAMP. These results suggest that collagen IV genes are regulated not only by the enhancer but also by methylation and chromatin structure in certain types of cells.

The collagen IV enhancer has been further characterized. Deletion analysis revealed that the active sequence for the enhancer was within 200 bp located 2.5kb downstream from the first exon of the $\alpha 1$ (IV) gene. Sequence analysis of this segment revealed several conserved motifs. Gel retardation assays and DNAase I foot printing analysis indicated two regions of 14 and 15 bp which interacted with nuclear factors prepared from EHS tumor cells. Those regions contains sequences similar to cAMP responsive sequences and drosophila homeotic protein "eve" binding sequence.



STRUCTURAL MODEL OF NIDOGEN





UV- crosslinking and South-Western blotting indicated two proteins of 40kD and 60kD interacting with the eve containing sequence. It appears that multiple DNA elements and several nuclear factors must be involved in the regulation of the collagen gene.

Peter Burbelo, Leslie Bruggeman, Paul Klotman, Satoshi Horikoshi, Makoto Sawada, Yoshi Yamada

Laminin

Expression of exogenous genes for the laminin chains has also been attempted to address questions on whether the individual chains have biological activity and how the chains are assembled and secreted. A full length B1 chain cDNA was assembled by recombining overlapping partial clones and placed under the control of the Baculovirus polyhedrin promoter. A recombinant Baculovirus containing the B1 cDNA sequence was obtained by in vivo recombination and subsequently infected into insect cells. A relatively large amount of the B1 chain (about 1 mg/l culture) was produced in these infected cells. Although a signal peptide was present in the construct, the majority of the protein remained intracellular. The B1 chain was purified by an antibody affinity column and its biological activities were examined. The B1 chain was glycosylated and had activity in promoting B16-F10 melanoma cell adhesion. It bound to heparin and to collagen IV but did not promote neurite outgrowth. EM-rotary shadowing showed a globular structure suggesting that the α -helical coiled-coil structure can be formed only in the presence of other chains. Expression of the B2 chain and the biologically active segments of the A chain has also been explored in the Baculovirus expression vector system. Various derivatives of human small cell lung carcinoma were examined and found to have different levels of the laminin chains. We have begun to examine if different biological properties of the variant cells are due to the different expression levels of the laminin chains. In this study, we have prepared recombinant retroviruses containing each laminin chain and introduced them into various lines of small cell lung carcinoma cells.

Rat astrocytes and glioma cells produce only the B2 chain. We have constructed a variety of the B2 promoter CAT plasmids and transfected these into cells to identify genetic mechanisms determining tissue-specific expression of the gene.

Kohei Ogawa, Ken Tashiro, Greg Sephel, Rafi Fridman, Hynda Kleinman, Yoshi Yamada

Diabetes Mellitus

Diabetic renal disease and hypertension continue to be the major causes of mortality in the diabetic patient population. One recent therapeutic approach has been the restriction of dietary protein largely based on animal models of diabetes. In the diabetic rat, we have demonstrated that high protein feeding accelerates the development of glomerulosclerosis and markedly alters arachidonic



acid metabolism. Despite the presence of prominent glomerular hyperfiltration, kidneys from diabetic rats fed a high protein diet (60% casein) produce more thromboxane A₂, an eicosanoid with potent vasoconstrictor properties that also induces platelet aggregation. In contrast, PGE₂ and prostacyclin are not increased. These data suggest that metabolic changes induced by high protein feeding may contribute to the appearance of glomerulosclerosis by mechanisms unrelated to either vasoconstriction or platelet aggregation. In order to test the hypothesis that thromboxane A₂ stimulates matrix protein production directly, we have evaluated the response of differentiated mouse teratocarcinoma cells (F9+) and human and rat renal mesangial cells to the stable thromboxane A₂ analog U44619. U44619 stimulates increased synthesis of type IV collagen and laminin, and increased production of mRNA for these genes. Studies are currently underway to determine whether this effect is due to an increase in transcription or stabilization of message. In addition, studies are in progress to determine the effects of glucose, glucagon, and insulin on expression of basement membrane genes in glomerular mesangial cells and glomerular endothelial cells. Finally, a line of transgenic mice has been created using the reporter gene chloramphenicol acetyl transferase under the control of the promoter and enhancer of the α 1 chain of type IV collagen gene. Diabetes will be induced in these animals using streptozocin and low level changes in expression of type IV collagen will be assessed by CAT assays and correlated with renal function and protein excretion.

Leslie Bruggeman, Satoshi Horikoshi, Peter Burbelo, Yoshi Yamada, Deirdre Collins, Derrick Grant, Paul Klotman

Hypertension

Hypertension remains a major risk factor for cardiovascular diseases including myocardial infarction, stroke, and kidney disease. In a genetic model of essential hypertension, the spontaneously hypertensive rat, we have found that isolated renal tubules have increased oxidative metabolism which is not due to Na⁺ transport. Instead, this increase in cellular respiration appears to be related to changes in phospholipid metabolism. Similarly, in a model of renovascular hypertension, we have demonstrated that increased thromboxane A₂ production by the contralateral kidney is associated with impaired renal functional hypertrophy and mediates, in part, the increase in systemic blood pressure. Chronic elevations in blood pressure lead to vascular damage particularly in the renal vessels and glomeruli. Studies are currently underway exploring the importance of vasoactive peptides and alterations in Na⁺ and K⁺ balance that may have a profound effect on collagen IV production by endothelial and mesangial cells.

Satoshi Horikoshi, Elizabeth Horigan, Paul Klotman

Renal Growth and Hypertrophy

Following unilateral nephrectomy, the single remaining kidney



increases in size and renal function returns to approximately 80% of the value prior to nephrectomy. However, in certain disease states such as renovascular hypertension, cyclosporine treatment after transplantation, and unilateral obstruction, renal hypertrophy does not occur normally. This failure of the remaining kidney to respond to a decline in function contributes to the pathogenesis of these diverse conditions. We have demonstrated that following birth, the steady state mRNA levels of collagen IV and laminin progressively decline in normal animals. However, following nephrectomy, mRNAs of collagen $\alpha 1(IV)$ and laminin B2 increase in the remaining kidney from 2-6 hours following the removal of the contralateral kidney. We are currently isolating nuclear factors from kidneys following contralateral nephrectomy which may be transcriptional regulators for type IV collagen and laminin genes.

Satoshi Horikoshi, Elizabeth Horigan, Yoshi Yamada, Paul Klotman

Polycystic Kidney Disease

Adult polycystic kidney disease is characterized by markedly increased and disordered growth of cysts from all tubular segments of the nephron. The signals for disordered growth are unknown, although a decrease in epidermal growth factor production by renal epithelial cells has been suggested as one possible explanation. We have now demonstrated that mRNA for EGF is decreased in the kidneys of cystic animals. However, growth factor activity is increased in the cyst fluid. This growth factor is inhibited by anti-EGF antibody but does not have the same molecular weight as EGF by Western analysis. Studies are currently in progress to better characterize and purify this growth factor.

Satoshi Horikoshi, Elizabeth Horigan, Yoshi Yamada, Paul Klotman

Inflammatory Mediators and Cytokines: Basement Membrane Gene Expression

The inflammatory response and the expression of basement membrane genes appears to be closely linked. A number of renal diseases such as lupus nephritis, allograft rejection, and immune complex glomerulonephritis are characterized by mononuclear cell infiltration, mesangial expansion, and glomerulosclerosis and fibrosis. Similar responses occur in inflammatory diseases of the lung (interstitial pneumonitis), the liver (primary biliary cirrhosis), the skin (scleroderma), and the salivary glands (Sjogrens syndrome). In all these diseases, a number of cytokines have been incriminated. For example, we have demonstrated increased production of thromboxane A2 in rejecting renal allografts, cyclosporine toxic kidneys, and kidneys from lupus mice. Furthermore, treatment with thromboxane receptor antagonists improves renal function in acutely rejecting renal allografts and cyclosporine nephrotoxicity, and appears to reduce glomerular basement membrane thickening in a rat model of chronic allograft rejection. In a study in patients following renal transplantation



and treatment with cyclosporine, acute administration of a thromboxane synthetase inhibitor, CGS 13080, increased renal blood flow and glomerular filtration rate. Based on these findings, we are currently investigating the role of these cytokines and mediators in initiating transcription of extracellular matrix proteins in a number of inflammatory disease models.

Satoshi Horikoshi, Leslie Bruggeman, Elizabeth Horigan, Ben Weeks, Yoshi Yamada, Paul Klotman

Wound Healing

Defects in wound healing are common in diabetics, patients with collagen vascular diseases, and the elderly. We have described two models of impaired wound healing in the guinea pig. In the first model, topical administration of calcium to an epidermal wound inhibits repair by stimulating increased collagenase production. In the second model, the addition of Suramin, a platelet derived growth factor (PDGF) antagonist, to a dermal incision, impairs wound healing by a different mechanism. In this model, collagenase activity is decreased and the expression of collagen I mRNA is inhibited. These data document the importance of collagenase and matrix gene expression during normal wound repair. We have applied these findings to small patient samples using polymerase chain reaction. In patients with keloids or hypertrophic wounds, mRNA for collagen I was markedly increased and collagen I was markedly decreased, suggesting that overexpression of collagen I and/or reduced production of collagenase contributes to the pathogenesis of these conditions.

Anthony Sank, Thomas Shima, Maria Chi, George Martin

Human Immunodeficiency Virus 1 (HIV 1)

Transactivation of matrix proteins and their receptors by human immunodeficiency virus (HIV) 1 may be responsible for some of the clinical manifestations of AIDS. Since the HIV-1 gene product Tat is such a potent transactivator and since Tat transgenic mice develop cutaneous tumors, we hypothesized that Tat or cellular factors induced by Tat might induce transcription of receptors for the matrix proteins. We have transfected a construct with a strong heterologous promoter (CMV) linked to Tat into human T lymphocytes (H9, CD4+). These lymphocytes normally grow in suspension. When H9 cells are transfected with Tat and plated on laminin, they develop marked cytoplasmic outgrowths and demonstrate increased cellular attachment when compared with H9 cells transfected with beta actin - CAT. We have now exposed these cells to Tat-containing media and have observed similar effects. These data suggest that Tat may transactivate the laminin receptor on CD4+T helper lymphocytes and, as a result, may markedly influence cellular immune responses and localization. Studies are currently in progress to evaluate whether laminin receptors are increased by



Tat and, if so, the functional implications of changes in laminin receptor number on immune function.

Jay Rappaport, Ben Weeks, Paul Klotman

Recent data have also suggested increasing importance for the renal disease associated with HIV-1 infection. For example, monkeys with SIV have been shown to have a form of renal failure of unknown etiology and mice transgenic for Tat, Rev, and Nef have a very active glomerulonephritis. We have transfected human mesangial cells (which are CD4+) with the CMV promoter - Tat construct and have evaluated type IV collagen production. Tat decreases type IV collagen production as assessed by immunoprecipitation. Cotransfection of CMV Tat with a construct containing the promoter and enhancer elements of type IV linked to CAT, decreases CAT activity when compared to non-Tat cotransfections. These data suggest that Tat decreases type IV collagen, in part, at the level of transcription. This alteration in mesangial type IV collagen production may contribute to the clinical manifestation of the AIDS-associated renal disease. We are presently examining whether this effect also occurs in other cells that are known to synthesize type IV collagen and whether other viruses trophic for the kidney (i.e. cytomegalovirus) have similar effects.

Jay Rappaport, Ben Weeks, Paul Klotman

Kaposi's Sarcoma is a highly vascularized tumor generally localized to skin and mucous membranes. Cells from Kaposi's appear to be mesenchymal in nature and are as invasive through basement membrane as are other malignant tumors. In addition, Kaposi's cells appear to secrete a factor that induces endothelial cell migration, possibly contributing to its vascularity. Fibroblast growth factor (FGF) also induces a similar response by endothelial cells. An oncogene with homology to FGF has been isolated from Kaposi's lesions and we have found that cells transfected with this oncogene also produce a potent angiogenic factor, although the cells are not invasive themselves. More recently, we have studied cells from transgenic mice developed by Gilbert Jay which express Tat. These animals develop skin lesions that are similar to Kaposi's sarcoma. Cells from these lesions also secrete an angiogenic factor,

suggesting that Tat transactivates FGF-like genes in transformed cells, thus inducing the Kaposi's lesion.

Eric Thompson, Ben Weeks, Thomas Shima, George Martin, S. Nakamura, Z. Salahuddin, Gilbert Jay

Cytomegalovirus

Cytomegalovirus (CMV) infection is often latent in the host but when activated, it produces serious morbidity. In the fetus, CMV produces often fatal cytomegalic inclusion disease. In young adults, CMV infection is associated with a form of acute mononucleosis, and in the immunocompromised host, CMV produces



severe life-threatening illness including retinitis and gastroenteritis in AIDS patients, and pneumonitis in transplant recipients. Using the polymerase chain reaction in a mouse model of latency, we have demonstrated that salivary glands, kidney, and spleen can harbor latent virus. Current studies are underway to develop an in vitro model of latency in renal epithelial and salivary gland cells in culture in order to study the molecular mechanisms of latency and the transcriptional factors responsible for reactivation. Of particular importance are the early genes of CMV which are responsible for transactivation of CMV as well as HIV-1. We will infect a variety of cell lines including lymphocytes and mesangial cells with the immediate early genes of CMV in order to evaluate viral and cell associated nuclear proteins that may bind to the promoters of these viruses and initiate transcription. In this way, we hope to identify a common pathway for activation that might be responsive to therapeutic inhibition as a strategy for decreasing the activation of AIDS or CMV in the host.

Mary Klotman, Leslie Bruggeman, Paul Klotman

Malignant Tumors Require Arachidonic Acid Metabolites for Invasion and Metastases

The metastatic potential of tumor cells is associated with a proteolytic ability to degrade basement membrane. This proteolytic activity can be modulated at the level of enzyme activity or at the level of enzyme production. We have found that blockage of this activity at either level reduces both invasion in vitro and metastases in vivo. A specific inhibitor of collagenase IV developed by G.D. Searle blocks the malignant phenotype and prevents tumor formation in vivo.

Intracellular interference with the metabolism of arachidonic acid modulated the secretion of collagenase IV. Exposure of tumor cells to a series of highly specific inhibitors of either cyclooxygenase or lipoxygenase resulted in loss of the invasive potential in vitro, reduced collagenase IV production, and reduced the number of metastatic lesions in vivo. Further, the regulation seems to be at the mRNA level as well. The observed inhibition was found to be reversible since the addition of specific metabolites to inhibitor-treated cells restored the invasive potential and the secretion of collagenase IV. Substitution of arachidonic acid metabolites with EPA-derived metabolites restored activity only partially, indicating the importance of arachidonic acid metabolites in the regulation of collagenolytic activity. Pretreatment of tumor cells with eicosapentanoic acid (EPA) (1-10 μ M) for several weeks reduced the invasive potential and the collagenase IV secretion. In vivo studies with EPA-treated cells show a similar reduction in tumor formation. Since EPA is found in certain fish oils, we are also testing the effects of high fish oil diets on tumor metastases.

Reuven Reich, Leah Royce, George Martin, Hynda Kleinman



Studies on Small Cell Lung Carcinoma (SCLC)

Small cell lung carcinoma is a highly metastatic tumor characterized by its poor prognosis and resistance to chemotherapy. SCLC cell lines grow in vitro as floating aggregates and are classified as variant and classic types. We have found that some SCLC cell lines attach and migrate in response to laminin. Adhesion of SCLC cells to laminin induces morphological changes and confers an enhanced resistance to cytotoxic drugs. Subcutaneous injection into nude mice of SCLC cells mixed with a reconstituted basement membrane (Matrigel) rich in laminin results in tumor formation, even at very low tumor cell inocula. The presence of a laminin-derived synthetic peptide, YIGSR, known to be involved in tumor cell adhesion in the mixture of cells and Matrigel inhibits tumor growth. Analysis of laminin production by SCLC cells showed that the SCLC tumor lines differ in their capability to synthesize the three chains of laminin. Whereas a variant type of SCLC cells produces both B1 and B2 chains, classic SCLC cells synthesize only B2, as determined by protein and mRNA analysis. To investigate the effects of laminin in SCLC cells, experiments are now in progress to express exogenous laminin genes in SCLC cells using a retrovirus vector. The goal of these studies is to determine whether over-expression of laminin results in phenotypic changes and/or an enhancement in tumorigenicity.

Rafael Fridman, Reuven Reich, Tamoko Kanemoto, George Martin

Studies on Ovarian Carcinoma

Ovarian carcinoma grows in the peritoneal cavity and usually responds to chemotherapy but remission generally lasts no more than 5 years. We are using a cell line (NIH:OVCAR-3), isolated from a patient with an adenocarcinoma of the ovary, which has been shown to form ascitic tumors in nude mice when injected intraperitoneally. We have tested the effect of a novel 5-lipoxygenase inhibitor (SC-44661) developed by G.D. Searle in this model of ovarian cancer. Lipoxygenase is a major enzyme involved in the metabolism of arachidonic acid and inhibitors of this enzyme block collagenase IV production. We have found that intraperitoneal administration of SC-44661 to nude mice inoculated with OVCAR-3 cells resulted in a drastic inhibition of ascites formation and a significant increase in median survival when the drug was administered daily. The inhibitor appeared not to have toxic effects on the mice and after cessation of the treatment the tumors did not reoccur. In vitro invasion assays using reconstituted basement membrane showed that the presence of SC-44661 inhibited the invasiveness of OVCAR-3 cells.

Rafael Fridman, Tamoko Kanemoto, George Martin, Reuven Reich

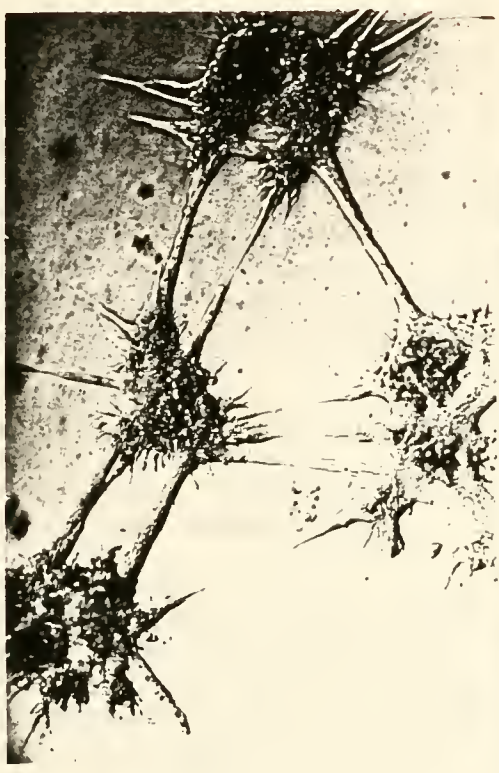
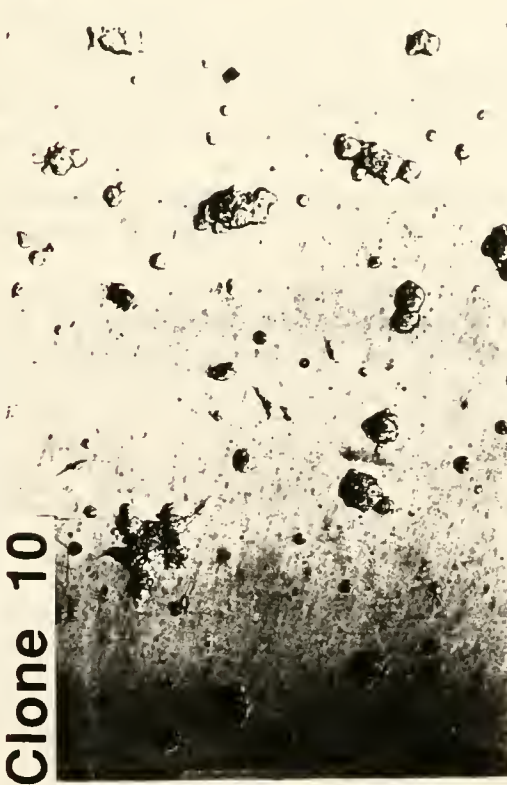


Non-Malignant Cells on Matrigel

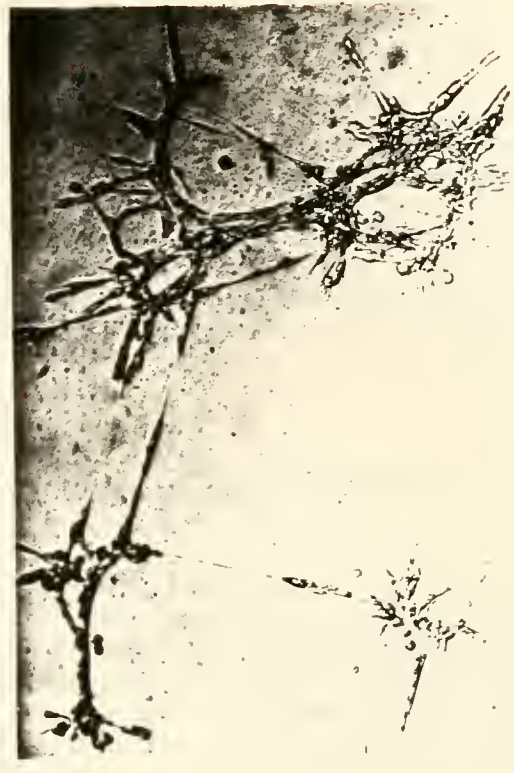
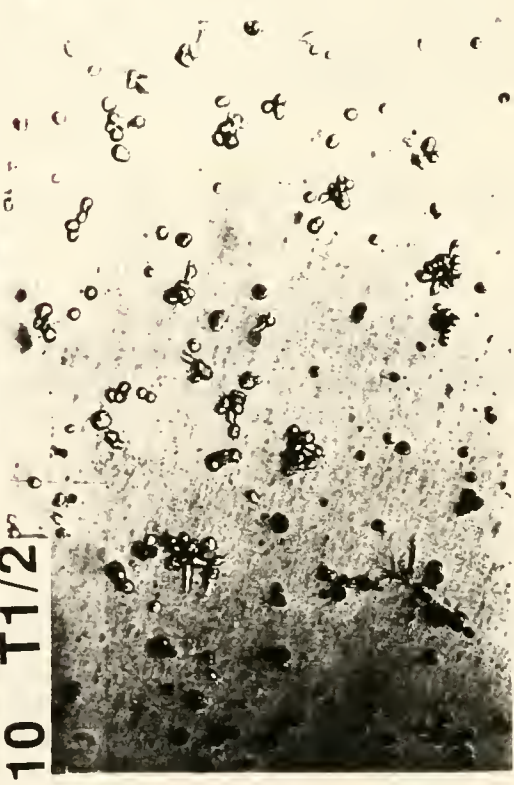
plus laminin A chain
peptide pa22-2

no additions

Clone 10



10 T1/2



18

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1850-1851

Metastases

It is well documented that metastases are formed from a primary tumor. Since the metastatic lesions are much more highly malignant than the parent tumor, we tested whether the secondary lesions were more likely to give rise to tertiary metastases or if the tertiary metastases arise from the primary tumor. Using a model involving tumor cell injection directly into the blood stream, we demonstrate that metastatic lesions do give rise to metastases. We find that the inhibitor of collagenase IV activity (when administered early) reduces the initial number of tumors but has no effect on the number of subsequent metastases that form. In related studies, we are also looking at the development of metastases in young and aged animals.

Leah Royce, Reuven Reich, George Martin

Studies on Laminin Peptide Active in Promoting Collagenase IV Activity and Tumor Metastases

The basement membrane glycoprotein laminin promotes tumor cell adhesion, growth, and metastases, as well as increased production of type IV collagenase. This protein has been cloned and sequenced at LDBA and active synthetic peptides have been identified. One peptide, PA22-2, from the A chain was found to increase the number (5-fold) of lung colonies in mice injected with B16F10 cells. This peptide in vitro also was found to increase collagenase IV activity to the same extent as laminin. In related studies with non-malignant cells, an increase in invasive behavior is observed in vitro when cultured with the peptide (Fig. 3). The expression of collagenase IV and oncogenes in response to this peptide in both benign and malignant cells is being tested.

Hynda Kleinman, Leah Royce, Tamoko Kanemoto, Shun Kubota, Yoshi Yamada, George Martin

TGF β (transforming growth factor) has remarkable effects on the growth and gene expression of many cells. We have found that TGF β reduces the invasive behavior of tumor cells in vitro. Collagenase IV activity and cell migration are blocked and B16F10 melanoma cell colonization of lungs is also reduced.

Shun Kubota, Yoshi Yamada

Collagen II Gene

We have studied the molecular mechanisms by which cartilage genes are regulated and expressed during development. Protein function has also been addressed by expressing cartilage genes in appropriate cells. We have explored the molecular basis of acquired and genetic human diseases associated with cartilage.

We previously showed that the enhancer within the first intron of the collagen II gene was required for a high level of transcription



of the gene in chondrocytes. The enhancer activity was localized to a 500 bp sequence. Gel retardation and DNase foot printing analysis revealed several cis-acting elements within the enhancer. Deletion analysis indicated a DNA sequence of about 30 bp in the promoter region was required for the high level of the enhancer mediated transcription. Gel retardation analysis suggested a common nuclear factor(s) was involved in interacting with both the promoter element and the enhancer. In addition to these positive regulatory elements, two negative regulatory elements (silencers) have been identified in the promoter region. These elements suppressed the expression of the collagen II gene in fibroblasts. However, the enhancer over-rode the silencer activity in chondrocytes. These results suggest that the levels of collagen II are determined by both positive and negative regulatory elements.

Pierre Savagner, Michael Chirigos, Yoshi Yamada

Cartilage Chondroitin Sulfate Proteoglycan (Aggrecan)

Large aggregating chondroitin sulfate proteoglycan (named aggrecan) is a major proteoglycan in cartilage. Aggrecan contains numerous glycosaminoglycan chains on the core protein and possesses affinity for hyaluronic acid. We previously isolated and sequenced full length cDNAs from the rat. Because of the potential applications to human diseases, we have used the rat cDNAs to isolate the corresponding human cDNA and gene. Most of the sequencing of these cDNA are now completed. Two notable differences were observed between the rat and human sequences. The human aggrecan is missing an internal portion of the c-terminal domain, just after the 3' end of the lectin homology. The shortened form of the sequence is a result of exon skipping. The other major difference is found in the amino-terminal half of the ser-gly repeat domain in which the human sequence shows a highly repetitious segment not present in the rat. This region in the human consists of a 19 residue sequence, making the ser-gly domain of human aggrecan some 240 residues longer than that of the rat.

The rat gene for aggrecan was isolated and characterized. The gene is about 100 kb in size and contains 15 exons. There is a striking correspondence between structural domain and exon, with the exception of the G3 domain.

The amino terminal, two globular domains (Domain 1 and Domain 3), of aggrecan have been proposed to bind hyaluronic acid. We have carried out functional analysis to determine the hyaluronic acid binding site of aggrecan by expressing a series of the corresponding segment of cDNA in bacteria. These studies suggest that subdomains, domain 1B and 1B', are essential for the binding of the first globule of aggrecan to hyaluronic acid.

Kurt Doege, Michelle Marks, Yoshi Yamada



Link Protein

Link protein binds hyaluronic acid and has affinity for the hyaluronic-acid binding region of aggrecan. Link proteins and aggrecan were known to share some structural features along with complementary binding activities, and it was of interest to compare them structurally, and with regard to gene regulation, since they share tissue and developmental specificity of expression. The rat link protein gene has been isolated and characterized. About a 2 kb segment of the 5' flanking DNA of the gene has been sequenced and its promoter activity has been examined by fusing it to the CAT gene and transfecting it into chondrocytes. The promoter activity of the construct was increased by 3-4 fold when a segment of the first intron was included, suggesting the presence of an enhancer in the first intron. Both cDNA and the gene for the human link protein have also been isolated and characterized. Comparison of the rat and human promoter region indicates the high degree of conservation.

Craig Rhodes, Pierre Savagner, Michael Chirigos, Yoshi Yamada

Human Cartilage Diseases

We have undertaken the molecular analysis of a wide variety of human diseases associated with cartilage. These include hereditary disorders such as the skeletal dysplasias as well as numerous acquired disorders, including arthritis. Patients with spondyloepiphyseal dysplasia congenital (SED), are characterized by short stature with retinal detachment and cleft palate, and have been shown to have a deletion of a portion of the collagen II gene. We have been trying to identify the exact position of the deletion. A number of other SED patient's DNA have been screened by southern blotting with a collagen II probe.

Yolanda Sanchez, Yoshi Yamada

Transgenic Mice With the Collagen II Gene

We have isolated and characterized a number of clones for cartilage and basement membrane components. Our objectives are to use the enormous potential of transgenic technology so that we can understand the molecular mechanisms which regulate the synthesis of cartilage and basement membrane matrix, and identify the role of the individual components during normal development and in pathologic states. We have been focusing on the following areas: (1) identification of regulatory elements of genes for cartilage and basement membrane proteins involved in tissue-specific expression; (2) creation of animals carrying foreign genes expressed in specific tissues; and (3) creation of animals carrying specific mutations in cartilage and basement membrane genes.

Previously we have isolated the type II collagen promoter and enhancer and showed by transfection into chondrocytes that the



enhancer was required for the high level of transcription of the collagen II gene. To further examine tissue specificity of these elements, the collagen II promoter-enhancer-CAT construct was micro-injected into embryos to generate transgenic mice. Several lines of transgenic mice have been obtained. The reporter gene (CAT) was expressed in tissues including joints, sterna, and eyes where collagen II was synthesized. A construct with the collagen II promoter-CAT without the enhancer showed little CAT expression in all tissues. These results suggest that the collagen II and enhancer can direct expression of foreign genes specifically in cartilage tissues. Transgenic animals with the collagen II promoter-enhancer-CAT were used in further studies of bone fracture repair. Initial expression of type II collagen in the formation of the fracture callus occurs at day 5 and is expressed through day 11. Femurs of transgenic animals were broken, removed at various stages of healing and assayed for CAT activity. CAT activity was found to dramatically increase at the time of mesenchyme differentiation into chondrocytes at day 5. These results suggest that the expression in transgenic mice of this construct was appropriately responding to tissue injury in the de-differentiation process of bone remodeling and repair. Since the CAT assay is simple and quick, and does not require a large amount of tissues, this transgenic mouse line will be useful to study the repair process in fractures.

The developmental regulation of collagen II gene expression has also been examined in transgenic mice using the techniques of cell lineage ablation. The technique involves the expression of the diphtheria toxin A chain gene under the control of the collagen II promoter and enhancer. When the toxin is expressed, it kills the cells and thus eliminates any tissue that would have been derived from the progenitor cell. Micro-injection of this construct did not produce any viable transgenic offspring. However, a high number of dead embryos, as well as abnormally developing embryos were observed. The abnormal embryos were observed at day 12 or later, which is coincidental with the onset of chondrogenesis. These transgenic embryos had physical abnormalities including cleft palates, shortened and underdeveloped limbs, no eyes, kinked tails and generally resembled the phenotype of chondrodystrophic mice which are defective in cartilage matrix. These results indicate that the expression of the toxin gene under the control of the collagen II promoter and enhancer has occurred not only in correct tissues, but also at the right time frame of development.

Since the collagen II promoter and enhancer can direct expression of foreign genes specific in cartilage, several constructs with these regulatory elements have been prepared and attempted to generate transgenic mice. These include: interleukin I, c-fos, bFGF, c-myc, SV40 T-antigen, collagenase, and stromelysin.

Ken Brown, Leslie Bruggeman, Xie Hou-Xiang, Wayne Little, Dave Strong, Yoshi Yamada



Transgenic Mice With Basement Membrane Genes

Several constructs have been prepared to generate transgenic mice. A line of transgenic mice with the $\alpha 1(\text{IV})$ collagen promoter-enhancer-CAT has been established. Preliminary results using the heterozygous mice showed CAT expression in both kidney and spleen. A line of transgenic mice with the laminin B2 promoter-CAT has also been generated. To create an animal model of diseases associated with collagen IV genes, we have constructed a full length cDNA which has a 110 bp deletion of the helical portion of the molecule. The cDNA was then placed under the control of the $\alpha 1(\text{IV})$ collagen promoter and enhancer. The mutated gene construct will be examined for its expression by transfection assay in culture and micro-injected into mouse embryos to generate transgenic mice.

Leslie Bruggeman, Xie Hou-Xiang, Peter Burbelo, Hisae Hori, Wayne Little, Dave Strong, Yoshi Yamada

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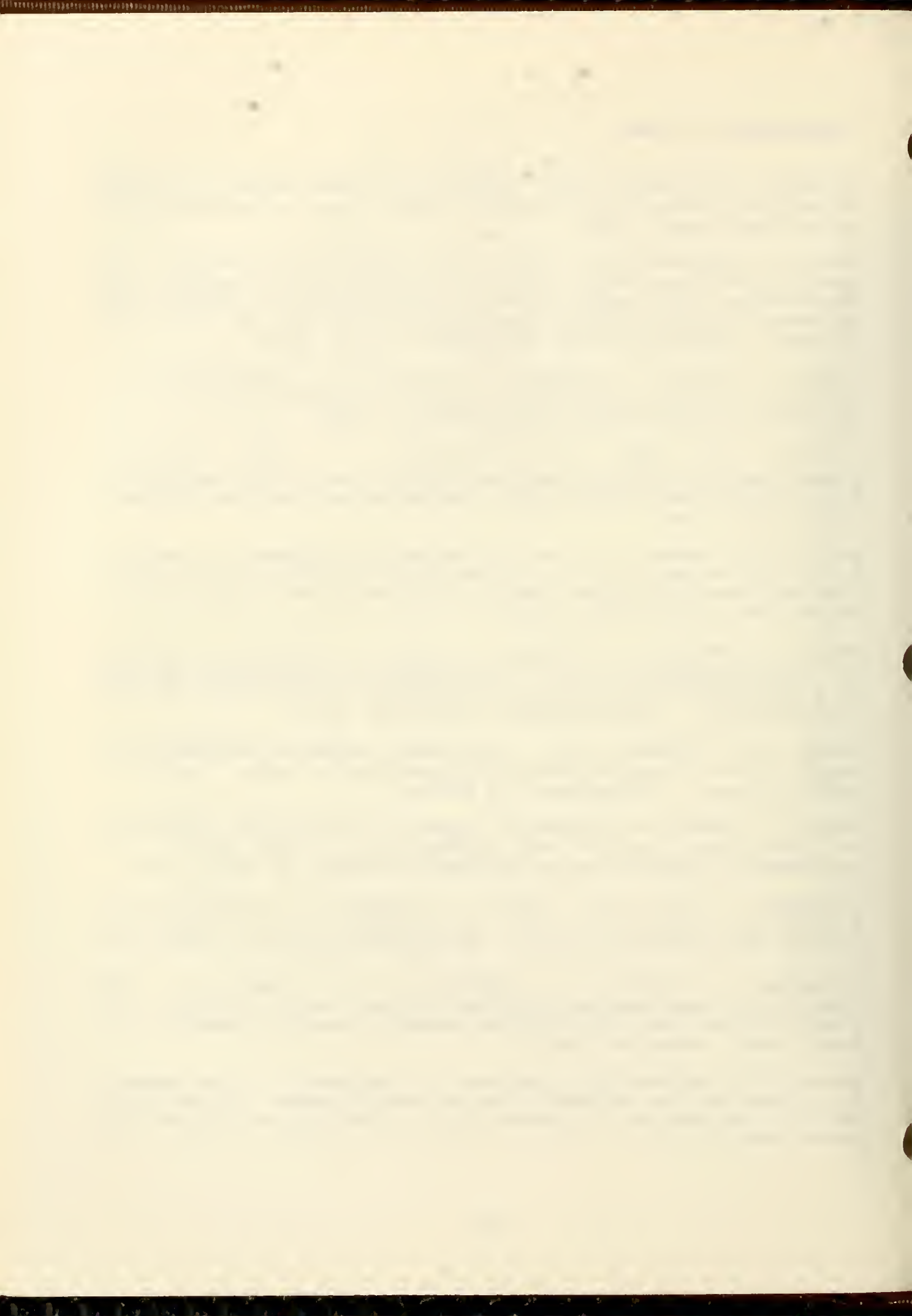
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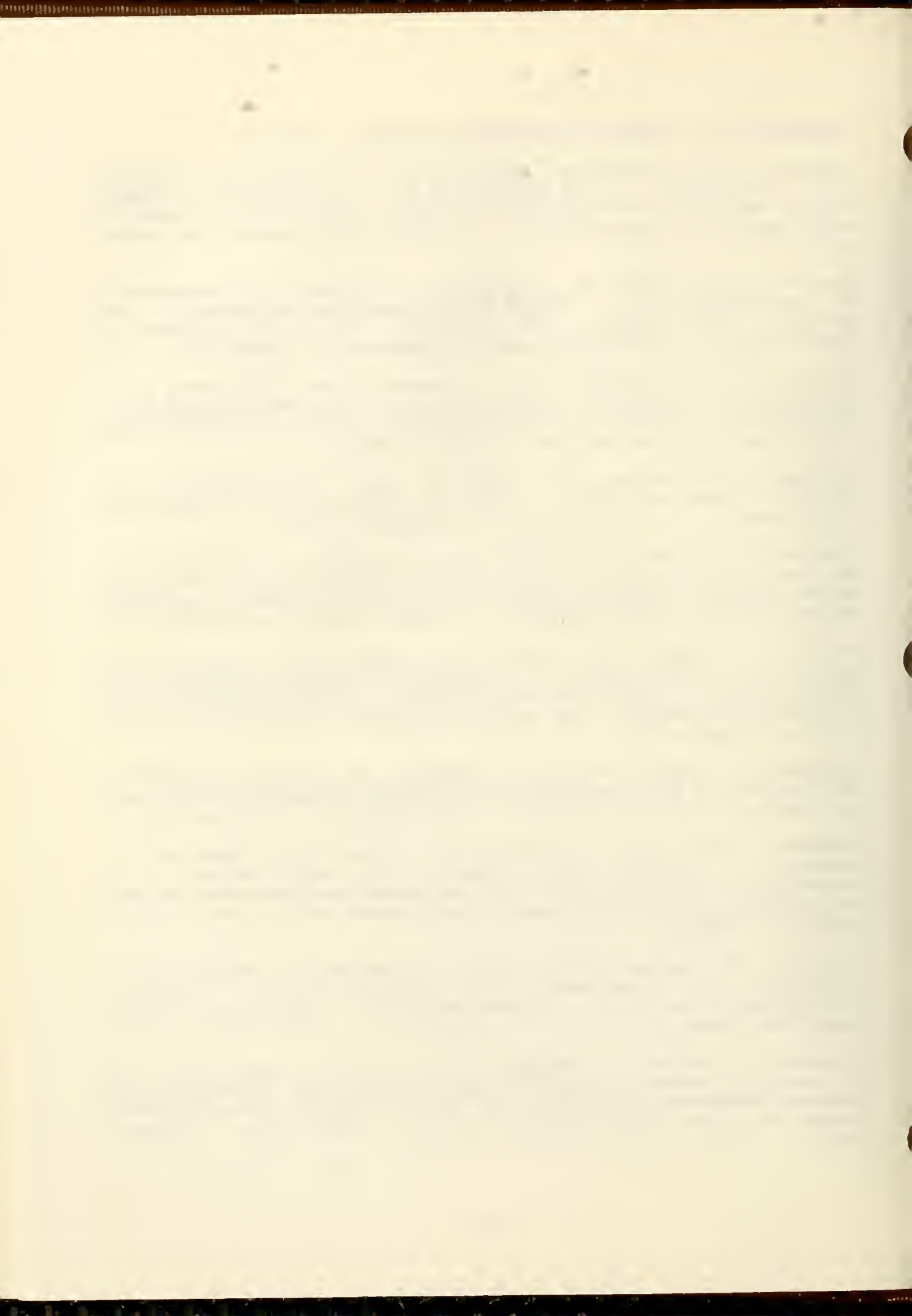
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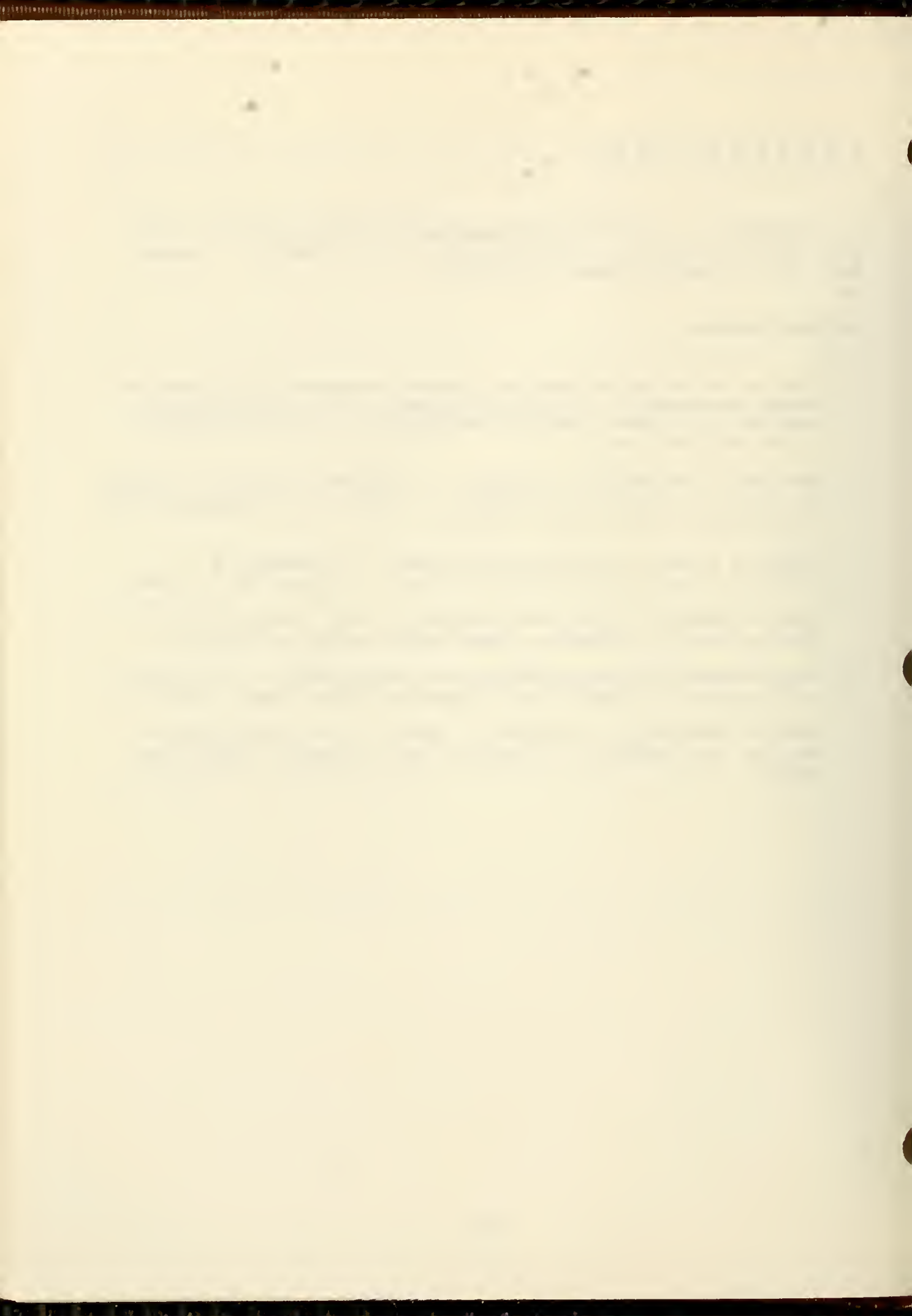


P A T E N T S 1 9 8 9

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PATENTS PENDING:

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- 3) "Laminin A chain and active peptides." Y. Yamada, M. Sasaki, H.K., Kleinman and G.R. Martin, filed, Nov 7, 1988.
- 4) "Use of minoxidil for wound healing." A.C. Sank, G.R. Martin, and S. Ledbetter, Application serial #07/281/129.
- 5) "Acylaminoalkylpyredineamides as of metastasis." G. Fuller, G.R. Martin, R. Reich, and R. Mueller, filed, May, 1989.
- 6) "Use of heterocyclic amides to inhibit tumor metastasis." Fuller, G.R. Martin, R. Mueller, and R. Reich, filed, May, 1989.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-13 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Proteins in Tissue Architecture and Cell Function

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

Kleinman, H.K.	Res. Chemists	DB NIDR
Martin, G.R.	Chief, LDBA	DB NIDR
Sephel, G.C.	Biologist	DR NIDR
Cannon, F.B.	Biologist	DB NIDR
Grant, D.S.	Visiting Fellow	DB NIDR
Tashiro, K.	Visiting Fellow	DB NIDR
Yamada, Y.	Visiting Scientist	DB NIDR

COOPERATING UNITS (if any)

NIA, NCI, NHLBI, NINCDS, University of Virginia, Harvard University, Georgetown University, Monsanto, John Hopkins University.

LAB/BRANCH

Laboratory of Development Biology and Anomalies

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

7.5

PROFESSIONAL:

4.75

OTHER:

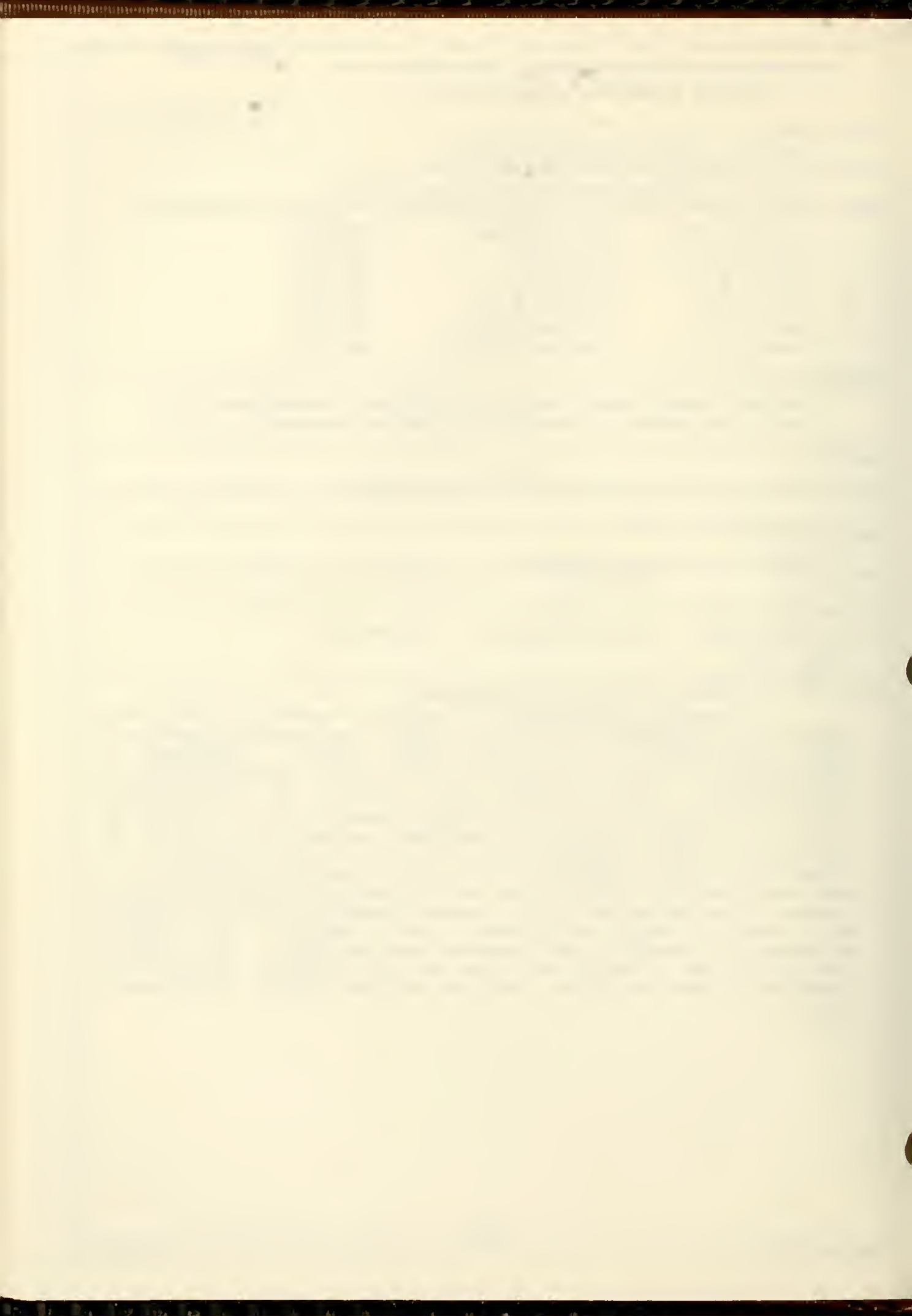
2.75

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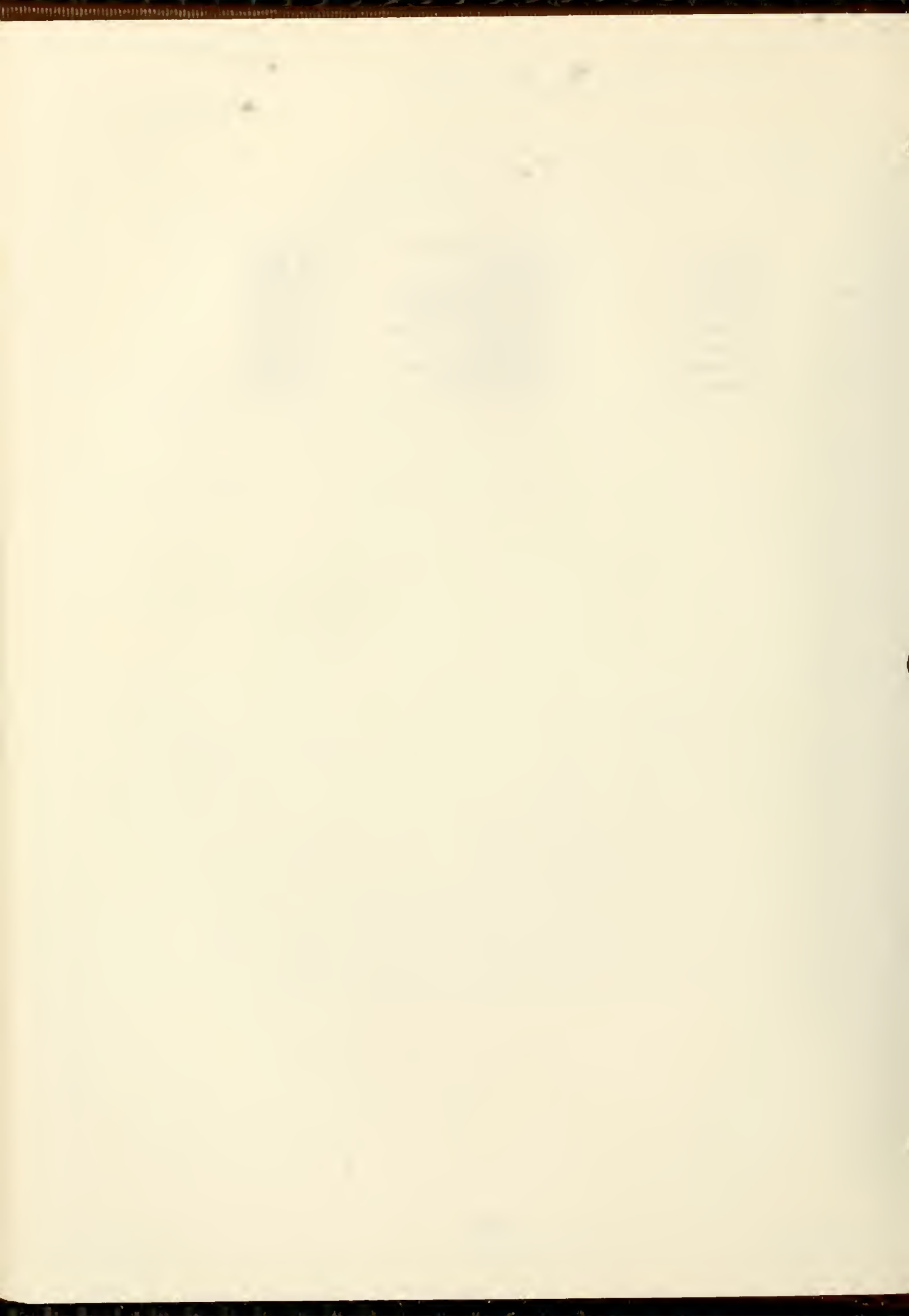
- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Cell-matrix interactions are important regulatory events during embryogenesis and repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate and differentiate in response to tissue- and cell-specific matrix molecules has been established. Our approach has been to identify the (1) biologically active matrix components, (2) localize active sites on the matrix component with site specific antibodies and synthetic peptides, (3) identify and characterize cellular receptors, and (4) gain an understanding of the intracellular events involved in the biological response. Specifically, we have identified three active sites on the basement membrane glycoprotein laminin. Of particular interest is a synthetic peptide of 18 amino acids from the A chain of laminin which promotes neurite outgrowth, cell adhesion, collagenase IV production, and increased tumor metastases. Other peptides from the B1 and A chains are active for cell adhesion, migration, and inhibition of tumor metastases. Such peptides have potential as therapeutic agents.



Adler, S.H.	Guest Researcher	DB NIDR
Weeks, B.J.	Biologist	DB NIDR
Dym, M.	Guest Researcher	DB NIDR
Shiraishi, N.	Visiting Scientist	DB NIDR
Clement, B.	Visiting Scientist	DB NIDR
Wilson, J.	Secretary	DB NIDR
Klotman, P.	Guest Researcher	DB NIDR
Dudek, D.	Clerk-Typist	DB NIDR



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00481-01 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Connective Tissue Gene Expression in Development and Disease

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

Klotman, Paul	Special Expert	DB NIDR
Bruggeman, Leslie	Visiting Scientist	DB NIDR
Burbelo, Peter	Staff Fellow	DB NIDR
Chi, Maria	Biologist	DB NIDR
Grant, Derrick	Visiting Scientist	DB NIDR
Horigan, Elizabeth	Research Biologist	DB NIDR
Horikoshi, Satoshi	Visiting Fellow	DB NIDR
Kleinman, Hynda	Research Chemist	DB NIDR

COOPERATING UNITS (if any)

Laboratory of Tumor Cell Biology/NCI (Jay Rappaport, Mary Klotman, Z. Salahuddin, Gilbert Jay). Nephrology Section, Duke University Medical Center (Deirdre Collins, Thomas Coffman)

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

7.58

PROFESSIONAL

3.25

OTHER

4.33

CHECK APPROPRIATE BOX(ES)

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

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

The spatial and temporal expression of specific proteins which constitute the extracellular matrix and the receptors for these proteins are critical for normal tissue organization, development and growth. Conversely, abnormal expression of these extracellular proteins and receptors as a result of either genetic or acquired diseases often represents the pathologic basis for clinical illness. For example, many renal, pulmonary, hepatic, and skin diseases are characterized by abnormal deposition of extracellular matrix proteins leading to impaired function and healing. The purpose of these studies is to understand the molecular mechanisms by which genes for the extracellular matrix are regulated during normal development and growth and to determine the molecular basis of various diseases associated with synthesis of their proteins. Using a combination of molecular, cellular and physiologic techniques, we are evaluating several normal and pathophysiologic conditions associated with changes in the extracellular matrix including renal growth and hypertrophy following unilateral nephrectomy, polycystic kidney disease, diabetes, hypertension, chronic transplantation rejection, cyclosporine toxicity, and wound healing. In addition, we are exploring mechanisms of viral latency and reactivation in renal epithelial cells and the importance of transactivation of matrix protein genes by human immunodeficiency virus 1. We are currently evaluating matrix gene expression in an attempt to identify important transcriptional factors during development and disease; we are exploring the specific peptide sequences of matrix proteins which are necessary for renal cell attachment, differentiation, and cell function; and we are creating transgenic mice in order to evaluate the importance of tissue-specific gene expression which may contribute to the clinical manifestation of disease.



Martin, George	Chief, LDBA	DB NIDR
Sank, Anthony	Staff Fellow	DB NIDR
Shima, Thomas	Guest Worker	DB NIDR
Thompson, Eric	Guest Worker	DB NIDR
Weeks, Ben	Biologist	DB NIDR
Yamada, Yoshihiko	Visiting Scientist	DB NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00482-01 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Tumor Growth and Metastases

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

Martin, George R.	Chief, LDBA	DB NIDR
Kleinman, Hynda	Res. Chemist	DB NIDR
Reich, Reuven	Visting Associate	DB NIDR
Fridman, Rafael	Guest Researcher	DB NIDR
Royce, Leah	Dental Staff Fellow	DB NIDR
Kubota, Shun	Guest Researcher	DB NIDR
Kanemoto, Tamoko	Guest Researcher	DB NIDR
Yamada, Yoshihiko	Visiting Scientist	DB NIDR

COOPERATING UNITS (if any)

McGill University, Canada; G.S. Searle Co., Skokie, IL; Washington University, St. Louis, FDA; NCI, Georgetown University, DC.

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

PROFESSIONAL:

OTHER

6.75	1.75	5.00
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CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to understand the mechanisms which regulate tumor cell growth and metastasis. Tumor cells must attach, degrade and migrate into tissues to become malignant. The activity of collagenase IV, an enzyme involved in the degradation of the blood vessel wall, is critical to this invasive behavior. Compounds which block its activity or its synthesis reduce the metastatic phenotype. In particular, we have shown that metabolites of arachidonic acid are involved in the synthesis of this degradation enzyme and in the malignant behavior of cells. The glycoprotein laminin, which is present in the blood vessel wall, is a potent promoter of collagenase IV activity. We find that a synthetic peptide of 18 amino acids can induce collagenase IV activity and synthesize it at the mRNA level. This peptide also renders non-malignant cells more invasive. A growth factor, TGF,β, is found in our studies to reduce the malignant phenotype. Our goal is to obtain a better understanding of the malignant phenotype and to devise new therapeutic strategies to reduce metastases.



Adler, Scott
Stratford, Bridget
Klotman, Paul
Thompson, Eric

Guest Researcher
Biologist
Guest Researcher
Guest Researcher

DB NIDR
DB NIDR
DB NIDR
DB NIDR

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00483-01 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Gene Regulation and Function of Cartilage

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

Yamada, Yoshihiko	Chief, MBS	DB NIDR
Savagner, Pierre	Visiting Fellow	DB NIDR
Doege, Kurt	Staff Fellow	DB NIDR
Chirigos, Michael	Biologist	DB NIDR
Rhodes, Craig	Biologist	DB NIDR
Becvar, Radim	Visiting Fellow	DB NIDR
Line, Sergio	Visiting Fellow	DB NIDR
Liebman, Jeff	Guest Researcher	DB NIDR

COOPERATING UNITS (if any)

John's Hopkins University, Shriner's Hospital, University of Texas,
University of Montreal

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

4.45

PROFESSIONAL

0.50

OTHER

3.95

CHECK APPROPRIATE BOX(ES)

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

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

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

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



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00484-01 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Animal Models of Connective Tissue Diseases in Transgenic Mice

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

Yamada, Yoshihiko	Chief, MBS	DB NIDR
Bruggeman, Leslie	Post-Doc	DB NIDR
Little, Wayne	Biologist	DB NIDR
Brown, Kenneth	Medical Director	DB NIDR
Hou-Xiang, Xie	Visiting Fellow	DB NIDR
Mosley, General	Bio. Lab. Tech.	DB NIDR
Strong, Dave	Bio. Lab. Tech.	DB NIDR

COOPERATING UNITS (if any)

Eli Lilly, Shriners Hospital, University of Texas, Osaka University

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

5.78

PROFESSIONAL:

4.60

OTHER:

1.18

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to understand the molecular basis of connective tissue components as well as their gene regulation in normal development and in disease state using transgenic mice as models. Transgenic mice created by injection of DNA into mouse embryos have been exploited for the elucidation of factors which determine tissue specificity of gene expression. Phenotypic changes due to expression of foreign gene center the control of tissue specific heterologous promoters have also been studied. Creation of transgenic animals which carry mutated exogenous genes as models for human genetic diseases of cartilage and basement membrane have been exploited. Recently developed technique of targeted homologous recombination makes it possible to suppress the function of a specific function. We have began establishing embryonic stem (ES) cell lines carrying a gene targeted by homologous recombinatic of an exogenous DNA construct and introducing recipient blastogenesis in an effort to obtain chimeric mice that contain the altered genetic information in their germ line. Several lines of transgenic mice carrying constructs of the collagen II promoter-CAT and the collagen II promoter plus enhancer-CAT have been generated. Developmental regulation of collagen II has been studied by introducing the diphtheria toxic gene under the control of the collagen II promoter and enhancer. The abnormal developed embryos were generated in these experiments. The phenotype of these animals is similar to chondrodystrophic mice which have deficiencies in cartilage matrix. The $\alpha 1$ (IV) collagen promoter-enhancer-CAT construct as well as the laminin B2 promoter-CAT construct have been used to generate transgenic mice to examine if they can direct expression of the CAT gene in tissue specific fashion. A construct containing a mutated $\alpha 1$ (IV) collagen cDNA under the control of the $\alpha 1$ (IV) promoter and enhancer has been constructed and introduced into mouse embryos to generate transgenic mice.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00485-01 DB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Gene Regulation and Function of Basement Membrane

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

Yamada, Yoshihiko	Chief, MBS	DB NIDR
Burbelo, Peter	Staff Fellow	DB NIDR
Bruggeman, Leslie	Post-Doc	DB NIDR
Klotman, Paul	Special Expert	DB NIDR
Hori, Hisae	Guest Researcher	DB NIDR
Ogawa, Kohei	Guest Researcher	DB NIDR
Noonan, Douglas	Post-Doc	DB NIDR

COOPERATING UNITS (if any)

John's Hopkins, Max-Planck Institute, Asalin Kasei Chemical Corp, NCI.

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

5.43

PROFESSIONAL

1.15

OTHER

4.28

CHECK APPROPRIATE BOX(ES)

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

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

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

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



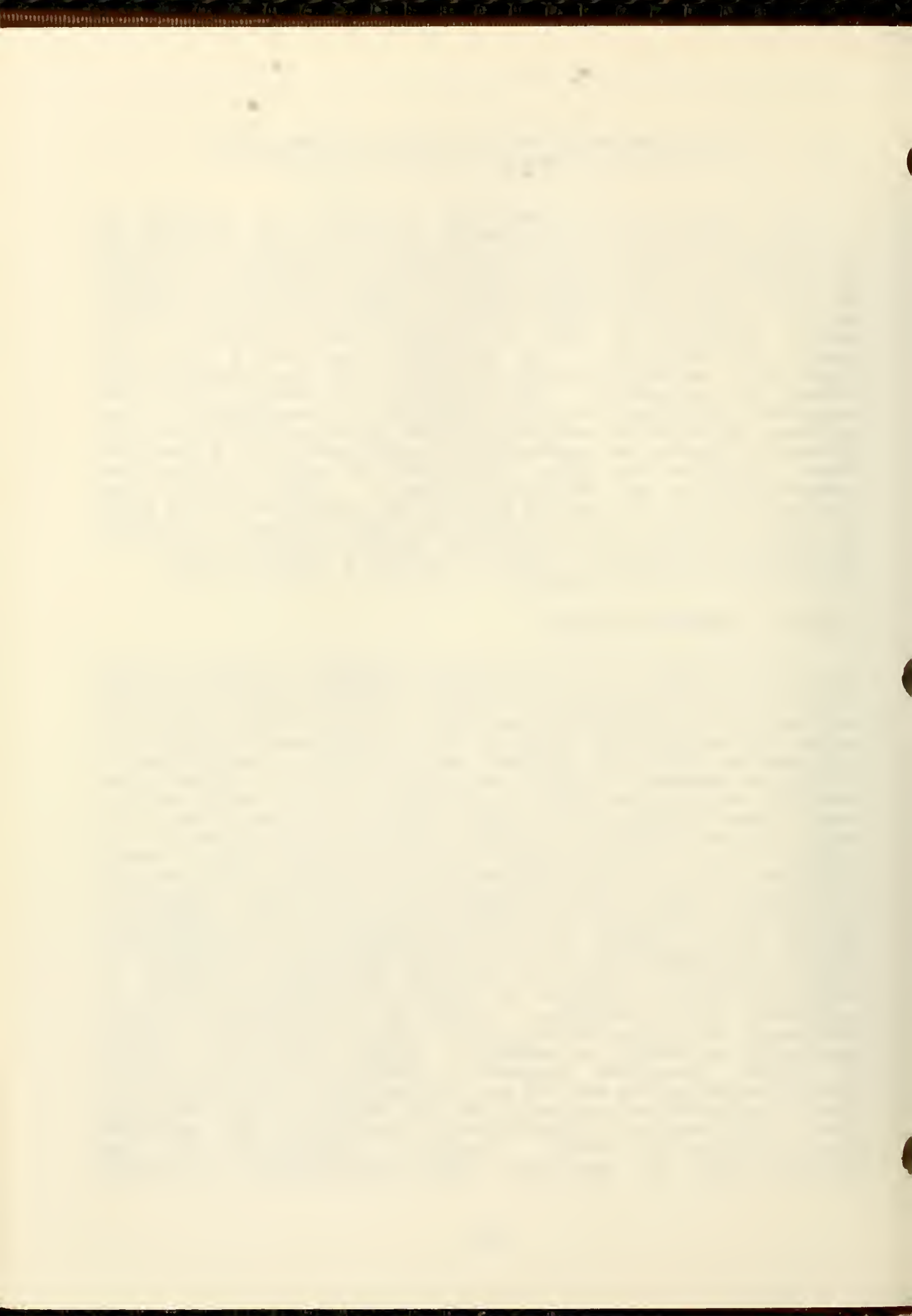
Sawada, Makoto	Visiting Fellow	DB NIDR
Horigan, Beth	Biologist	DB NIDR
Tashiro, Kenichiro	Visiting Fellow	DB NIDR
Laurie, Gordon	Visiting Associate	DB NIDR
Fukuda, Katsunori	Visiting Fellow	DB NIDR
Kleinman, Hynda	Chief, CBS	DB NIDR
Sephel, Greg	Guest Researcher	DB NIDR
Clement, Bruno	Visiting Fellow	DB NIDR

Annual Report of the Laboratory of Immunology
National Institute of Dental Research

The Laboratory of Immunology continues its research on defining the cellular and molecular bases of acute and chronic inflammatory diseases. Our programs seek to elucidate the nature of immunoglobulin receptors and biochemical pathways in basophils and mast cells which trigger immediate hypersensitivity reactions and the intercellular activities in immune and chronic inflammatory responses in order to modulate these events for the benefit of the host. The Laboratory has made a number of significant research advances during the past year despite major losses in personnel. The transfer of several full-time equivalent positions from the Laboratory of Immunology to other programs within NIDR, necessitated the complete elimination of a productive program on hematopoietic growth factors at a time when these cytokines are being recognized as extremely important immunomodulators and therapeutic agents. Other projects have also had to be curtailed and some may be discontinued. In spite of these set backs, our scientists have been eminently successful in recruiting talented postdoctoral fellows through various non-FTE support mechanisms in order to sustain a vigorous multidisciplinary research program.

CELLULAR IMMUNOLOGY SECTION

A focal point in the research program of the Cellular Immunology Section is the mononuclear phagocyte population which is pivotal in inflammation and immune responses. Upon activation and maturation of precursor monocytes into macrophages, the cells become bactericidal and cytotoxic and also elaborate a variety of inflammatory mediators, enzymes, and monokines. Recognition of foreign substances, microbes and tumor cells is dependent upon specific recognition sites or receptors on the cell surface. Among these receptors are binding sites for the constant Fc region of immunoglobulin G (Fc γ R). There are three distinct Fc γ R species which mediate uptake of opsonized particles, immune complexes and antibody-dependent cellular cytotoxicity. Circulating human monocytes express Fc γ RI and Fc γ RII, identified by specific monoclonal antibodies, but only express Fc γ RIII in tissues during maturation into macrophages or at inflammatory sites. The appearance of Fc γ RIII on monocytes in inflammatory lesions prompted a search for cytokines which might be present in such sites and be capable of upregulating these receptors on peripheral blood monocytes. Among the inflammatory cytokines evaluated, only transforming growth factor beta (TGF- β) induced expression of Fc γ RIII on monocytes. Within 24 hr after exposure to TGF- β 1, monocytes expressed enhanced levels of Fc γ RIII identified using the mAb 3G8 and FACS analysis. Upregulation of Fc γ RIII appeared to be specific in that Fc γ RI (mAb 32) and Fc γ RII (mAb IV.3) were not significantly elevated by the picomolar concentrations of TGF- β . Receptor augmentation was dose dependent and not associated with other markers of monocyte



maturation including enhanced HLA-DR and IL-2R expression. These novel data suggest that TGF- β concentrated in a localized inflammatory lesion may contribute to monocyte phagocytic activity by its ability to promote Fc γ RIII levels on the cell surface.

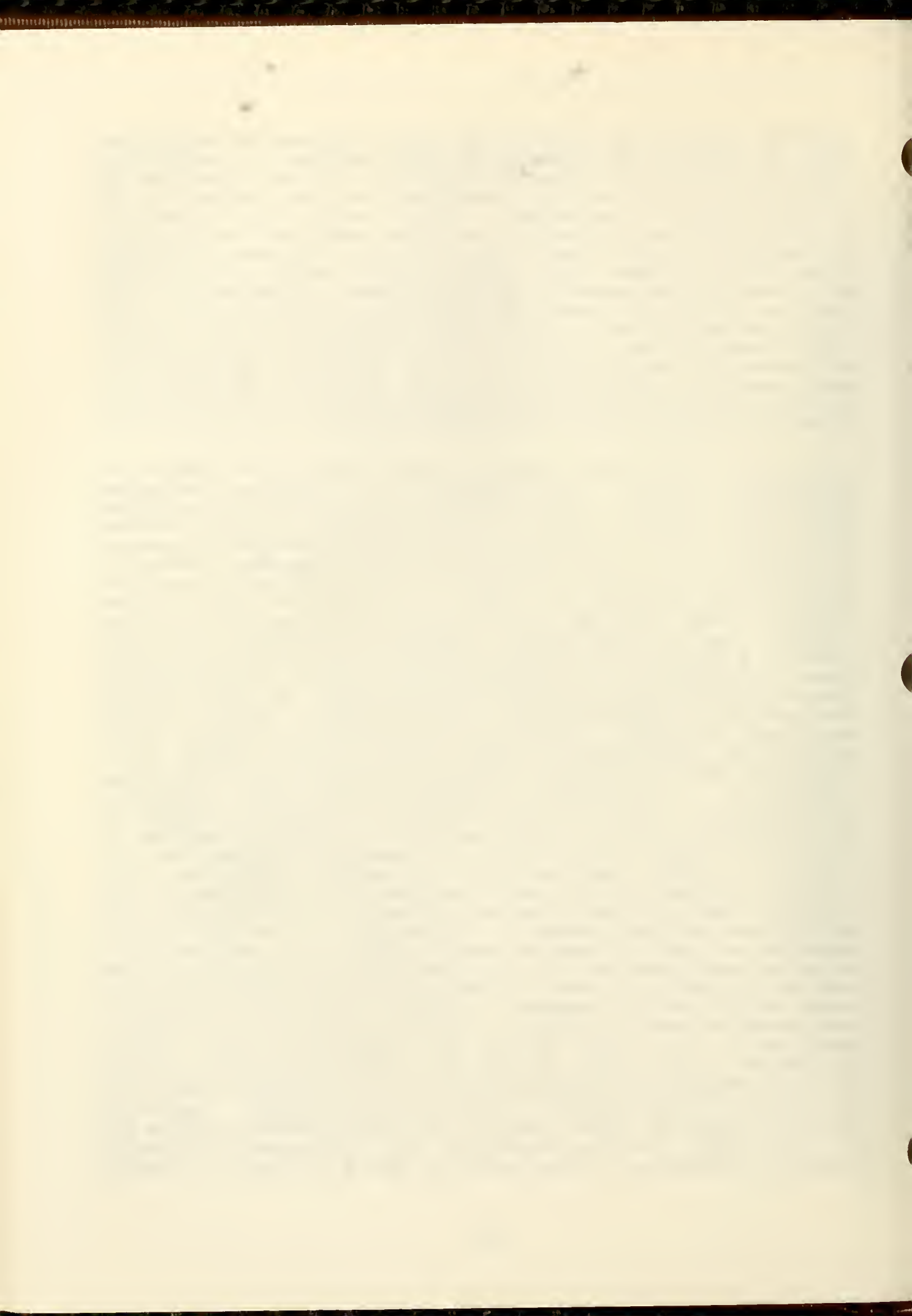
At similar concentrations, TGF- β induces monocyte synthesis of IL-1, TNF, PDGF and FGF. In addition, TGF- β 1 upregulates the steady-state expression of monocyte TGF- β 1 mRNA, but not TGF- β 2 mRNA, suggesting that TGF- β 1 can further amplify an inflammatory response via an autocrine mechanism. To further investigate the mechanism of the changes in the steady-state levels of TGF- β 1 mRNA, the effect of cycloheximide (CX), an inhibitor of peptide bond formation, on the accumulation of TGF- β 1 mRNA was analyzed. CX caused a superinduction of TGF- β 1 mRNA without changing its half-life, suggesting the involvement of a labile protein repressor at the transcriptional level. In contrast, superinduction of TGF- β 1 mRNA in TGF- β 1-stimulated monocytes by CX occurred through posttranscriptional stabilization of the TGF- β message. Moreover, nuclear runoff experiments revealed a 2-fold increase in transcription following stimulation with TGF- β 1. Thus, it appears that the autoinduction of TGF- β 1 is regulated by both transcriptional and posttranscriptional mechanisms.

In related studies, identification of immunoreactive TGF- β in inflamed synovium of rodents with erosive polyarthritis pointed toward a role for this polypeptide in chronic inflammatory lesions. To define the potential role of TGF- β in chronic inflammatory lesions such as arthritis, TGF- β 1 was injected intra-articularly in the joints of Lewis rats. Following injection of 1 μ g TGF- β , marked swelling and erythema of the treated joints were apparent within 12 hours, reaching a peak after 3 daily injections. There was no detectable swelling in the contralateral vehicle-injected control joint. Cessation of TGF- β resulted in a gradual decline of the swelling. Histopathologic evaluation revealed an infiltration of neutrophils, large numbers of Ia⁺ monocytes/macrophages and some T cells into the synovial space, consistent with active inflammation. Extensive synovial fibroblast hyperplasia occurred within 48 hours. TGF- β 2 which is greater than 70% homologous to TGF- β 1 was found to induce a similar level of synovitis. This is consistent with the in vitro ability of both TGF- β 1 and β 2 to induce both rat macrophage chemotaxis and transcription and translation of monocyte/macrophage-derived growth factors for mesenchymal cells. These data demonstrate that TGF- β , released by platelets and/or activated inflammatory cells, plays a direct role in leukocyte recruitment and activation in arthritic and other chronic inflammatory lesions.

TGF- β is not only a modulator of inflammatory cell activity but is also a potent chemoattractant for fibroblasts, and activates fibroblasts to synthesize a variety of matrix components including collagen. As a first step towards defining a role for TGF- β as a regulator of bacterial cell wall-induced hepatic granuloma formation and fibrosis, a rabbit antibody

directed against the NH₃-terminal 1-30 residues of TGF-β₁ was used to examine the presence of TGF-β peptide in sections of granulomatous livers. Specific staining for TGF-β peptide was readily detectable within granulomas, but was absent in the surrounding liver parenchyma. High levels (5-25 ng/ml) of soluble TGF-β were found in cultured granuloma supernatants. Consistent with this observation, little or no TGF-β₁ mRNA was detectable in normal liver, whereas high levels of expression were found in RNA prepared from granulomas. Interestingly, two distinct transcripts were expressed by granulomas: the typical 2.5 kb transcript and a novel 1.9 kb transcript. The functional significance of the 1.9 kb transcript expressed in granulomas is not known, but the expression of this transcript has previously been associated with tissue injury and hypoxia. The expression of high levels of TGF-β by granulomas may represent an important signal directing granuloma-associated fibrosis.

Chronic inflammatory lesions such as periodontal disease and rheumatoid arthritis characterized by activated monocyte-macrophages are also often associated with loss of bone and cartilage. Important studies in the Cellular Immunology Section have focused on the role of guanine nucleotide binding proteins (G proteins) in the prostaglandin-cyclic AMP dependent pathway of collagenase production by monocytes. G proteins are a family of intracellular proteins that play a key role in signal transduction. These proteins are heterotrimers composed of α, β, and γ chains, and are distinguished principally by their structurally distinct α chains which bind and hydrolyze GTP. The function of these G proteins can be modulated and analyzed using bacterial toxins which catalyze covalent modifications of the α chains. The addition of cholera toxin to concanavalin A (Con A) treated monocytes, but not unstimulated monocytes, resulted in a significant enhancement of the release of PGE₂, other cyclooxygenase and lipoxygenase pathway products, and the enzyme collagenase. This evidence suggested that Con A induced or translocated a cholera toxin-sensitive G protein coupled to phospholipase which liberated arachidonic acid, in addition to its direct stimulatory effects on adenylyl cyclase and the increase in cAMP required for collagenase production. In contrast, pertussis toxin, which induces ADP-ribosylation of G_{21α}, inhibits Con A-induced monocyte synthesis of arachidonic acid metabolites and collagenase. Documentation that the effects mediated by cholera and pertussis toxins were related to G proteins was obtained in ADP-ribosylation experiments using radioactive NAD⁺. In order to further identify the ribosylated bands in the monocyte membrane proteins as specific G proteins, the proteins were analyzed by Western blots in which the G proteins G_{sα}, G_{1α1}, G_{1α2}, and G_{1α3} were identified with specific antibodies. In ongoing experiments, these G protein subunits have been shown to be modulated by Con A, LPS and PMA stimulation as well as with relevant pharmacological agents, suggesting specific roles for these proteins in the activation process and in particular, in the regulation of collagenase synthesis. Moreover, alteration of the ratio of the G_{sα} to the G_{1α} subunits



in chronic inflammatory disease may result in abnormal production of collagenase. Continued definition of the role of G proteins in the intracellular events leading to collagenase production may provide new therapeutic approaches.

Whereas a prolonged monocyte-macrophage response in chronic inflammatory lesions can have pathologic consequences, impaired or deficient monocyte function can also be detrimental to the host. In continuing studies, the laboratory has been involved in elucidating the contribution of monocytes to the immune dysregulation in AIDS. A population of circulating mononuclear cells from patients with AIDS was identified which expressed interleukin 2 receptors (IL-2R). By dual fluorescence flow microfluorometry, the patients' IL-2R⁺ cells were further identified as Leu M3⁺ monocytes, whereas Leu M3⁺ monocytes from normal subjects were uniformly IL-2R negative. By Northern analysis, monocytes from AIDS patients, but not control subjects, constitutively expressed steady state levels of IL-2R mRNA. Functionally, the IL-2R⁺ monocytes were capable of depleting biologically active IL-2 from culture supernatants, suggesting a mechanism for the reduced IL-2 levels commonly seen in AIDS patients. IL-2R⁺ monocytes also expressed increased levels of surface HLA-DR which may favor monocyte T-cell interactions and the transmission of HIV. In related studies, normal monocytes were infected with a macrophage-tropic HIV isolate in vitro and monitored for IL-2R and HLA-DR expression. By 48 hrs after exposure to HIV in vitro, but prior to evidence of productive infection, >25% of the monocytes became IL-2R⁺ with increasing numbers of IL-2R⁺ cells and HLA-DR levels through day 6. These early signalling effects of HIV could be mimicked by adding purified HIV envelope glycoprotein gp120 to the monocytes. This stimulation of monocytes prior to or independent of productive infection of the cells by HIV is consistent with in vivo observations of activated and/or abnormal functions by monocytes that do not appear to be infected with HIV in AIDS patients.

Aberrant or inappropriate expression of cytokines and cell surface antigens may also play an important role in AIDS and other chronic diseases. Previous studies in this Laboratory revealed dramatic differences in growth factor expression, most notably TNF- α , by human peripheral blood monocytes following exposure to HIV-1. Enhanced cytokine gene expression occurred during the period of increasing viral production, lending support to the role of cytokines in the infection cycle and the contribution of TNF- α in the upregulation of HIV-1 expression providing a positive regulatory circuit favoring viral replication. Cytokine RNA levels declined when peak viral expression was achieved. In addition, transcription of other cellular genes was also downregulated during this period of the infection cycle, suggesting that the regulatory circuit favors viral replication at the expense of host gene expression.

Since the gastrointestinal tract is the initial route of infection and often the site for presenting symptoms in many AIDS

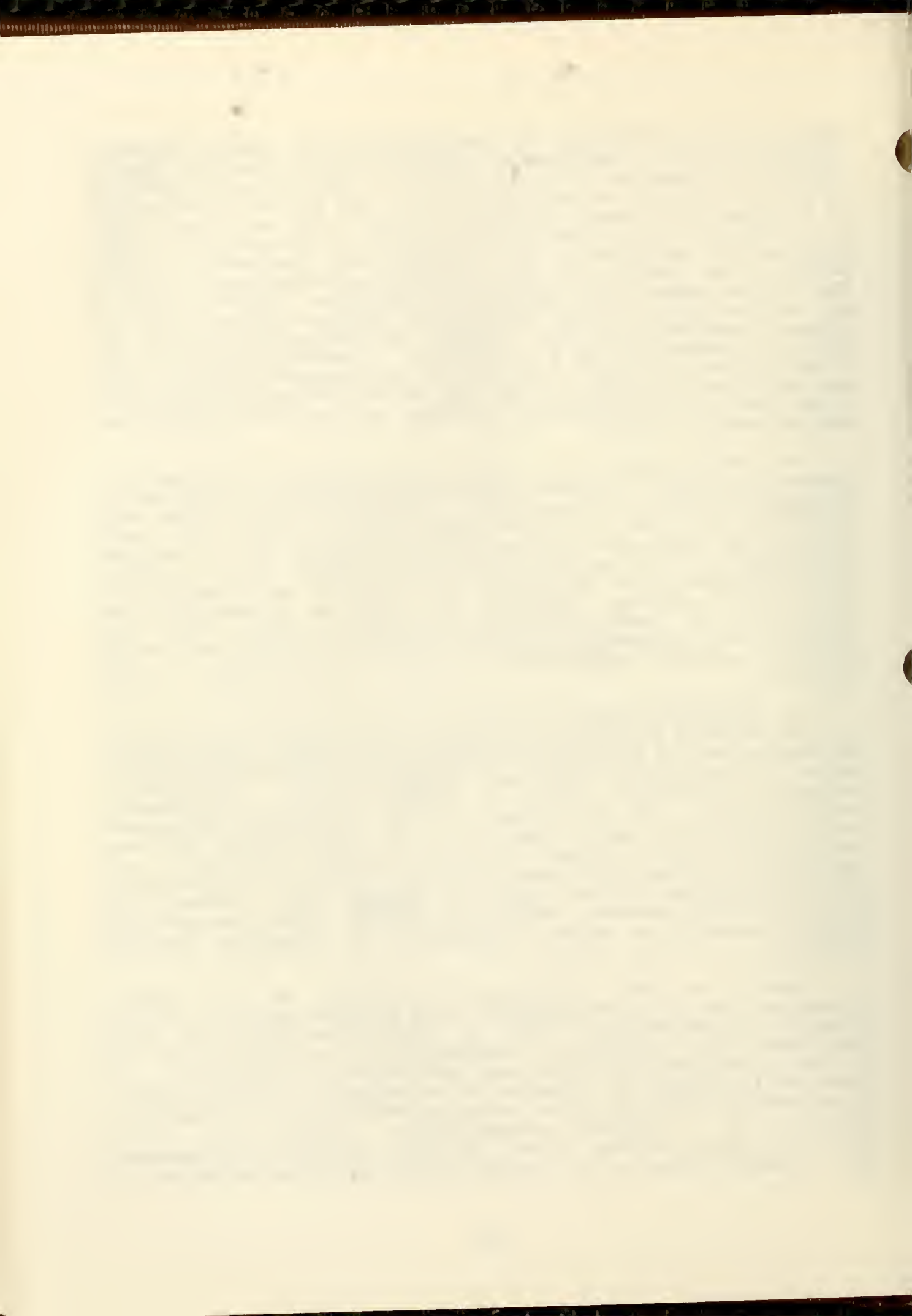


patients, the Laboratory is investigating the mucosal system's response to HIV. Preliminary evidence suggests that HIV can be taken up by mucosal M cells and that the secretory antibody response to HIV in the gut (IgG but not IgA anti-HIV antibodies) is strikingly different from that in oral cavity secretions (IgA, but little or no IgG anti-HIV antibodies). In addition, major progress is being made in isolating and characterizing the phenotype and function of mucosal monocytes/macrophages, possibly the initial immune cell that virus encounters. The functions of mucosal macrophages being investigated include effector activity (oxygen dependent killing), cytokine production and antigen (C. pylori) presentation to T lymphocytes. Understanding the state of activation and function of mucosal macrophages will facilitate our anticipated studies of the role of the mucosal macrophage in infectious and inflammatory diseases of the oral cavity and gastrointestinal tract.

Following acquisition of HIV and subsequent development of immunosuppression, the host acquires pathogens such as CMV and C. albicans. During the past year, members of the Section have discovered that CMV can induce tumor necrosis factor (TNF) gene and peptide expression by monocytes in vitro and in mucosal macrophages in AIDS patients with CMV colitis. This important finding suggests that CMV-induced TNF secretion may contribute to the organ pathology (colitis as well as pneumonitis and retinitis) in immunocompromised persons. Since TNF can upregulate HIV expression, our finding also offers an explanation for the mechanism whereby CMV may serve as a cofactor in HIV infection.

C. albicans is another important pathogen of the oral cavity in AIDS patients and other immunosuppressed persons. Importantly, the mechanisms of macrophage killing of C. albicans by reactive oxygen intermediates have been characterized. Furthermore, macrophage fungicidal killing of Candida, as well as accessory cell function, can be upregulated by granulocyte-macrophage colony-stimulating factor (GM-CSF). This upregulation has been confirmed in AIDS patients receiving GM-CSF therapy in our collaborative study with investigators in the NCI. In addition, an investigation of the type of Candida that infects AIDS patients is in progress, comparing isolates from AIDS patients with isolates from normal volunteers, by biotyping and DNA analysis.

Based on the information obtained in the above investigations in which monocytes from AIDS patients as well as monocytes infected with HIV in vitro were shown to have functional and phenotypic abnormalities, our scientists in collaboration with NCI scientists have focused on potential therapeutic modalities directed at monocytes. In this regard, dipyridamole (DPM) has been shown to potentiate the inhibitory effects of 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxycytidine against HIV-1 in human monocyte-macrophages. DPM, commonly used as a coronary vasodilator and inhibitor of



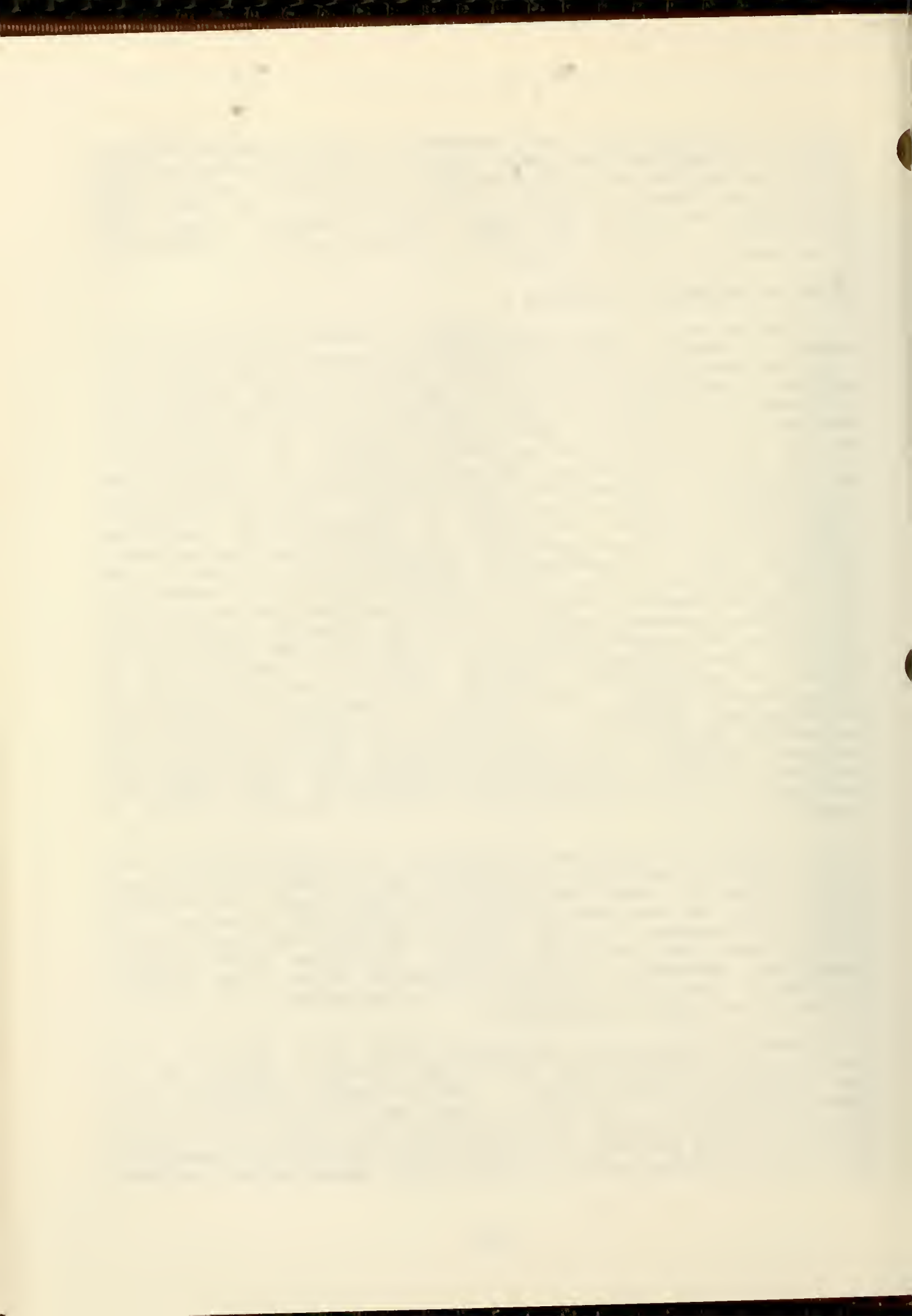
platelet aggregation in the treatment of cardiovascular disease, does not potentiate the toxic effects of AZT on monocytes or on human bone marrow (granulocyte-monocyte) progenitor cells. Since monocyte-macrophage lineage cells appear to be the major reservoir for HIV-1 in vivo, these findings suggest the possibility of using DPM or its analogues in combination chemotherapy of HIV infections.

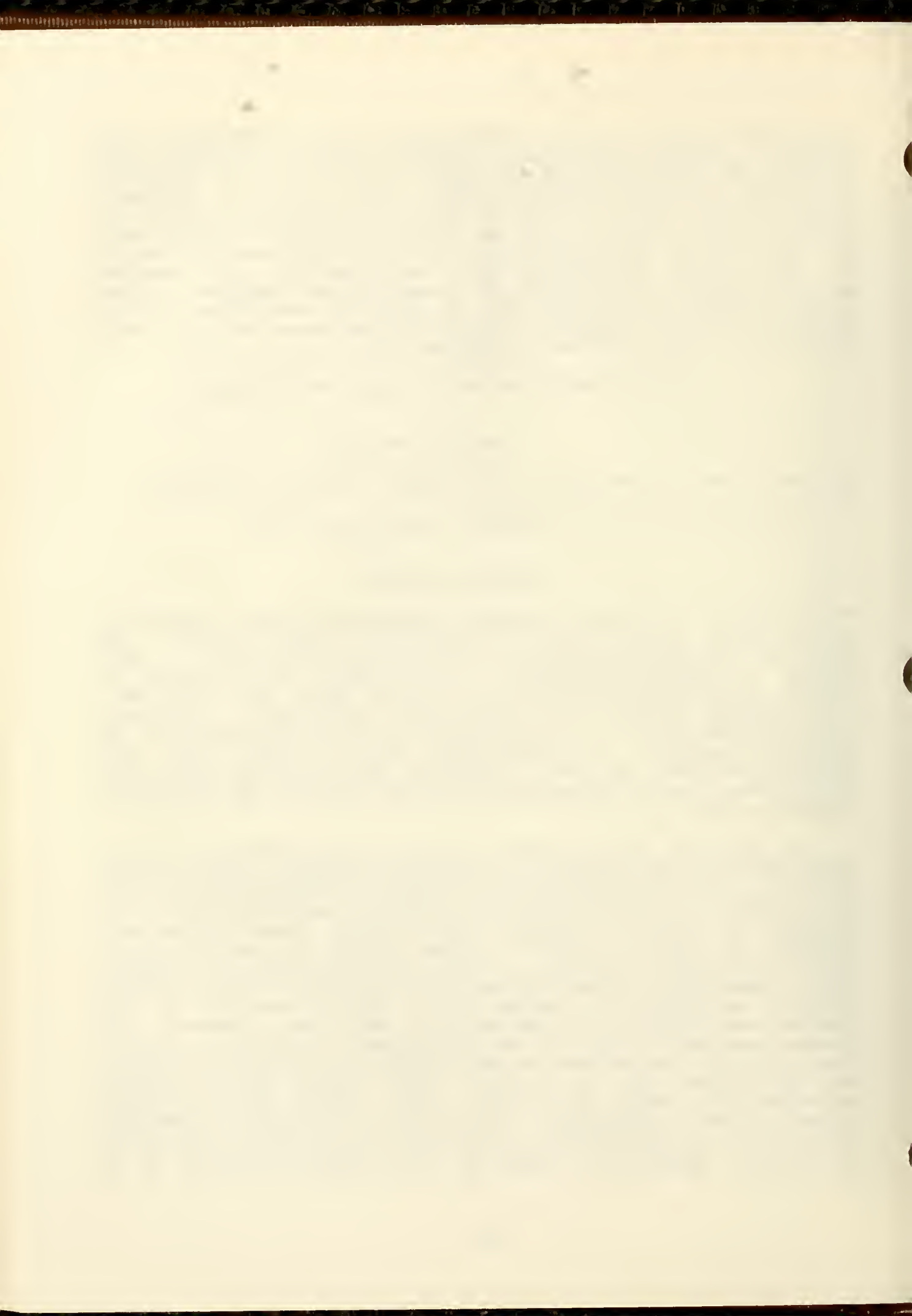
CLINICAL IMMUNOLOGY SECTION.

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

A number of monoclonal antibodies have been isolated that bind to the high affinity IgE receptor on rat basophilic leukemia cells. One of these antibodies has been used for affinity purification of the receptor and the associated proteins. The amino acid sequence from the purified protein was then used to isolate the cDNA for both the rat and human high affinity receptors. Presently affinity purification of the other receptor proteins is underway to identify other components that might be critical for signal transduction.

Several monoclonal antibodies have been isolated that inhibit the binding of IgE to its high affinity receptor on the rat basophilic leukemia cell. One of these antibodies, AA4, inhibits histamine release, but does not block the binding of other anti-IgE receptors to the cell surface. The number of AA4 molecules bound per cell is 14 times the number of IgE receptors. As shown by immunostaining of thin layer chromatograms, antibody





increase in the amount of polymerized actin within the cells. This action is associated with the plasma membrane, and is concentrated in the surface folds and ruffles. Using monoclonal antibodies against two other cytoskeletal components, tubulin and vimentin, it has been shown that while there is a redistribution of microtubules and intermediate filaments following AA4 binding, both of these cytoskeletal components remain confined to the cell body and show no direct association with the plasma membrane. Additional studies are now in progress to further define the relationship of both the Fc_γ receptor and the antigen for AA4 to the cytoskeleton, especially actin and actin binding proteins. Examination of RBL-2H3 cells stimulated with phorbol esters, which activate protein kinase C (PKC), revealed that the phorbol esters produced morphological changes almost identical to those seen with AA4, suggesting that AA4 may be acting through a similar mechanism. Studies using staurosporine, a specific inhibitor of PKC, prevent the AA4 induced changes in a dose dependent manner. The distribution of PKC in the RBL-2H3 cells has also been investigated by fluorescent image analysis using a fluorescent phorbol ester derivative, and the Meridian ACAS 470 Interactive Laser Cytometer. When the cells are exposed to the phorbol ester, it binds to the free PKC in the cytosol. Following activation of the Fc_γ receptor, the PKC is translocated to the plasma membrane where it binds, and is no longer available to bind the dye. In control RBL-2H3 cells the majority of the fluorescent dye is concentrated in the cell body in association with secretory granules. Thirty minutes following stimulation of IgE sensitized cells with antigen, the average fluorescence intensity per cell is reduced to $44\% \pm 7.7\%$ of control values. In comparison, when the cells are exposed to AA4 for 30 minutes before staining, the average fluorescence intensity per cell drops to $27\% \pm 2.5\%$ of controls. This indicates that binding of AA4 to the cell surface is affecting PKC distribution. Studies are in progress to determine if binding of AA4 also influences other biochemical processes such as myoinositol turnover and diacylglycerol production. Another aspect of AA4 which was investigated during the past year, was its distribution in vivo. Light microscopic immunocytochemical techniques were employed to determine the specificity of binding of AA4 in adrenal glands, brain, bone marrow, epididymis, kidney, liver, lung, pancreas, parotid glands, skin, small intestine and testis from adult rat. The distribution of AA4 in both fixed and unfixed tissue sections was examined by immunofluorescence, immunoperoxidase and immunogold methods. Plastic embedded sections examined by immunogold were counterstained for mast cells. In all tissues examined, with the possible exception of bone marrow, the only cells which stained with AA4 were mast cells. In the bone marrow, cells which could not conclusively be identified as mast cells, but which may be mast cell precursors, were also stained with the AA4. Studies are in progress to identify the cells in the bone marrow which stain with the AA4 and to determine the distribution of AA4 in fetal tissue.

The changes in the cytoskeleton of RBL-2H3 cells following

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

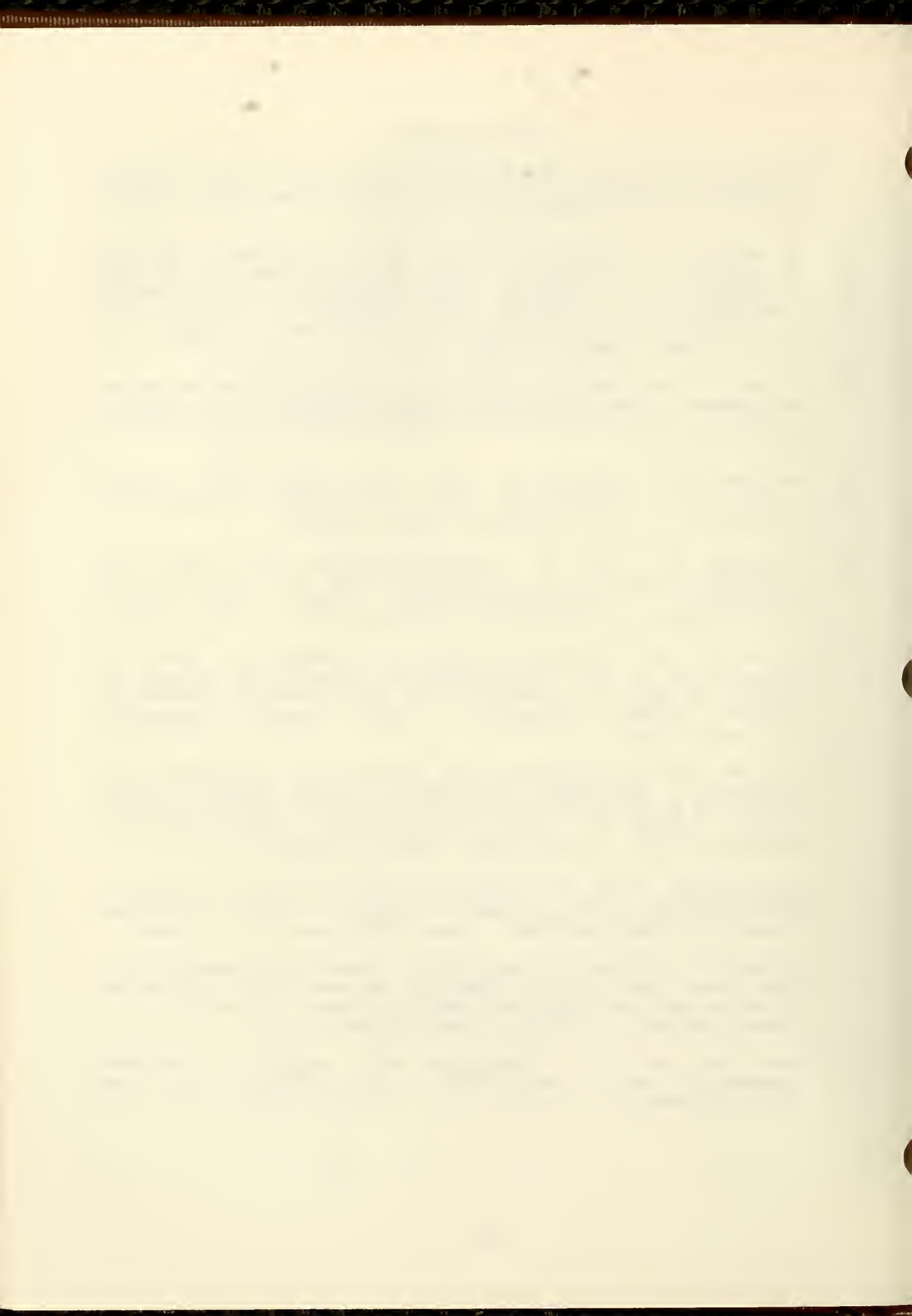
The internalization of ligands to the Fc_γ receptor has been followed both by electron microscopy and by density gradient centrifugation. The fate of an antibody to the receptor (BC4) and either IgE or its antigen (DNP-HSA) were followed. Both ligands were internalized in a receptor mediated fashion and by 1-2 hours after exposure to the tracers, essentially all of it was localized in lysosomes. Current emphasis is on determining if just the ligand or if both receptor and ligand are delivered to lysosomes. RBL-2H3 cells were iodinated, in order to label cell surface receptors, prior to exposure to BC4. The cells were allowed to internalize the BC4 for two hours and then fractionated on Percoll density gradients. BC4 was then immunoprecipitated from the lysosomal fraction, using anti-mouse IgG. Following separation on polyacrylamide gels, ¹²⁵I labeled Fc_γ receptor internalized from the cell surface was present along with the BC4 in the lysosomal fraction. Therefore following stimulation with antibody, receptor and ligand are both delivered to the same compartment. Studies are now in progress to determine if the Fc_γ receptor is also delivered to lysosomes following IgE mediated histamine release.

A monoclonal antibody, mAb AD1, was found that recognizes a novel cell surface protein on rat basophilic leukemia cells (RBL-2H3). At high concentration this antibody caused direct histamine secretion (30% release at 100 μg/ml). When cells were exposed to lower concentrations the mAb AD1 inhibited IgE-mediated but not calcium ionophore-induced histamine release (50% inhibition at 3 μg/ml). It also inhibited the IgE-mediated increase in cell-associated Ca²⁺, arachidonic acid release and the breakdown of phosphatidylinositol. F(ab)₂ fragments of this mAb inhibited histamine release, but the Fab was not active. This antibody also inhibited histamine release from rat

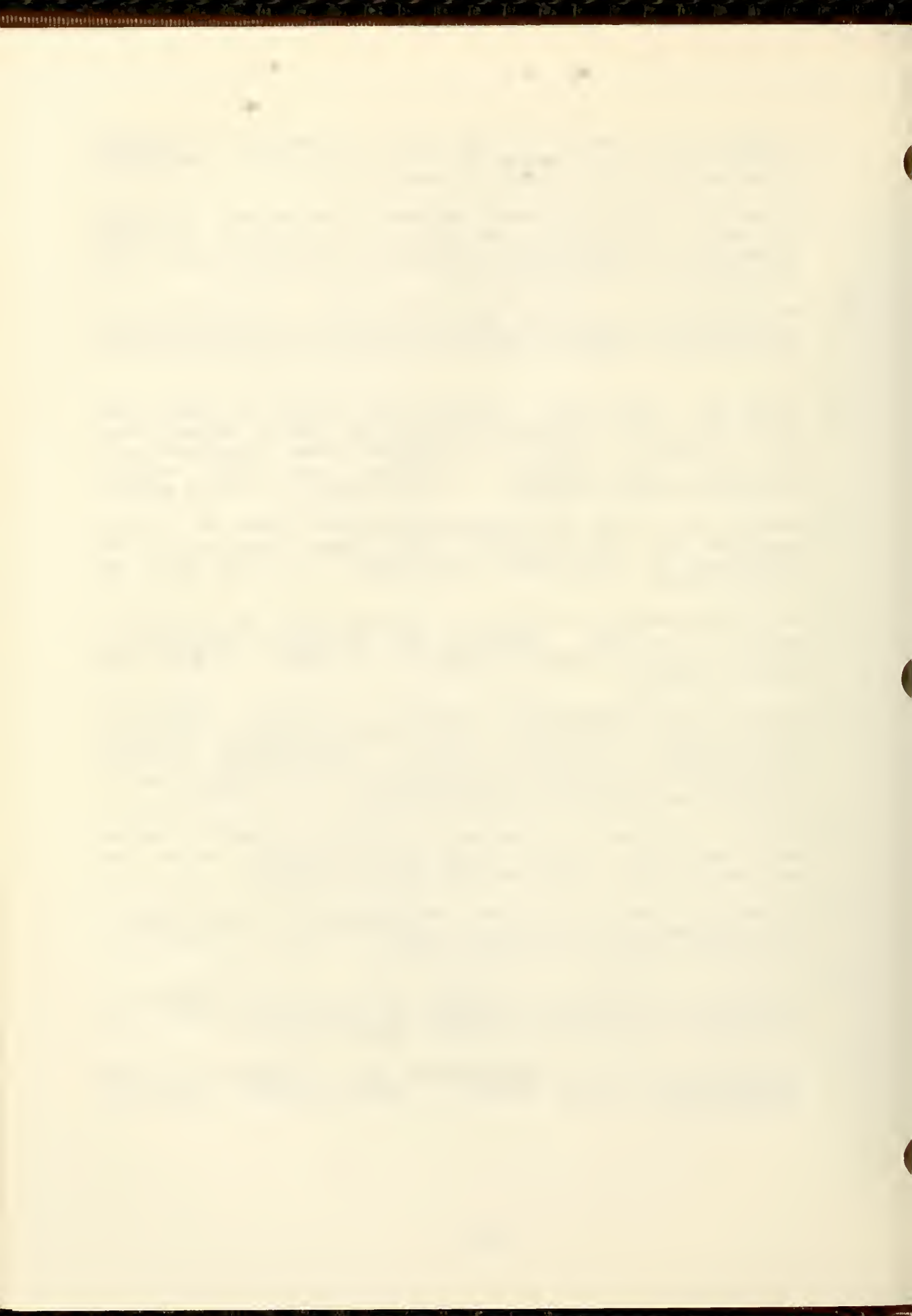
peritoneal mast cells and the in vivo passive cutaneous reaction. The mAb AD1 did not inhibit the binding of IgE nor of several anti-IgE receptor antibodies. In reciprocal binding experiments, IgE did not inhibit mAb AD1 binding; but several other anti-receptor antibodies inhibited binding only when intact but not as Fab fragments. Therefore, the sites on the cell surface to which this antibody binds are probably close to the high affinity IgE receptor. The mAb AD1 immunoprecipitated a 50-60 kDa broad band from ¹²⁵I-surface labeled RBL-2H3 cells that was different from the previously recognized components of the high affinity IgE receptor. Therefore, this newly recognized cell surface protein is different from known IgE receptor proteins and plays a role in the IgE-mediated histamine release from RBL-2H3 cells.

PUBLICATIONS

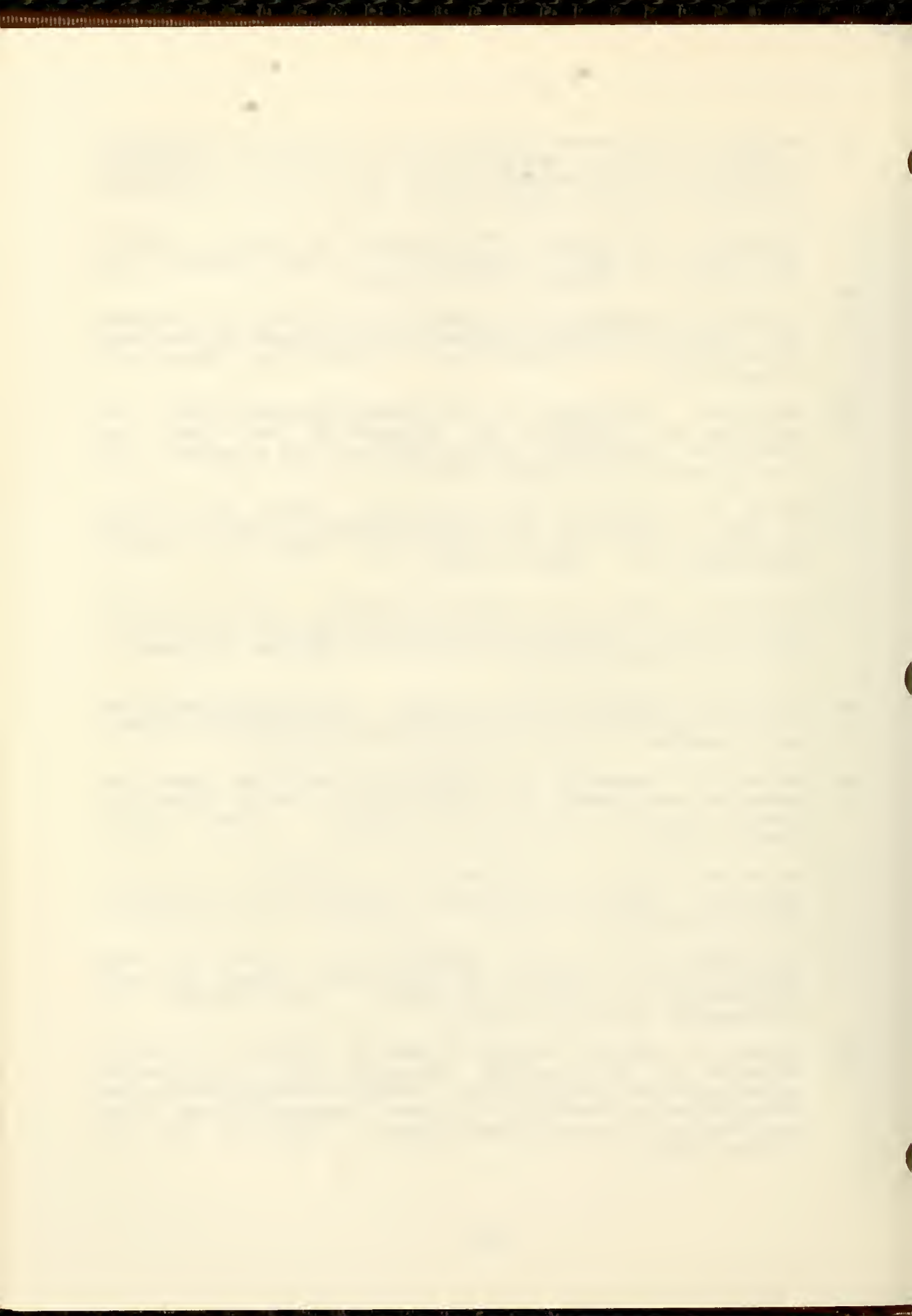
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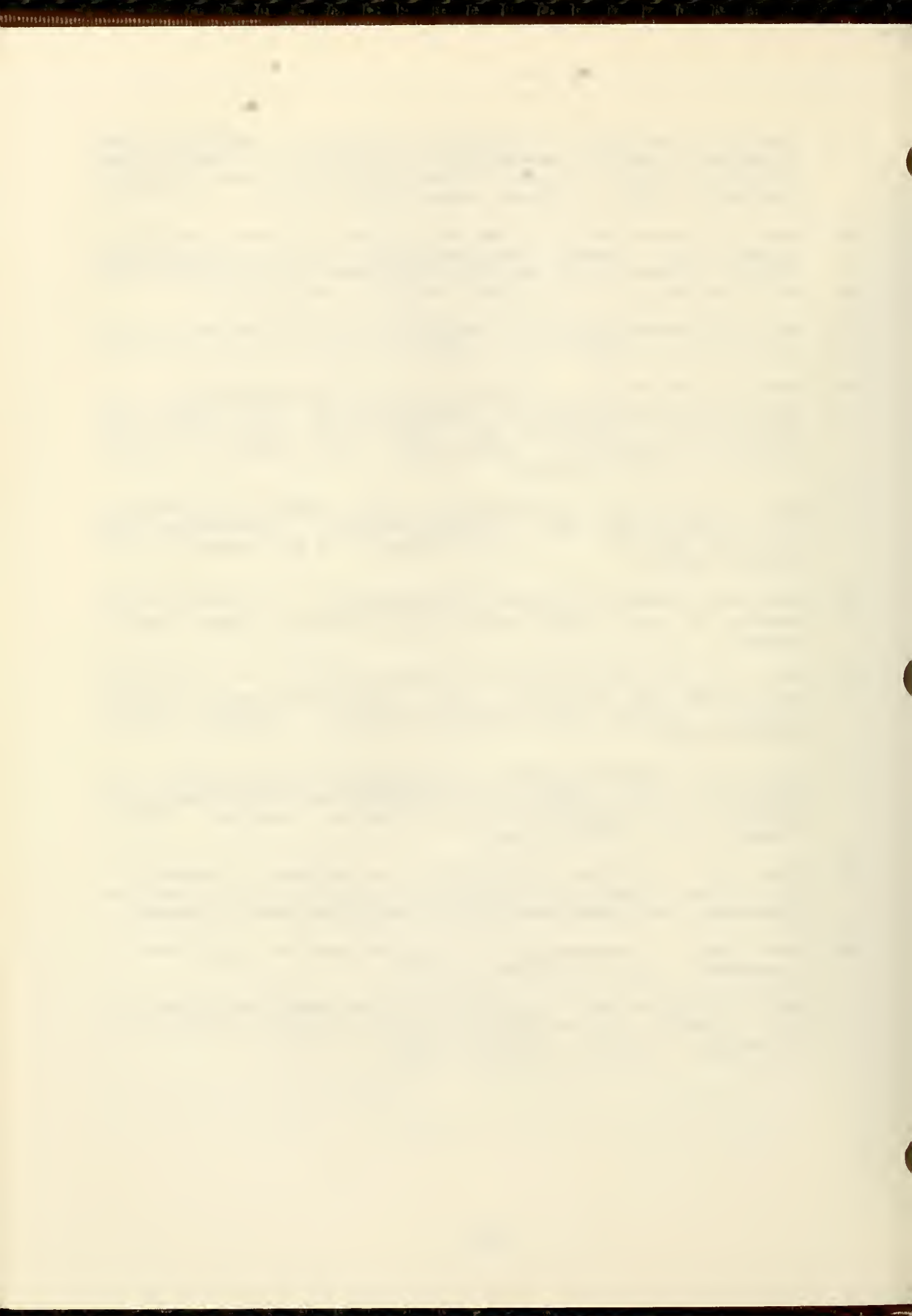
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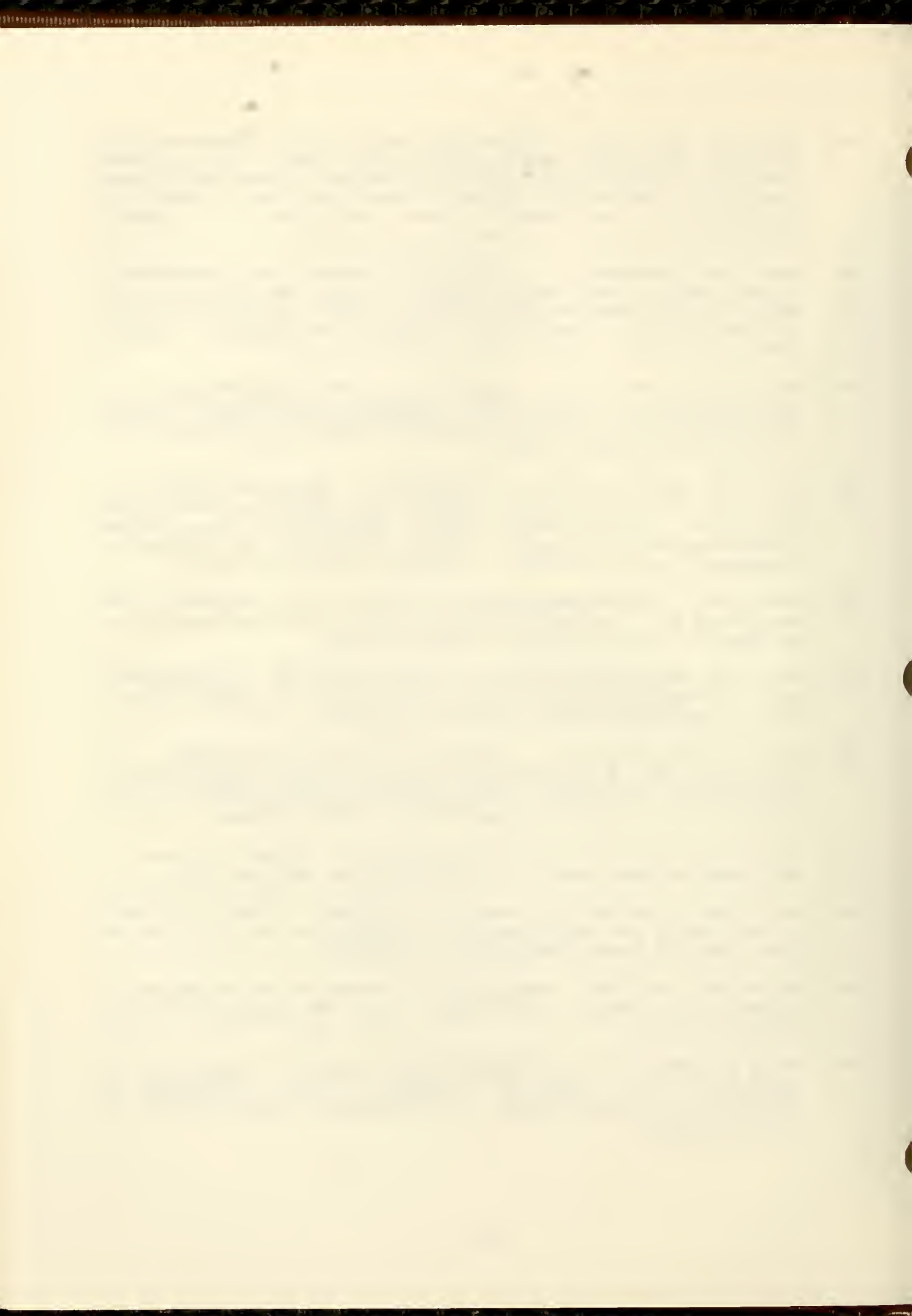
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00034-21 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (60 characters or less Title must fit on one line between the borders)

Mechanisms of Histamine Release

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

Siraganian, Reuben P	Chief, Clinical Immunology	LI NIDR
Book, William A	Research Microbiologist	LI NIDR
Berenstein, Elsa H	Microbiologist	LI NIDR
Kitani, Seiichi	Visiting Fellow	LI NIDR
Benhamou, Mark	Visiting Fellow	LI NIDR
Stephan, Volker	Guest Worker	LI NIDR
Jamawy, Majed M	IRTA	LI NIDR
Osuji, Shizuka	Guest Worker	LI NIDR

COOPERATING UNITS (if any) M. Beaven, LCP, NHLBI, NIH.

I. Karten, IDCPR, NICHD, NIH

W. Reinhold and G. Her, Harvard School of Public Health, Div. of Biol. Sci.

J. Guo and V. Ginsburg, LSB, NIDDK, NIH.

LAB BRANCH

Laboratory of Immunology

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.55

PROFESSIONAL

3.65

OTHER

.90

CHECK APPROPRIATE BOX(ES)

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

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

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, the anaphylatoxins, LHRH peptides, and the Ca²⁺ ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DE-00046-18 LI

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Chronic inflammation and immunomodulation

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

Wahl, Sharon	Chief, Cellular Immunology Section	LI NIDR
Allen, Janice	Chemist	LI NIDR
Dougherty, Suanne	Microbiologist	LI NIDR
Wong, Henry	Staff Fellow	LI NIDR
Welch, Glenn	Biologist	LI NIDR
Venkateshan, C.	Visiting Scientist	LI NIDR

COOPERATING UNITS (if any)

I. Katona, USUHS; L. Ellingsworth, Collagen Corporation, S. Gartner, M. Popovic, NCI, J. Weinstein, J. Szebeni, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

4.5

PROFESSIONAL.

2.60

OTHER.

1.90

CHECK APPROPRIATE BOX(ES)

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

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

This project focuses on elucidating the contributions of lymphocytes and monocytes to the initiation, perpetuation and resolution of an immune response in order to define potential mechanisms of modulation of cellular immune sequelae. Enhanced phagocytic activity, essential to host defense, is dependent upon recognition of foreign substances, microbes and tumor cells by receptors for the Fc region of IgG (Fc γ R). Circulating monocytes express Fc γ RI and Fc γ RII, identified by specific monoclonal antibodies, but only express Fc γ RIII during maturation. The appearance of Fc γ RIII on monocytes in inflammatory lesions prompted a search for cytokines which might be capable of upregulating these receptors. Among the inflammatory cytokines evaluated, only transforming growth factor beta (TGF- β) induced gene expression and translation of Fc γ RIII on monocytes.

Whereas normal circulating monocytes do not express Fc γ RIII, circulating monocytes from AIDS patients have Fc γ RIII which may facilitate uptake of HIV by enhancing antibodies. Additionally, AIDS patients' monocytes expressed interleukin 2 receptors (IL-2R), while monocytes from normal subjects were IL-2R negative. By Northern analysis, monocytes from AIDS patients, but not control subjects, constitutively expressed steady state levels of IL-2R mRNA. Functionally, the IL-2R monocytes could deplete IL-2 from culture supernatants, suggesting a mechanism for the reduced IL-2 levels commonly seen in AIDS patients. IL-2R monocytes also expressed increased levels of surface HLA-DR which may favor monocyte T-cell interactions and the transmission of HIV. In addition, targeting of these HIV-1 infected monocytes with anti-viral agents suggests a new approach to therapy.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00199-13 LI

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

In Vitro Studies of Secretory Cell Structure and Function

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

Oliver, Constance	Research Biologist	LI NIDR
Waters, Judith F	Biologist	LI NIDR
Weedon, Lynda L	Biologist	LI NIDR
Fujimura, Akira	Visiting Fellow	LI NIDR
Banks, Sandra K	Special Volunteer	LI NIDR

COOPERATING UNITS (if any)

Dr. A. Robbins, LBM, NIDDK

LAB/BRANCH

Laboratory of Immunology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.58

PROFESSIONAL:

1.00

OTHER:

1.58

CHECK APPROPRIATE BOX(ES)

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

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

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



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00290-10 LI

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Production of Hybridomas

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

Siraganian, Reuben P	Chief, Clinical Immunology	LI NIDR
Mergenhagen, Stephan E	Chief, Laboratory Immunology	LI NIDR
Hook, William A	Research Microbiologist	LI NIDR
Berenstein, Elsa H	Microbiologist	LI NIDR
Fischler, Cynthia	Bio. Lab. Tech. Microbiology	LI NIDR
Zinsser, Frank U	Guest Worker	LI NIDR
Kitani, Seiichi	Visiting Fellow	LI NIDR
Bishop, Brian	Biologist	LI NIDR

COOPERATING UNITS (if any) P. Tempro, F. Cassels and J. London, LI, NIDR, NIH.
S. Shaw, Y. Shimizu and G.A. Van Seventer, EIB, NCI, NIH.
L. Wahl, LI, NIDR, NIH; A.R. Hand, CIPC, NIDR, NIH.
M. Mednieks, Dept. Mol. Biology, Northwestern Univ. Med. School., Chicago, Ill.

LAB/BRANCH
Laboratory of Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.55	1.90	2.65

CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

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

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fc_γ receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00392-06 LI

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Monocyte/Macrophage Function in the Acquired Immunodeficiency Syndrome

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

Smith, Phillip D., M.D.	Senior Staff Fellow	LI, NIDR
Wahl, Sharon M., Ph.D.	Chief, Cellular Immunology Section	LI, NIDR
Wahl, Larry M., Ph.D.	Microbiologist	LI, NIDR
Lamerson, Cindy L.	Biologist	LI, NIDR
Janoff, Edward N.	Visiting Scientist	LI, NIDR
Saini, Romi	Biologist	LI, NIDR
Mai, Uwe, Ph.D.	Guest Researcher	LI, NIDR

COOPERATING UNITS (if any) Dr. J.M. Orenstein, George Washington Medical Center, Dr. M. Popovic, NCI; Dr. S. Gartner, NCI; Dr. R. Yarchoan, NCI; Dr. S. Broder, NCI; Dr. A. Suffridini, NIAID; Dr. H. Masur, NIAID; Dr. M.S. Chernick, NIDDK

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

6.05

PROFESSIONAL

3.35

OTHER

2.7

CHECK APPROPRIATE BOX(ES)

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

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

The monocyte/macrophage plays a critical role in host defense against many intracellular and extracellular pathogens. The focus of this laboratory has been to elucidate mechanisms of interaction between this cell and certain pathogens including human immunodeficiency virus (HIV), cytomegalovirus (CMV), Candida albicans and Campylobacter pylori. In related studies, we are investigating mechanisms for augmenting monocyte/macrophage effector and accessory cell functions in in vitro studies and in patients.





1111 1 1000

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00424-04 LI

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Regulation of Cytokine Expression in Immunological Disorders

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

McCartney-Francis, Nancy	Senior Staff Fellow	LI	NIDR
Wahl, Sharon	Chief, Cellular Immunology	LI	NIDR
Wahl, Larry	Microbiologist	LI	NIDR
Mizel, Diane	Chemist	LI	NIDR
Dougherty, Suanne	Microbiologist	LI	NIDR
Wong, Henry	Staff Fellow	LI	NIDR

COOPERATING UNITS (if any)

M. Norcross, FDA; S. Gartner, M. Popovic, A. Roberts, M. Sporn, NCI, NIH.

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

3.5

PROFESSIONAL

1.2

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The aim of this research program is to define the cytokines elaborated by the cells involved in the inflammatory process and in various disease states and to describe the molecular mechanisms that regulate cytokine gene expression. The transforming growth factor betas (TGF- β) belong to a family of multifunctional factors which can affect critical cellular functions either directly or indirectly through the induction of other mediators. In addition, TGF- β 1 upregulates the steady-state expression of monocyte TGF- β 1 RNA, suggesting that TGF- β 1 can further amplify an inflammatory response via an autocrine mechanism. This autoinduction of TGF- β 1 appears to be regulated by both transcriptional and posttranscriptional mechanisms. A closely related homologue, TGF- β 2, also induces monokine expression and upregulates TGF- β 1 RNA expression. TGF- β 2 RNA, like TGF- β 1, is expressed constitutively in monocytes; however, the expression of TGF- β 2 mRNA is unchanged by TGF- β 1 or β 2 stimulation. These studies suggest that TGF- β 1 and TGF- β 2 expression in monocytes are controlled by separate regulatory pathways. Inappropriate expression of cytokines may play an important role in disease conditions. Dramatic differences in growth factor expression, most notably tumor necrosis factor (TNF- α), by human peripheral blood monocytes occur following exposure to HIV-1, the etiological agent of AIDS. Computer analyses of the DNA sequences of regulatory regions of HIV-1 and the TNF- α gene revealed a 75% sequence similarity in the enhancer regions including the NF- κ B binding site, suggesting that the regulation of TNF- α (and possibly other cellular genes) and HIV-1 expression share a common mechanism. Analyses of nuclear proteins demonstrated that resting monocytes constitutively express NF- κ B binding proteins and the level of expression is relatively unchanged by the state of activation. Thus it appears unlikely that NF- κ B expression is solely responsible for the functional changes in monocyte activity following HIV-1 infection. However, the constitutive synthesis of NF- κ B binding proteins in circulating monocytes may explain the persistence of HIV-1 in macrophages throughout the clinical course of AIDS.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DE-00441-03 LI

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Characterization and Immunoregulation of Experimentally Induced Inflammation

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

Allen, Janice Benson	Chemist	LI, NIDR
Wahl, Sharon M.	Chief, Cellular Immunology Section	LI, NIDR
Feldman, Gerald	NRSA Research Associate	LI, NIDR
Mergenhagen, Stephan	Chief, Laboratory of Immunology	LI, NIDR
Wright, Lori	Stay-in-School Student	LI, NIDR
Skaleric, Uros	Visiting Associate	LI, NIDR
Manthey, Carl P.	PRAT	LI, NIDR

COOPERATING UNITS (if any)

E. Amento, Genentech; J. Coffey, Hoffman LaRoche; A. Hand, CIPCB, NIDR;
L. Ellingsworth, Collagen Corporation; R. Simmons, Syntex.

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

5.13

PROFESSIONAL.

1.53

OTHER:

3.6

CHECK APPROPRIATE BOX(ES)

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

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

A single intraperitoneal injection of Group A streptococcal cell walls (SCW) induces a biphasic pattern of polyarthritis and hepatic granulomas in susceptible rats. We have continued to explore the cellular and molecular events leading to these chronic inflammatory lesions in order to provide insight into potential modulation of these pathologic events. To determine the role of reactive oxygen intermediates in the development of joint pathology, polyethylene glycol (PEG)-conjugated superoxide dismutase (SOD) or PEG-catalase was injected directly into one of the hind joints. A single injection of SOD given with the SCW significantly suppresses the acute and chronic synovitis. Furthermore, SOD given 10 days after SCW inhibits the subsequent mononuclear cell-mediated pathology. Catalase was less effective than SOD. In additional studies, based on the demonstrated antiproliferative and immunomodulatory effects of gamma-interferon (γ IFN), the acute and chronic phases are suppressed. This decreased inflammation and tissue destruction by γ IFN is attributed to decreased arachidonic acid metabolism and a significant inhibition of monocyte chemotactic activity, with a >80% decrease in cell surface C5a receptors. γ IFN is currently undergoing phase I clinical trials in arthritis patients. In related studies, identification of a potent immunomodulatory cytokine, transforming growth factor-beta (TGF- β), in inflamed synovium and granulomas of SCW-injected rats suggested a role for TGF- β . TGF- β 1 and - β 2 were injected intraarticularly and monitored for their effects on cellular recruitment and activation and potential modulation of pathogenic effects. Swelling and erythema were apparent within 12 hrs, peaking after 3 daily injections. Cessation of TGF- β gradually resulted in a clinically normal joint. Histological examination revealed infiltration of mononuclear cells, activated to express growth factors, which likely regulate the pronounced synovial hyperplasia. Thus, these studies reflect the contributions and regulation of cell-cell interactions in inflammatory lesions, and the potential for therapeutic intervention.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DE-00456-02 LI

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Cell activation: relationship to connective tissue and immunodeficiency diseases

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

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

COOPERATING UNITS (if any)

I. Katona, USUHS; D. S. Finbloom, FDA; W. L. Farrar, NCI; L. O. Arthur, NCI; A. Spiegel, NIDDK; O. Nahor, NIDR; J. Weinstein and J. Szebeni, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.22

PROFESSIONAL

.82

OTHER

1.4

CHECK APPROPRIATE BOX(ES)

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

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

Our research on the biochemical pathways involved in the signal transduction leading to collagenase production by monocytes has focussed on the role of guanine nucleotide proteins (G proteins). Treatment of monocytes with cholera toxin greatly enhanced the induction of PGE2 and collagenase in monocytes stimulated with Con A or LPS. However, cholera toxin had no effect on monocyte PGE2 or collagenase in the absence of Con A or LPS. Cholera toxin is known to ADP-ribosylate the α subunit of Gs and autoradiograms of SDS-PAGE gels of plasma membrane proteins from monocytes demonstrated specific ribosylated bands. Moreover, in stimulated monocytes, but not controls, an additional ADP-ribosylated band was detected. When monocytes were exposed to pertussis toxin and stimulated with Con A or LPS there was a significant suppression of PGE2 and collagenase. ADP-ribosylation experiments revealed that pertussis toxin also ribosylated specific monocyte membrane proteins. Pertussis toxin is known to ribosylate the α subunits of Gi proteins. With antibodies to the specific subunits of G proteins, the monocyte membrane was shown to be positive for Gs α , Gi α 1, Gi α 2, and Gi α 3. In ongoing experiments, these G protein subunits have been shown to be modulated by Con A, LPS and PMA as well as with pharmacological agents suggesting specific roles for these proteins in the activation process in monocytes, particularly as it pertains to the synthesis of collagenase.



with 1 pp. 2



ANNUAL REPORT OF THE LABORATORY OF MICROBIAL ECOLOGY
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Laboratory of Microbial Ecology presently consists of the microbiology and microbial pathogenesis groups whose missions are to identify and characterize cell-to-cell interactions among oral microorganisms, interactions between oral microorganisms and host tissue, and host responses to the oral flora.

MICROBIOLOGY SECTION

Microbial Ecology Group

The microbial ecology group has exploited new initiatives to more completely define the extent of the bacterial interactions that mediate colonization of and plaque formation in the oral cavity and the mechanisms responsible for these interactions. Expanded coaggregation studies have concentrated on the role of three groups of gram negative bacteria, Fusobacterium nucleatum, Porphyromonas (Bacteroides) gingivalis and Veillonella species in establishing a stable oral microflora. F. nucleatum and P. gingivalis are potential pathogens and the Veillonella species occupies an important position in the food chain consuming lactic acid. In contrast to fusobacteria strains, which interact with members of at least ten genera of oral bacteria, P. gingivalis coaggregates only with fusobacteria. The failure of P. gingivalis to interact with other oral bacteria may explain why this microorganism is seen in the later stages of periodontal disease, after fusobacteria have established themselves.

A major effort is underway to identify, isolate and describe adhesins on the surface of oral bacteria. The lactose sensitive adhesin on Bacteroides loeschei fimbriae which recognizes receptors on procaryotes (Streptococcus sanguis 34) and eucaryotic cells has been purified in quantity and is currently being physically and biochemically characterized. The protein can exist as soluble aggregates or in a non-aggregated state and as such agglutinates erythrocytes and streptococcal partner cells or blocks coaggregation with streptococcal cells, respectively. The S. sanguis H1 carbohydrate receptor which interacts with Capnocytophaga ochracea has been purified and its substituent hexasaccharide units completely characterized. Lactose-inhibitable adhesins on F. nucleatum, C. ochracea and V. atypica are currently being identified and characterized as are the lactose non-inhibitable adhesins on Capnocytophaga gingivalis and Actinomyces naeslundii.



Physiology Group

The physiology group has purified to homogeneity a novel NADP-dependent oxidoreductase which, in the presence of pyruvate, converts ornithine or lysine into N⁵-(L-1-carboxyethyl) ornithine or N⁶-(L-1-carboxyethyl) lysine, respectively. This enzyme which is unique to certain group N streptococci (genus Lactococcus) has been characterized physically and its amino acid composition and N-terminal amino acid sequence have been determined. The gene for this unusual synthase appears to be linked to nisin production, nisin resistance and sucrose metabolism. Polyclonal antibodies produced against the enzyme are being used to screen for expression of the cloned synthase gene.

Studies on sugar transport in the gram negative bacterium, Fusobacterium nucleatum, revealed that in addition to the energy source, glutamate, sugar uptake was also dependent on the presence of sodium ion. Li⁺, K⁺, Rb⁺ or Cs⁺ had little or no effect on the accumulation of the hexose. Since lysine could be substituted for glutamate and the lysine driven reaction required no sodium ion, it appeared that the ion requirement was related to glutamate metabolism rather than hexose transport. This unusual hexose transport system has also been demonstrated in other species of fusobacteria including F. varium, F. necrophorum, F. russii and F. gonidiformans.

Molecular Genetics Group

Studies directed at understanding the PTS process by which oral streptococci and lactobacilli transport and phosphorylate sugars continued. This year research focused on an attempt to determine the amino acid residue in the integral membrane protein, EnzymeII, which acts as both a permease and a kinase. The amino acid residue that participates in transphosphorylation at the active center EnzymeII^{lac} of Lactobacillus casei was determined. Replacement of each of the histidine and cysteine residues in EII in turn with other amino acids by site-directed mutagenesis, followed by an analysis of their ability to phosphorylate lactose using an in vitro assay system, revealed that only cysteine-483 is required for enzymatic activity. This residue occurs in the only region of the EnzymeII^{lac} which shares significant sequence conservation with its analogues isolated from streptococci and staphylococci. The conclusion that only cys-483 is required for catalytic activity is the first demonstration of a catalytic cysteine residue in an EnzymeII. No other EnzymeII has been systematically analyzed in this fashion. Current studies are directed at an examination of the transport process per se; that is, a determination of the mechanism by which sugars are bound to, and moved across, the membrane.



In other research, a synthetic operon was constructed from the lac-PTS genes and a strong constitutive promoter isolated from the β -gal gene of L. bulgaricus. The recombinant operon was introduced into lactose negative lactobacilli using electroporation and the HOST-VECTOR system previously developed in this laboratory. Transformants containing the recombinant operon were able to develop on lactose and contained higher levels of the PTS gene products than wild-type cells. In these, and other studies, it was demonstrated that recombinant DNA could be introduced and expressed in oral lactobacilli and streptococci. There have been no previous reports of this kind of recombinant strain construction in lactobacilli. The use of lac genes will allow selectable "food grade vectors" (i.e. those not containing antibiotic resistance markers) to be developed. These, in turn, can be used for in vivo examination of the efficacy of lactobacilli as gene delivery vehicles to mammalian hosts.

PATHOGENIC MECHANISMS SECTION

The research activities of this Section continue to focus on characterization of oral bacterial adhesins and their complementary receptors on tooth surfaces, mammalian cells and other bacteria as well as the biological consequences and intervention of these recognition processes.

New approaches have yielded considerable information concerning the receptors for the Gal/GalNAc reactive lectin associated with the type 2 fimbriae of Actinomyces viscosus T14V on bacteria and mammalian cells. Studies were initiated to examine the receptors for this lectin on HL-60 cells. This cell line can be differentiated towards polymorphonuclear leukocytes (PMNs) by dimethylsulfoxide and this process was monitored by expression of C3b/C3bi receptors as well as morphological changes. A. viscosus T14V bound to both undifferentiated and differentiated HL-60 cells that were treated with sialidase to expose the receptors for the actinomyces lectin. Binding was inhibited by lactose and did not occur to non-sialidase treated cells. A mutant, A. viscosus 147, that lacks fimbriae failed to adhere. A. viscosus T14V, but not A. viscosus 147, was ingested by differentiated HL-60 cells. A 110 kDa putative glycoprotein receptor on the HL-60 cells was identified by binding of radioiodinated plant lectins with specificities similar to that of the bacterial lectin to sialidase treated nitrocellulose transfers of cell extracts separated by SDS-PAGE. The 110 kDa band was detected in extracts of both differentiated and undifferentiated cells indicating that it is a constitutive component of the HL-60 membrane. The radioiodinated plant lectins also detected this band on sialidase treated nitrocellulose transfers of extracts of peripheral blood PMNs but extracts of these cells contained, in addition, lectin reactive bands at 140 and 85 kDa. The 140 kDa band is not considered to be a receptor since it is not labeled by radioiodination of PMN

surface glycoproteins. The absence of the 140 and 85 kDa components in extracts of HL-60 cells offers a distinct advantage for further characterization and purification of this putative receptor. Use of a cell line will also obviate the difficulties associated with donor variability and obtaining large numbers of cells.

Nuclear magnetic resonance spectroscopy has been applied to establishing the structures of the receptors for the actinomyces fimbrial lectin on oral strains of streptococci. These receptors are present on Streptococcus sanguis 34, S. sanguis J22 and S. sanguis 10557. Mutants of these strains selected by their inability to agglutinate with Gal/GalNAc specific plant lectins also lack the receptor for the actinomyces fimbrial lectin. The receptors on the three parent strains are all repeating phosphodiester linked oligosaccharides. Of major interest was the finding that either Gal β 1 \rightarrow 3GalNAc or GalNAc β 1 \rightarrow 3Gal, both of which are recognized by the actinomyces lectin, is found at the reducing end of the oligosaccharide of each strain. This domain, therefore, probably constitutes the receptor portion of the polysaccharide. Structural differences have been detected at the non-reducing ends of the oligosaccharides and these probably reflect the antigenic dissimilarities between the three streptococcal strains.

The distribution of receptors for the actinomyces lectin on more than sixty strains of oral streptococci that represent defined taxonomic groups has been delineated. Many of these strains were obtained from Dr. Mogens Kilian whose extensive studies have resulted in the classification of these streptococci on a genetic basis and have demonstrated distinctive localization of different taxonomic groups within the oral cavity. Of major significance was the finding of the receptor for the actinomyces lectin on all strains of S. oralis. It was also detected on 25% of the S. mitis strains and on 67% of the strains in S. sanguis taxon 4. Generally, similar receptors were absent in S. sanguis taxons 1, 2 and 3 as well as the newly described species S. gordonii. In extending these investigations all of these streptococcal strains were assessed for the presence of a sialic acid binding lectin by hemagglutination of erythrocytes from several mammalian species. The lectin was detected on numerous strains of S. sanguis, S. gordonii and S. oralis but not on S. mitis strains. These findings provide strong support for a correlation between the adherence properties of oral bacteria and their distribution in the oral cavity.

Emphasis has also been placed on the interaction of bacteria with receptors other than the glycoconjugates recognized by bacterial lectins. Studies in this area have resulted in the development of new concepts concerning the role of cleavage products of the third component of complement (C3) in the initiation and enhancement of phagocytosis of actinomyces by PMNs. Previous investigations in



other laboratories have not clarified the consequences of PMN recognition by bacteria opsonized with C3 fragments (C3b/C3bi) in the absence of other attachment mechanisms. C3b/C3bi was bound to the actinomyces following activation of the classical complement pathway by IgM, an antibody class for which there are no PMN receptors. PMNs, on which the receptors for the actinomyces lectin remained blocked by the omission of sialidase, killed C3b/C3bi coated A. viscosus T14V. Moreover, A. viscosus 147, a mutant that lacks the lectin, was also killed when opsonized with these C3 fragments. The latter strain does not interact with PMNs prior to opsonization as determined by its inability to bind to the phagocytic cells or to stimulate the production of superoxide anions, release the contents of secondary granules, generate hydrogen peroxide or chemiluminesce. These findings indicate that C3b/C3bi can be the primary initiating factor involved in phagocytosis by PMNs.

A previously undescribed concept concerning cooperativity between a bacterial lectin and C3b/C3bi in the initiation of PMN mediated bactericidal activity has also been detected. Lectin dependent ingestion of A. viscosus T14V resulted in a marked decrease in the number of viable bacteria. If, in addition, the bacteria were opsonized with C3b/C3bi killing was dramatically increased. Thus, the complement system is clearly implicated in PMN mediated host defense against the oral actinomyces. In vivo, C3b/C3bi could be bound to the bacteria as a result of IgM activation of the complement sequence, as shown experimentally, or these C3 fragments could be deposited through activation of the alternative complement pathway in the absence of immunoglobulins, an activity that has been attributed to the oral actinomyces.

Previous studies in this laboratory have clearly demonstrated that the PMN responses evoked by different bacterial lectins are not identical. Thus, the Gal/GalNAc reactive lectin of the oral actinomyces initiates ingestion and killing of the bacteria and also stimulates the release of superoxide anions and the contents of secondary, but not primary, granules. In contrast, the sialic acid reactive lectin of certain oral streptococci initiates ingestion and the release of superoxide anions and secondary granule contents but the bacteria are not killed. Additional evidence for divergent pathways of PMN activation by bacterial lectins has been obtained. The actinomyces lectin stimulates PMN chemiluminescence that is inhibitable by lactose. No specific chemiluminescence is observed following the incubation of the streptococci with PMNs. Examination of potential PMN microbicidal systems revealed that the actinomyces lectin stimulated the release of hydrogen peroxide but the streptococcal lectin was devoid of this activity. Thus, the responses initiated by the actinomyces lectin contribute both to inflammation of host tissues and destruction of the bacteria whereas the streptococcal lectin can induce inflammation without affording any protection to the host.



Quantitative immunochemical studies relating to the potential intervention of bacterial attachment by immunoglobulins have advanced significantly. Antibodies were raised against the subunits of the two types of fimbriae on A. viscosus T14V that mediate attachment to the tooth pellicle (type 1) or to glycoconjugates on other bacteria and mammalian cells (type 2). These subunits, each of which is a 54 kDa protein, were obtained by cloning and expression of the respective genes in Escherichia coli. The antibodies were characterized and compared with antibodies against each type of intact fimbriae for their effects on fimbriae mediated adherence processes. The antibody against the type 1 subunit inhibited adsorption of A. viscosus T14V to saliva treated hydroxyapatite but its Fab fragment was inactive. Identical results were obtained with an antibody to the intact type 1 fimbriae. This antibody appeared to be directed exclusively against the subunit. However, the Fab fragment of a second antibody raised against the intact fimbriae in a different rabbit did inhibit attachment. This antibody was not completely neutralized by the type 1 subunit. These latter findings were also observed with antibodies against the type 2 fimbriae in that the Fab fragment of an antibody against the intact fimbriae blocked the fimbrial associated lectin activity whereas the Fab fragment of an antibody against the type 2 subunit was inactive. Thus, subpopulations of antibodies against the intact type 1 or type 2 fimbriae apparently recognize, in addition to epitopes present on the respective subunits, structural features exposed only on the intact fimbriae or minor components associated with the intact fimbriae that mediate adherence. Studies are in progress to define the specificities of these antibody subpopulations that apparently react with the adhesins of the two types of fimbriae.



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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00042-19 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Molecular biological characterization of oral bacteria

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

Chassy, Bruce M.	Research Chemist	LME, NIDR
Flickinger, Jeannette L.	Microbiologist	LME, NIDR
Alpert, Carl-Alfred	Visiting Associate	LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

3.00

PROFESSIONAL:

2.00

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

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

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

This project seeks to use genetic, biochemical and physiological approaches to investigate the pathogenicity of oral bacteria. The two specific areas of investigation are: 1) characterization of genes isolated from oral bacteria; and 2) development of systems for genetic exchange in oral bacteria. The amino acid residue that participates in transphosphorylation at the active center EnzymeII(lac) of Lactobacillus casei was determined. Replacement of each of the histidine and cysteine residues in EII with other amino acids by site-directed mutagenesis, followed by an analysis of their ability to phosphorylate lactose using an in vitro assay system, revealed that only cysteine-483 is required for enzymatic activity. Using the HOST-VECTOR system previously developed for the genetic manipulation of lactobacilli, the expression of a number of heterologous genes has been examined. Of particular interest, the genes encoding the EnzymeII(lac), P-β-gal and Factor III(lac) of L. casei were transcriptionally fused to the strong constitutive β-galactosidase promoter isolated from L. bulgaricus to create an operon-like structure which was inserted into the shuttle vector pBS19 forming pBS914. This construction was transformed into lactose negative L. casei strains; transformants were selected by their ability to grow on lactose. Efforts are now directed at construction of food grade vectors with lactobacilli used in food fermentations, or isolated from the gastrointestinal tract, for gene delivery and antigen presentation to humans.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00043-19 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Physiological and genetic studies on pathogenic oral microorganisms

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

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

COOPERATING UNITS (if any)

Georgetown University School of Medicine, Washington, DC; University of Maryland Dental School, College Park, MD

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

1.70

PROFESSIONAL

.70

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

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

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

The fimA gene, which encodes a subunit of type 2 fimbriae from the oral gram-positive microbe Actinomyces viscosus T14V, has been expressed at a high level using the bacteriophage T7 promoter/RNA polymerase system in Escherichia coli. The 53-kDal protein has been purified to apparent homogeneity from such recombinant cells and used to raise antibody in order to more precisely delineate the relationship between the functional and antigenic properties of type 2 fimbriae and the cloned subunit. In Western immunoblots of partially dissociated type 2 fimbriae anti-subunit IgG revealed the same ladder-like pattern of bands as seen with IgG against type 2 fimbriae, which indicates that these fimbriae consist of only one major component. Enzyme-linked immunosorbent assays showed that the cloned subunit and native type 2 fimbriae were antigenically very similar in their reactivity with homologous and heterologous Fab fragments (Fabs) directed at either intact fimbriae or the cloned subunit. Competitive binding studies revealed that most of the epitopes recognized on A. viscosus by anti-fimbriae Fabs also were detected by anti-subunit Fabs. However, and in contrast to results obtained with anti-fimbriae Fabs, anti-subunit Fabs did not inhibit the lectin-mediated coaggregation of A. viscosus with Streptococcus sanguis. Thus, it appears that the lectin activity associated with actinomyces type 2 fimbriae is not expressed by the subunit and may well be a minor, but distinct, component of these fimbriae.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00061-18 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Complement activation and inflammation

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

Sandberg, Ann L.	Chief, Pathogenic Mechanisms Section	LME, NIDR
Lyman, Caron A.	Staff Fellow	LME, NIDR
Joralmon, Richard A.	NRSA Fellow	LME, NIDR
Mudrick, Linda L.	Microbiologist	LME, NIDR
Cisar, John O.	Research Microbiologist	LME, NIDR
Cureton, Charlette P.	Secretary (Typing)	LME, NIDR

COOPERATING UNITS (if any)

University of Colorado

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Pathogenic Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

3.70

PROFESSIONAL

2.15

OTHER.

1.55

CHECK APPROPRIATE BOX(ES)

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

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

New concepts concerning the function of the third component of complement (C3) in phagocytosis of oral actinomyces by PMNs have been developed. Actinomyces viscosus T14V carrying C3b/C3bi deposited by IgM and the earlier acting complement components was killed by PMNs. The apparent absence of IgG in this system and killing of a similarly treated mutant that lacks the Gal/GalNAc lectin strongly suggest that destruction of the bacteria is initiated by recognition of PMN C3b/C3bi receptors. A previously undescribed cooperative effect between a bacterial lectin and C3 in the initiation of bactericidal activity was also delineated. Lectin dependent killing of A. viscosus T14V was dramatically enhanced if the bacteria were opsonized with C3b/C3bi. Utilization of the HL-60 cell line that can be differentiated towards PMNs has provided a new approach to identifying and characterizing the receptors for bacterial lectins. Extracts of HL-60 cells were subjected to SDS-PAGE, transferred to nitrocellulose filters and developed with radioiodinated plant lectins with specificities similar to that of the actinomyces lectin. A single major lectin reactive band was detected at approximately 110kDa. Additional parameters of PMN activation have further delineated the divergent pathways of phagocytic cell stimulation initiated by the actinomyces lectin and a sialic acid reactive lectin on certain strains of oral streptococci. Both lectins stimulate the release of inflammatory mediators from PMNs but only the actinomyces are killed by the phagocytic cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
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PROJECT NUMBER

Z01 DE 00254-12 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Microbial antigens associated with specific adherence

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

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

COOPERATING UNITS (if any)

University of Florida; University of Colorado; University of Maryland;
 Royal Dental College Aarhus, Aarhus, Denmark

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Pathogenic Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

2.10

PROFESSIONAL.

1.10

OTHER.

1.00

CHECK APPROPRIATE BOX(ES)

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

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

Rabbit antibodies against the 54 kDa structural subunits of Actinomyces viscosus T14V type 1 and type 2 fimbriae have now been characterized for their effects on fimbriae-mediated bacterial adherence. Immune IgG formed against the type 1 fimbrial subunit blocked bacterial adherence to saliva-treated hydroxyapatite but the Fab of this antibody did not. Similarly, the Fab of a rabbit antibody directed against the 54 kDa type 2 fimbrial subunit failed to inhibit the lectin mediated coaggregation of A. viscosus with Streptococcus sanguis 34. In contrast, this coaggregation was inhibited by the Fab of an antibody against type 2 fimbriae. This activity involved a minor subpopulation of the total anti-fimbriae antibody. The coaggregation receptor polysaccharide of S. sanguis J22 was identified, isolated and its structure determined. The polysaccharide was composed of a repeating, phosphodiester-linked heptasaccharide. The site of lectin recognition appeared to be Gal β 1-3GalNAc at the reducing end of the oligosaccharide chain. The adherence properties of over 60 oral streptococcal strains representing well defined taxonomic groups was also examined. Many strains previously identified as S. sanguis were reclassified as S. oralis. An ecological property of the latter species is the expression of receptors for lactose-sensitive coaggregations with Actinomyces spp. The association specific adherence properties with distinct taxonomic groups of oral streptococci may play a significant role in determining the distribution of these bacteria within the oral cavity.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00273-11 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Cell-cell interactions between oral actinomyces and other bacteria

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

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

COOPERATING UNITS (if any)

Virginia Polytechnic Institute and State University, Blacksburg, VA; University of British Columbia, Vancouver, Canada; Tel Aviv University, Tel Aviv, Israel; and Georgetown University, Washington, D.C.

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

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2.00

OTHER:

1.25

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

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

As additional information regarding cell-to-cell recognitions among oral bacteria is discovered, it is becoming increasingly clear that a dynamic but organized microbial community exists in the oral cavity. The concept of highly specific partnerships advanced by this laboratory gained additional support with the evidence that Porphyromonas (Bacteroides) gingivalis and Selenomonas spp. coaggregate only with Fusobacterium nucleatum and not with the other ten genera tested.

Coaggregation between F. nucleatum PK1594 and P. gingivalis PK1924 is inhibited equally well by lactose, galactose, and N-acetylgalactosamine (50% inhibition at 2 mM), which is a third general kind of lactose-inhibitable coaggregation observed with oral bacterial partnerships. Coaggregation between S. sanguis PK488 and A. naeslundii PK606 appears to be mediated by an adhesin of 38 kD, which also recognizes salivary receptors. This is the first report of a dual adhesive function of a surface protein which recognizes a cellular and noncellular receptor. Coaggregation between V. atypica PK1910 and Streptococcus spp. appears to occur by three distinct mechanisms of which one is lactose-inhibitable.

The results of each of these investigative approaches are focused on understanding the relationship of cell surface recognitions among oral bacteria and their role in microbial ecology.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00341-08 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Regulation of sugar transport and metabolism in lactic acid and oral bacteria

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

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

COOPERATING UNITS (if any)

DMNB, NINDS, NIH

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

1.10

PROFESSIONAL.

1.10

OTHER:

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

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

1. N(5)-(L-1-Carboxyethyl)-L-ornithine: NADP oxidoreductase (EC.1.5.1-) has been purified to homogeneity for the first time from cells of Streptococcus lactis. The NADPH-dependent enzyme mediates the biosynthesis of two previously unknown amino acids: N(5)-(L-1-carboxyethyl)-L-ornithine and N(6)-(L-1-carboxyethyl)-L-lysine.
2. Polyclonal antibodies have been prepared against the purified enzyme. The first 37 amino acids from the NH(2)-terminus have been determined by stepwise Edman degradation.
3. A mutant of Streptococcus lactis K1 has been isolated which is N(5)-(CE)ornithine synthase(-), nisin(-), nisin resistance(-) and sucrose(-). Studies with (32)P-labeled oligonucleotide probes indicate a chromosomal locus and a genetic linkage for these traits.
4. The obligate requirement for Na ion for glutamate-dependent sugar accumulation by Fusobacterium nucleatum has been established by the findings that Na is required for: (a) the translocation, and (b) intracellular fermentation of the amino acid (energy source).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00382-06 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Growth and interaction of oral microorganisms

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

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

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

2.20

PROFESSIONAL

1.20

OTHER

1.00

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Sodium and sugar transport: Ammonium glutamate failed to stimulate glucose transport in F. nucleatum until Na was added to the assay mix. Na proved to be the only alkali metal ion which was needed for glutamate stimulated glucose transport by F. nucleatum. Some sugar transport activity could be restored by the addition of Li, however, none was found by adding K, Rb, or Cs. The Na dependency of sugar incorporation by F. nucleatum was associated with glutamate transport and use but was unrelated to sugar transport itself. Sugar was transported independently of Na when lysine was used for energy instead of glutamate. Lysine was used by a washed cell suspension of F. nucleatum without added Na, however, no ammonium glutamate was used by the same cell suspension unless NaCl was added. Ammonium glutamate-C-14 was not retained by cells of F. nucleatum until Na was added to the assay mix. Cells supplied with lysine for energy failed to retain labeled ammonium glutamate in the absence of added Na. On addition of Na, labeled glutamate was rapidly retained by the cell suspension, and the rate of entry of labeled glutamate was much faster with lysine added for extra energy.

Sugar transport in other Fusobacteria: Amino acid dependent sugar transport, previously shown by us for a single strain of F. nucleatum, has now been extended to other F. nucleatum strains as well as other Fusobacterium species. Glutamate stimulated the incorporation of C-14 labeled glucose and galactose into F. varium, F. necrophorum, F. russii and F. gonidiformans.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00454-03 LME

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Role of surface molecules in metabolism and ecology of oral bacteria

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

London, Jack P.	Microbiologist	LME, NIDR
Allen, Janet R.	Microbiologist	LME, NIDR
Cassels, Fred J.	Warner-Lambert Fellow	LME, NIDR
Citron, Jean	Staff Fellow	LME, NIDR
Kolenbrander, P.E.	Microbiologist	LME, NIDR

COOPERATING UNITS (if any)

LC, NHLBI, NIH; PICPB, NIDR, NIH; LI, NIDR, NIH; University of Connecticut; and Tel Aviv University, Tel Aviv, Israel

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

3.25

PROFESSIONAL

2.25

OTHER

1.00

CHECK APPROPRIATE BOX(ES)

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

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

The fimbriae-associated adhesins of Bacteroides loescheii which mediate the coaggregation with Streptococcus sanguis 34 has been purified to homogeneity and the Actinomyces israelii PK14 is being further purified. Minor modifications in the purification procedure increased the yield of the S. sanguis-specific adhesin between 4 to 5 fold providing sufficient material for extensive binding studies and a complete chemical characterization. The adhesive protein was shown to be a hexamer having a native M.W. of 450 kD and basic in nature with a pI of between 7.4 and 8.4. Its N-terminal amino acid sequence (28 residues) and amino acid composition has been determined, the protein is rich in Glx, Asp and Lys residues. The protein aggregates as the pH of the solution approaches its pI and at pH 8.0 the protein forms an insoluble precipitate. Thus, under appropriate conditions the protein either blocks coaggregation between B. loescheii and S. sanguis at or agglutinates streptococcal cells and neuraminidase-treated RBCs.

A gene library of 9 to 23 kb fragments of B. loescheii cDNA was created in λ phage GEM 11. Using a 59 membered nucleic acid probe representing the preferred codon usage for the amino acid residues of the first 20 N-terminal amino acids, the gene encoding the adhesin has been identified on a 2 kb restriction fragment.



ANNUAL REPORT OF THE NEUROBIOLOGY AND
ANESTHESIOLOGY BRANCH

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Neurobiology and Anesthesiology Branch (NAB) is concerned with the study of oral-facial sensation with particular emphasis on mechanisms of pain and the development of new methods of pain control. Correlative multidisciplinary approaches are used to answer questions about the functional organization of nociceptive systems in normal and pathological states. The clinical component of the Branch focuses on pain assessment and new pain treatment methods and often follows up advances made in the laboratory to determine their applicability to the clinical situation.

The Branch was established in 1974 and has grown from a small group of physiologists and anatomists studying nociceptive pathways to a major program spanning basic and clinical research on pain and its control. The research conducted has direct relevance to our understanding of mechanisms of neuronal function, in general. In the last last few years, there have been major technical advances in the neurosciences that have given us the opportunity to move our research into new and exciting directions. Our previous studies of the organization and neurochemistry of nociceptive systems have provided a foundation for recent studies on the function of the system following tissue inflammation and nerve injury in animal models. Our clinical models have also focused on chemical mediators associated with surgical trauma and neurohumoral mechanisms of pain and analgesia following the stress and discomfort of surgical procedures. The NAB continues to provide the leadership and coordination of the activities of the NIDR/NIH Pain Research Clinic in the ACRF.

In the last few years we have developed animal models of peripheral tissue inflammation and nerve injury in the rat in order to study neuronal plasticity following acute and persistent behavioral hyperalgesia associated with these conditions. We have also developed a reliable method of assessing hyperalgesia in the rat. With these models and tools, a number of NAB investigators have focused their attention on neurochemical and electrophysiological changes in primary afferents and in the spinal dorsal horn following tissue and nerve damage.

Investigators in the Branch have received considerable recognition for their research accomplishments. A number of investigators organized symposia and workshops at the American Pain Society and Society for Neuroscience meetings in Toronto. Drs. Max and Iadarola were invited to present papers at a conference on, "New Strategies in Pain Control" in Princeton, New Jersey, and Drs. Ruda and Dubner were invited to present papers at a symposium on "Serotonin and Pain" in France. In addition, Dr. Bennett was invited to the annual meeting of the Israel Pain Society in Tel Aviv and Dr. Dubner presented a paper at the IUPS satellite symposium on Information Processing in the Somatosensory System. Finally, Dr. Dubner was the recipient of the second annual Bristol-Myers Award for Distinguished Achievement in Pain Research.

Research accomplishments of the Branch are presented in detail below.



Chemical Mediators of Inflammation and Pain

This year we have continued to study the effects of peripheral tissue inflammation and resulting pain. Inflammation is a common component of many acute and chronic pain conditions including postsurgical acute pain, cancer pain and arthritis. Using our acute carrageenan model of inflammation in the rat that mimics acute pain produced by tissue damage from surgery, we have continued to examine changes in the inflammatory exudate as well as neurochemical mediators of stress and pain released following inflammation. Studies have continued on the pharmacology of corticotropin releasing factor (CRF), a hypothalamic peptide which mediates the stress-induced release of pituitary beta endorphin and ACTH. In previous clinical studies, we have shown that CRF produces analgesia when administered to oral surgery patients. We have examined the mechanism of action of CRF in the rat in the carrageenan model. CRF significantly blocked carrageenan-induced hyperalgesia, edema and hyperthermia and was approximately 1,000 times more potent than indomethacin. CRF retained its analgesic and anti-inflammatory potency in hypophysectomized rats but its anti-inflammatory effects were absent in adrenalectomized animals. The analgesic effects at 30 min were blocked by the opiate receptor antagonist, naltrexone. When injected directly into inflamed tissue, CRF had an analgesic effect, but no anti-inflammatory effects were determined. These studies indicate that CRF produces analgesia and anti-inflammatory effects by multiple mechanisms of action. The analgesic effects are due to an initial opioid mechanism and to a later mechanism that is pituitary-, adrenal- and opioid-independent. The later analgesic mechanism may be due to a direct peripheral effect of CRF on peripheral nociceptors. The anti-inflammatory effects of CRF are due to the actions of adrenal glucocorticoids.

We have also continued our studies on the release of bradykinin in the inflammatory exudate in both the rat carrageenan model and in the human subject oral surgery model. In the oral surgery studies, the use of microdialysis probes has shown that tissue levels of bradykinin during oral surgery increase nearly ten-fold, and that these levels are more than 200-fold greater than blood levels of bradykinin. The findings suggest that drugs which alter bradykinin levels may have important analgesic and anti-inflammatory properties. For these reasons, we have examined the effects of opioids on bradykinin release in the rat carrageenan model. We found that levorphanol significantly reduced local levels of bradykinin as compared to the administration of saline or the inactive stereoisomer, dextrorphan, and that these effects parallel the analgesic and anti-inflammatory effects of the drug. These studies demonstrate that the alteration of peripheral inflammatory mediators represents a mechanism of action of analgesic and anti-inflammatory agents and can be used as a marker of their efficacy.

Neuronal Plasticity Following Peripheral Tissue Inflammation and Nerve Damage

Inflammation also results in central nervous system changes that involve the activation of specific opioid peptides. We have previously shown that inflammation induced by various inflammatory agents results in significant increases in the dorsal horn content of the opioid peptide, dynorphin, and a rapid increase in preprodynorphin mRNA. We have now shown that a similar increase occurs in a rat model of peripheral neuropathy. These data demonstrate that an increase in dynorphin synthesis may represent a common central nervous system response to injury with resultant hyperalgesia and pain, since the inflammation and neuropathy models produce hyperalgesia via different mechanisms.

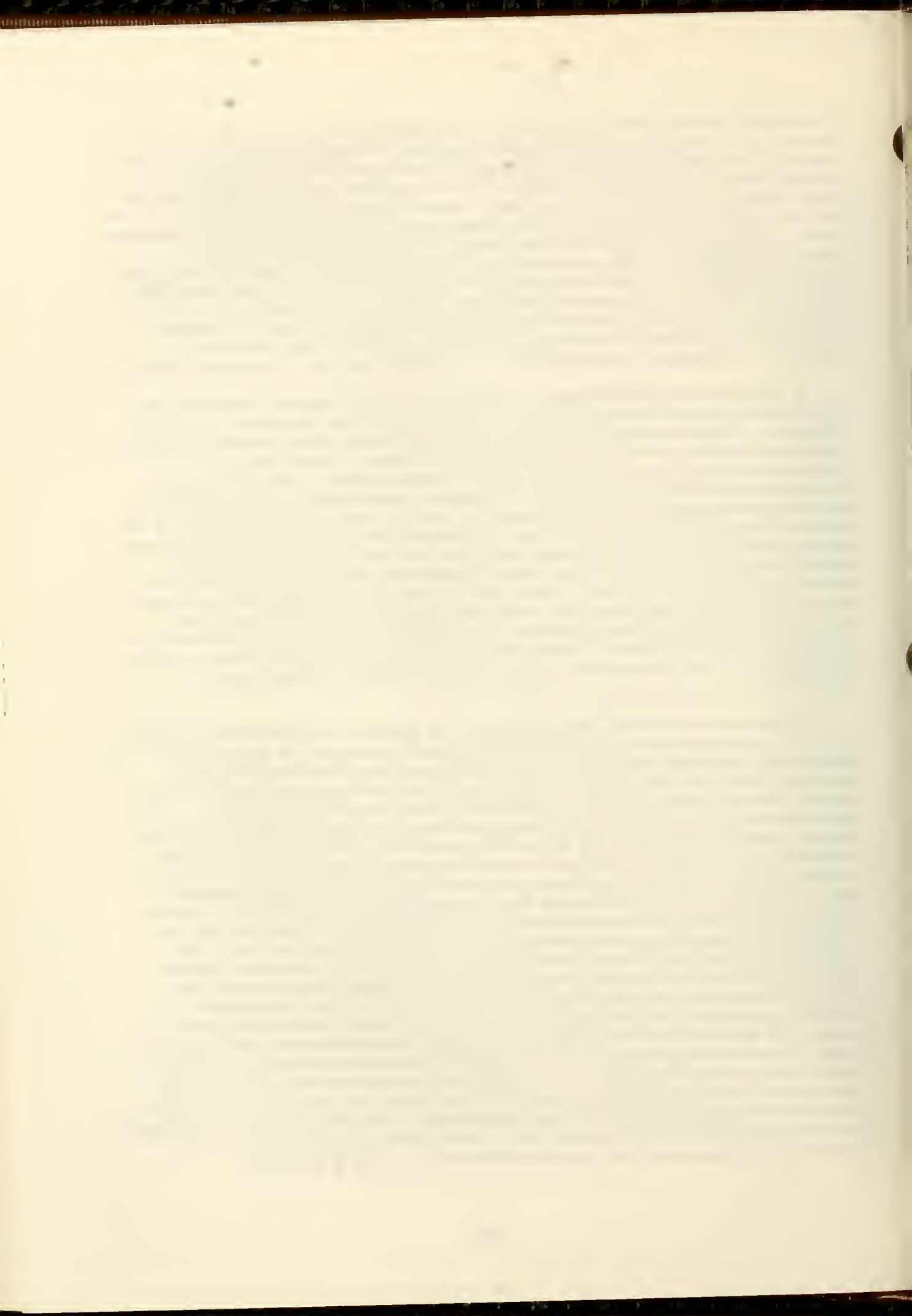
This year we have studied the mechanisms of dynorphin gene regulation



by examining the time course of expression of the proto-oncogene, c-fos, a nuclear protein involved in the transcriptional control of gene expression. There is a marked and very rapid increase in c-fos mRNA in spinal cord during the inflammation. With an antibody to a synthetic peptide corresponding to a region of the c-fos protein, we have identified multiple, specific immunoreactive bands by quantitative western blotting during inflammation. We find that fos proteins have a prolonged half-life which suggests that these proteins may be involved not only in triggering transcriptional events but in sustaining them as well. Using immunocytochemistry, we have determined the location of neurons expressing the fos protein in laminae I, II and V-VI. These correspond to the laminae where the dynorphin-containing neurons are located. We are presently determining whether dynorphin and c-fos are co-localized in the same cells. These findings point to the possible usefulness of dynorphin increases in the central nervous system as a marker for hyperalgesia and the possible therapeutic effectiveness of agents that act at the dynorphin receptor in the spinal dorsal horn.

In the dorsal horn, calcitonin gene-related peptide (CGRP) originates exclusively from small diameter primary afferents, most of which play a role in the transmission of nociceptive information. This year we have followed up earlier observations on the relationship between dynorphin-containing neurons and CGRP in the rat inflammation model using immunocytochemical double-labeling techniques. Previous studies at the light microscopic level demonstrated CGRP immunoreactive varicosities on approximately one-half of the dynorphin immunoreactive neurons in laminae I, II and V of the dorsal horn ipsilateral to the peripheral tissue inflammation. The contacts were located on both the cell soma and proximal dendrites and typically numbered greater than 25 per cell. At the ultrastructural level, synaptic contacts were found between lamina V dynorphin-containing neurons and CGRP terminal varicosities. These results confirm that the upregulation of dynorphin peptide seen during inflammation is associated with neurons that receive direct synaptic input from presumptive nociceptive primary afferents. We have also shown that CGRP terminals in the spinal dorsal horn make synaptic contact with neurons that project to the midbrain, and that a subpopulation of these neurons show an upregulation of dynorphin content during inflammation.

We have continued to study the physiology of the neurons in the superficial spinal dorsal horn in the same inflammation models in order to examine changes in the physiological properties of the neurons associated with hyperalgesia and changes in dynorphin gene expression. Our initial observations of lamina I projection neurons studied ipsilateral to an inflamed hindpaw revealed an increased incidence of large receptive fields, mixed responsiveness to stimulation of both cutaneous and deep tissues, and increased spontaneous activity. These changes occurred as early as 5-6 hours and persisted for days. Additional experiments were designed to determine whether these changes were related exclusively to peripheral receptor-mediated mechanisms or included central nervous system neuronal plasticity at the level of the spinal dorsal horn. By studying the receptive fields of primary nociceptive afferents following inflammation, by mapping receptive fields with electrical stimulation in inflamed and control animals, and by anesthetizing large portions of the expanded receptive fields to reduce central summation produced by spontaneously active nociceptive afferents, we were able to conclude that the expanded receptive fields must depend on central nervous system disinhibitory mechanisms in addition to peripheral nociceptor sensitization. In preliminary studies we have attempted to determine whether the expansion of the receptive fields is related to dynorphin upregulation during the same time period. We have studied neurons before and after the spinal administration of dynorphin peptide and a selective kappa agonist. The data to date suggest that there are at least two populations of dorsal horn neurons influenced by these agents: one type exhibits an analgesic response and the other exhibits a net hyperresponsiveness to mechanical stimuli with expansion of the receptive fields. Future *in vitro* studies will be necessary to parcel out the role of selective opiate receptors in these puzzling observations following inflammation.



In order to examine the effects of nerve injury on neuronal activity and behavior, we have developed a model of hyperalgesia, allodynia and spontaneous pain in the rat. This is the first model in animals that appears to adequately mimic neuropathic pain in humans with nerve injuries due to trauma or disease. Nerve function studies 1-3 days after constriction injury of the sciatic nerve reveal that most of the rapidly conducting axons cannot conduct impulses across the injured region. However, nearly all of the very slowly conducting nerve fibers, associated with unmyelinated axons, conduct normally. Light and electron microscopic observations made 8 days after the injury confirm the nerve function studies and show that almost all of the myelinated axons are interrupted at the site of the injury and degenerating distally. Unmyelinated axons, on the other hand, are nearly all intact and appear normal. Recent studies have also shown that there is a depletion of norepinephrine and neuropeptide Y in the sympathetic vasomotor supply to the planter artery and vein on the side of the injury. By 30 days post-injury, there was nearly complete depletion. There was no consistent relationship between the loss of sympathetic neurochemical innervation and the cutaneous temperature of the denervated foot, an unexpected finding.

Nerve function studies in the nerve injury model also indicate that the nerve fibers conducting in the A-beta and A-delta range have spontaneous discharges that appear to originate from the dorsal root ganglia. This is an unexpected finding since spontaneous discharges have been shown by others to originate from the neuroma that forms at the central end of a completely severed nerve. The nerve injury also is associated with a decrease in the spinal levels of the peptides SP and CGRP, and an increase in the spinal levels of the opioid peptide, dynorphin. Recently, we have found that there is a large increase in the number of GABA neurons in the spinal cord associated with the injury. This increase is seen in laminae I, II, V, VI, VII, VIII and the region around the central canal. These findings suggest that there are marked changes in neurochemical specificity associated with nerve injury and that central changes at the dorsal horn level may be important in the mechanism of the hyperalgesia associated with the model.

Another model of neuronal plasticity is the neonatal administration of capsaicin, a neurotoxin selective for primary afferents. Such treatment results in decreases in spinal CGRP and SP and an alteration in the threshold to some nociceptive stimuli. We have previously discovered a time-dependent return of thermal sensitivity, and an increase in the density of CGRP immunoreactive axons in neonatal capsaicin-treated animals between 8 and 16 weeks of age. Our observations have been extended to the ultrastructural level showing that some unmyelinated and small myelinated axons remain in Lissauer's tract and in the neuropile of laminae I/II and V in the spinal dorsal horn. There is also an increase in the density of CGRP immunoreactive profiles in the dorsal horn and there appears to be an increased number of unmyelinated axons in the area around the central canal. These observations suggest that a subpopulation of unmyelinated primary afferent axons are involved in the plasticity changes occurring in the adult central nervous system following neonatal injury.

Nociceptive Circuitry

Recent studies have identified a second messenger system localized to a subpopulation of trigeminal and primary afferent neurons. Protein kinase C (PKC) is a complex family of enzymes that appears to play a role in many cellular processes including sensory transduction, transmitter release and gene expression. PKC is one of the targets of the phosphoinositide second messenger system involved in cellular communication. Using an antibody to beta-PKC, immunocytochemical studies revealed that PKC was localized to numerous neurons in the lumbosacral dorsal root ganglia, the trigeminal ganglion and the mesencephalic nucleus of V. The cells were mainly large- and medium-sized and large myelinated fibers were labelled peripheral and central to the ganglia. Few neurons co-localized both PKC and CGRP. These are the first studies to demonstrate the presence of PKC in ganglion cells and in the



mesencephalic nucleus of V and the observations suggest that beta-PKC is preferentially localized to large diameter primary afferent neurons with minimal overlap with presumed nociceptive afferents. Such a marker for PKC may be useful in the identification of unknown transmitters localized in myelinated non-nociceptive afferents.

As mentioned above, CGRP is a marker for presumed nociceptive afferents and is located exclusively in primary afferent neurons. In earlier studies, we used CGRP as a marker to determine the rostral projections of small myelinated and unmyelinated axons and found that some CGRP immunoreactive afferents projected at least 5 segments rostral to their segment of entry. In order to further characterize this long afferent projection, we utilized electron microscopy and analyzed the terminal arbors of these projections. Most of the CGRP immunoreactive fibers were unmyelinated and made synaptic contact with unlabeled dendritic profiles, indicating that these long projections likely originate from C fibers and that they establish functional connections in the dorsal horn. Quantification of the extent of this system has begun by using a double-label technique in which a retrograde marker was used in combination with immunocytochemistry. Preliminary results indicate that as many as 30% of the CGRP-labelled ganglion cells in the SI dorsal root ganglion project rostrally to the L4 segment.

In previous years we developed a thermal discrimination task in which monkeys make fine thermal discriminations in the noxious heat range. With this behavior model, we have studied the role of different neuronal populations in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. In addition, we employ this model to examine the effect of different chemical mediators on the monkeys' discriminative capacities. This year we have continued our study of cerebral cortex neurons involved in pain discrimination. Nociceptive neurons, mainly of the wide-dynamic-range type, were found in somatosensory cortex area I. The neuronal discharge of these cortical nociceptive neurons was correlated with the monkeys' ability to detect noxious thermal stimuli. We recorded from 15 cortical nociceptive neurons while the monkey was given small stimuli of 0.2 to 0.8 deg C. A linear regression of individual trials revealed a significant relationship between neuronal discharge and behavioral detection speed in the task. Responses to threshold stimuli also provided evidence that these neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. These results put to rest the long held view that the cerebral cortex does not participate in the encoding process of stimulus features in the noxious range.

Descending noradrenergic systems are known to exert powerful inhibitory effects on nociceptive transmission in the dorsal horn. We examined the effects of ST-91, a selective alpha-2 agonist, microinjected into the medullary dorsal horn, on the monkeys' ability to perceive the intensity of noxious heat stimuli, using our thermal discrimination behavioral task. ST-91 produced a dose- and stimulus intensity-dependent reduction in the monkeys' ability to detect small increases in noxious heat intensity. There was no effect on the monkeys' ability to detect visual or cooling stimuli indicating that the microinjection of ST-91 impaired noxious heat detection without altering motor function or motivational and attentional components of the monkeys' performance. Systemic injections of idazoxan, a specific alpha-2 antagonist, but not prazosin, an alpha-1 antagonist, produced a significant attenuation of the effect of ST-91. We conclude that alpha-2 agonists are capable of attenuating the sensory discriminative component of pain in a receptor-specific manner. These studies are important in the development of new and adjunctive agents in the control of acute and chronic orofacial pain.

Pain Assessment

The purpose of these human pain studies is to develop psychophysical and behavioral models of pain perception in humans and to utilize these models in the understanding of pain



mechanisms in humans and the development of new methods of pain control. We have developed an interactive staircase method which is a major refinement of the multiple random staircase method of psychophysical assessment. This method provides information about perceived pain in units of stimulus intensity rather than in units of perceptual magnitude, provides information about an individual's scaling abilities, and provides information about the time course of an analgesic effect. This year we have determined the sensitivity of the interactive staircase method to opioid potentiation. The method was capable of distinguishing small doses of fentanyl from saline on top of a preloading small dose of fentanyl. The finding suggests that the staircase method can detect the analgesia produced by a simulated opioid potentiator and supports the use of the method for the assessment of novel agents that may potentiate opioid analgesia. The staircase method also has been used to assess the efficacy of 50% nitrous oxide on thermal sensitivity. In comparison to oxygen, nitrous oxide significantly increased the stimulus temperatures required to maintain the same responses. Thus, the method is sensitive to the effects of a mild analgesic agent that produces rapid, phasic changes in pain sensitivity.

In another study, examining cognitive measures of pain responsivity, the Marlow Crowne social desirability scale was used to divide subjects into defensive (tendency to suppress negative affect) and non-defensive subjects. Using verbal descriptors of pain, subjects high in defensiveness rated heat stimuli to be equally intense but significantly more unpleasant than subjects scoring low in defensiveness. These findings are contrary to previous results by other groups with less-controlled methods, and suggest that the effect of defensiveness may be sensitive to differences in pain assessment methods.

Assessment and Treatment of Chronic Pain

These clinical trial studies evaluate new methods of studying pain and its relief, with a particular focus on pain syndromes often refractory to standard treatments. We presently are evaluating the effects of agents that increase the availability of serotonin or norepinephrine in the brain and thereby presumably suppress pain. This year we completed a study of the effects of desipramine in painful diabetic neuropathy and postherpetic neuralgia. Analgesia in both groups of patients was superior with desipramine than with placebo. In postherpetic neuralgia patients, the analgesic effect appears to be independent of the drug's antidepressive actions. In painful diabetic neuropathy patients, however, there was a tendency for patients assessed as "depressed" to have greater pain relief than patients who were not depressed. Our clinical trials suggest that desipramine, which appears to increase the availability of norepinephrine in the brain, has an analgesic action on neuropathy pain similar to that of amitriptyline, the agent we have studied previously. Clinicians can use desipramine instead of amitriptyline, sparing many patients the sedation and anticholinergic effects of the latter drug. We are now completing a study which compares these drugs and other agents directly, in order to determine whether desipramine is as effective a pain reliever as amitriptyline, the standard in clinical use.

Relief of Acute Pain

These studies consist of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as the model. The existence of multiple classes of opiate receptors in the brain suggests that opioid analgesics which selectively bind to only one of these receptors may result in analgesia with reduced side effects. This hypothesis is being tested by the evaluation of spiradoline, a kappa receptor agonist. Injection of spiradoline into the deltoid region results in analgesic activity comparable to 10 mg of morphine. Additional studies are needed to confirm its analgesic activity and to assess its side effect liability.



A parallel series of studies evaluate the safety and efficacy of drugs used for anxiety relief using the same oral surgery model. A study of oral triazolam, a benzodiazepine, in combination with nitrous oxide, indicated that 0.25 mg of oral triazolam in combination with nitrous oxide resulted in therapeutic benefit, patient safety and recovery within two hours postoperatively. A factorial comparison of the two drugs to placebo and diazepam suggests that the triazolam and nitrous oxide combination results in efficacy comparable to IV diazepam with less signs of sedation and less residual impairment in psychomotor function. These results may provide a rational basis for the use of an oral benzodiazepine and nitrous oxide as an alternative to parenteral sedation with diazepam or similar drugs.

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October 1, 1988 - September 30, 1989

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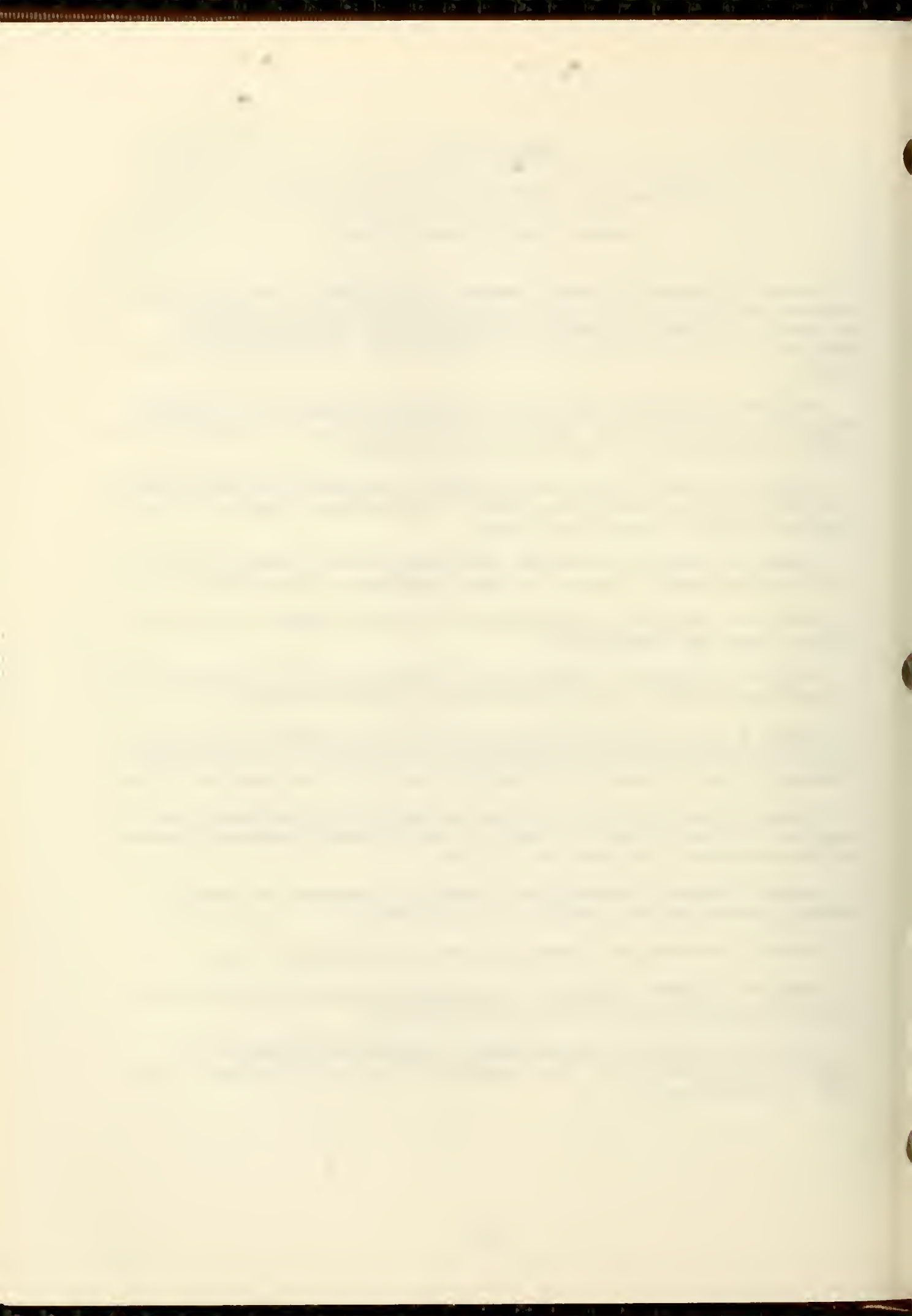
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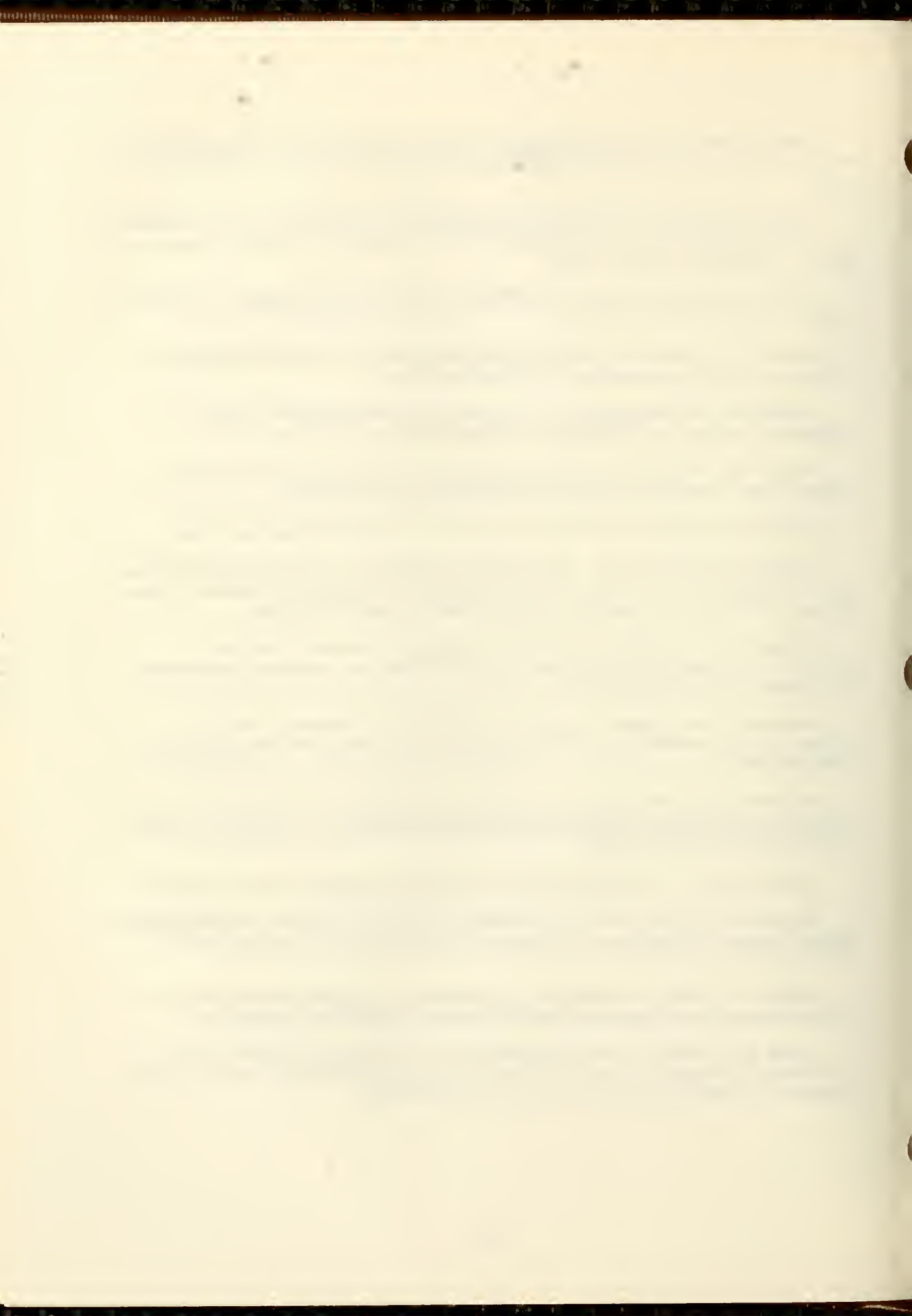
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00132-15 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

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

Hargreaves, Kenneth	Staff Fellow	NAB NIDR
Dionne, Raymond	Medical Staff Fellow	NAB NIDR
Costello, Ann	Post Doctoral Fellow	NAB NIDR
Dubner, Ronald	Chief, NAB	NAB NIDR

COOPERATING UNITS (if any)

Goldstein, David	Staff Scientist	ETB NHLBI
Jackson, William	Medical Staff Fellow	LOM NIDR
Schafer, Susan	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pharmacology Unit, Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.05

PROFESSIONAL:

1.8

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators in an established animal model of inflammation, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials.

This year, we have extended our research into the pharmacology of corticotropin releasing factor (CRF) by determining its mechanisms of action in the rat carrageenan model of inflammation. CRF was approximately 1,000 times more potent than indomethacin for blocking hyperalgesia, edema and hyperthermia. The analgesic effects of CRF are due to both an initial mechanism which involves release of endogenous opioids (possibly pituitary beta endorphin) and to a second mechanism which is pituitary-, adrenal- and opioid-independent. This second mechanism is due to a direct, peripheral effect of CRF in inflamed tissue, possibly by acting on peripheral nociceptors. In addition, the anti-inflammatory effects of CRF are due to the actions of adrenal glucocorticoids. CRF may represent a prototype of a novel class of peripherally-acting analgesic drugs for treating acute pain.

We have also extended our research on bradykinin by developing microdialysis probes which, for the first time, permit accurate measurement of the levels of inflammatory mediators in inflamed tissue. In oral surgery patients, tissue levels of iBK increase nearly 10-fold over a 4 hour observation period. The peak in tissue concentrations of iBK (approx. 16,000 fm/ml) precedes the peak in pain report and the two factors are significantly correlated. In a parallel study using microdialysis probes in rats, tissue levels of iBK were approximately 6-fold greater in carrageenan-treated paws than control paws. Tissue levels of iBK were significantly reduced by administration of the opioid levorphanol, while its inactive stereoisomer was without effect. Pre-treatment with dexamethasone blocked the increase in iBK during carrageenan inflammation. The dexamethasone effect is due to activation of glucocorticoid receptors leading to de novo synthesis of anti-inflammatory protein(s) since the effect was blocked by both a glucocorticoid receptor antagonist and a protein synthesis inhibitor.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00133-15 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Assessment of Experimental and Clinical Pain

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

Gracely, Richard H.

Research Psychologist

NA NIDR

Dionne, Raymond A.

Research Pharmacologist

NA NIDR

Dubner, Ronald

Chief, NAB

NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.55

PROFESSIONAL

1.00

OTHER

1.55

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are (1) to assess psychophysical methods of experimental pain measurement; (2) to assess clinical pain measures in a dental setting; (3) to use these methods to evaluate underlying mechanisms of clinical pain syndromes; and (4) to evaluate the mechanisms and efficacy of pharmacological and non-pharmacological pain-control agents. The interactive computer-based staircase scaling method was used in six experiments.

The first experiment simulated opioid potentiation by administering a small dose (.55 ug/kg fentanyl) of narcotic double-blind after an initial narcotic infusion. The model demonstrated sufficient sensitivity for planned evaluation of opioid potentiators.

The second experiment manipulated baroreceptor activity by reclining or standing postures. Reclining resulted in analgesia, suggesting cardiac activation of opioid analgesic systems.

The third experiment assessed the fast time course of nitrous oxide analgesia. Results support the sensitivity of the model and evaluated both the onset and offset of nitrous oxide analgesia.

The fourth experiment provides evidence that the absence of cardiac chest pain during "silent ischemia" may represent a central analgesic state detectable by experimental methods.

The fifth and sixth experiments demonstrated the validity of the staircase method with verbal scales that discriminate between sensory intensity and unpleasantness.

A seventh experiment found that subjects scoring high in "defensiveness" rated sensations evoked by thermal stimuli as significantly more unpleasant in relation to subjects low in defensiveness, explaining a large portion of the variability associated with subjective pain judgments.

An eighth experiment compared clinical thermal pain threshold procedures and found that latency to a slowly increasing continuous stimulus was superior if less than 3 stimuli can be delivered and a single staircase method was superior if more than 3 stimuli can be used. These methods are part of an assessment battery used to evaluate the pain mechanisms mediating clinical neuropathic pain and experimental models produced by topical capsaicin or lidocaine. Experimental results indicate that small unmyelinated C-fibers modulate myelinated A-delta perceived pain sensation, that spontaneous pain and pain evoked by light touch or usually non-painful stimuli can be evoked experimentally, and that partial A-deafferentation may result in pain produced by A-beta fibers which usually signal non-painful touch sensations.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-11 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Brain Stimulation Analgesia in the Control of Chronic Pain

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

Gracely, Richard H.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Max, Mitchell B.	Neurologist	NA NIDR

COOPERATING UNITS (if any)

Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.50

PROFESSIONAL:

.35

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

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

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

The purposes of the study are (1) Assess the effectiveness of chronic electrical stimulation of midbrain sites for the relief of chronic pain in humans; (2) Evaluate the efficacy and mechanisms of traditional narcotic analgesia and compare these to chronic electrical stimulation of midbrain sites; (3) Validate experimental models of pain and their potential diagnostic use in chronic pain patients; and (4) Determine and compare the impact of both traditional narcotic and chronic electrical stimulation therapies on the functional, intellectual and emotional well being of these patients. The effects of chronic brain stimulation in surgical patients will be compared to the effects of narcotics previously administered to patients and to effects of narcotic regimes in non-surgical chronic pain patients. In addition, the effects of narcotics on perceptual and neural mechanisms of experimental induced pain will be assessed in pain-free volunteers.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00286-10 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Experimental Therapeutics for Acute Pain and Apprehension in Ambulatory Patients

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

Dionne, Raymond	Research Pharmacologist	NA NIDR
Costello, Ann	Postdoctoral Fellow	NA NIDR
Hargreaves, Kenneth	Senior Staff Fellow	NA NIDR
Kaufman, Eliezer	Visiting Scientist	NA NIDR

COOPERATING UNITS (if any)

Samaha, Ramona	Nurse	CC Nursing
Thompson, Margaret	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.55

PROFESSIONAL:

2.35

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

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

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

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model for minor surgical procedures with associated intraoperative and postoperative pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety.

Administration of 0.5 mg of proglumide, an antagonist of cholecystokinin, was demonstrated to significantly potentiate and prolong the analgesic efficacy of 4 mg of morphine for postoperative pain while higher doses of proglumide were without effect. A dose response study evaluating SCH 34826, a novel agent which inhibits the breakdown of enkephalin in the central nervous system, failed to demonstrate analgesic activity, possibly due to poor penetration into the central nervous system. Preliminary results of a comparative study with spiradoline, a novel analgesic which acts at the kappa opioid receptor, suggests potency comparable to 10 mg of parenteral morphine.

A parallel series of investigations are evaluating the safety and efficacy of drugs used for anxiety relief in patients undergoing minor surgical procedures with local anesthesia. A dose-range study of the combination of oral triazolam, a benzodiazepine, and nitrous oxide indicated that 0.25 mg of triazolam in combination with nitrous oxide resulted in therapeutic benefit, patient safety and recovery within two hours postoperatively. Preliminary results of a second study suggest that the combination of triazolam and nitrous oxide results in therapeutic benefit comparable to IV diazepam on several dependent measures but with less signs of sedation and less residual impairment in psychomotor function. A third study consists of a multi-center collaborative study evaluating the efficacy and safety of intravenous midazolam to the combination of midazolam plus methohexital, medazolam plus fentanyl and methohexital, and placebo. Data collection is approximately 50% complete. The results of this investigation will provide a basis for selecting the parenteral agent or combination which provides the optimal balance between efficacy and safety for use in ambulatory patients.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00288-10 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Neuropharmacological Characterization of Synaptic Circuitry in Dorsal Horn

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

Ruda, Maryann	Research Biologist	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Humphrey, Emma L.	Biol. Lab. Tech (Elec. Mic.)	NA NIDR
Takahashi, Osamu	Fogarty Visiting Scientist	NA NIDR
Solodkin, Ana	Guest Researcher	NA NIDR
Traub, Richard	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS (if any)

Donna Hammond, University of Chicago, Dept. Anesthesiology and Critical Care, Chicago, Ill.

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neurocytology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

5.10

PROFESSIONAL

2.50

OTHER:

2.60

CHECK APPROPRIATE BOX(ES)

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

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

The neural circuitry of the dorsal horn of the spinal cord forms the basis for the mechanisms of pain and analgesia. Our lab has made significant inroads in understanding the neuronal connectivity which subserves these sensory phenomena through experiments involving multiple markers to identify interactions between neural elements.

Afferent input to spinal dynorphin neurons which exhibit an up-regulation during peripheral inflammation and hyperalgesia was examined using the electron microscopic mirror technique. Direct synaptic input between a subpopulation of dynorphin neurons and presumed nociceptive primary afferents containing CGRP was observed.

Ultrastructural analysis of primary afferent axons which return in adulthood following neonatal injury demonstrated that the plasticity in neuronal organization occurs in part, in the unmyelinated population of primary afferent axons.

Analysis of long projecting primary afferent axons demonstrated that axons projecting at least five segments from their root of entry include a subpopulation of unmyelinated axons which are part of the synaptic circuitry in those segments.

β -Protein Kinase C was localized to a subpopulation of trigeminal, mesencephalic V and spinal dorsal root ganglia neurons. They likely represent large, myelinated, non-nociceptive afferents.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-10 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Microinjection of analgesic agents into the Medullary Dorsal Horn of the Behaving Monkey

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

Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David A.	Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

1.8

PROFESSIONAL

1.4

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

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

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

We examined the effects of ST-91 microinjected into the medullary dorsal horn (MDH) on the ability of monkeys to detect small temperature increases in the noxious heat range. Behavioral, detection latency and the percentage of correct detections were used as measures of the monkeys perceived intensity of noxious thermal stimulation. The monkeys were trained to detect temperature changes of 0.4, 0.6 and 1.0°C (T2) superimposed on an initial temperature shift to 46°C (T1). ST-91, an alpha-2 adrenergic agonist, was microinjected (1, 3, 10 and 30 micrograms) into the MDH and produced dose- and stimulus-dependent increases in the detection latencies to T2 noxious heat stimuli. There was no effect of ST-91 on the detection of innocuous cooling or visual stimuli indicating that ST-91's effects on the detection of noxious heat are independent of motivational, motoric or attentional factors. Systemic idazoxan (an alpha-2 receptor-specific antagonist), but not prazosin (an alpha-1 receptor specific antagonist) or saline, significantly attenuated the effects of ST-91 on all noxious T2s. These data demonstrate a pharmacologically-specific effect of an alpha-2 agonist on the perceived intensity of noxious heat stimuli at the MDH, the earliest central relay for noxious information.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00329-08 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Discrimination of Thermal Stimuli Applied to the Face in Monkey

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

Dubner, Ronald	Chief, NAB	NA NIDR
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

.4

PROFESSIONAL:

.2

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

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

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

This project correlates behavioral responses with neural responses of thalamic projection and non-projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) produced by noxious thermal stimuli in the behaving monkey. Medullary dorsal horn neurons encode thermal discriminative information used by the monkey to perform a thermal detection task. Many medullary dorsal horn neurons encode thermal intensity in a manner which allows the detection of small changes in noxious temperatures. The role of dorsal horn wide-dynamic-range (WDR) and nociceptive-specific (NS) neurons in the encoding of the perceived intensity of noxious stimuli was determined while monkeys detected near-threshold changes in the intensity of noxious heat stimuli. Behavioral detection latencies were a reliable measure of the perceived intensity of these stimuli. There was a significant correlation between behavioral detection latency and neuronal discharge of WDR, but not NS neurons. In addition, WDR neurons exhibited greater activity on correctly-detected versus non-detected trials, whereas NS neurons did not. We conclude that WDR neurons are involved in the encoding process by which monkeys perceive the intensity of noxious heat stimuli near detection threshold. Some thermally sensitive neurons also respond to other stimuli used by the monkey for the successful completion of the task. This task-related activity occurs in characteristic patterns of excitation and/or inhibition and some neurons which exhibit such activity project to the thalamus. The task-related responses exhibited by some of these neurons may modulate sensory activity and thereby influence the perception of and response to oral-facial pain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-07 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Analgesic Mechanisms in Patients with Chronic and Acute Postoperative Pain

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

Max, Mitchell	Neurologist	NAB NIDR
Lynch, Sue	Medical Staff Fellow	NAB NIDR
Zeigler, Daryl	Visiting Fellow	NAB NIDR
Gracely Richard H.	Research Psychologist	NAB NIDR

COOPERATING UNITS (if any)

Benjamin, Janice	Nurse	CC Nursing
Craig, Bradene E.	Nurse	CC Nursing
Muir, Joanne	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.15

PROFESSIONAL

2.95

OTHER

.2

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to elucidate the principles of treatment of acute and chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury and surgery.

In a randomized, double-blind, crossover study, 19 patients with postherpetic neuralgia and 20 patients with painful diabetic neuropathy were treated with 6 weeks each of placebo and desipramine, an antidepressant that selectively blocks synaptic reuptake of norepinephrine. In both groups, desipramine was superior to placebo in relieving pain, and caused fewer side effects than had been observed in previous studies with amitriptyline, the standard medication. Magnitude of pain relief was comparable to that which had been observed in previous trials using amitriptyline. These results support the hypothesis that the potentiation of noradrenergic neural activity is sufficient to inhibit some neuropathic pain states, and that acute stimulation of serotonergic receptors may not be sufficient for analgesia. To directly examine these questions, a comparison of amitriptyline, desipramine, and fluoxetine (a specific blocker of serotonin reuptake) is nearing completion, and a chronic study of the adrenergic agonist clonidine will soon begin.

Several new research programs are underway: a post-operative pain program with the Bethesda Naval Hospital Departments of Surgery and Anesthesiology studying the effects of pre-operative treatment with prostaglandin inhibitors on post-operative pain, and the specific potentiation of morphine analgesia by desipramine; and a program of detailed sensory testing to elucidate the mechanisms of neuropathic pain states.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00377-06 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Role of the Primate Primary Somatosensory Cortex in Nociception

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

Kenshalo, Jr., Dan R.	Senior Staff Fellow	NA NIDR
Chudler, Eric H.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Iwata, Koichi	Visiting Fellow	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.3

PROFESSIONAL:

2.6

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

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

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

The role played by the primary somatosensory cortex (SI) in the perception of painful stimuli is poorly understood. We have used two approaches to resolve this problem. In the first, SI neuronal activity was correlated with the ability of monkeys to detect small increments in noxious thermal stimulation. In the second approach, the ability of the monkey to detect and to discriminate noxious thermal stimulation was examined during a reversible lesion, produced by cooling the somatosensory cortex.

The activity of nociceptive SI neurons was recorded while the monkey performed a psychophysical task. The monkeys detected small (0.2°-0.8°C) increases in skin temperatures superimposed on noxious levels of thermal stimulation (45-49°C) applied to the face. The detection latency, expressed as detection speed, was used as a measure of the perceived intensity of sensation. Two-thirds of the neurons that responded to noxious thermal stimulation increased their discharge rate with increases in stimulus intensity. The remaining neurons responded to noxious thermal stimulation, but did not grade their discharge rates with increases in stimulus intensity. The neuronal discharge of nociceptive SI neurons was correlated with the monkeys' ability to detect noxious thermal stimulation. A significant correlation was found between the peak neuronal discharge and the monkeys' detection speed. In addition, the discharge of nociceptive SI neurons was significantly greater on correctly detected vs non-detected trials. Increases in foreperiod length produced an increase in both the detection speed of the monkey as well as an increase in the peak neuronal discharge of nociceptive SI neurons.

In the cortical cooling experiments, the monkeys were required to detect small increments in noxious thermal stimulation (detection task). On some trials the monkeys were required to discriminate that the thermode temperature was higher than on detection trials, to release the button and to escape the noxious thermal stimulus. After cooling SI, the monkey exhibited clear deficits in the detection and the discrimination tasks. We conclude that nociceptive SI neurons are involved in the process through which monkeys perceive the intensity of noxious thermal stimulation.



NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 DE 00413-04 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Experimental Neuropathy of Peripheral Nerve in Rats

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

Bennett, Gary J.	Research Biologist	NA NIDR
Kajander, Keith C.	Postdoctoral Fellow	NA NIDR
Wakisaka, Satoshi	Visiting Fellow	NA NIDR

COOPERATING UNITS (if any)

Prof. B. Munger; Hershey Medical Center; Hershey, PA
 Dr. T. Sugimoto; University of Osaka; Osaka, Japan
 Dr. V. Seybold; University of Minnesota; Minneapolis, MN

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.4

PROFESSIONAL

3.0

OTHER

.4

CHECK APPROPRIATE BOX(ES)

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

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

A chronic constriction injury to the sciatic nerve of the rat produces a neuropathic pain syndrome that is very similar to the neuropathic pain states seen in man. In the experimental model, the abnormal sensations begin within 2 days, reach peak intensity in about 10 days, and persist for 2-3 months. Anatomical, neurochemical, and electrophysiological methods were used to search for abnormalities in the peripheral and central nervous system that might be associated with the pathogenesis of the neuropathic pain. In the peripheral nervous system, light- and electron-microscopy showed that within 8 days the injury results in a partial and differential deafferentation that interrupts nearly all of the myelinated axons but spares a large majority of the small unmyelinated axons. In addition, the neuropathy has been found to produce, within 2-4 weeks, a near total depletion of norepinephrine, dopamine-beta-hydroxylase, and neuropeptide Y in the axons that comprise the sympathetic perivascular plexus. Lastly, electrophysiological experiments have documented that at 1-3 days post-injury about one-third of the A-beta axons and one-fifth of the A-delta axons in the injured nerve are sending spontaneous impulses into the spinal cord. This spontaneous discharge does not originate at the site of nerve injury, but rather in the dorsal root ganglion that contains the damaged axon's cell body. In the central nervous system, the regions of the spinal cord that are innervated by the damaged nerve display several abnormalities. There is a marked increase in the incidence of atrophic neurons, which are rare in the normal spinal cord. The incidence of atrophic neurons is further increased when the animals are given subconvulsive doses of strychnine, a drug which blocks inhibitory synaptic transmission. In addition, there are marked changes in the levels of receptor binding for several substances. Decreases are found for receptor binding for substance P and for the peptide that acts at the delta opioid receptor. There is an increase in binding for the mu opioid receptor. No changes are found for binding of the calcitonin gene-related peptide receptor or for the kappa opioid receptor.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-04 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

CNS Neurotransmitter Regulation During Peripheral Inflammatory States

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

Iadarola, Michael J., Ph.D.

Senior Staff Fellow

NA NIDR

Draisci, Gaetano, M.D.

Guest Researcher

NA NIDR

Yeung, Choh Lun

Biologist

NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanism Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.05

PROFESSIONAL:

2.9

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

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

This project concerns the role of central nervous system (CNS) neurons in sensory processes especially as they relate to pain and the control of pain. A model of peripheral inflammation has been developed to investigate the relationship between spinal cord neurons, mainly those containing the opioid peptides enkephalin and dynorphin but also other neurotransmitters and neuropeptides, and abnormal primary afferent input. Alterations in gene expression are assessed by measurement of peptide and mRNA levels and in situ hybridization techniques. Having characterized the time course and stimulus requirements for the increase in spinal cord dynorphin gene expression we are now turning our attention on understanding the controls governing the increase. What are the molecular mechanisms, what are the physiological mechanisms?

We have shown that the inflammation also induces an increase in mRNA levels coding for the c-fos proto-oncogene which precedes the increase in dynorphin. C-fos is a nuclear protein involved in transcriptional regulation. We have raised a highly selective antibody to this protein and have characterized the location of the protein increase with immunocytochemistry and the molecular species that are elevated by quantitative western blotting. The elevation in c-fos protein is prolonged and the protein(s) involved undergo marked alterations in amount and several immunoreactive bands are observed. Whether these proteins are products of new fos-related genes is a question we are currently addressing. Regardless, our data demonstrate a new fact not revealed by measurement of the mRNA. We find that, in vivo, fos proteins can have a prolonged half-life which suggests that these proteins may be involved not only in triggering transcriptional events but in sustaining them as well. We have also obtained preliminary evidence that c-fos mRNA is elevated in the periaqueductal grey (PAG), a brain region involved in pain processes. This implies that the PAG is activated during inflammation and that some gene regulatory process is engaged here as in the spinal cord.

The significance of these studies is that they reveal which opioid neurons in spinal cord are activated in response to inflammatory pain and, possibly, pain associated with arthritis and cancer. Further elucidation of the pivotal role of the spinal dynorphin system in nociceptive processes and mechanisms underlying the increase in gene expression may provide new avenues for the pharmacotherapy of pain and new insights into chronic opioid abuse and tolerance.



NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 DE 00440-03 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Dorsal Horn Circuitry Related to Pain: Inflammation-induced Plasticity

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

Hylden, Janice L.K.	Staff Fellow	NA NIDR
Nahin, Richard L.	Staff Fellow	NA NIDR
Traub, Richard J.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

1.65

PROFESSIONAL.

1.45

OTHER

.2

CHECK APPROPRIATE BOX(ES)

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

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

In the present research project, we have employed a combination of physiological, pharmacological and behavioral approaches to the study of somatosensory systems related to pain and hyperalgesia.

The activity of lumbar dorsal horn lamina I projection neurons was studied in normal rats and in rats with an inflamed hindpaw. Inflammation was produced by injecting Freund's adjuvant into the plantar surface 4 hours to 5 days prior to electrophysiological recording. The majority of cells recorded in rats with inflamed limbs demonstrated properties uncharacteristic of this cell population in control rats, including large receptive fields, discontinuous receptive fields, responsiveness to deep as well as cutaneous tissues, and ongoing or bursting spontaneous activity. Cells with complex receptive fields were encountered from less than 6 hours to 5 days after induction of inflammation. This time course correlates with the occurrence of hyperalgesia to thermal stimuli. The contributions of nociceptive afferent sensitization and alterations in the physical environment of peripheral receptors to the observed enlargement of receptive fields were examined by testing the responses of cells to localized electrical and thermal stimuli in the absence and presence of local anesthesia. The results employ that the enlargement of receptive fields cannot be accounted for by sensitization of peripheral nociceptors or physical changes in the environment of peripheral receptors and must therefore involve changes within the central nervous system. We have begun to address the nature of inflammation-induced central changes by pharmacological manipulations.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00460-02 NA

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Neuronatomical mechanisms of pain transmission and modulation

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

Nahin, Richard L.	Staff Fellow	NA NIDR
Hylden, Janice L.K.	Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.4

PROFESSIONAL

1.0

OTHER

.4

CHECK APPROPRIATE BOX(ES)

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

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

In the present research, we have employed a variety of anatomical techniques to study the neurocircuitry of the spinal cord as it relates to pain and hyperalgesia.

Lamina I neurons were retrogradely labeled after injections into the caudal midbrain of normal rats. Spinal cord sections from these animals were processed for Calcitonin Gene Related Peptide (CGRP) or dynorphin (DYN) immunoreactivity. It was found that a number of lamina I projection neurons were contacted by either CGRP or DYN immunoreactive varicosities.

Spinal cord sections of animals with a hyperalgesic hindpaw were processed for GABA or GAD immunoreactivity. It was found that the number of neurons immunoreactive for GABA or GAD was substantially greater in spinal cord sections ipsilateral to the hyperalgesic hindpaw.

Alterations in the peptide content of primary afferents innervating an inflamed/Hyperalgesic hindpaw were studied in the rat. It was found that there is a large increase in the amount of CGRP found in primary afferents innervating the skin (dermis and epidermis) of the inflamed paw vs. normal paws.



ANNUAL REPORT OF THE LABORATORY OF ORAL MEDICINE
NATIONAL INSTITUTE OF DENTAL RESEARCH

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

The Laboratory of Oral Medicine is involved in a number of collaborative projects including: (1) preparation of human monoclonal antibodies to HIV (NIAID); (2) preparation of human monoclonal autoantibodies with different specificities (Cetus Immune Research Laboratories, Palo Alto, CA); (3) characterization of antibodies to human thyroglobulin and microsomes (Mount Sinai School of Medicine, New York); (4) human monoclonal antibodies to rabies virus (The Wistar Institute of Anatomy and Biology, Philadelphia, PA); (5) characterization of lymphocyte subsets using monoclonal antibodies (The Wistar Institute, Philadelphia, PA); (6) studies related to the function of the antigen binding site of polyreactive human monoclonal antibodies (McGill University, Montreal, Quebec, Canada); (7) sequences of human V_H and V_L genes (University of Texas, Dallas, TX); (8) studies related to EBV (NCI and FDA); (9) studies on the interaction of HTLV-1 with various lymphocyte subsets (NCI and NHLBI); (10) development of transgenic mice carrying the HIV provirus (NIAID); (11) development of transgenic mice carrying human melanoma gene (The Wistar Institute, Philadelphia, PA); (12) development of transgenic mice carrying diabetes associated polypeptide cDNA (The University of Chicago, Chicago, IL); (13) development of transgenic mice carrying EBV (C₃D) receptor (University of Washington, St. Louis, MO); (14) cloning of diabetes-related autoantigens (University of Washington, Seattle, WA; Toukai Sangyo-Iryodan Chuuou Hospital, Tokai-shi, Japan); (15) cloning and characterization of thyroid-related autoantigens (NIDDK); (16) RFLP, genomic cloning and chromosomal localization of the gene for the 70 kDa autoantigen (NCI); (17) isolation and characterization of genes for myocardial autoantigens (University of Nebraska Medical Center, Omaha, NE); (18) detection and cloning of oncogenes from human endocrine tumors (NICHD and NCI); (19) viral etiology of autoimmune diseases (NIAMS); (20) clinical studies on factors that trigger and agents that inhibit reactivation of HSV (NIAID); and (21) investigation of topical indomethacin as a blocking agent for reactivation of HSV and investigation of epithelial irritants as triggers of HSV reactivation (UCLA School of Medicine, Los Angeles, CA).

Since last year, a number of new techniques were introduced into the laboratory and existing ones modified. These include: (1) polymerase chain reaction (PCR) to amplify HIV sequences in the saliva of AIDS patients; (2) utilization of PCR for amplifying specific sequences in the human genome, inserting new restriction sites, and synthesizing radioactive DNA probes of high specific activity for detecting HSV and human retroviral sequences in a number of clinical conditions; (3) biotinylated probes for detecting immobilized nucleic acids; (4) in situ hybridization using non-radioactive

probes for detecting mRNA in tissues; (5) cloning of cDNA into eukaryotic expression vectors; (6) liposome mediated DNA transfection of various cells; (7) Southwestern blotting to detect DNA binding proteins; (8) DNA cellulose chromatography for detecting the affinity of DNA binding; (9) utilization of peptides to define the DNA binding domains of proteins; (10) utilization of anti-peptide antibodies to map the functional domains of cellular proteins; (11) fluorescein-activated cell sorting to study the binding of biotinylated TSH to various cells; (12) in vitro protein phosphorylation using labeled inorganic phosphates; (13) nuclear matrix extraction; (14) analysis of N-linked glycosylation using tunicamycin; and (15) isolation of HeLa cell plasma membrane "ghosts".

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

I. AUTOANTIBODIES AND THEIR PROPERTIES

- A. Role of CD5⁺ B Lymphocytes in Rheumatoid Arthritis (RA): In last year's Annual Report we showed that polyreactive antibodies are made by CD5⁺ B lymphocytes. This past year we studied the role of CD5⁺ B lymphocytes in RA. We showed that in patients with RA, circulating CD5⁺ B lymphocytes, but not CD5⁻ B lymphocytes, are increased in number and size, exist in an activated state, spontaneously proliferate, and secrete Ig that binds to the Fc fragment of IgG. By constructing continuous mAb-secreting cell lines from CD5⁺ B lymphocytes, the properties and dissociation constants (K_d) of these antibodies were determined. Two types of rheumatoid factors (RFs) with discrete reactivities were produced. The first type is polyreactive and binds with relatively low affinity (K_d , 10^{-7} mol/liter) to the Fc fragment of IgG. These antibodies are similar to those produced by CD5⁺ B cells from healthy subjects. The second type of RF is monoreactive and binds with higher affinity (K_d , 10^{-9} mol/liter) to the Fc fragment of IgG. These latter autoantibodies are produced by CD5⁺ B cells of RA patients, but not healthy subjects. It is concluded that both monoreactive high affinity and polyreactive low affinity RFs are produced by CD5⁺ B cells from patients with RA.
- B. Frequency of B Cells Producing Monoreactive High Affinity Autoantibodies in Patients with Hashimoto's Disease and Systemic Lupus Erythematosus: The frequency of cell precursors producing Ig of different classes and Ag-binding activities were determined, using EBV-infection and limiting dilution assays, in healthy subjects and patients with autoimmune disease. A large proportion of circulating B cells from healthy subjects were committed to the production of IgM antibodies that were polyreactive and bound a variety of self- and exogenous Ag, i.e., IgG Fc fragment, ssDNA, thyroglobulin, thyroid microsomal Ag, insulin, and tetanus toxoid. Similar frequencies of these polyreactive antibody-producing cells were found in patients with Hashimoto's disease and SLE. In contrast, significantly higher frequencies of cell precursors producing monoreactive IgG autoantibodies to thyroid Ag (thyroglobulin

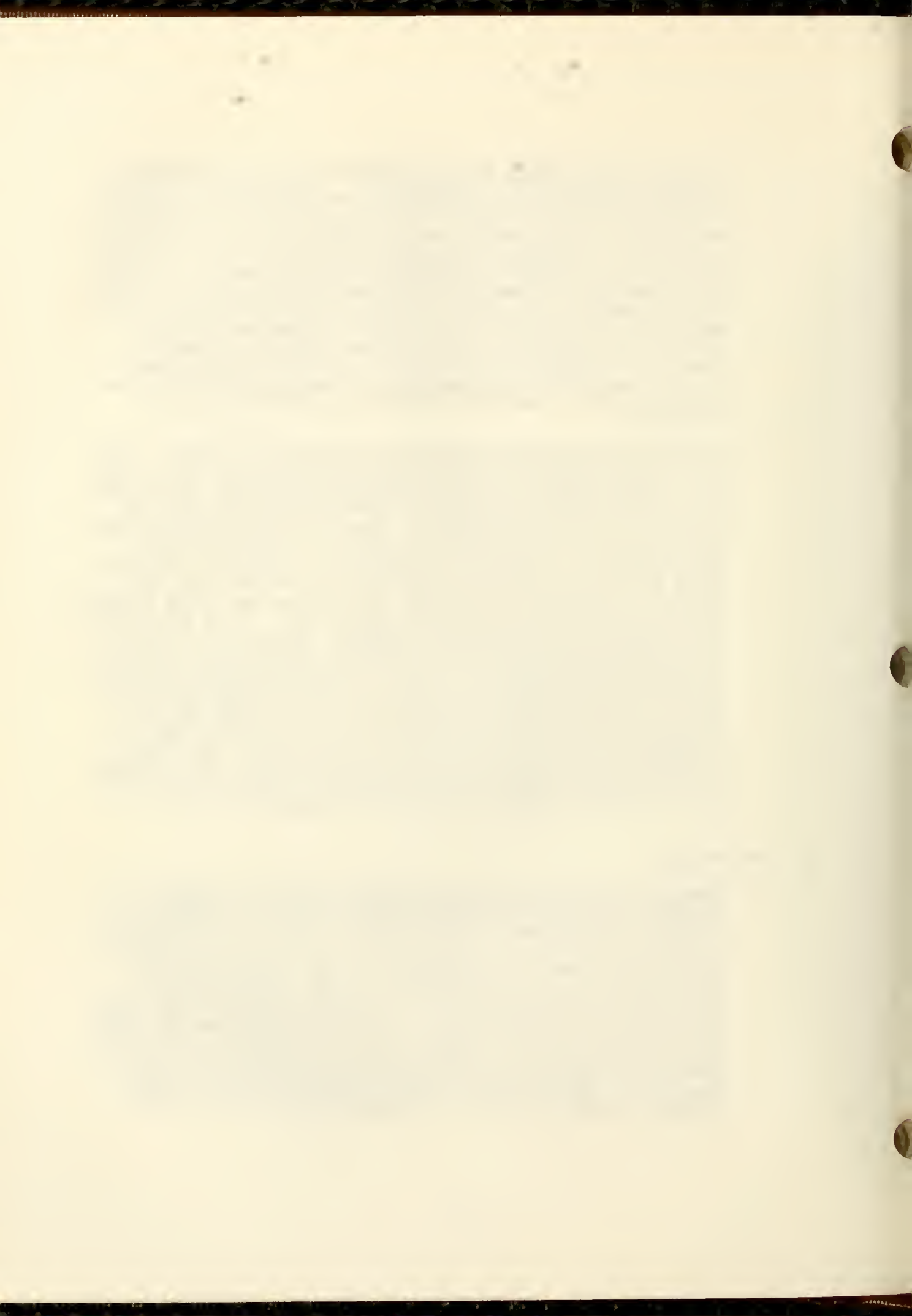


and thyroid microsomal Ag) and ssDNA were found in Hashimoto's disease and SLE patients, respectively. Calculation of the K_d revealed that monoclonal polyreactive antibodies were in general low affinity (K_d , 10^{-3} to 10^{-7} mol/liter), whereas monoclonal monoreactive autoantibodies were high affinity (K_d , 10^{-9} to 10^{-11} mol/liter). The detected frequency and high affinity of the monoreactive autoantibodies in Hashimoto's disease and SLE patients were comparable to those of anti-tetanus toxoid and anti-insulin IgG mAb produced by B cell clones from vaccinated healthy subjects and insulin-treated patients with insulin-dependent diabetes mellitus, respectively. These findings support the hypothesis that the autoimmune B cell repertoire in patients with organ-specific and systemic autoimmunity is shaped by Ag-driven clonal responses rather than merely reflecting a polyclonal B cell activation.

- C. Polyreactive Antibodies Are Derived from Germ-Line Genes: Eight full length cDNA were isolated from EBV transformed human peripheral blood lymphocytes derived from different normal individuals. Five were derived from antibodies with the characteristics of natural polyreactive antibodies. Three were either monoreactive or bireactive. The most striking feature of the structure of these molecules was their utilization of V_H families. Although three used the large V_{HIII} family and one used the large V_{HI} family, the other four used genes derived from two of the recently defined small human V_H families, V_{HIV} and V_{HV} . Three of the molecules represent V_{HIV} expressed sequences and one is the first example of a V_{HV} gene used in an antibody of defined specificity. The nucleotide sequences of some of the molecules were remarkably similar in their V_H gene segments to previously described V_H genes. The data suggest that natural autoantibodies may use a restricted portion of the V_H repertoire, and, in addition, that polyreactive antibodies may be germ-line encoded. The immunoglobulins coded by these germ-line sequences appear to be similar to the classical "natural antibodies" and may serve as the first line of defense against invading micro-organisms.

II. AUTOANTIGENS

- A. Demonstration That the 70 kDa Protein Is a Nuclear Binding Protein: Last year we isolated a novel human 2.1kb cDNA which encodes an autoantigen. We have now expressed this cDNA using a baculovirus vector. Large amounts of a 70kDa protein were produced in insect cells infected with the recombinant virus. The protein was not glycosylated or phosphorylated and was localized in the nuclear matrix. By Southwestern blotting and DNA-cellulose chromatography, it was demonstrated that the 70kDa protein binds to DNA in buffer containing 0.75M NaCl, indicating high affinity binding. Moreover, the protein binds with equal affinity to double-stranded and single-stranded DNA. These properties suggest that the protein might be involved in DNA metabolism, DNA replication or transcription.



Immunoprecipitation was used to show that 10/20 patients with Graves' disease and 0/20 normal donors contained IgG antibodies to the 70kDa protein. A report from another laboratory suggests that the 70kDa protein may be the same as the 70kDa component of the Ku autoantigen, the target of autoantibodies in some patients with systemic lupus erythematosus and other diseases. This autoantigen, therefore, may have a wider importance in autoimmunity than has been previously realized.

- B. Identification of Region of Protein to Which DNA Binds: In order to identify the region of the protein that interacts with the DNA, we tested by Southwestern blotting the ability of genomic DNA to bind to various peptides. Initially, 17 individual peptides (synthesized on the basis of the deduced amino acid sequence of the 2.1kb cDNA) were blotted onto nitrocellulose membrane. The blots were hybridized with ³²P-labeled total human genomic DNA. The blots were washed with buffers containing different amounts of salt and the bound DNA quantitated. The studies revealed that the DNA bound with a high affinity to a peptide which is 15 amino acids in length. Peptides covering other regions of the protein did not show any binding to DNA. The ability of this peptide to inhibit the binding of native protein is currently being tested. The current work shows that synthetic peptides are useful in identifying DNA binding domains of proteins. This may lead to a better understanding of the cellular function of DNA-binding proteins.
- C. Cellular Localization of 70 kDa Protein: In other experiments, transcripts hybridizing with the 2.1kb cDNA were found in all normal and transformed human tissues and cell lines tested. The 70kDa protein was demonstrated in several cell lines by Western blotting. Immunofluorescence microscopy and fluorescence-activated cell sorting were used to show that the 70kDa protein was present in the nucleus of normal and transformed human cells. Surprisingly, the protein also was found in the plasma membrane. This was confirmed by Western blot analysis of isolated plasma membrane "ghosts" from HeLa cells. This is one of the first studies to demonstrate the simultaneous expression of a cellular protein both in the nucleus and the plasma membrane. These observations could be important in understanding the role of the 70kDa autoantigen in autoimmune diseases. The expression of nuclear autoantigens on the plasma membrane has significant implications for understanding the pathogenesis of human autoimmune diseases.

III. HERPES SIMPLEX VIRUS (HSV)

- A. Clinical Trial on Prevention of Reactivation: We have previously reported on the development of a novel system for studying reactivation of herpes simplex virus (HSV) infection in humans. In this model, patients with a history of recurrent HSV infection are exposed to ultraviolet light (UV) to produce a "sunburn" at a site of a previous HSV recurrence. About 50% of the patients develop an HSV recurrence at the site of exposure within 6 days.

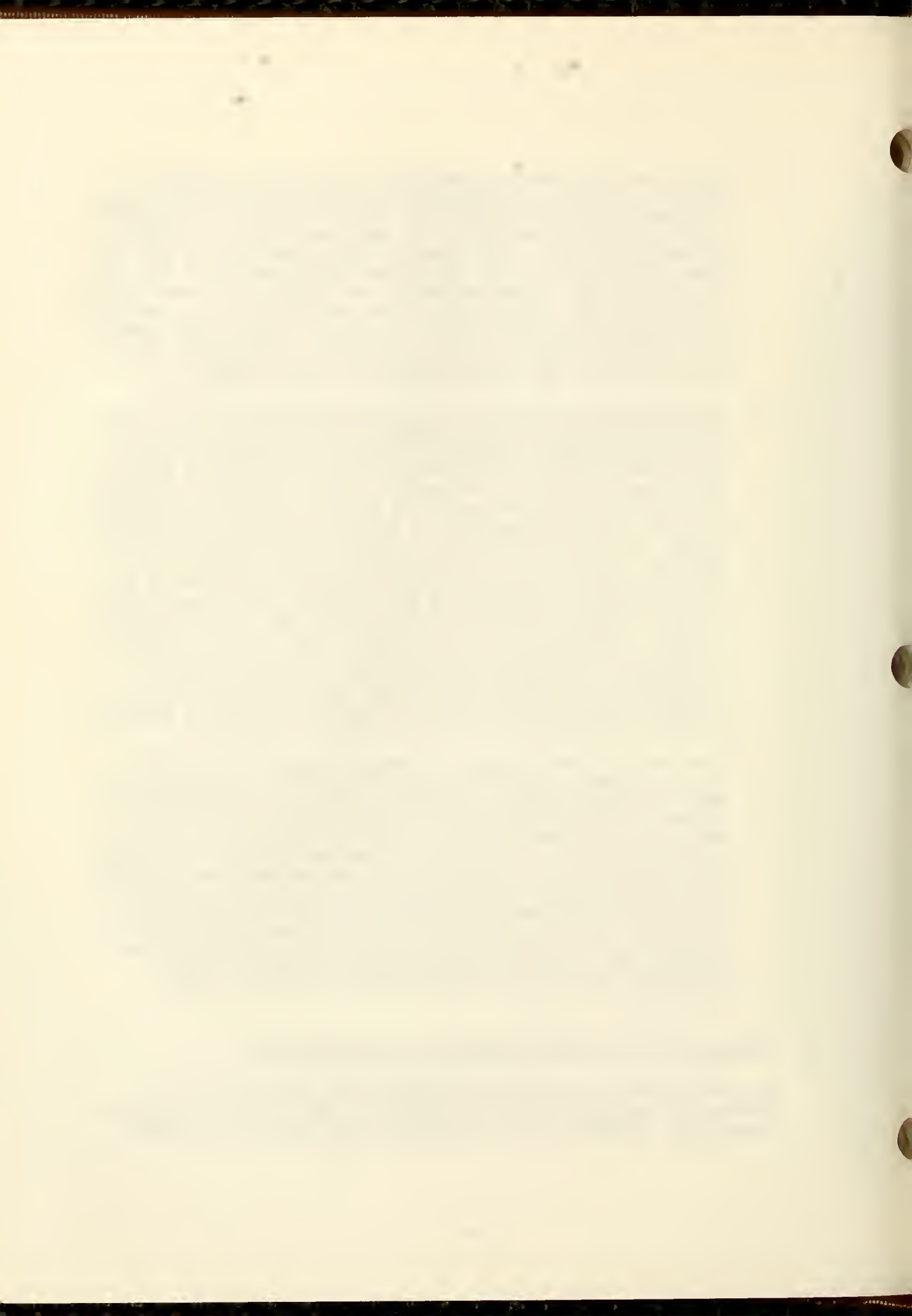
During the past year, we completed a double-blind placebo-controlled trial to evaluate the ability to acyclovir to prevent UV light-induced HSV recurrence. Acyclovir proved very effective in preventing reactivation of HSV when administered 24 hours before UV exposure. This suggests that patients may be able to take acyclovir to prevent recurrences during periods of high risk of reactivation. This represents a new approach in preventing HSV lesions when the inducing stimuli can be identified. In addition, this study demonstrates the effectiveness of the UV-light model for evaluating antiviral agents. Additional studies are underway to determine if sun-blocking agents or anti-inflammatory drugs also can prevent UV light-induced reactivation of HSV.

- B. Immunized Mice Challenged with Herpes Simplex Virus by the Intra-nasal Route Show Protection against Latent Infection: We previously described a recombinant vaccinia virus that expresses the glycoprotein D (gD) gene of herpes simplex virus 1 (HSV-1). Mice immunized with this recombinant showed substantial protection ($\geq 70\%$) against the development of a latent trigeminal ganglionic infection when challenged with a sublethal dose of HSV by the lip route. In this model, lips are abraded, disrupting the epithelial surface, and a drop of saline containing HSV is placed on the abraded surface. Virus enters the peripheral sensory nerve endings and travels retrograde up the trigeminal nerve trunk to the trigeminal ganglion where a productive and then latent infection ensues. Although the lip-abrasion mouse model has proven useful in studying the pathogenesis, prevention and treatment of HSV infection, this model may not precisely parallel orolabial infection in man, where infection may occur through transfer of infected secretions, by social acts such as kissing, to epithelial surfaces where abrasions are not clinically apparent.

Over the last year we chose to study vaccine efficacy by intranasal challenge a model that does not involve tissue abrasion and may more closely parallel infection in humans. Our experiments showed that vaccinated mice challenged by the intranasal route were protected almost as well as mice challenged by the lip route, indicating that the lip abrasion method was not yielding falsely high protection data. In the case of the non-abrasive intranasal route of challenge, it would appear that the virus is neutralized by circulating antibodies either shortly after the virus penetrates the mucosal surface or after it produces a mild local infection. In either case, the antibody generated by vaccination prevents the virus from entering the nerve where it would be protected from neutralization and could establish a latent infection.

IV. TRANSGENIC MICE AND HUMAN IMMUNODEFICIENCY VIRUS (HIV)

We have completed and published (Science) the first phase of our studies on creating a small animal model for HIV by use of transgenic technology. Transgenic mice containing intact copies of the human



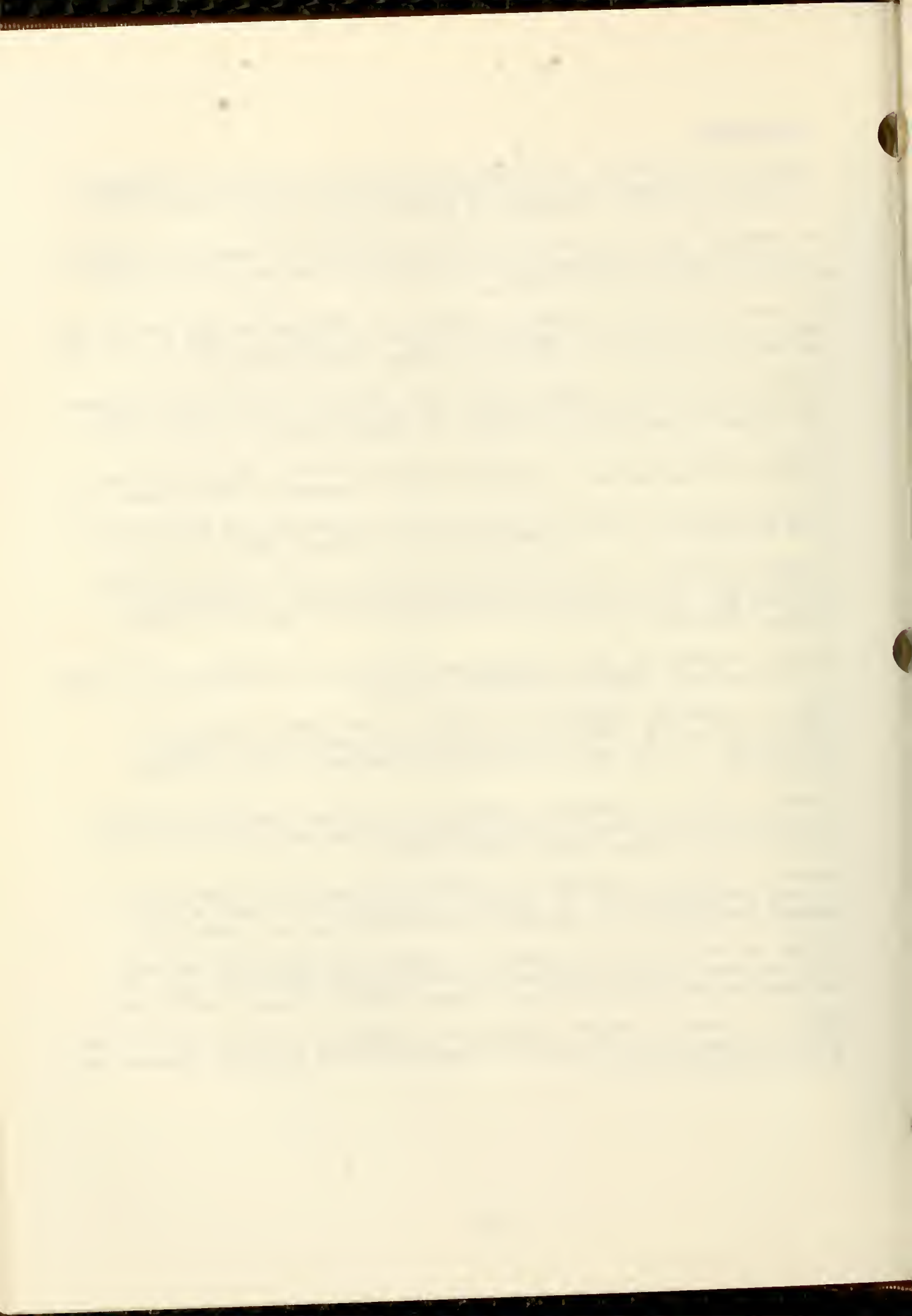
immunodeficiency virus (HIV) proviral DNA were constructed. Founder animals were not viremic for HIV and remained healthy during a 9-month observation period. After being mated with nontransgenic animals, one founder mouse (No. 13) gave rise to F₁ progeny that developed a disease syndrome characterized by marked epidermal hyperplasia, lymphadenopathy, splenomegaly, pulmonary lymphoid infiltrates, growth retardation, and death by day 25 of life. Infectious HIV, indistinguishable from parental virus by immunoblot analysis, was recovered from the spleen, lymph nodes, and skin of five of five affected animals.

The second phase of our study has been initiated and involves molecularly dissecting the pathogenesis of HIV by using the individual genes of HIV to make transgenic mice. Thus far, two heterozygous FVB/N mouse lines have been developed which bear the HIV-1 nef gene as an integral component of their genome. Mice transgenic for nef demonstrate a high rate of fetal death or abortion. Implications for the mechanism of HIV-1 pathology and fetal abortion in non-transgenic mothers are being studied.

Several transgenic mouse lines that carry a truncated derivative of the HIV-1 proviral genome have been shown to develop lethal kidney disease and associated skeletal and heart muscle tissue pathology. Permanent animal lines are being developed to serve as an animal model for the study of kidney failure and myositis. The possibility is being explored that this is an autoimmune disorder associated with viral gene expression.

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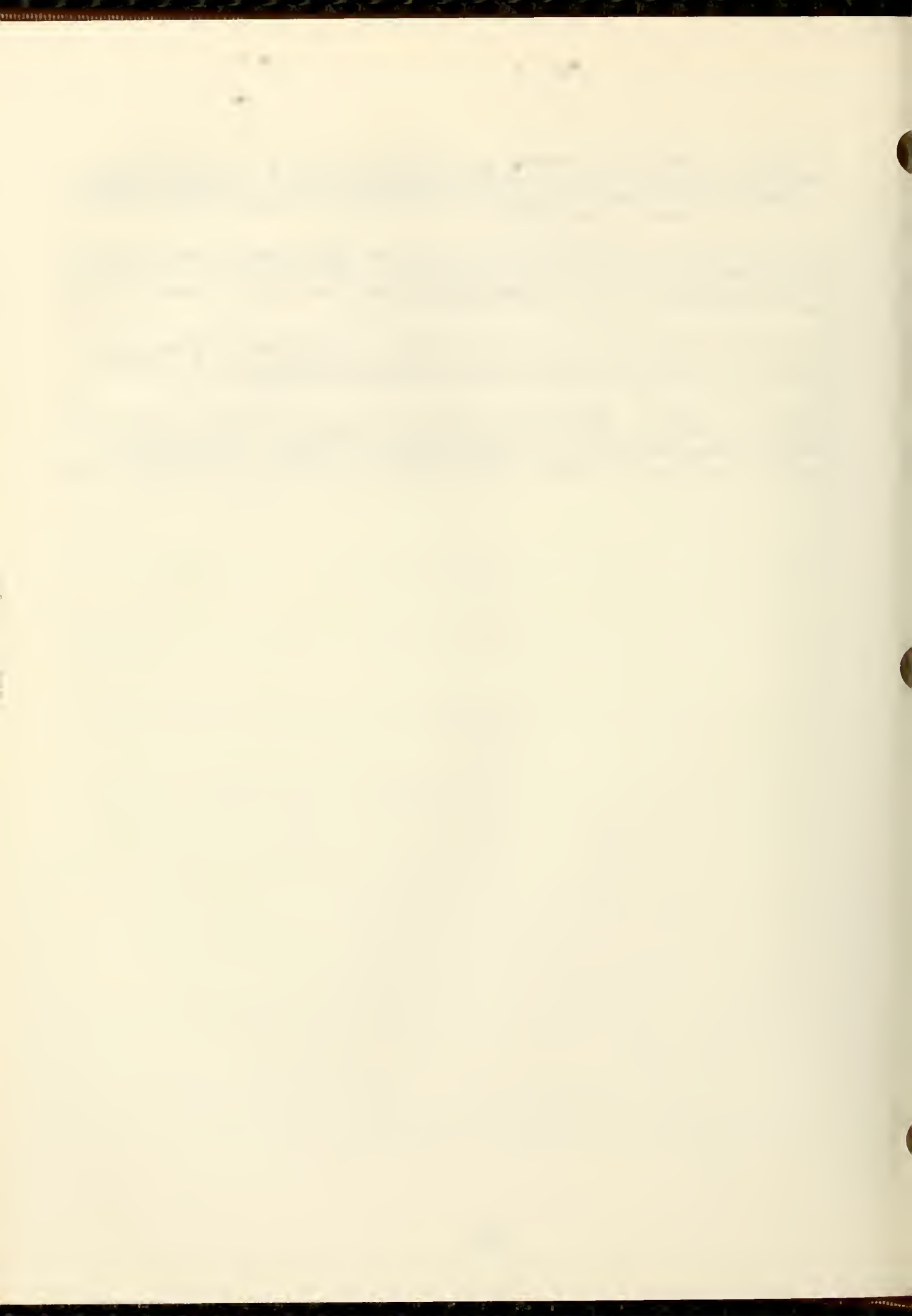


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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00421-04 LOM

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Herpes Simplex Virus and Persistent Infections

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

PI:

Rooney, James F. Special Expert LOM NIDR

OTHERS:

Notkins, Abner L. Medical Director LOM NIDR

COOPERATING UNITS (if any)

Laboratory of Clinical Investigation, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

CHECK APPROPRIATE BOXES)

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

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

We have continued studies utilizing ultraviolet light-induced reactivation of herpes simplex virus (HSV) in humans. This novel model system has been used to study the pathogenesis of HSV reactivation and to evaluate various agents for their ability to prevent recurrences of HSV. During the past year, we have completed a double-blind placebo controlled trial evaluating the ability of acyclovir to prevent ultraviolet light-induced recurrences of HSV. Acyclovir proved very effective in preventing recurrences, suggesting that patients may be able to take acyclovir to prevent HSV infection during periods of high risk for reactivation. This represents a new approach toward preventing HSV lesions in patients with frequent recurrences. In addition, this study is the first demonstration of the utility of this model for evaluating antiviral agents. Additional studies are underway to determine if sun-blocking agents or anti-inflammatory drugs can prevent UV light-induced reactivation of HSV.

Ongoing studies with a recombinant vaccinia virus which expresses HSV glycoprotein D have demonstrated that vaccination with this recombinant provides protection against intranasal challenge with HSV, a route of challenge which closely mimics the natural route of infection in man.

Additional experiments have been carried out in the lab to determine the immune response in mice to individual HSV glycoproteins. In these experiments, the glycoprotein D gene of HSV-1 (HSV-1 gD) was expressed in mouse cells and those cells were then injected into control (non-immune) or HSV-1 immunized syngeneic mice to determine what, if any, immune response would develop. A potent inflammatory response developed in mice that had previously been immunized with HSV-1 but not in non-immune control mice. These studies indicate that transfected murine cells expressing HSV-1 gD can be used to study the in vivo immune response to single viral proteins and argues that the immune response contributes to the pathogenesis of HSV infection.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00423-04 LOM

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Cloning, Expression and Characterization of Human Autoantigens

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

Prabhakar, Bellur S.	Microbiologist	LOM NIDR
OTHERS:		
Allaway, G.P.	Visiting Associate	LOM NIDR
Goto, Yasuhiro	Visiting Fellow	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR
Oates, Edward L.	Staff Fellow	LOM NIDR
Srinivasappa, Javaraiah	Visiting Associate	LOM NIDR

COOPERATING UNITS (if any)

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NCI, NIH, Bethesda, MD 20892	

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

11.00

PROFESSIONAL

7.45

OTHER:

3.55

CHECK APPROPRIATE BOX(ES)

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

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

Recently, a 2.1kb cDNA which encodes a 70kDa human autoantigen was isolated. This protein has now been expressed in insect cells, in large amounts, using a recombinant baculovirus vector. The 70kDa protein expressed in these cells is neither glycosylated nor phosphorylated and is localized in the nuclear matrix. The protein binds to both single-stranded and double-stranded DNA with high affinity. Using synthetic peptides, we have identified one site, consisting of 15 aminoacids, on the 70kDa protein which is responsible for DNA-binding. A large proportion of patients with Graves disease have antibodies to this protein. The 70kDa autoantigen appears to be identical with the 70kDa component of the Ku autoantigen, the target of autoantibodies in systemic lupus erythematosus and other diseases. The 70kDa protein is expressed in all human cells and tissues examined. Moreover, we have found this autoantigen both in the nucleus and on the cell surface. The 2.1kb cDNA has been expressed in NIH-3T3 cells using a variety of promoters in an attempt to reveal the normal cellular function of the protein.

Studies are continuing on cloning of autoantigens involved in diabetes mellitus. Several clones have been isolated from insulin producing beta cell cDNA expression libraries by screening with patient sera. These clones are being sequenced and analyzed. In another approach, an anti- β cell monoclonal antibody was used to isolate a partial cDNA clone encoding a novel β cell antigen which might be involved in the disease. Isolation of the full-length cDNA is in progress.

We are also examining the role of diabetes associated polypeptide (amylin) expression in diabetes. This protein is found as amyloid deposits in β cells of diabetic patients. The amylin gene has been expressed using several promoters in NIH-3T3 and rat insulinoma cells. Preliminary studies indicate that the transfected cells have a slightly different morphology and that their growth rate is reduced. These clones are currently being used to develop transgenic mice. These studies should help in understanding the role this protein plays in the etiology of diabetes.



Continuation - Principal Investigator and Other Personnel

Z01 DE 00423-04

Takai, Osamu	Visiting Fellow	LOM NIDR
Toscani, Anthony	IRTA	LOM NIDR
Vivino, Alfred	Staff Fellow	LOM NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE 00467-02 LOM

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Human B Cell Repertoire and Autoantibodies

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

PI:	Casali, Paolo	Visiting Scientist	LOM NIDR
OTHERS:	Goldfarb, Inna	Visiting Associate	LOM NIDR
	Harindranath, Nagaradona	Visiting Associate	LOM NIDR
	Ikematsu, Hideyuki	Visiting Fellow	LOM NIDR
	Notkins, Abner L.	Medical Director	LOM NIDR
	Ueki, Yuji	Visiting Fellow	LOM NIDR

COOPERATING UNITS (if any)

Mount Sinai School of Medicine, New York, New York	Cetus Company, Palo Alto, California
Texas Southwestern Medical School, Dallas, Texas	
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania	

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

6.23

PROFESSIONAL.

4.07

OTHER.

2.23

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

EBV transformation in conjunction with limiting dilution culture and somatic hybridization techniques have been used to establish cell lines capable of making human monoclonal antibodies to a number of self-antigens, e.g., ssDNA, thyroglobulin, insulin, IgG, Fc fragment and exogenous antigens, e.g., tetanus toxoid, HIV. These technologies have been applied to the study of the human B cell repertoire in patients with systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's disease and insulin-dependent diabetes mellitus. B lymphocytes producing two groups of autoantibodies were detected in these patients. The first one includes autoantibodies binding to multiple self and exogenous antigens, in general, with low affinity. These polyreactive antibodies also can be detected in healthy subjects. The second one includes high affinity monoreactive autoantibodies and is found only in autoimmune patients. Using fluorescence-activated cell sorter, the B lymphocytes devoted to the production of polyreactive "autoantibodies" were identified as a discrete (CD5+) B cell subset. In most cases, lymphocytes devoted to the production of high affinity antibodies were identified as CD5-B cells. The gene segments coding for the V region of polyreactive antibodies have been sequenced and found to be in germ-line configuration.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00471-02 LOM

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Transgenic Mice as a Model for Studies of AIDS and autoimmunity

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

PI:		
Abramczuk, Jan W.	Visiting Scientist	LOM NIDR
OTHERS: Dillon, Patrick J.	IRTA	LOM NIDR
Dickie, Peter	Visiting Associate	LOM NIDR
Dhawan, Subhash	Senior Staff Fellow	LOM NIDR
Dorfman, Nickolas A.	Expert	LOM NIDR
Rooney, James F.	Expert	LOM NIDR
Notkins, Abner L.	Medical Director	LOM NIDR

COOPERATING UNITS (if any)

Laboratory of Molecular Microbiology, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

6.38

PROFESSIONAL

4.63

OTHER

1.75

CHECK APPROPRIATE BOX(ES)

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

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

Lines of transgenic mice bearing stably integrated copies of various HIV-1 genes have been established. They are being characterized for the purpose of developing animal disease models and to study HIV-1 gene regulation. In three lines bearing a functional HIV-1 envelope gene construct, focal segmental glomerulosclerosis has developed in transgenic animals. In two lines bearing an HIV-1 *nef* gene construct, transgenic animals display a high incidence of stillbirths and fetal abortion. Other transgenic animals under study bear the HIV-1 *tat* gene and the human CD4 (HIV-1 receptor) gene. Additional founder animals, transgenic for the entire HIV provirus were generated, to continue the development of a small animal model of AIDS.

The studies on autoimmune responses in mice transgenic for the herpes virus glycoprotein D continued. Transcription of the transgene was confirmed for several tissues of three, of four tested, pSVgD lines. Two pSVgD lines, tolerant to gD were challenged with herpes virus: the observed mortality was similar to that observed after a challenge on non-transgenic, immunologically naive, controls. These findings are leading to new insight into the immunological response to herpes virus.



EXTRAMURAL PROGRAM

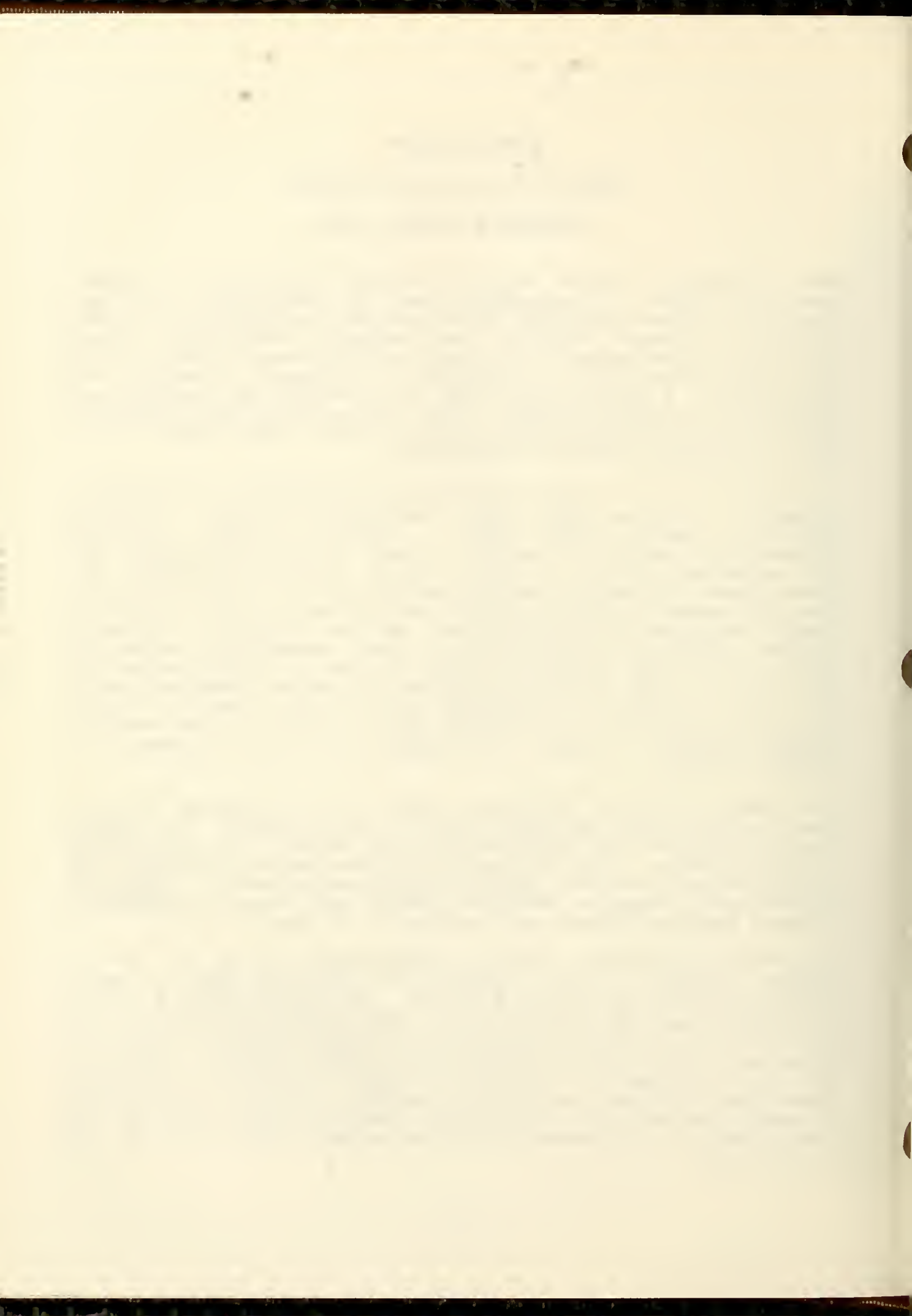
ANNUAL REPORT
REPORT OF THE ACTING DIRECTOR
EXTRAMURAL PROGRAM, NIDR

The Extramural Program of the National Institute of Dental Research (NIDR) is responsible for the development, review, funding, and management of grants seeking Institute support for research, research training, and manpower development. Three program branches support a wide spectrum of research that extends from basic research to new methods of diagnosis, treatment and prevention. A special assistant is in charge of training and manpower development programs, while review and grants management are the responsibility of a Special Review Branch (SRB) and a Grants Management Section respectively.

A broad array of award mechanisms is available to extramural scientists ranging from small feasibility grants to regular grants to large center grants. During FY 1989, the NIDR Extramural Program (EP) made 643 research and training awards for an estimated total of almost \$ 93 million including 389 investigator-initiated research project grants (\$61.5 million). The 389 research project grants accounting for 66% of the EP budget comprised 305 regular grants for \$44.3 million; 16 program project grants for \$8.9 million; 1 young investigator award for \$50,000; 35 FIRST awards for \$3.1 million; 18 MERIT awards for \$3.6 million; and 14 small business innovative research awards for \$1.5 million, an increase of \$330,000 over the funds specifically set aside for that purpose. Of the 389 research project grants, 53 were new awards and 46 were renewals of existing awards.

The NIDR also funded 19 center grants for an estimated total of \$14.5 million which was about 15.5% of the EP budget. The center awards supported four multi-categorical centers, one Oral Health in Aging Center, five Periodontal Diseases Research Centers, two Caries Research Centers, and one Pain Research Center. Three new Craniofacial Anomalies Research Centers and three new Materials Science Research Centers were awarded in FY 1989.

The "Other Research" budget, approximately 12.5% of the EP budget, funded 31 small grants for \$647,414 and 17 small instrumentation grants for \$307,032. It also provided \$ 55,890 as full or partial support for 2 conferences and \$145,000 for the Division of Research Resources Minority Biomedical Research Program. Seventy six percent of the Other Research budget or \$8.9 million funded 17 career development awards, 25 individual physician scientist for dentist awards, 29 individual dentist scientist awards and 9 institutional dentist scientist awards (84 individuals), all together providing support for a total of 155 individuals.



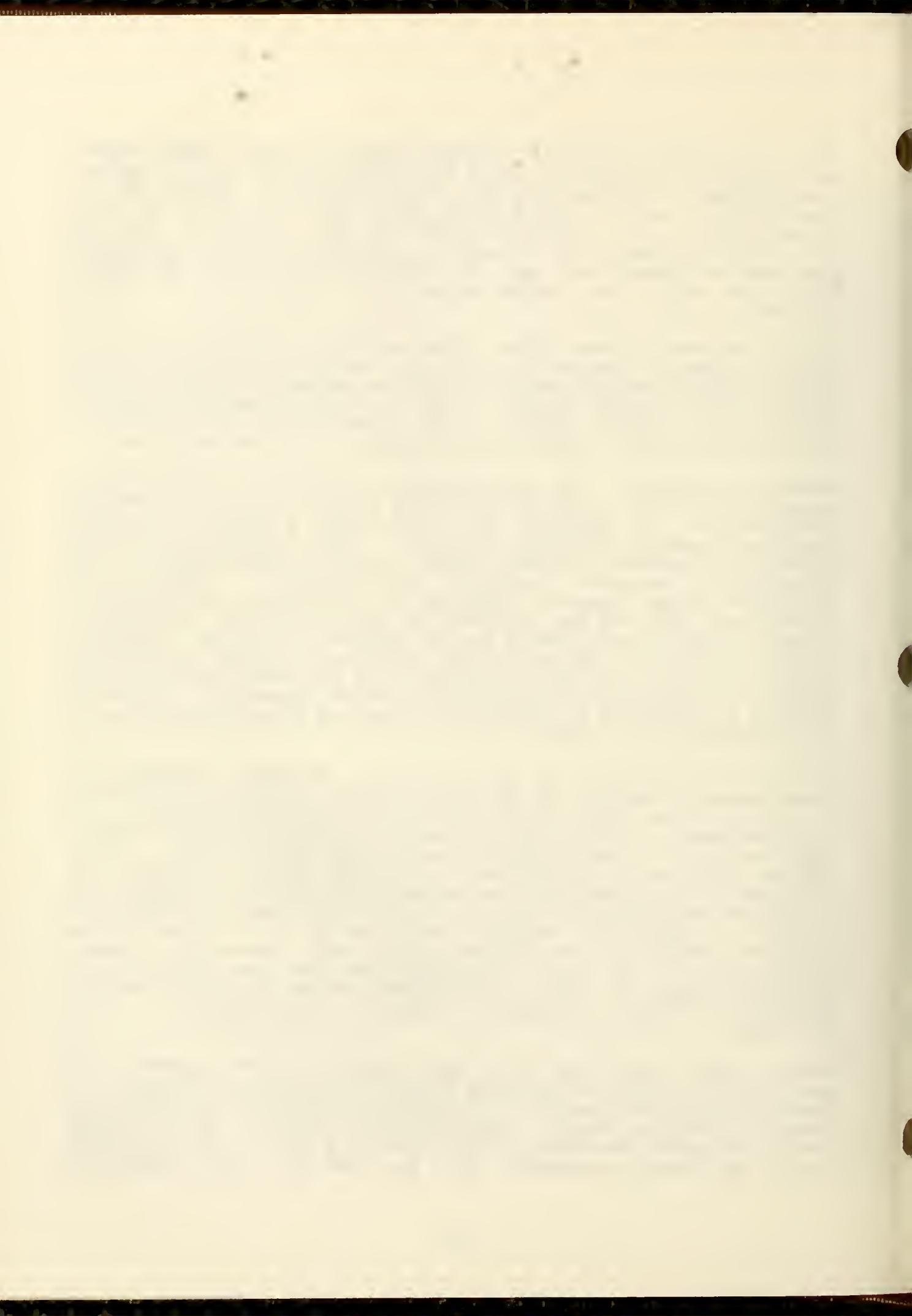
The \$5.3 million National Research Service Award (NRSA) budget was disbursed as follows: \$3.8 million in support of 31 regular institutional training programs and \$379,563 in support of 24 institutional awards to provide short-term (summer) training opportunities for dental students; and \$951,164 for 35 individual fellows. The 31 regular institutional training grants provided 16 predoctoral and 98 postdoctoral training positions while the short-term awards supported a total of 153 dental students.

Special Emphasis Areas: AIDS: NIDR extramural support for AIDS-related research reached \$2 million. Included in the portfolio were 2 program project grants, 6 regular research grants, 1 FIRST award, and 1 career award. AREA: NIDR awarded 12 Academic Research Enhancement Awards for the first time in FY 1989 for a total of \$1 million, almost \$600,000 above the NIDR requirement.

Minority Programs: The NIDR provided \$158,802 in direct grant support (1 regular research grant and 1 small grant) to minority institutions. In addition, the NIDR funded 4 minority research supplement awards totalling \$195,755. The contribution to the Minority Biomedical Research Support Program administered by the Division of Research Resources was \$145,514. These funds supported dental research projects at 3 different institutions. In addition, \$66,545 was provided the Minority Access to Research Careers program administered by the National Institute of General Medical Science in support of 11 ancillary training activities. Funding (\$13,500) was also provided for a new short-term training program intended only for minority dental students. The total support for minority programs in FY 1989 was \$580,116 which was a 22% increase over FY 1988.

EP activities were varied and extensive. The most demanding and time-consuming efforts of the year involved review by the SRB of 5 applications for Caries Research Centers support, 5 applications for Craniofacial Anomalies Research Centers, and 16 applications for Materials Science Research Centers. These reviews utilized the "applicant interview" format. However, in the case of the Materials Science Research Centers, the applicant interview was preceded by a "triage" review which reduced the number of competitive applications from 16 to 7. Two Caries Research Centers were funded which were competing renewal applications. Of the 5 applications for the Craniofacial Anomalies Research Centers three were successful and of the 16 Materials Science Research Centers applications, 3 were successful.

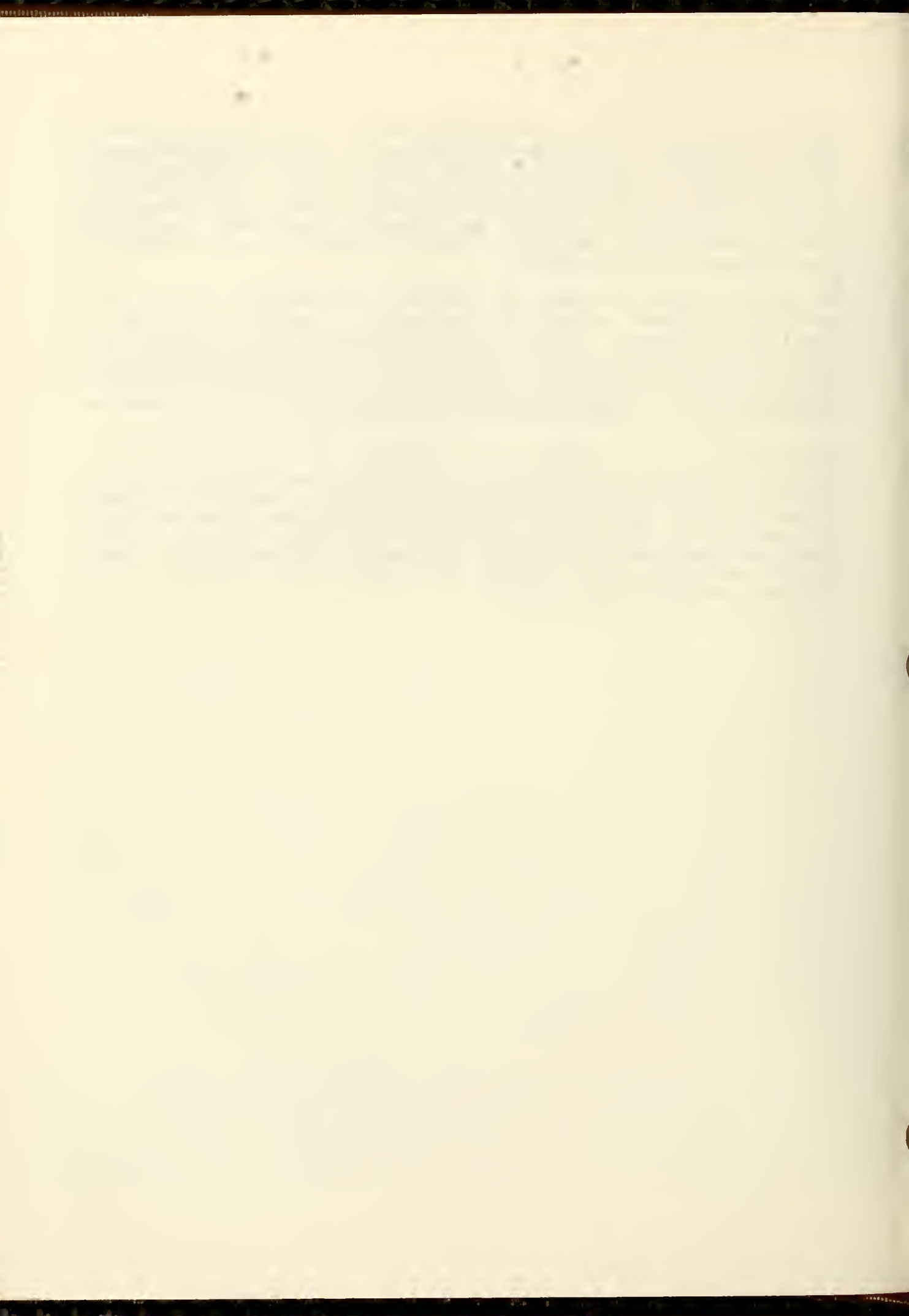
Other major activities were the development of requests for applications (RFA) for Clinical Dental Research Core Centers, Dentist Scientist Award - Institutional Grants, and National Research Service Award - Institutional grants. The Clinical Dental Research Core Centers RFA represents both a new initiative and a new funding mechanism (P30) for the NIDR. The submission



deadline is in FY 1990. Other RFAs were: "Evaluation of Commonly Used Orthognathic Treatment Procedures", "Human Immunodeficiency Virus Inhibitory Factors in Human Saliva", and "Development and Characterization of Immortalized Salivary Gland Epithelial Cell Lines". Program Announcements which were issued were: "Basic and Clinical Research on Normal and Impaired Oral-Motor Function", "The Effects of Oral Factors on Taste and Smell", and "Biology of Tooth Movement and Eruption".

EP staff visited a number of grantee institutions as part of a continuing effort to keep the scientific community informed of institute programs. Staff also participated in numerous site visits and attended scientific meetings using these occasions as opportunities for further contact with the scientific community. In addition, EP staff played a major role in the planning, organization and conduct of several conferences.

The success of our efforts are dependent on the existence of a strong partnership between the extramural community and the NIDR. The willingness of the extramural scientific community to serve on peer review committees and advisory committees is essential to the success of our many initiatives and last, but not least, their own research is the force that moves science forward. This report reflects some, but by no means all, of the progress achieved during the past year.



ANNUAL REPORT

RESEARCH TRAINING AND MANPOWER DEVELOPMENT

EXTRAMURAL PROGRAM - NIDR

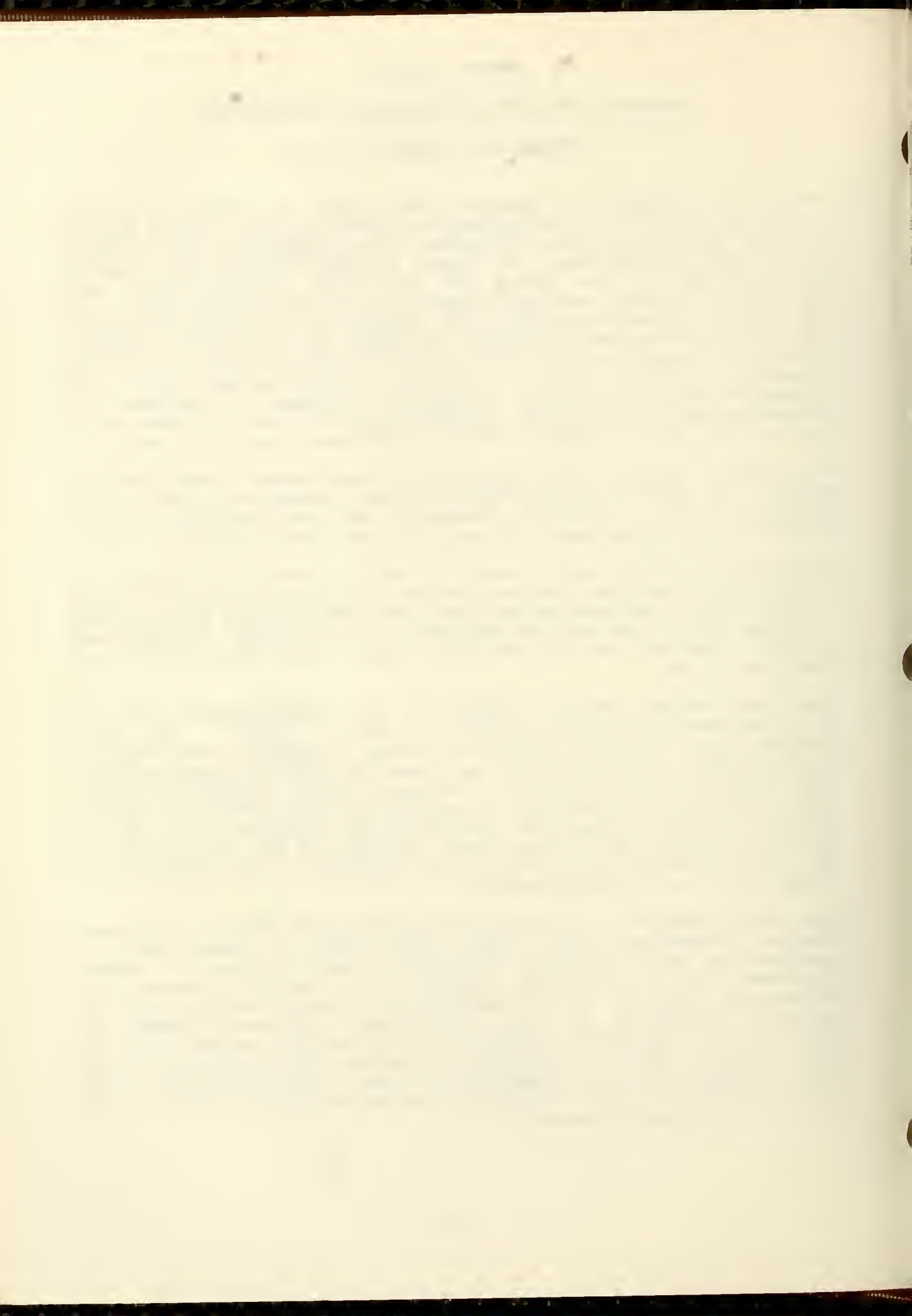
The first Request for Applications (RFA) for training grants under our new policy and guidelines (noted in last year's annual report) produced 23 applications: five competing continuation and 18 new applications. We expect to fund three of the renewal applications in the areas of nutrition, epidemiology, and salivary sciences, and approximately five new applications, in the areas of neuroscience, soft tissue diseases/oral biology, salivary sciences/oral biology, epidemiology and mineralization. From the 23 applications reviewed, twelve were in the potentially fundable range, and of these, approximately six had molecular/cellular biology components to them. The need for molecular/cellular biology training prompted much discussion at one of our Dental Research Programs Advisory Committee meetings.

The second RFA for institutional training grants under our new policy was issued in the NIH Guide for Grants and Contracts on March 17, 1989. This RFA requested new and competing renewal applications in the areas of cariology and periodontology.

Last year's annual report noted our initial funding of a training grant in the area of "primary medical and dental care." Due to a departmental reorganization, our one funded dental primary care training grant has been reassigned to the original primary care funding component of the PHS, the Health Resources and Services Administration.

Our last annual report noted the RFA announcement for the institutional Dentist Scientist Award (K16). All nine institutional awardees submitted renewal competing continuation applications. In addition, we received six new applications. The September 1989 National Advisory Dental Research Council approved continued funding for six (of the nine) currently active K16s and funding three new institutional programs. It is the NIDR's intention to provide funds for each of the individuals on the three programs that will not be renewed to the completion of their five-year training period.

The fourth meeting of the program directors of the institutional Dentist Scientist Award (K16) took place in March 1989 in conjunction with the AADR meeting in San Francisco. Issues important to the efficient operation of the K16 program were discussed, such as, the integration of a basic science and the clinical component in each individual's program, funds for supplies and equipment, minority participation, recruitment and the applicant pool, and starting salaries. The "cost-of-living" differences between the locations of each of the K16 award sites and the effect of the starting salaries offered at each of the institutions were discussed.



There was discussion that an attempt should be made to convene all the individuals being supported by the K11, K15, and K16 programs at some time during the IADR/AADR meeting. It was generally agreed that it would be beneficial to have these individuals socialize and learn about each other's progress and experiences. The program directors commented that this meeting was very useful and should continue to be held once a year.

The Physician Scientist Award (PSA) for Dentists (K11) and the Dentist Scientist Awards (K15-individual and K16-institutional) continue to receive interest in the academic community. As a result, we reviewed and approved eight K11 and twelve K15 applications. We were able to award seven new K11s, and eight new K15s this year. The nine institutional awards were each allowed to add two more additional individuals to their programs. Therefore, the NIDR is currently supporting approximately 115 individuals at some stage of their research/clinical development in these three programs. These individuals are colloquially referred to as "Clinical Research Scientists" (CRSs).

The first "graduates" from the PSA (K11) program entered the research community during the summer/fall of this fiscal year. All were successful in obtaining academic appointments and all had a number of offers from which to choose.

The first group of CRSs from the DSA program will "graduate" next fiscal year (summer/fall '90). With the collaboration of the Office of Planning, Evaluation, and Communications, we are in the process of initiating a system to monitor the progress of the CRSs upon the completion of their training.

At the conclusion of this fiscal year, the NIDR will be supporting approximately 280 trainees, fellows, and clinical research scientists, at some stage of their research/clinical development. These individuals are being trained in the following program areas:

<u>Program Area</u>	<u>Number of Individuals</u>	<u>Dollars</u> ^{1/}
Caries	26	797
Nutrition	4	140
Materials	17	969
Salivary Sciences	18	801
Periodontal Diseases	71	3,329
Stomatology	27	1,426
Craniofacial Anomalies	67	3,113
Pain/Neurosciences	20	966
Behavioral Sciences	16	583
Epidemiology	<u>14</u>	<u>542</u>
Totals	280	12,575

^{1/}
The dollar amounts (in thousands) are meant only to indicate the approximate order of magnitude of manpower development and training funds in the respective program areas. They were obtained by multiplying the number of positions by the "average" dollar amount per position for each funding mechanism, i.e., training grants, fellowships, and CRSs.



Of the funds being expended for manpower development and training, approximately \$6.0 million (47%) are in the Periodontal and Soft Tissue Diseases Research Branch, \$3.3 million (26%) in the Craniofacial Anomalies, Pain Control, and Behavioral Research Branch, and \$3.4 million (27%) in the Caries, Restorative Materials and Salivary Research Branch.





ANNUAL REPORT

CARIES, RESTORATIVE MATERIALS AND SALIVARY RESEARCH BRANCH

Extramural Program, NIDR

Progress in dental caries research included epidemiological and clinical studies on coronal and root caries experience in the elderly and minority populations and on the effect of malnutrition on tooth development and dental caries in children, studies on the etiology of root surface caries, development of new agents and/or vehicles to enhance the anticaries effects of fluoride and to test the cariogenicity of dietary items, the application of molecular genetic approaches to produce novel caries vaccines, and studies to elucidate the mechanisms of fluoride absorption from the gastrointestinal tract. Progress in research on restorative materials included studies to determine if the human tumor cloning assay could be used to evaluate cytotoxicity of various dental alloys, studies on in vivo mercury and methyl mercury levels in relation to dental amalgam restorations, studies to evaluate long term clinical failures in posterior composites, studies to assess quantitative x-ray diffraction of leucite changes from thermal treatments of dental porcelain, and the effect of isocyanate content and molecular weight of adhesive on bonding. Recent progress in research on salivary glands and their secretions included studies on bacterial agglutinin activity in the saliva of human identical and fraternal twins, mediation of cyclic AMP production and mucin release by vasoactive intestinal peptide receptors in dispersed acini from rat submandibular gland, the role of salivary epidermal growth factor in the maintenance of the physicochemical characteristics of the rat oral and gastric mucosal mucus coat, characterization of in vivo salivary-derived enamel pellicle, and adsorption and transpeptidation of salivary components to buccal epithelial cells.

- A01 Determine the incidence and prevalence of dental caries covering all target populations.

Epidemiologists at the University of North Carolina and the University of Iowa have examined coronal caries experience in North Carolina adults aged 65+. The Piedmont Dental Study is a longitudinal study of 1,019 adults age 65+ in five N.C. counties. They report baseline data on the relationships among sociodemographic and microbiologic characteristics with coronal caries prevalence. 819 dentate subjects were examined in their homes by a team of calibrated examiners. Examiner reliability for DFS (decayed and filled tooth surfaces) status exceeded 90% for all pairs of examiners. Half of the participants had 20 or more teeth. The mean number of teeth among black persons was

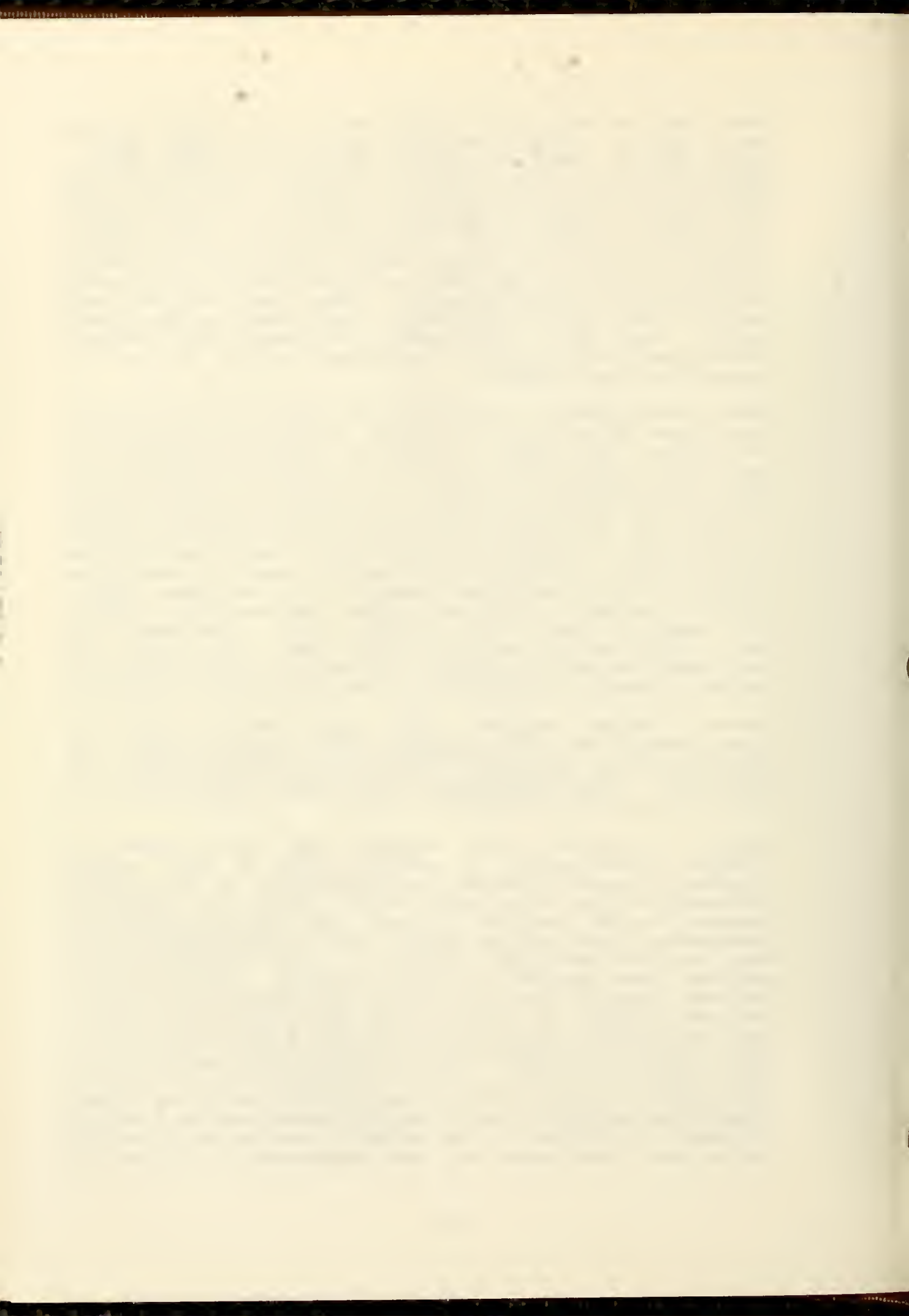


less than for whites and blacks were more likely to have fewer than 12 teeth. Mean DFS scores for whites were 3 times higher. High levels of Lactobacillus (LB) and S. mutans (SM) were associated with unfilled caries, but not with high DFS levels. Multiple regression analyses of DFS and decayed surface (DS) scores indicated that race, gender, socioeconomic status (SES), SM, and time-since-last-visit-to-dentist (TLVD) were significantly associated with caries history, and race, SES, LB, and TLVD were significantly associated with unfilled caries. - These data indicate that age 65+ blacks are significantly more likely to have fewer teeth, lower DFS, and higher DS scores than whites, differences only partially explained by SES, SM and LB scores, TLVD, and gender.

These investigators also looked at factors in root caries experience in the same population. Root caries (RC) data are limited and little is known of factors related to RC experience. They report RC data in relation to demographic, and microbiologic variables in baseline exams of the Piedmont Dental Study. Subtracting filled surfaces related to abrasion, and controlling for education and SES, white subjects were significantly more likely than blacks to have RC experience. However, black seniors were 1.7 times more likely to have untreated decayed root surfaces (DRS). Multiple regression analyses on DRS indicated that coronal DS, number of teeth, sex, lactobacillus, attachment loss were significantly associated with DRS. Age, place of residence, buffering capacity, and salivary flow rates were not significantly related to root caries experience or DRS.

These findings suggest that older whites have had significantly more RC experience than older blacks, but black seniors currently need significantly more RC treatment. These differences remain when controlling for education, SES and residence.

During a clinical trial testing the effectiveness of fluoride mouthrinsing in adults, data on root caries incidence were collected by clinicians at the State University of New York, Stony Brook. Since the difference between the DFS and DFT (decayed and filled teeth) root increments of the placebo control and F-mouthrinsing groups were not statistically significant, the results of all subjects were pooled for this analysis. 796 subjects received baseline and 3-yr. follow-up visual-tactile exams for root caries. All were working adults or their spouses. They ranged from 20-65 years old with a mean age of 39.9 years, and they resided in fluoride-deficient communities on Long Island, New York. 81.4% of subjects did not develop root caries or have root fillings placed during the 3-year observation period. The 18.6% that developed root caries averaged 0.8 DFS/year. The subjects' age and baseline DFS status were associated with the development of a root DF

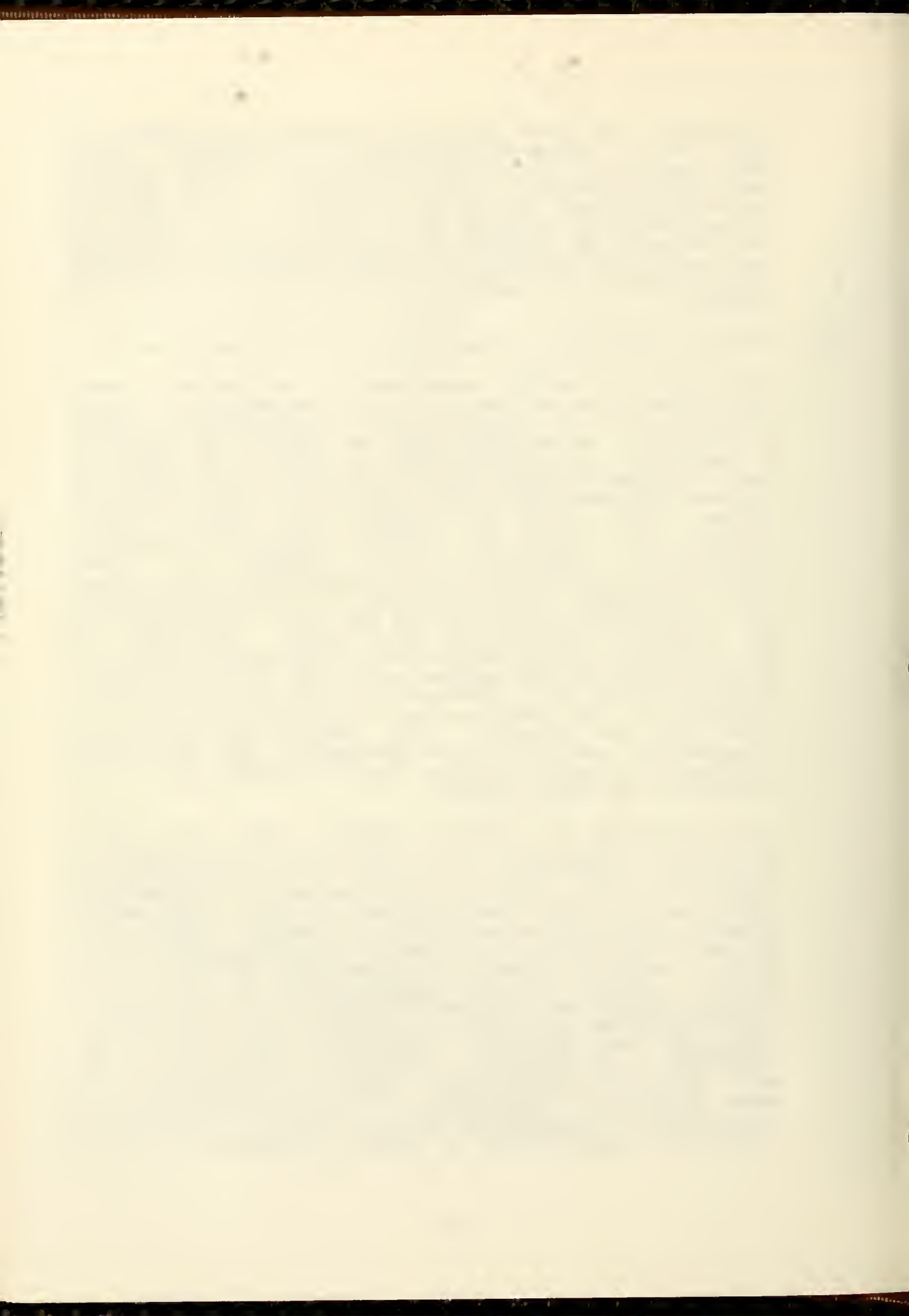


increment. No 20-24 year olds developed root caries and only 2 of 217 25-34 year olds had a DF increment. 13% of 35-44 year olds; 27% of 45-54 year olds; and 57% of 55-65 year olds demonstrated a root DF increment. Of the subjects who developed root caries, 50% of the 35-44 year olds, 66% of the 45-54 year olds and 82% of the 55-65 year olds had a root DF score at baseline. - Age and previous history of root caries are indications of root caries risk and should be considered when designing anticaries clinical trials for adults.

A03 Further elucidate the causes of all types of dental caries.

Cariologists in Boston, Massachusetts, carried out a study to evaluate the presence of total streptococci, mutans streptococci, S. sanguis, S. salivarius, "other streptococci," enterococci, total actinomyces, A. viscosus, A. naeslundii, A. odontolyticus and total lactobacilli in dental plaques obtained from sound and carious (incipient and more advanced) individual root surfaces (522 samples) of subjects (276) with and without root surface caries. Plaques from sound and carious surfaces contained predominant proportions of S. sanguis and "other streptococci" and somewhat lesser proportions of A. viscosus and A. naeslundii; the proportions of other groups/species of organisms were generally less than 1%; the prevalence of the organisms followed the same trend. Prevalence and proportions of the groups/species of organisms in plaque on sound surfaces of root surface caries-free and caries-active subjects were only different in the case of mutans streptococci, the prevalence and proportions of which were lower in the former. The proportions of mutans streptococci, enterococci and lactobacilli were also lower in plaque on sound surfaces than in plaque on carious surfaces in the same subjects.

The association of selected bacteria with root surface caries was also studied by scientists at the Dows Institute for Dental Research, Iowa, and at the University of Manitoba, Canada. Plaque from root surfaces of 165 subjects (mean age 65 years) was analysed to show relationships between caries lesions and specific bacteria. Samples were grouped according to the presence of Lactobacillus and S. mutans. Only 18/165 samples contained both bacteria, 27/165 had S. mutans without Lactobacillus and 12/165 had Lactobacillus alone. Samples containing both bacteria had a higher isolation frequency of S. mitis 1 and no typical A. naeslundii were isolated. Isolation frequencies for A. naeslundii in groups with S. mutans and Lactobacillus alone were 33% and 50% respectively. Typical A. viscosus did not show significant differences. The results confirm an association of S. mutans and Lactobacillus with root surface lesions and suggest that S. mutans, Lactobacillus and S.



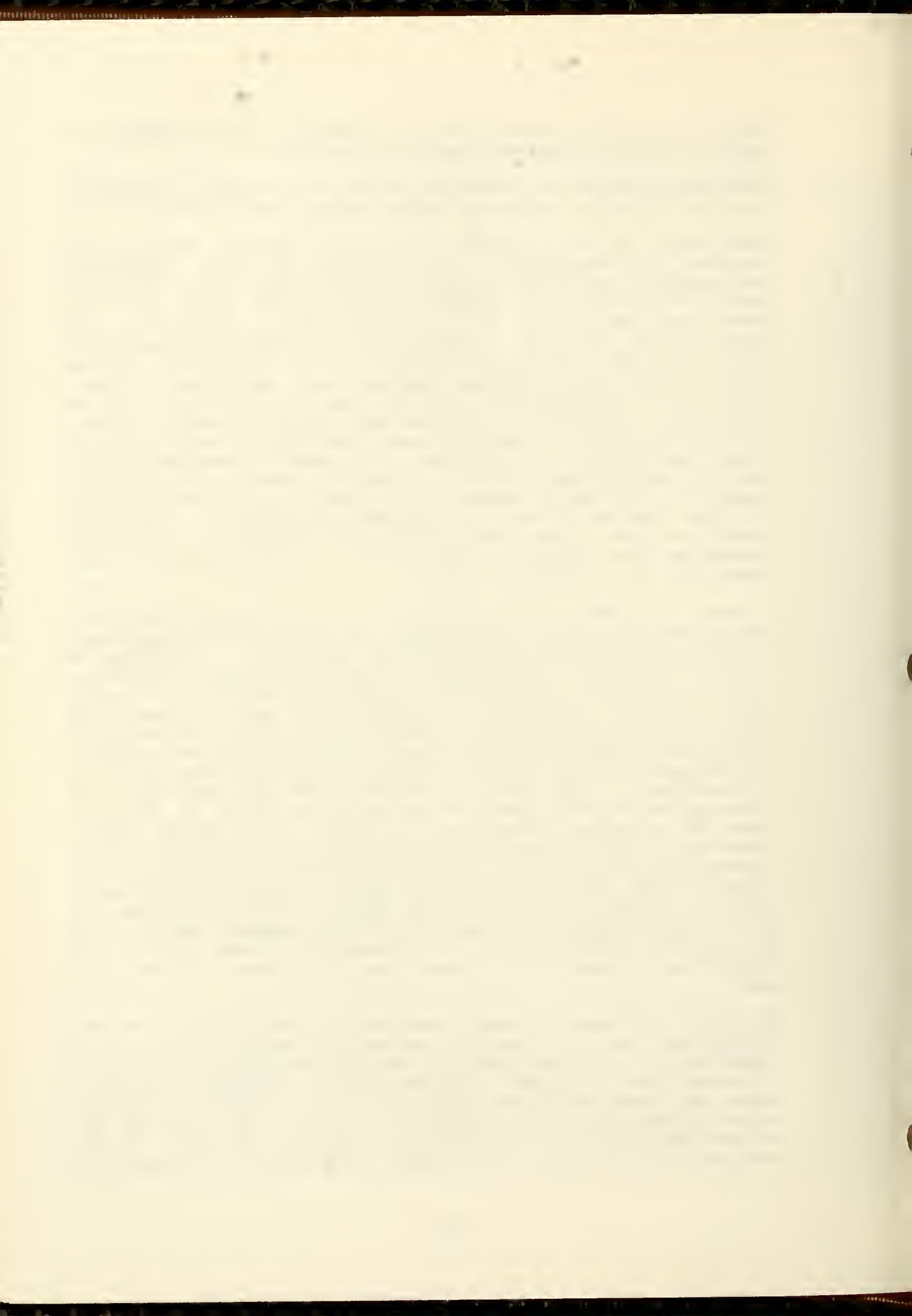
mitis 1 in the absence of A. naeslundii may represent a specific community associated with lesions.

A08 Develop and test new fluoride agents and vehicles and apply existing fluoride regimens to new target populations.

Scientists at the Medical College of Georgia carried out studies to determine if caffeine is the component responsible for elevated plasma F peaks when NaF is given to rats in caffeinated drinks rather than water and to determine its effect on bioavailability, retention, and excretion of fluoride. Female rats were given intragastric (ig) solutions containing either NaF or NaF with caffeine dissolved in decaffeinated coffee, tea, or Classic Coca-Cola. Plasma samples were collected at 20-60 minute intervals. Fluoride levels peaked at significantly higher levels in animals given beverages containing caffeine than those containing no caffeine. In similar studies, urine samples were collected at 3, 6 and 24 hour intervals from animals in metabolic cages. The animals receiving caffeine in the metabolic cage experiments exhibited increased retention and later excretion of fluoride. The data indicates that caffeine increases the bioavailability and retention of fluoride in rats.

A novel F rinse which consists of two solutions has been developed by research scientists at the ADAHP Paffenbarger Research Center in Gaithersburg, Maryland. Solution A contains calcium and solution B contains Na_2SiF_6 , phosphate (P), and acetate. When equal volumes of solutions A and B were combined, the total F concentration was 240 ppm, and the free F ions produced by hydrolysis of SiF_6^{4-} caused CaF_2 precipitation which in turn removed free F from the solution and allowed hydrolysis of SiF_6^{4-} and precipitation of CaF_2 to continue. The added P assured that the solution was supersaturated with respect to hydroxyapatite so that no loss of the tooth mineral would occur. The acetate buffer consumed the H^+ released from the SiF_6^{4-} hydrolysis. The F uptake produced from the experimental rinse was over 15 times greater than that produced by a 250 ppm conventional F rinse. Since the cariostatic effects from F rinses are believed to derive from their ability to deposit labile F in the oral cavity, the two-component rinse should be significantly more efficacious than the rinses currently in use.

In vivo F uptake by enamel lesions from application of NaF dentifrice and a fluoride releasing device (FRD) were compared in a study carried out by cariologists at the University of Michigan. Matched sets of enamel specimens were cut from extracted human molars. Subsurface lesions were produced in a lactate buffer, containing 1% carboxymethylcellulose with Ca, and, at pH 4.5. Eight specimens were worn in removable mandibular appliances by



six subjects for each 15 and 30 day control and two test periods. A F-free dentifrice and NaF dentifrice were used 3x/d for a control and test period respectively. A second experimental regimen was conducted using a FRD, placed in the midline of the appliance. The specimens were evaluated for Ca content. Significant differences were found in the fluoride content in both NaF dentifrice treated and FRD exposed specimens to a depth of 100um. A significantly greater amount of F was deposited at all levels of the lesion in the NaF dentifrice treated specimens compared with the FRD exposed specimens. The F content in both of the test groups was significantly different from the controls.-

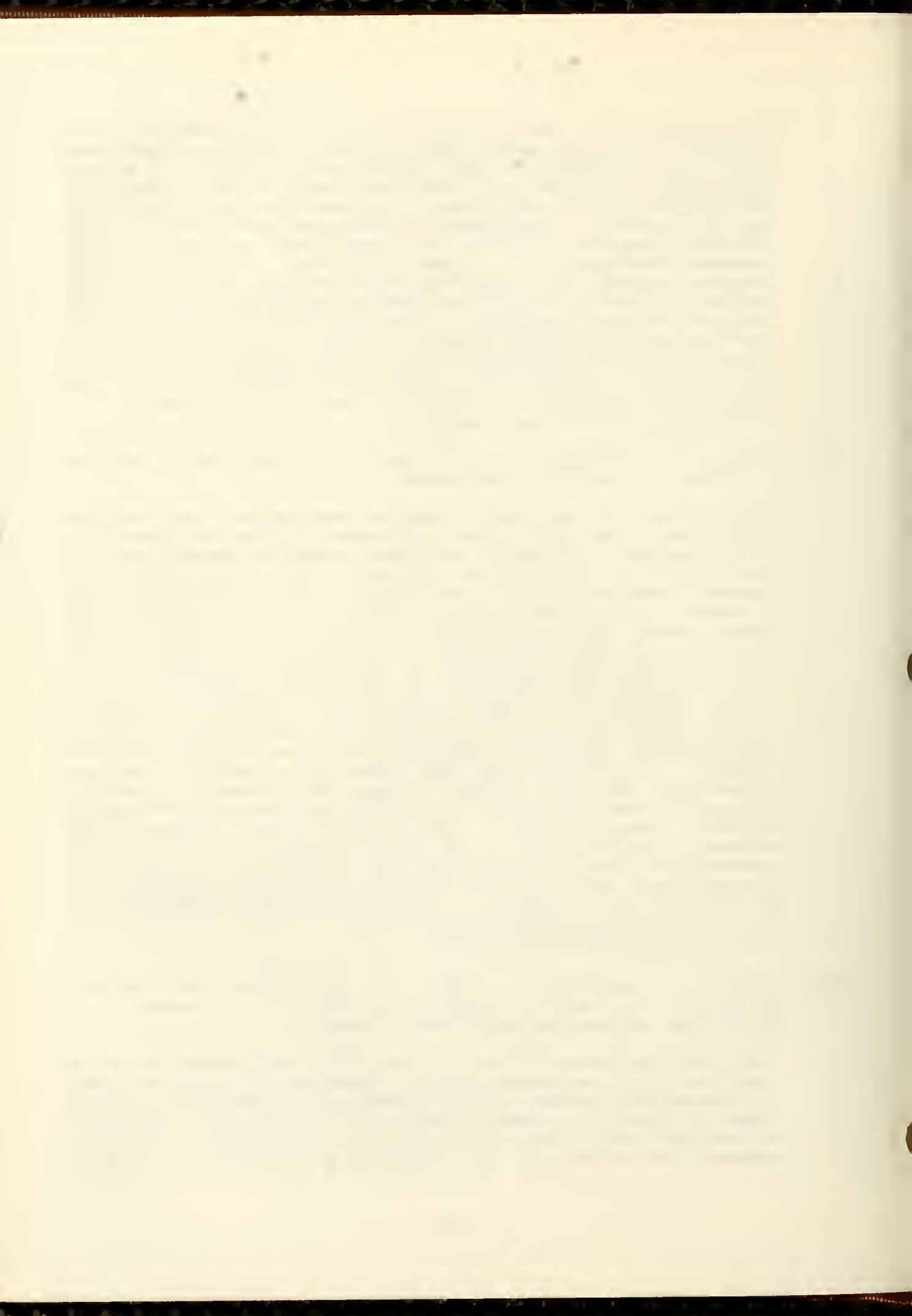
The findings indicate that both a NaF dentifrice or a FRD can result in significant fluoride uptake and that the longer time period (30 days) resulted in more fluoride in the depth of the enamel lesion.

- A12 Purify and characterize all potentially important S. mutans antigens for vaccine development

Application of molecular genetic approaches has led to isolation of several genes implicated in the cariogenicity of S. mutans, utilization of these genes to create specific mutants of S. mutans for further study, the ability to produce relatively large quantities of highly purified gene products for caries vaccine studies, and introduction of these genes into heterologous bacteria as a novel way of inducing secretory antibodies. This latter approach has been utilized by scientists at Washington University, St. Louis, and at the University of Alabama to immunize animals by the gastric route with a recombinant vaccine in which S. mutans genes for surface proteins are incorporated into a nonpathogenic strain of bacteria which has affinity for gut tissue. Expression of the genes has led to antigen stimulation of gut lymphoid tissue and release of anti-S. mutans IgA antibodies in the saliva of mice via the common mucosal immune system. The investigators indicate that although society may not be ready for administering a live recombinant avirulent vaccine to children to control a disease such as caries that is not life-threatening, it may be possible in the future to add an anticaries component to a vaccine system expressing multiple antigens and designed for life-threatening diseases.

- A16 Develop a sequence of tests for estimating the caries-producing or caries-inhibiting potential of members of particular categories of dietary items.

The true cariogenicity or caries inhibitory properties of a food can only be established by experimentally determining in humans the extent of tooth decay associated with a given food. Since such experiments are not ethically feasible, scientists must rely on carefully standardized indirect methods to establish the cariogenic potential of foods.



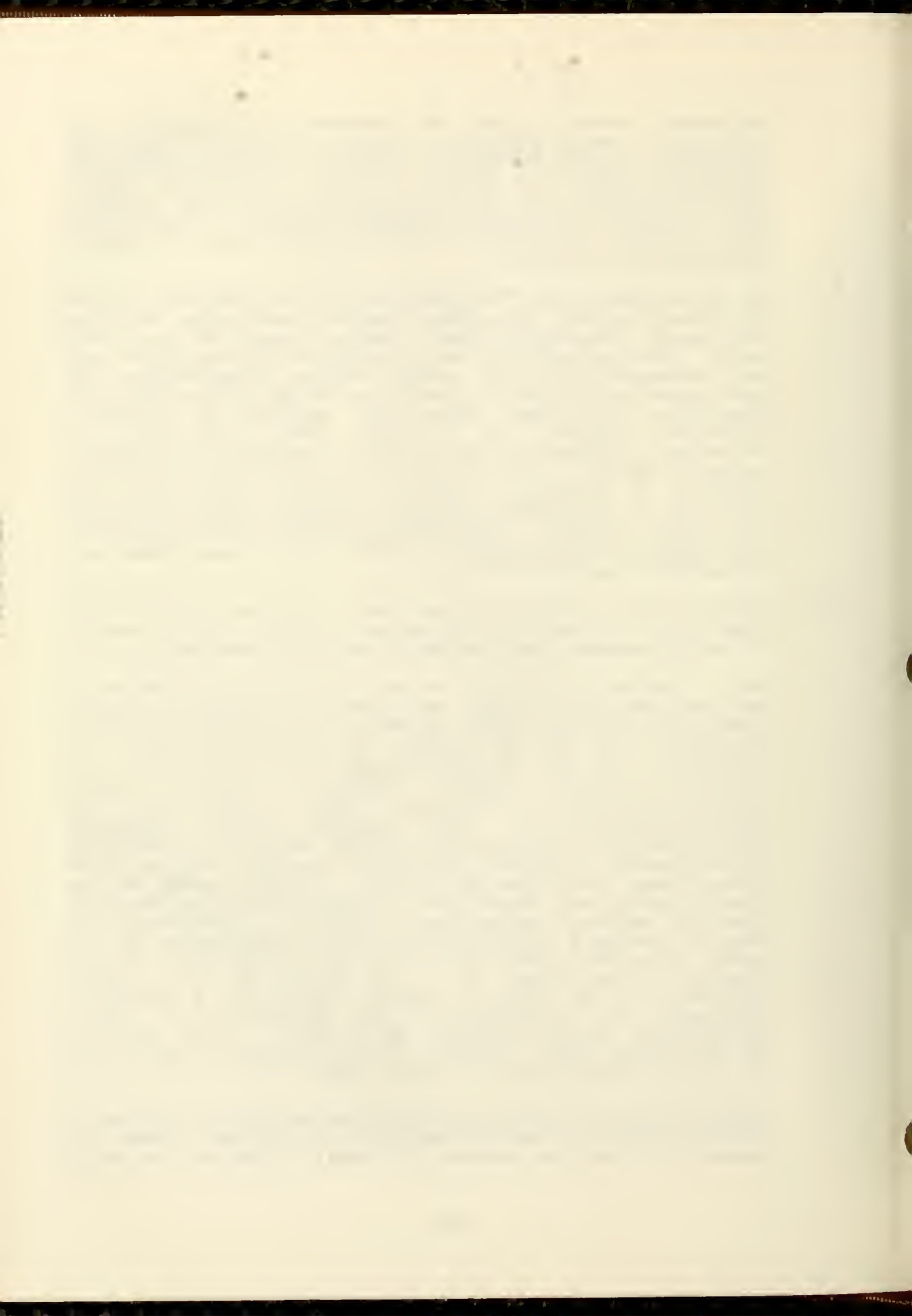
Cariogenic potential may be assessed by measuring acid formation in dental plaque, by enamel demineralization and by animal caries formation. Safe, in vivo, short-term intraoral models to measure plaque acidity and enamel demineralization have been developed, with NIDR support, that can assess foods for cariogenic potential. The use of these methods dramatically decreases the need for animal experiments in this area.

Recent studies on the demineralization and remineralization of enamel lesions have been carried out using in situ appliances containing enamel pieces placed in the human mouth. The demineralization potential of dietary items and the remineralization of early lesions were studied by physicochemical methods. Solutions, mouth rinses and gels containing different forms and amounts of fluoride, different regimens for delivery, have been found to effectively remineralize early enamel lesions or to prevent lesion formation. The uptake of fluoride into lesions produced a modified apatite structure that was more resistant to subsequent demineralization. The effects of pH, other minerals accompanying fluoride and the total composition of the fluoride delivery system were important for optimal remineralization.

- J12 Determine the characteristics and mechanisms of fluoride absorption from the gastrointestinal tract and the transport of the ion across other epithelia and cell membranes.

While fluoride (F) passage across individual cell membranes and some epithelia occurs as a weak electrolyte, fluoride absorption from the small intestine is unaffected by pH. Scientists at the University of Minnesota School of Dentistry investigated the alternative possibility that fluoride movement across intestinal mucosa occurs predominantly as the ionic form rather than the undissociated acid HF. Small segments of small intestine from fasted rats were mounted as flat sheets in a modified Ussing chamber. Mucosal and serosal buffers were modified Krebs Ringer solutions at pH 7.4. F was added to the mucosal buffer and the serosal F concentration was measured after 30 minutes incubation at 37°C. Substitution of chloride ion by a non-diffusible anion (isethionate) in the buffer increased mucosal to serosal F transfer, and voltage clamping in the range -50 mV to +50 mV led to a 4-fold difference in F transfer (greater with more + voltage). Decreased Na⁺ concentration and inhibition of active transport by ouabain reduced (F) transfer. - The findings are consistent with F passage across intestinal epithelium as an anion rather than as a weak acid.

Fluoride is one of few nutrients absorbed from the stomach, but the extent of gastric absorption is not well known. F absorption from the stomach vs small intestine was also

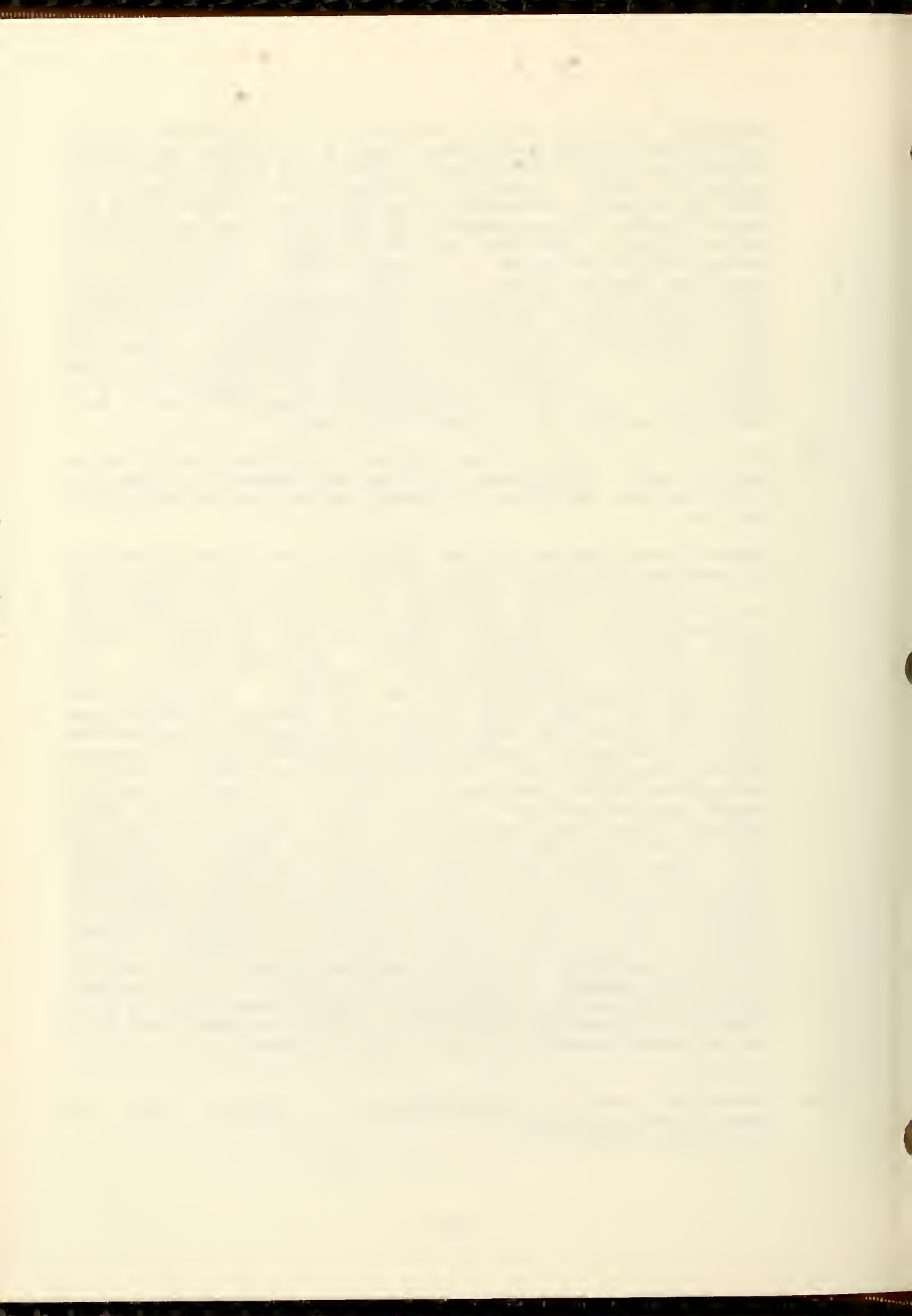


examined in rats by the University of Minnesota group. Fasted adult male rats were given F in water by stomach intubation, with ^{14}C -labeled polyethylene glycol (PEG) as a marker of gastric emptying. F analysis and ^{14}C counting was done on stomach, duodenum, jejunum, ileum, distal ileum and cecum tissues. Approximately 75% of the F dose was absorbed in 40 minutes and 90% in 120 minutes. Peak plasma F concentration occurred 10 minutes after intubation, and began to decline after 40 minutes. At 10 minutes after intubation, when the bulk of the F remained in the stomach, only approximately 25% of the F absorption had occurred from the stomach, and 75% from the small intestine. After 120 minutes, 20.4% of total F absorption had occurred from the stomach. - Although the stomach is unquestionably a significant site for F absorption, its contribution is much smaller than that of the small intestine.

- L06 Establish the relationships between nutritional deficiencies during tooth development in the development of specific tooth lesions which may increase caries susceptibility in children.

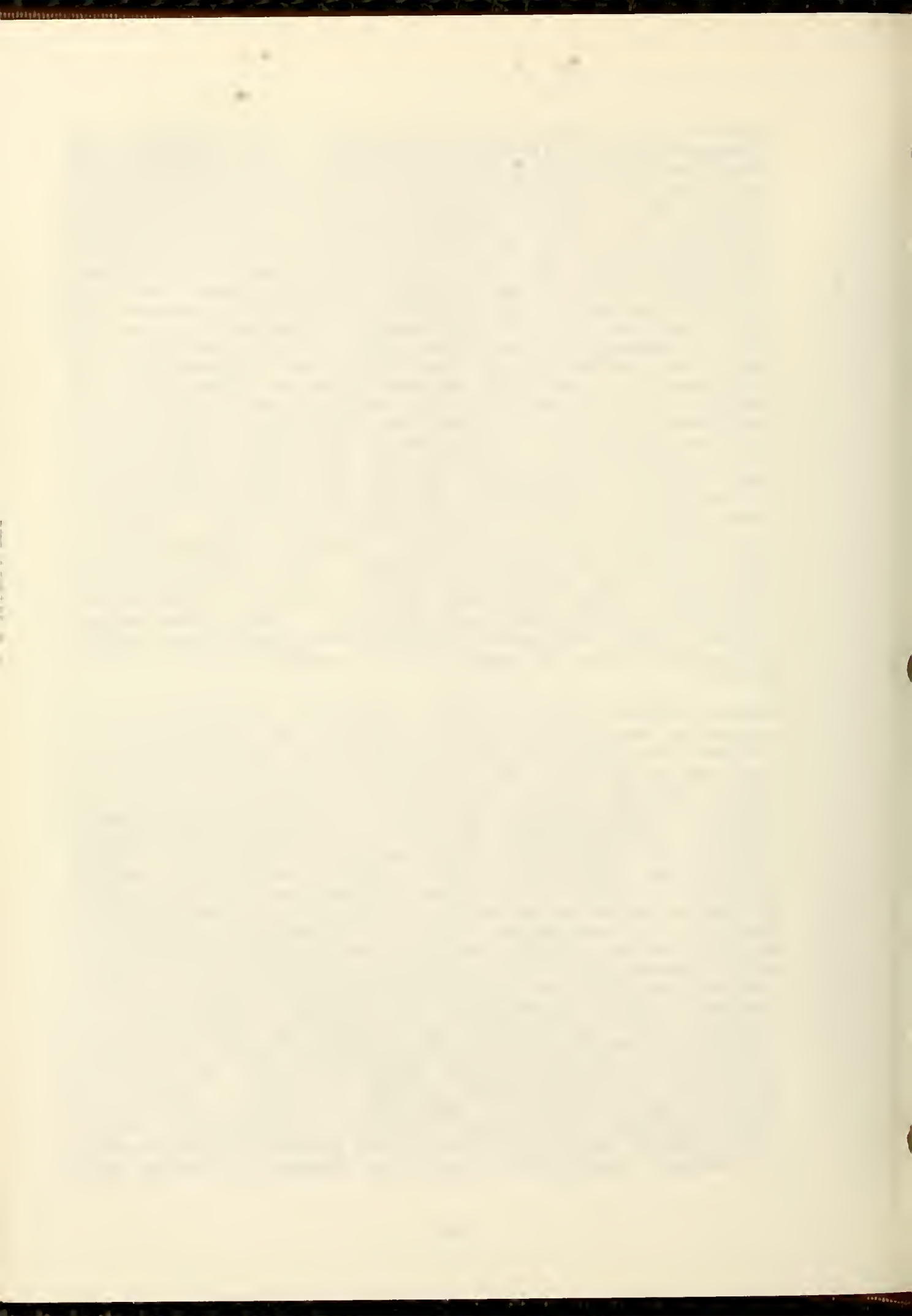
Dental investigators at the University of Alabama and the Universidad Peruana Cayetano Heredia, Peru, are carrying out a longitudinal study designed to evaluate the effect of early malnutrition on oral tissues development and susceptibility to dental caries in the deciduous teeth. Three hundred nineteen Peruvian children from a low socioeconomic, non-fluoridated, community in the city of Lima are participating in the study. Children were recruited into the study at age 5-11 months at which time they were classified according to anthropometric parameters into: a) normal, b) wasted (acute malnutrition), c) stunted (chronic malnutrition), or d) stunted and wasted. Dental evaluations were conducted at 12, 18, 24 and 30 months. These evaluations have revealed that children who have suffered from both chronic and acute malnutrition during their first year of life have a rate of caries attack that is 7 times greater than that of normal children. Children who had suffered from either acute malnutrition or chronic malnutrition during the second semester of life appear to have a caries attack rate similar to that of normal children. The three groups of malnourished children showed a delayed eruption of their teeth compared to the control group. In summary, preliminary data from this longitudinal study indicates that nutritional injury resulting in both stunting and wasting by age 5-11 months is associated with an increased susceptibility to dental caries later in life.

- P04 Determine the biocompatibility of metals used for restorative materials.



Several in vitro methods exist for testing the biocompatibility of dental materials. To determine the effect of solid materials such as the alloys used in crowns and bridges on individual cells, most investigators use one or both of two tissue culture methods. Both methods (agar diffusion and direct contact) assess the amount of cell damage by using dyes to determine cell viability and/or to note general cell appearance. As such, these methods appear to recognize only a severe form of cell damage, one that prevents the cell from retaining vital stains because its cell membrane is severely damaged. A potentially serious form of damage but one which might not be recognized by these two methods would be injury which prevents cell proliferation. Although methods such as those employing extracts of the solids being examined can measure cell proliferation, most utilize cell monolayers which makes counting difficult. An assay which does give a measure of a cell's ability to proliferate is the human tumor cloning assay (HTCA). To determine whether this assay could be used to evaluate cytotoxicity of various dental materials, investigators at the VA Medical Center, Ann Arbor, Michigan, have utilized HTCA with the P3J (Burkitt's Lymphoma) cell line to test six different alloys. The initial testing indicated a wide range of results with some alloys having no effect on colony formation by P3J cells, while others completely inhibited such growth. Early conclusions indicate that the human tumor cloning assay does appear promising for use in testing the biocompatibility of dental alloys.

Recent reports tend to raise questions in regard to the safety of mercury in amalgam fillings of dental patients. It has been reported that mercury can be released from amalgams by chewing. In addition, it has been reported that methyl mercury can be formed from a conventional and high copper admixed amalgam by action of either Streptococcus sanguis, Streptococcus mutans, or Streptococcus mitior. However, no studies have been reported to determine if oral indigenous microflora will convert mercury to methyl mercury. This is of great importance to the dental profession since methyl mercury is more toxic than inorganic mercury. Investigators at the University of Nebraska Medical Center, Lincoln, studied the amount of mercury and methyl mercury in the blood and urine samples of dental patients at different time periods (2 days, 2, 4, 6, and 12 months) after placements of amalgam restorations to determine if the amount of mercury or methyl mercury detected in the blood and urine samples exceed the maximum allowable concentration (<50 ng/ml in plasma, <20 ng/ml in urine and <20 ng/ml methyl mercury in blood). Thirty one patients participated in the study. At the first visit, each patient was given an oral examination and a questionnaire soliciting information concerning the presence of amalgam restorations and any unusual contacts with

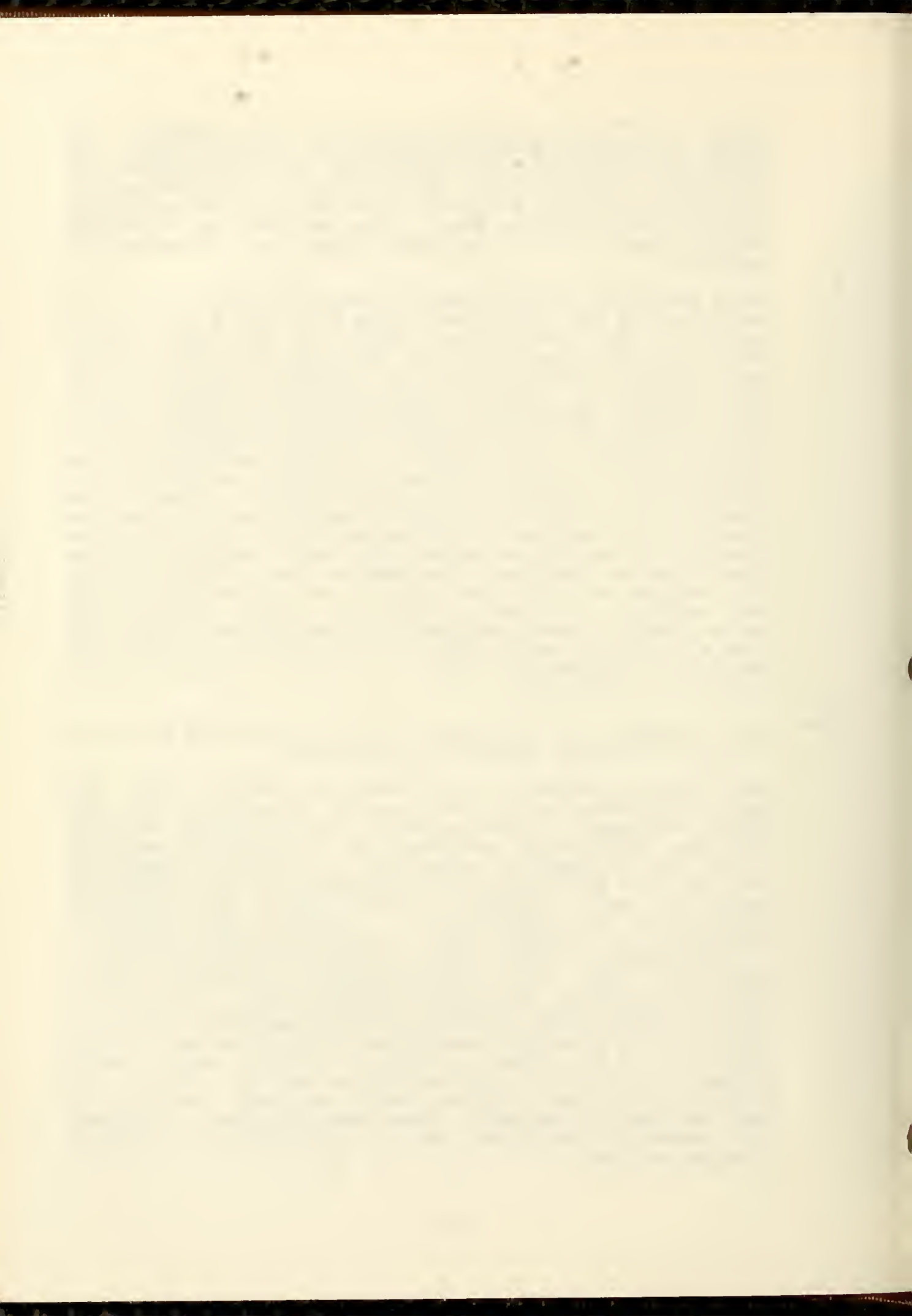


mercury, such as from dietary or occupational exposure. The number of current amalgam restorations were recorded. The design and potential risk factors of collecting blood was explained to each patient. Each patient was provided with a container to collect a 12 hour urine sample which was to be returned the following day. This was used as a patient control sample. A 20 ml blood sample was collected for analysis.

Blood and urine samples were collected at 2 days, 2, 4, 6, and 12 months for a duration of one year from a total of 35 patients. The specimens were frozen at -70°C until assay. Blood and urine specimens were analyzed for total mercury and inorganic mercury after wet digestion by cold vapor atomic absorption spectrophotometric method. Total mercury and inorganic mercury were determined and organic mercury was calculated as the difference between total mercury and inorganic mercury. Both mercury and methyl mercury could be detected in standard samples containing various known concentrations of mercury or methyl mercury. However, the presence of mercury (>20 ng/ml) or methyl mercury from the blood or urine samples of the patients studied could not be detected. Since neither mercury nor methyl mercury could be found from the blood and urine samples from patients with up to a maximum of eight new amalgam restorations over a one year period, the results from this study suggest that mercury used in dental amalgam is probably safe and will not pose a potential health hazard to the patients. Thus, the removal of amalgam for fear of mercury or methyl mercury toxicity is unnecessary.

P05 Develop better wear and color stability properties for known and newly developed composite formulations.

The current perception is that the failure rate for posterior composites is considerably higher than that for dental amalgams. Investigators at the University of North Carolina, Chapel Hill, have measured the incidence and type of failure for several categories of posterior composites over the last 5 to 10 years. Seventeen posterior composite materials were examined using direct and indirect evaluations at 0-5+ years. Clinical failures were categorized as excessive wear, recurrent caries, fracture, or other causes. Failure was compared statistically across materials types and failure causes by using Poisson regression methods with proportional hazards. Results of the 899 restorations tested at 5 years exhibited a failure level of 9.2% from all causes (wear = 0.4%, caries = 3.2%, fracture = 2.8%, other = 2.8%). Failures varied among products from 0% to 11.8% for caries and 0% to 11.2% for fracture. The total failure level was less than half of that for dental amalgam. These results seem to indicate that posterior composites can provide excellent long-term clinical service.



P15 Develop new and/or improved intraoral prosthetic materials.

The majority of single and multiple unit dental restorations (crowns and bridges) placed in this country are fabricated from porcelain-fused-to-metal. The choice of this treatment modality enables the dentist to restore lost tooth structure and replace missing teeth with a system of materials which provides an unsurpassed combination of esthetics and strength. Because a porcelain-metal restoration must be fabricated by fusing the porcelain to the metal at high temperatures, manufacturers of these products design their porcelains and alloys to be closely matched in thermal expansion characteristics. The goal of such a design is to avoid the development of transient or residual stresses in the porcelain upon cooling of the restoration from the firing temperature to room temperature. Considering that there are over 200 brands of alloys designed for porcelain bonding and numerous dental porcelains on the commercial market, it is not surprising that incompatible porcelain-metal combinations exist. But even in cases where the expansion coefficients (α) of the porcelain and metal are closely matched, the opportunity exists for a mismatch to develop if α of one of the components were to change during the various firing processes involved. A number of investigators have presented evidence that certain dental porcelains do in fact exhibit changes in the average α when these porcelains are subjected to repeated firings or to certain heat treatments. To obtain a better understanding of the mechanisms for these changes, investigators at the Medical College of Georgia, Augusta, are studying whether changes in the leucite content of a dental porcelain as a result of thermal treatments could be detected via quantitative x-ray diffraction. The thermal treatments investigated were isothermal soaks for 0, 4, 8, and 16 minutes at 500° and 750°C, respectively, or multiple firings of 1, 2, 4, 8 and 16 times. Four porcelain coupons were prepared for each thermal treatment. The order of fabrication of each specimen was randomly assigned. Quantative x-ray diffraction was performed on the heat-treated porcelain specimens using leucite standards containing 11.1, 22.3, 33.4 and 44.5% leucite. The data were subjected to linear regression analysis. A significant negative correlation was found between leucite wt. % and the number of firings. No relationship was found between the leucite content and the duration of the heat soak at 500°C. These data indicate that the amount of leucite is dependent on both the heat soak time and temperature, as well as the number of firings. Thus, leucite variations may result in changes in porcelain-metal compatibility.

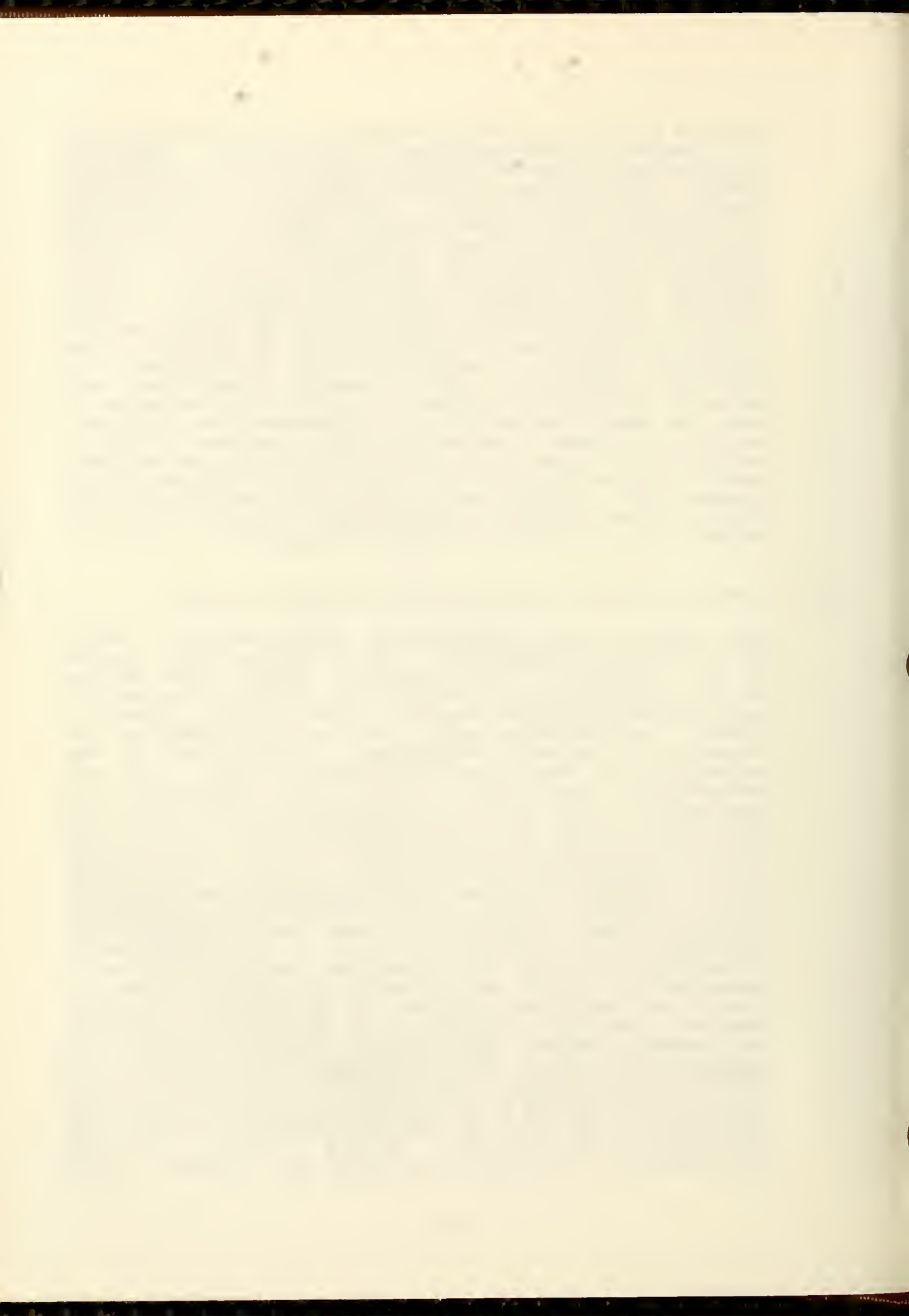
P99 Study of adhesion and adhesion promoting agents to dentin.



Previous studies by investigators at the National Institute of Standards and Technology, Gaithersburg, Maryland, indicate that Oligomers (low molecular weight polymers) with pendant isocyanate groups adhere strongly to glutaraldehyde treated hard and soft tissues. In this study the dependence of isocyanate content and molecular weight of adhesive and its bond strength to tissue was determined. Oligomers were synthesized and their bond strength to bone or dentin-composite was determined as previously described. Higher molecular weight adhesives were synthesized by reacting the monomeric mixtures at lower temperature for longer periods of time and using smaller concentrations of initiators than previously employed. Molecular weight of oligomers and polymers having the same monomeric composition were estimated from their intrinsic viscosity. Bond strength to bone and dentin varied but was independent of isocyanate content. Increase in molecular weight resulted in both an insignificant higher bond strength and isocyanate content. These findings indicate that for minimal diffusion into tissues and optimum biocompatibility, adhesives should contain a low percent isocyanate and a maximum molecular weight subject to adequate working properties.

H03 Further delineate salivary gland regulatory factors.

Human parotid and submandibular glands secrete a high-molecular-weight glycoprotein that aggregates many strains of oral bacteria. This agglutinin rapidly binds to the surface of susceptible bacteria in a calcium-dependent, temperature-independent reaction. After binding, there is a slower, temperature-dependent reaction that leads to the formation of bacterial aggregates. Investigators at the University of Pennsylvania-Philadelphia have previously demonstrated that there is a wide distribution of aggregating activity in the general population, that an individual's level of aggregating activity is stable over time, and that the level of activity is high or low for all of the susceptible microorganisms. While several other salivary proteins are capable of aggregating oral bacteria (e.g., lysozyme, sIgA), agglutinin-mediated aggregation accounts for most of the activity seen when streptococci are incubated with parotid saliva. An individual's characteristic level of aggregating activity may be a consequence of either environmental or genetic factors. A direct and efficient approach to evaluating a genetic component involves a comparison of the trait in identical and fraternal twins. Accordingly, agglutinin activity for Streptococcus sanguis and Streptococcus mutans in whole and parotid saliva obtained from identical and fraternal twins was recently compared by the above investigators in order to determine if the level of this glycoprotein in saliva was genetically determined. Strong evidence for the heritability of agglutinin activity was obtained. This



evidence may prove useful in exploring the biosynthesis of this glycoprotein. Much evidence points to carbohydrate moieties as functional determinants on the agglutinin molecule. These may be associated with other well-known genetic markers such as blood group and/or secretor status.

Vasoactive intestinal peptide (VIP) has been identified as an important regulator of submandibular salivary gland function, consistent with its co-localization with acetylcholine in parasympathetic neurons innervating this gland. Enzymatically dispersed acini from rat submandibular gland are a useful system in which to study gland regulation at the cellular level. In this study, grantees at the University of Missouri-Columbia examined three aspects of VIP interactions with acini: inhibition of binding of the radioligand, ^{125}I -VIP, stimulation of cyclic AMP production, and enhancement of mucin release. VIP and the VIP receptor agonist, peptide histidineisoleucineamide (PH), inhibited ^{125}I -VIP binding to intact acini with IC_{50} values of 16 ± 3 nM and 46 ± 17 nM, respectively. This rank order of potency agrees with that observed previously in assays using rat submandibular gland membranes and is similar to values obtained in assays measuring increases in cyclic AMP production in which the ED_{50} values for VIP and PHI were 3.1 ± 1.8 nM and 29 ± 13 nM, respectively. Although VIP stimulation of cyclic AMP production was only about 10% of that seen in response to the sympathomimetic drug, isoproterenol, mucin release levels induced by the two agents were more similar. The ED_{50} for VIP-stimulated mucin release was 0.12 ± 0.05 nM. Thus, the potency of VIP for stimulating mucin release is approximately 50- to 100-fold higher than for inhibiting ^{125}I -VIP binding or for stimulating cyclic AMP production. These results suggest a "spareness" in the VIP receptor-coupled signal transduction pathway at a point between adenylate cyclase activation and mucin release such as protein kinase A or protein kinase A target proteins involved in this cascade system. Current studies are aimed at delineating the mechanisms underlying these observations. The results of this study provide the first radioligand binding evidence for VIP receptors on intact, dispersed acini of the rat submandibular gland coupled to cyclic AMP production. In addition, the stimulation of mucin release in response to VIP is the first functional response described for this neuropeptide in dispersed submandibular gland acini. This preparation may also be a useful system in which to study other exocrine effects of VIP, the mechanisms by which VIP acts, and the regulation of the VIP receptor at the cellular level.

- H05 Develop improved procedures for characterizing the molecular structure and function of the salivary proteins and other macromolecules important in oral health maintenance.

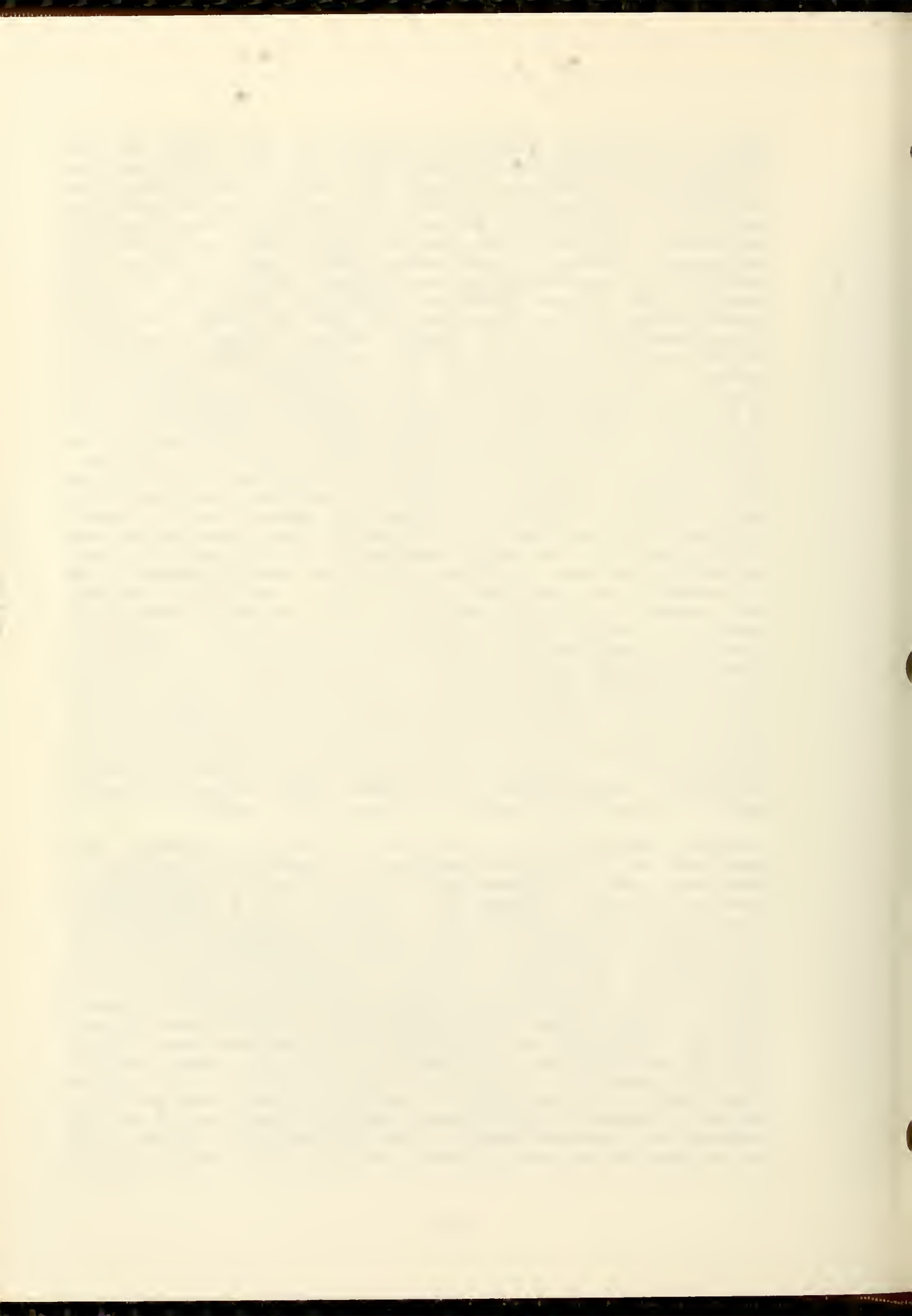


The epithelial surfaces of the alimentary tract elaborate copious quantities of viscous secretions which play an important role in the physiological functions along the digestive tract and are considered to be primary factors in the maintenance of the health of the oral cavity and gastrointestinal mucosa. In the oral cavity, the constituents of saliva not only protect the oral mucosa and soft gingival tissue, but also are essential for the defense of teeth against erosion and caries while, in the stomach, the secreted mucus constitutes the first line of mucosal defense against the damaging effects of acid and pepsin. The protective qualities of the mucus coat depend upon the nature of its constituents, tenacity of interactions occurring within the mucus gel, and the gel dimension. Among the factors implicated in the control of these events is epidermal growth factor (EGF). EGF is a 53 amino acid peptide elaborated in large quantities by submaxillary salivary glands. Although it has widespread biological effects, including stimulation of cellular growth and differentiation, regulation of specific gene expression, inhibition of gastric acid secretion, and mucosal protection, its precise physiological roles remain unknown. In the gastrointestinal tract, whereas duodenum and small intestine are known to elaborate EGF, the major source for this peptide in the stomach is saliva. Indeed, investigations have shown that removal of salivary glands causes weakening of gastric mucosal defense as evidenced by the increased susceptibility of the mucosal tissue to injury. However, the role of salivary EGF in the maintenance of the mucosal mucus layer remains poorly understood. Using sialoadenectomized rats, investigators at the University of Medicine and Dentistry of New Jersey-Newark have recently studied the involvement of EGF in the maintenance of oral and gastric mucosal mucus coat dimension and chemical characteristics. Examination of the oral and gastric mucosal surface revealed that deprivation of salivary EGF caused a 31-36% reduction in mucus coat thickness and a 38-43% reduction in adherent mucin content. Chemical analyses indicated that the mucus coat of sialoadenectomized rats exhibited a 21-28% increase in protein and a 67% decrease in covalently bound fatty acids, a 30% decrease in carbohydrates, and a 32-37% decrease in lipids. Sialoadenectomy also evoked changes in the chemical composition of the mucus glycoprotein component of oral and gastric mucus coat reflected in the lower content of sulfate (25-26%), associated lipids (24-25%), and covalently bound fatty acids (67-75%). Intragastric supplementation of EGF had no effect on the physicochemical changes caused by sialoadenectomy in the oral mucosal mucus coat, while nearly complete restoration to normal characteristics occurred in the gastric mucosal mucus coat. These results suggest that salivary EGF is essential for the maintenance of mucus coat dimension and quality needed in the protection of alimentary tract epithelium.

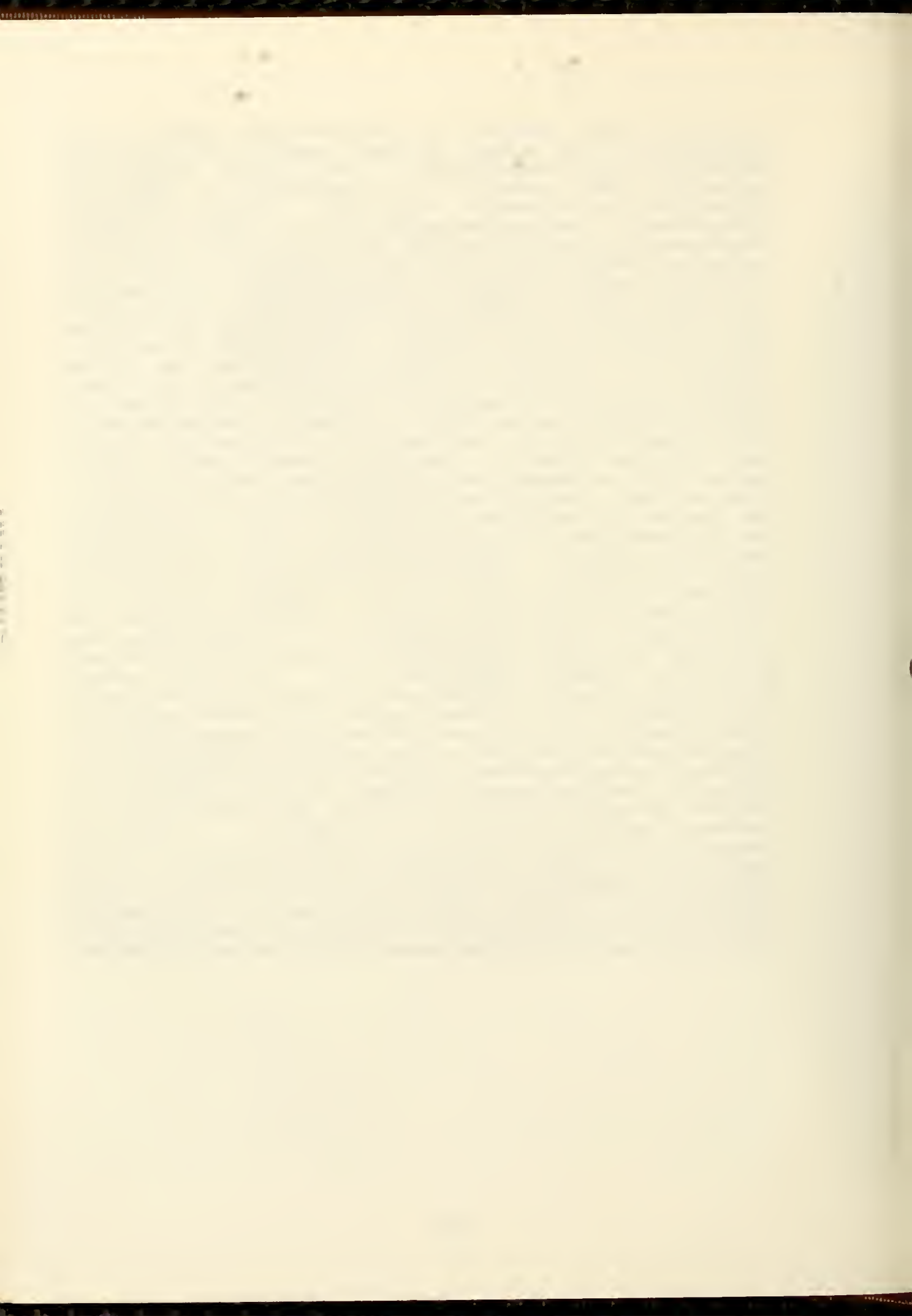


One of the major roles of saliva in the mouth is the formation of the acquired enamel pellicle (EP). EP provides a protective interface between the tooth surface and the external environment. It acts as a selective permeability barrier, regulates mineralization/demineralization processes, and modulates the microbial flora on the tooth surface. Several approaches have been utilized to characterize the molecular constituents of in vivo EP. The results of these studies indicate that the major constituents of the acquired EP are salivary proteins, glycoproteins and, to a lesser extent, serum components. Saliva comprises several protein families whose individual members are structurally and, possibly, functionally distinct. Previous studies on EP have not specified which of the individual members of these families contribute to EP formation. To better understand the role of saliva in modulating the environment surrounding the tooth surface, grantees at the State University of New York-Buffalo have now utilized Western transfer analyses and specific radiolabeling techniques to identify members of different salivary families that participate in the formation of the 2-h in vivo EP. The major components of this pellicle were salivary α -amylase, cysteine-containing phosphoprotein (CCP or cystatin), salivary mucin, and sIgA. Glycosylated amylase was present in larger quantity than the non-glycosylated species. Only CCP1 (cystatin SA-I) of the cysteine-containing phosphoprotein family was identified. The higher molecular-weight salivary mucin (MG1), but not the lower molecular-weight species (MG2), was detected. These results extend earlier observations regarding the selective nature of salivary protein adsorption to enamel surface by demonstrating that only specific members of salivary protein families are involved in 2-h in vivo enamel pellicle formation. The findings also suggest that individual family members may have different functions in the mouth.

Salivary pellicle is a film which coats oral surfaces and functions as a moisture retainer, a protective barrier, a lubricant, and a determinant for microbial colonization. Previous studies suggest that pellicle is a multilayered film which is formed initially by the selective adsorption of salivary molecules to oral surfaces followed by homo- or heterotypic complexing of these molecules with other molecules in the ambient saliva. Salivary components which adsorb to oral mucosal epithelial cells comprise the mucosal pellicle. The forces which mediate the interactions between salivary molecules and epithelial cell surface most likely include non-covalent interactions involving electrostatic and hydrophobic forces. Preliminary data from investigators at the State University of New York-Buffalo, however, suggest that these interactions may also be mediated by covalent bonds. Previous authors suggest that the surface of mucosal squames results from a metamorphosis



in which plasma membrane is simultaneously lined by the synthesis of an inner protein matrix and partially hydrolyzed by phospholipases. The matrix, termed the epithelial cell envelope, is synthesized by epidermal transglutaminase (endo -glutamyl -lysine aminoacyl transferase). Transglutaminases function through a double displacement mechanism in which the -carboxamide group of an endo-glutamine reacts with the enzyme thiol active site to yield a thiolester bond and free ammonia. Subsequently, the enzyme binds a primary amine which effects amino-lysis of the thiolester intermediate and participates in an amide bond with the glutamine residue. Amide bond (cross-link) formation is primarily between endo-glutamine and lysine residues of cytosolic proteins; however, epidermal transglutaminase is capable of utilizing non-epithelial proteins and polyamines such as putrescine for substrates. This suggests that the surface of oral epithelial squames may be characterized by a partially denuded protein matrix replete with an associated transpeptidase which may cross-link salivary proteins during mucosal pellicle formation. The purpose of the present study by the above investigators was to determine if salivary molecules can be covalently coupled to epithelial surfaces and explore the mechanisms of these interactions. Their data demonstrate the apparent covalent incorporation of [¹²⁵I]-parotid saliva and [¹⁴C]putrescine into high molecular weight complex(es) in the presence of buccal epithelial cells. This reaction was inhibited by EGTA, iodoacetamide, heat inactivation and putrescine, thereby suggesting that: 1) oral mucosal pellicle is formed by the selective adsorption of saliva to the epithelial cell plasma membrane and its associated cytoskeleton; and 2) the adsorbed salivary components may be cross-linked to each other or the epithelial cytoskeleton by epidermal transglutaminase. Clinically, the presence of an epithelial derived transglutaminase may have implications for the design of artificial salivas for individuals with decreased salivary function or xerostomia. A prominent symptom of xerostomic individuals is the sensation of a dry mucosa which is only transiently abated by present topical replacement therapies. Based upon the observations of the present study, it may be possible to enhance the substantive effect of artificial salivas by designing preparations which contain substrates for covalent cross-linking to mucosal epithelium.



ANNUAL REPORT

PERIODONTAL & SOFT TISSUE DISEASES RESEARCH BRANCH

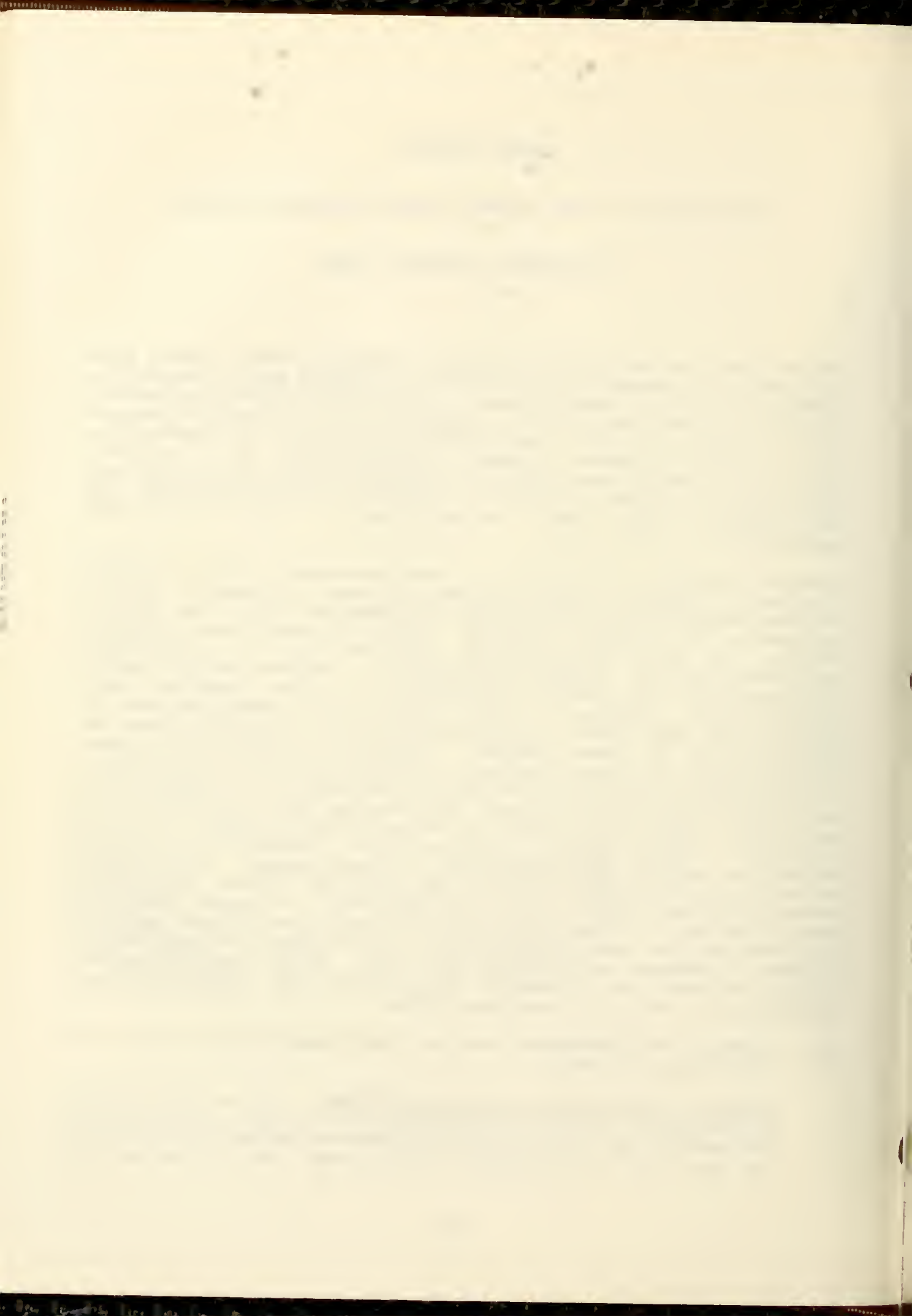
EXTRAMURAL PROGRAM, NIDR

During FY89 the Periodontal and Soft Tissue Diseases Branch made 112 awards for research on periodontal diseases and 43 awards for research on soft tissue diseases. The cost of the periodontal research and related projects supported in the Research Centers on Oral Biology totalled approximately \$23 million. Research on soft tissue diseases consumed approximately \$6.1 million. Training and development support related to both periodontal and soft tissue diseases totalled \$4.4 million, which supported 103 individuals. Thus, total expenditures for these activities approached \$33.5 million.

Selected research highlights in the periodontal area included clinically oriented studies on the influence of genetic factors on the composition of the human periodontal flora and on the expression of clinical disease. Also included were clinical trials of the efficacy of antibiotics in controlling periodontitis both in normal adults and in patients with juvenile periodontitis or mental retardation. Also reported were epidemiology findings of the periodontal complications in diabetes and data from a reexamination of a population examined periodontally 28 years before. Research in the soft tissue diseases and AIDS areas consisted of projects with heavy emphasis on laboratory work as well as clinical orientation. Included was an interesting basic project to develop gene therapy using epithelial tissue, as well as several other disease oriented efforts. These efforts included basic research related to protection against HSV infections via chemotherapy, vaccination, or salivary inhibition, and to the role of viruses and growth factors in oral cancer. Also included was a clinical report of oral findings in offspring of HIV-seropositive mothers and new findings on the oral microbiology and virology of periodontal disease in humans and simians with AIDS. Brief descriptions of these projects are presented below under the classifications developed for the NIDR Long Range Plan.

BO1 Identify the microbial species that cause various forms of periodontal diseases.

Genetic factors and the oral flora: In a very basic microbiology study, investigators at Virginia Commonwealth University are attempting to determine whether differences in the oral flora between individuals are influenced by

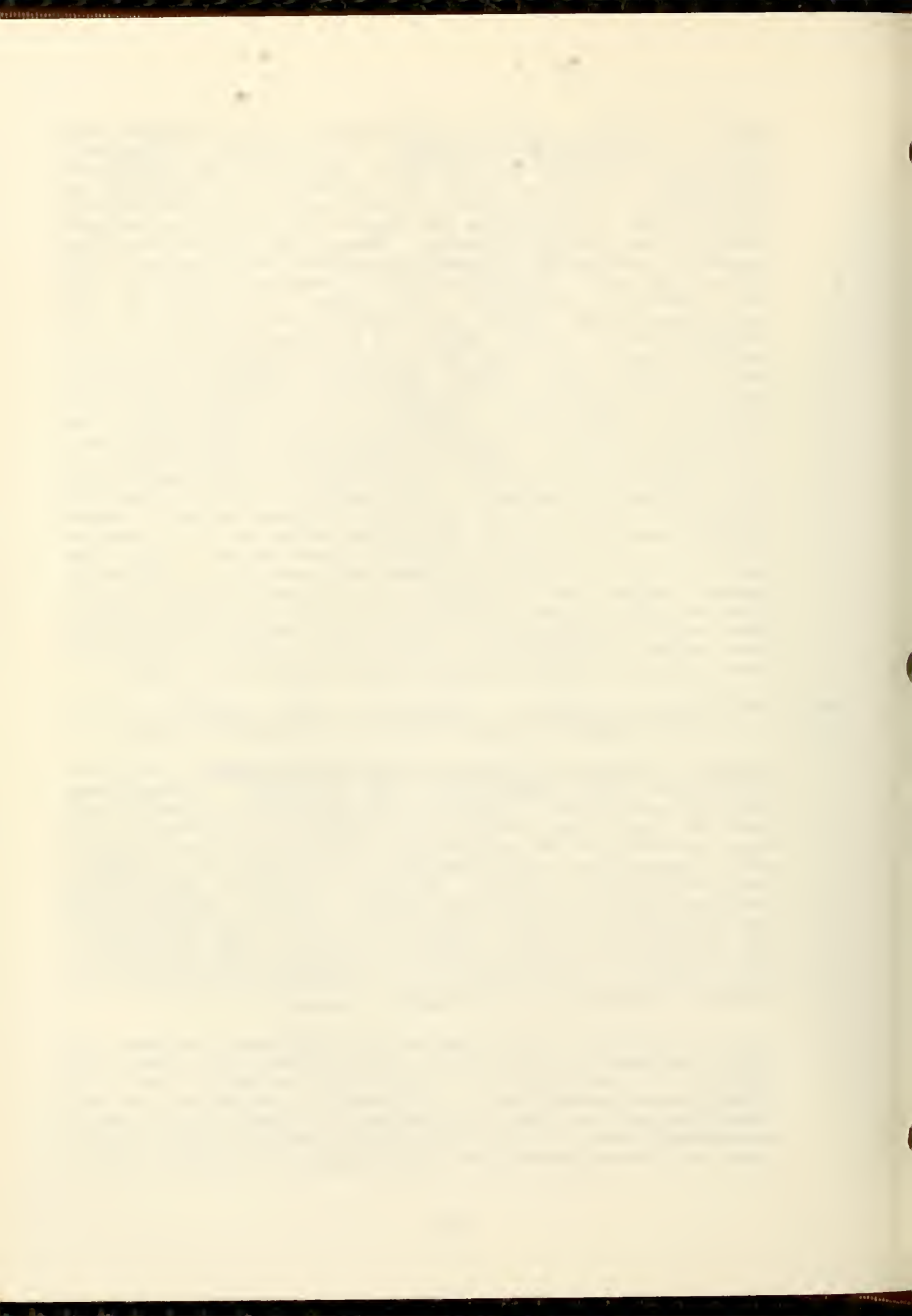


genetic factors. These investigators are culturing the flora of monozygous (identical) and dizygous (fraternal) twins and combining bacterial samples from different periodontal sites to arrive at results which represent an individual. They then do a series of statistical calculations to determine the degree of similarity between samples from two individuals. Thus, they can compare the degree of similarity between monozygous and dizygous twins of similar age. Theoretically, if genetic factors are the only factors involved, the degree of similarity of monozygous twins should be exactly twice that of the dizygous twins. In this study, it has been found that at 11 years of age, both types of twins show more similarity in their oral flora than randomly studied unrelated individuals, but do not differ from each other in their degree of similarity. However, at 12 1/2 years, the monozygous twins show greater similarity in their flora than the dizygous twins, and this difference in similarity is statistically significant. The numerical data indicating these differing degrees of similarity are 38.24 for the monozygous versus 24.18 for the dizygous twins. These figures suggest that the monozygous twins are 1.8 times as similar in their flora than the dizygous twins. Data from the 12 1/2 year old twins suggest that genetics is the major factor causing the differences in similarity, because the dizygous twins were no more alike than unrelated people. This data is among some of the first data published that goes beyond conjecture in implicating genetic factors as determinants in regulating the composition of the flora.

B02 Investigate genetic, biochemical and stress factors influencing susceptibility to the periodontal diseases.

Genetic Factors and Clinical Manifestations: Although clinicians have repeatedly made anecdotal observations linking periodontal disease susceptibility to hereditary factors, there has been little definitive information on this subject and no systematic studies have been carried out to assess the role of genetics in periodontal diseases. The preliminary findings from the Virginia twin study outlined above suggest that genetic factors influence the basic composition of the oral flora, but does not address the question of disease. However, another study of twins at the University of Minnesota is providing data suggesting that genetic factors may play a substantial role in the clinical expression of periodontal disease.

In the Minnesota study, periodontal disease findings are being compared in monozygous and dizygous twins, and the degree of periodontal similarity measured and its significance ascertained. The working assumption of the investigators is that the degree of similarity between monozygous twins is twice that between dizygous twins. Last year the investigators had completed data analysis from a



few pairs of twins. They have now provided additional data which considerably strengthens their previous conclusions that genetic factors appear to exert a critical impact on the evolution of periodontal disease.

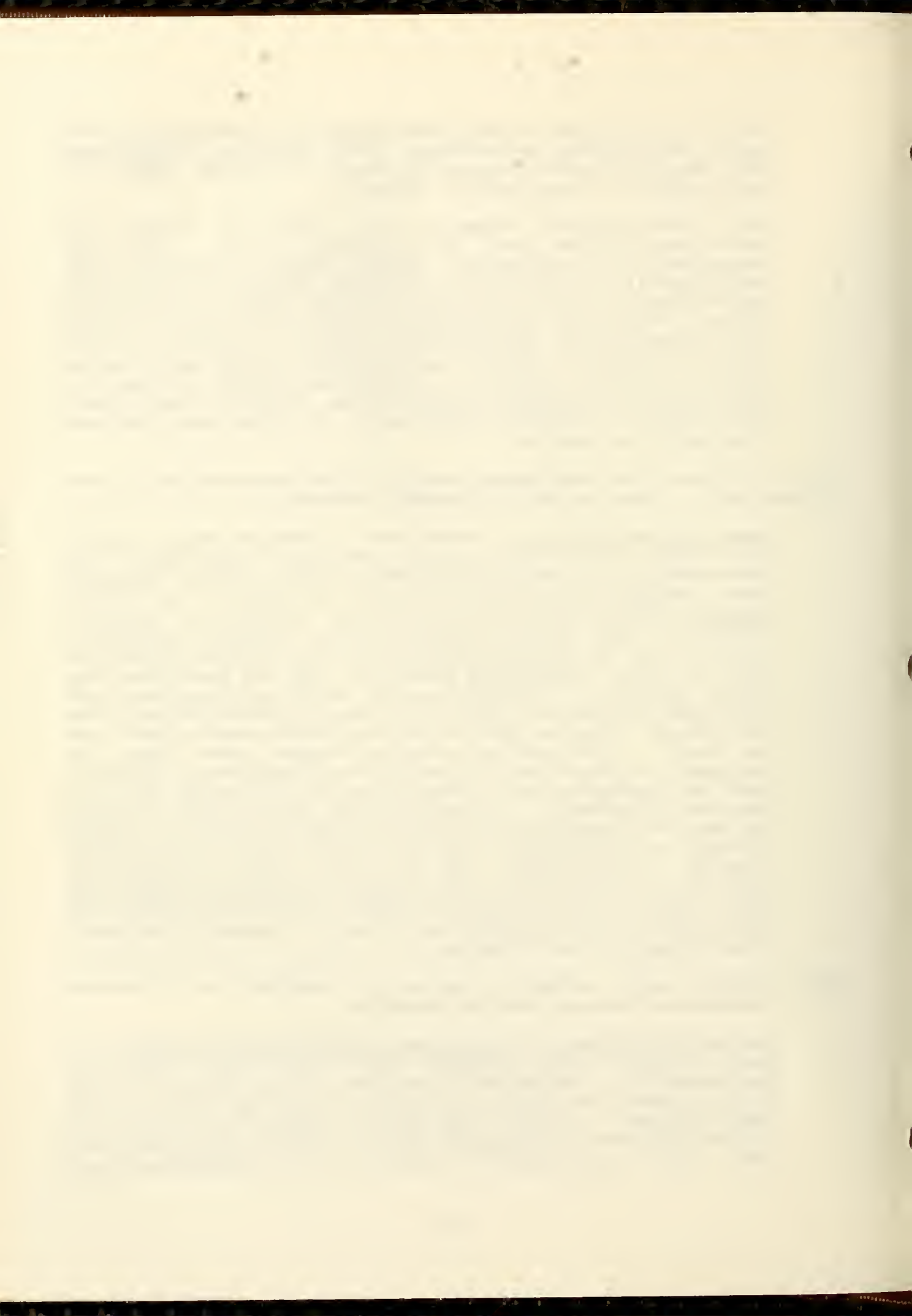
The investigators examined 104 pairs of twins-- 68 monozygous pairs and 36 dizygous pairs. Clinical measurements of periodontal pocket depth, attachment loss and gingivitis within the monozygous pairs of twins were approximately twice as similar as those taken from dizygous pairs. From their statistical analysis of these findings, the investigators estimate that about 68 per cent of the variation in periodontal assessments among these subjects is due to genetic factors. Continued study of genetic influences may enable clinicians to identify individuals with genetic susceptibility early in life and initiate programs of prevention.

B04 Elucidate the mechanisms leading to the destruction of soft tissue and bone in the periodontal diseases.

Bone Loss Mechanisms: Scientists at Forsyth Dental Center have shown that the immune cytokines interleukin 1b, interleukin-1a, and tumor necrosis factor (TNF) uncouple bone resorption/formation in vivo as well as in vitro. Coupling means that the resorption and formation of bone are linked, so that bone formation usually follows resorption. Coupling is the pattern observed in the regular metabolic cycle of bone. Uncoupling means that the two phenomena have become separated. In this case the cytokines interfere with the formation process, but do not seem to affect normal bone resorption. The net effect of this imbalance is that bone is lost. Uncoupling usually occurs when there is inflammation near bone. Tissue culture experiments indicate that the cytokines are effective in the following order of decreasing activity: Interleukin-1b > Interleukin-1a > TNF, and that the suppressive effects of these agents are exerted directly on osteoblasts. In a collaborative effort with an clinical investigator from the New Jersey College of Medicine and Dentistry, the Forsyth scientists showed by mono- and polyclonal antibody that Interleukin-1b and TNF are present in inflamed periodontal tissues from human cases of periodontal disease.

B08 Develop more effective means to control or eliminate periodontal disease-causing bacteria.

Antibiotic Efficacy In Localized Juvenile Periodontitis: A new therapy was devised for localized juvenile periodontitis, a relatively rare form of periodontal disease which affects the molar and incisor teeth of teenagers and young adults. Familial and genetic factors seem important in the disorder, and epidemiologic evidence indicates that the main pathogenic organism is Actinobacillus



actinomycetemcomitans (Aa), a Gram negative anaerobe. With successful therapy and maintenance, the localized disease does not progress, but in approximately 25 percent of the cases, treatment is unsuccessful, because it is difficult to eradicate the offending bacteria. As a result, the disorder progresses to a severe form, with loss of supporting bone, and loosening and eventual loss of teeth. At SUNY at Buffalo, investigators tested a new treatment which uses a combination of the two antibiotics, metronidazole and amoxicillin. This combination completely eradicated the bacteria involved in the local infection. In the study, 15 patients who had been previously treated by other means, but still suffered from localized juvenile periodontitis were given 250 mg. tablets of metronidazole and amoxicillin orally three times a day for one week. All of the patients showed immediate improvement, with decreased gingivitis and complete elimination of Aa.

Metronidazole Efficacy in Adult Periodontitis: The efficacy of metronidazole alone in eradicating anaerobic periodontopathic organisms was evident in clinical trials being carried out by University of Michigan investigators. These trials were designed to determine whether an optimized schedule of metronidazole coupled with traditional mechanical scaling and root planing can reduce the need for periodontal surgery. These studies represent one of the few instances in periodontal research where a completely double blind approach was implemented. In one trial, it was found that one week of metronidazole followed by debridement reduced the need for surgery by 5 teeth per patient, compared to the controls which received placebo drug plus debridement. This difference was maintained throughout the 2-3 year recall period. In a second study the protocol was similar, except that the metronidazole was given after debridement had been completed. This approach was also successful and the findings suggest that administering the metronidazole after debridement gives better results. However, this tentative conclusion awaits statistical verification.

Compliance Monitoring In Clinical Trials: To aid them in conducting their clinical trials, the Michigan investigators used plaque levels of spirochete organisms to indicate whether the patients under study were actually taking their metronidazole as instructed, since metronidazole is known to have a specific anti-spirochete effect. With careful patient monitoring, the investigators determined that with full compliance in taking the metronidazole, the spirochetes in plaque become reduced to negligible or nondetectable levels. Thus, it was possible to classify the patients as compliant or noncompliant by assaying spirochete levels. The results showed that the compliant patients needed significantly less periodontal surgery than either the noncompliant or the positive control patients.



Chlorhexidine Spray for Mentally Retarded Patients: Maintaining periodontal health is extremely difficult for patients with severe mental retardation, because they are often simply unable to perform oral hygiene procedures. Findings from a study at the University of Florida showed that the antibacterial mouth rinse chlorhexidine applied as a spray was unusually effective in reducing periodontal plaque in institutionalized adults with severe mental retardation. In the Florida study, staff nurses applied the chlorhexidine spray twice daily for 8 weeks to the mouths of 10 institutionalized adult patients with severe mental retardation and varying degrees of gingivitis and dental plaque. The patients who received the chlorhexidine spray showed healthier gingival tissues, dramatically decreased plaque and reduced bleeding. Ten other patients with gingivitis who brushed their teeth and received a placebo spray showed no improvement over the eight week period. Not only was the spray efficacious, but it was also cost-effective. This study is believed to be the first study to test chlorhexidine as a treatment for gingivitis in high risk institutionalized patients with mental retardation.

B12 Conduct well-designed epidemiological studies to determine the incidence and prevalence of the periodontal diseases among various age groups in America

Reexamination of Tecumseh Population After 28 Years: In an interesting epidemiologic study on periodontal disease in residents of Tecumseh, Michigan, investigators from McGill University reexamined 167 dentate individuals who had been examined in an earlier epidemiologic study in 1959, 28 years ago. All teeth present were examined and scored for periodontal parameters. Sixty-six percent of the tooth sites had not changed, or only changed within 1.0 mm during the 28 years. Loss of periodontal attachment had progressed very slowly except in approximately 13 per cent of the subjects, who showed an average loss of attachment of 2mm or more per person. When the data was subjected to logistic regression analysis, the following risk markers were significantly related to the loss of attachment increases: age, smoking and tooth mobility at the initial examination. Twenty-eight individuals had lost all of their teeth, but it was not clear what proportion had been lost because of periodontal disease.

Periodontal Disease in Noninsulin-Dependent Diabetic Pima Indians: During the past 5 years a longitudinal study on the oral complications of Type II diabetes has been conducted by investigators from SUNY Buffalo in collaboration with the diabetes epidemiology program of the NIDDK and the Phoenix Area Indian Health Service. During this period nearly 3000 subjects of varying age have been studied. Some of the highlights of this project are



presented in the paragraphs to follow.

Tooth Loss: After adjustment for age and sex, diabetics have an average of 12 missing teeth compared to 8 for nondiabetics and patients with impaired glucose tolerance (IGT). Moreover, diabetics are 15 times as likely to be totally edentulous than nondiabetics. With regard to duration, it was found that in Indians with diabetes of 5 years duration, 7 percent were edentulous, 10 years duration, 14 percent edentulous, and 20 years duration, 75 percent edentulous. The major reason for the tooth loss was periodontal disease and its complications.

Relationship of Periodontal Disease to Other Diabetic Complications: Diabetic patients with retinopathy were 4.6 times as likely to have periodontal disease than patients without retinopathy. No relationship was found with nephropathy or urine albumin concentrations. Diabetic patients with rheumatoid arthritis had significantly lower alveolar scores than those without arthritis. This finding could be due to the fact that these patients are probably taking nonsteroidal anti-inflammatory agents.

Relationship of Periodontal Disease to Metabolic control: After controlling for age, sex, and duration of diabetes, the odds of having periodontal disease for subjects with a fasting plasma glucose of ≥ 400 mg/dl were 2.1 times that of a subject with a fasting glucose of ≤ 200 mg/dl. Individuals with glycosylated hemoglobin (Hb) of 12 percent were 2.3 times more likely to develop periodontal disease than individuals with a glycosylated Hb of 6 percent.

F04 Identify and test antiviral compounds and biological inhibitors such as interferon that might control infection, prevent the development of latency, or prevent reactivation of latent herpes simplex virus (HSV) infection

Antiviral Chemotherapy Research: Because HSV can become reactivated after a prolonged latent period and because of its ability to mutate, research has focussed on the development of antiviral agents to control recurrent infections. Researchers at Ohio State University are investigating the enzyme, deoxyuridine triphosphate nucleotidohydrolase (dUTPase) as a potential target site for developing a specific class of anti-viral compounds. Specifically, their studies have been directed towards determining structural differences between human dUTPase and HSV-encoded dUTPase. They have purified both human and HSV-encoded dUTPase and have distinguished them on the basis of differences in biochemical, biophysical and immunological properties. In addition, they have synthesized compounds that specifically inhibit HSV-encoded dUTPase but not the cellular form. These compounds exert their HSV-encoded dUTPase inhibitory activity irreversibly and it appears to



be directed towards the active site of the enzyme. This feature of an active-site-directed inhibition might allow the rational development of specific compounds based on steric binding and structural configuration which could inhibit the replication of HSV. In addition, the investigators have demonstrated that the dUTPases are partly responsible for the chemotherapeutic effectiveness of fluorodeoxyuridine, a compound used in cancer chemotherapy. Approximately 95 percent of the U.S. population has been exposed to HSV and many of these individuals develop recurrent infection, both oral and genital. In addition to these more common types of infections by HSV, newborns, patients undergoing organ transplants and AIDS patients are among HSV-susceptible groups. While there is presently a compound (Acyclovir) that is effective in preventing reactivation of latent HSV, mutants of HSV which no longer respond to Acyclovir are beginning to emerge. Thus, new compounds must be developed for both antiviral and cancer chemotherapy. This study has demonstrated that HSV-encoded dUTPase is a useful target site for specific antiviral agents and is potentially useful for the development of agents effective against the entire class of herpesviruses.

Herpes Simplex Virus Vaccine Research: The development of a vaccine against Herpes Simplex Virus 1 & 2 (HSV-1 & 2) has been the focus of much of the recent virology research. Recent progress by investigators at the University of Pennsylvania indicates that this goal may not be too distant. Their efforts have been directed to HSV glycoprotein D(gD) one of the key components of the virion envelope. Earlier experiments showed that purified gD of HSV-1 (oral form) or HSV-2 (genital form) stimulates high titers of neutralizing antibody. This glycoprotein is highly conserved in sequence both within and between serotypes and is a candidate for a subunit vaccine against both virus types. One goal of the current studies is to define the functions of gD in virus infection and to relate the functions to its structure. Employing the approach of constructing gD mutations using the cloned genes, investigators have developed a series of deletion and point mutations. They have further localized a precise region of gD, site Ib, that is conformational in nature, is essential for gD function and is also correlated with the ability of the molecule to induce protection in animals. A further goal is to duplicate this region synthetically and to test whether the peptide retains biologic activity. Further plans are to confirm that gD is an essential protein and to determine whether specific changes and deletions in the molecule prevent it from functioning. Toward this end, they have developed a complementation assay to test directly the effect of mutations on virus infectivity. They have shown that: 1) removal of all the N-asparagine-linked carbohydrates from gD has little effect on virus infectivity; 2) deletion of antigenic site Ib abolishes the



ability of the virus to penetrate cells; 3) gD from the genital strain is interchangeable with the protein from the oral strain and 4) site-directed mutations of certain cysteine residues destroy infectivity while changes in other cysteine residues yield gD molecules with a temperature-sensitive phenotype. Within the past year, three U.S. and one foreign patent has been received for the technology related to the use of gD as a subunit vaccine. Two firms have scaled up the technology to commercially produce gD-1 and have tested the vaccine in animal models. gD produced in several different fashions also constitutes the basic immunogen for every proposed human vaccine.

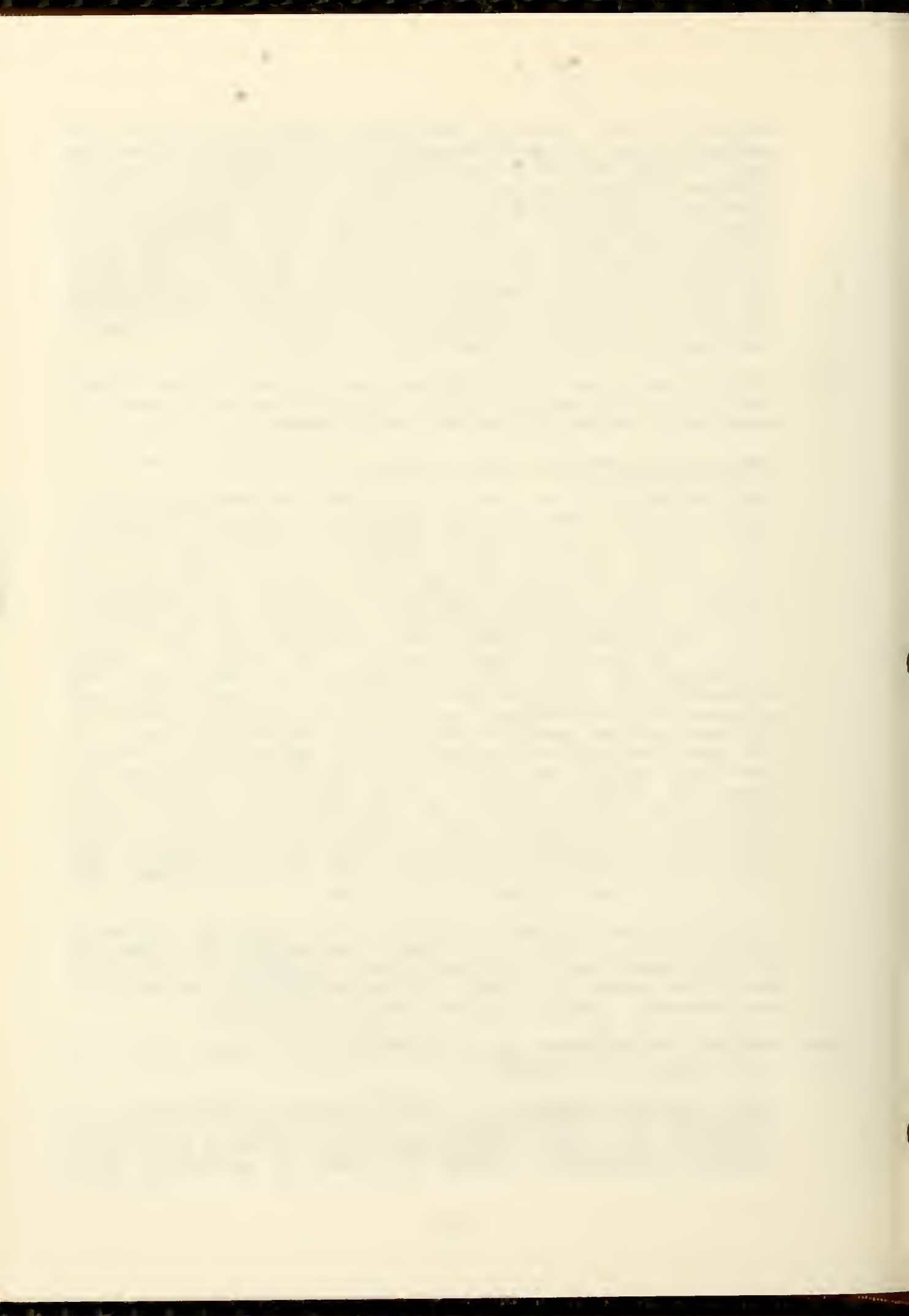
- F06 Examine the roles of microorganisms such as viruses, fungi and other environmental host factors in the development of neoplastic lesions of the oral soft tissues.

Papilloma Virus In Oral Cancer: In previous studies, investigators at the University of North Carolina demonstrated human papillomavirus (HPV) in both benign and malignant tumors of the cervical and mucosal epithelium. Using the Southern Blot assay system, they found genomic DNA of the oral/genital types of HPV in 60 percent of head and neck squamous cell carcinoma specimens. However, in subsequent studies, using the more sensitive polymerase chain reaction (PCR) amplification assay, they detected HPV DNA in 87 percent of head and neck squamous cell cancers. These investigators are also attempting to determine the expression of specific HPV functions in head and neck squamous cell carcinomas, using in situ hybridization with HPV mRNA probes specific for certain virus functions. So far they have obtained evidence of the expression of 8 virus functions in oral warts which were analogous to the pattern found in genital warts. They are now carrying out experiments to determine the expression of these virus functions in head and neck cancers particularly of the cheek, tongue and pharynx and attempting to determine how the virus affects cell growth and differentiation and eventually steps leading to oral cancer.

In yet another system, in vitro cell cultures are being developed in order to investigate the effects of virus DNA on cell growth and differentiation. The plan is to further use this system to study the co-carcinogenic effects of environmental factors such as tobacco and alcohol.

- F99 The role of epidermal keratinocytes using a rodent model for gene replacement therapy

Gene transfer therapy is rapidly gaining acceptance as a technique for ameliorating a variety health conditions. During the past year, investigators at SUNY Stony Brook have studied keratinocyte biology pertinent to epithelial gene



therapy. Research has focussed primarily on whether proteins secreted by epidermal keratinocytes can reach the systemic circulation. To answer this question, the investigators have employed athymic rats and nude mice grafted with human skin cells to follow the fate of the epidermal apolipoprotein E (Apo E). Apo E is a lipoprotein particle in blood which transports cholesterol to the liver and other organs. Previously, the investigators showed that keratinocytes in culture do synthesize and secrete Apo E. In the current experiments, nude mice received grafts of cultured keratinocytes. When serum was tested as early as four days following the graft and up to 47 days, Apo E levels were as high as 47 ng/ml. Removal of the graft resulted in an immediate drop in serum Apo E. Apo E serum levels were also measured in athymic rats bearing grafts of split thickness human skin. The Apo E levels in venous blood from graft tissue was found to be 11.3 and 11.8 ng/ml while levels in venous blood from non-graft bearing areas of the same rat were 8.4 and 8.9 ng/ml respectively. This implies that other epidermally secreted proteins having a similar fate may regulate the behavior of epithelial and other cells in the epidermis and that epithelial cells communicate with the rest of the body via chemical messengers. These experiments suggest that keratinocytes are highly promising as potential cells for gene therapy.

F99 The potential antiviral non immune properties of saliva

The importance of saliva in protecting oral tissues has been intensively investigated during the past two decades, with emphasis on the ability of salivary secretions to modulate the pathogenesis of the oral bacteria. However, the role of saliva in viral pathogenesis has not been well characterized. The presence of cell-protective as well as virus neutralization activity in human submandibular/sublingual saliva (HSMSL) with respect to the infection of cell cultures with Herpes Simplex Virus-I (HSV-I KOS strain) was previously reported by this group. The primary aim of this research is to determine the effect of pH on the viral neutralization activity of HSMSL and human parotid saliva (HPS) using a titer reduction assay. At pH 6.0, HSMSL reduced the viral titer by 98.5 percent, whereas at pH 7.0 and 8.0, the reduction was no more than 46 percent. A similar reduction could not be detected with HPS. To identify the salivary constituents involved, photoaffinity labeling techniques were employed. Partially purified HSV-I particles labeled with an iodinated photoaffinity probe (SASD) were incubated for one hour with HSMSL or purified salivary constituents in phosphate buffered saline (pBS), pH 6.0. The salivary/viral molecules were subjected to SDS-Page and labeled salivary components visualized by autoradiography. This procedure identified the selective interaction of viral particles with several components of HSMSL. The low molecular weight constituents

in HSMSL were selected for initial studies for viral neutralization activity. These proteins (1mg/ml of pBS, pH6) reduced the viral titer by 90 percent, suggesting that these salivary constituents in HSMSL may modulate the in vitro infectivity of HSV-I. Further fractionation of saliva resulted largely in salivary cystatins which were further reduced to one neutral (SA-I) and two anionic (SA-II and SA-III) constituents which were later purified to homogeneity by Fast Protein Liquid Chromatography. The virus neutralization activity of all three were then determined, using viral titer reduction assay. SA-II and SA-III effected a 30 and 37 percent reduction in viral titer but SA-I showed no neutralization activity. These studies will be extended to other herpesviruses, such as Epstein Barr virus and cytomegalovirus. The data obtained could contribute to the understanding of the pathogenesis of recurrent herpesvirus infections and provide new insights into the nonimmune salivary protective mechanisms which could be of benefit to severely immunocompromised individuals.

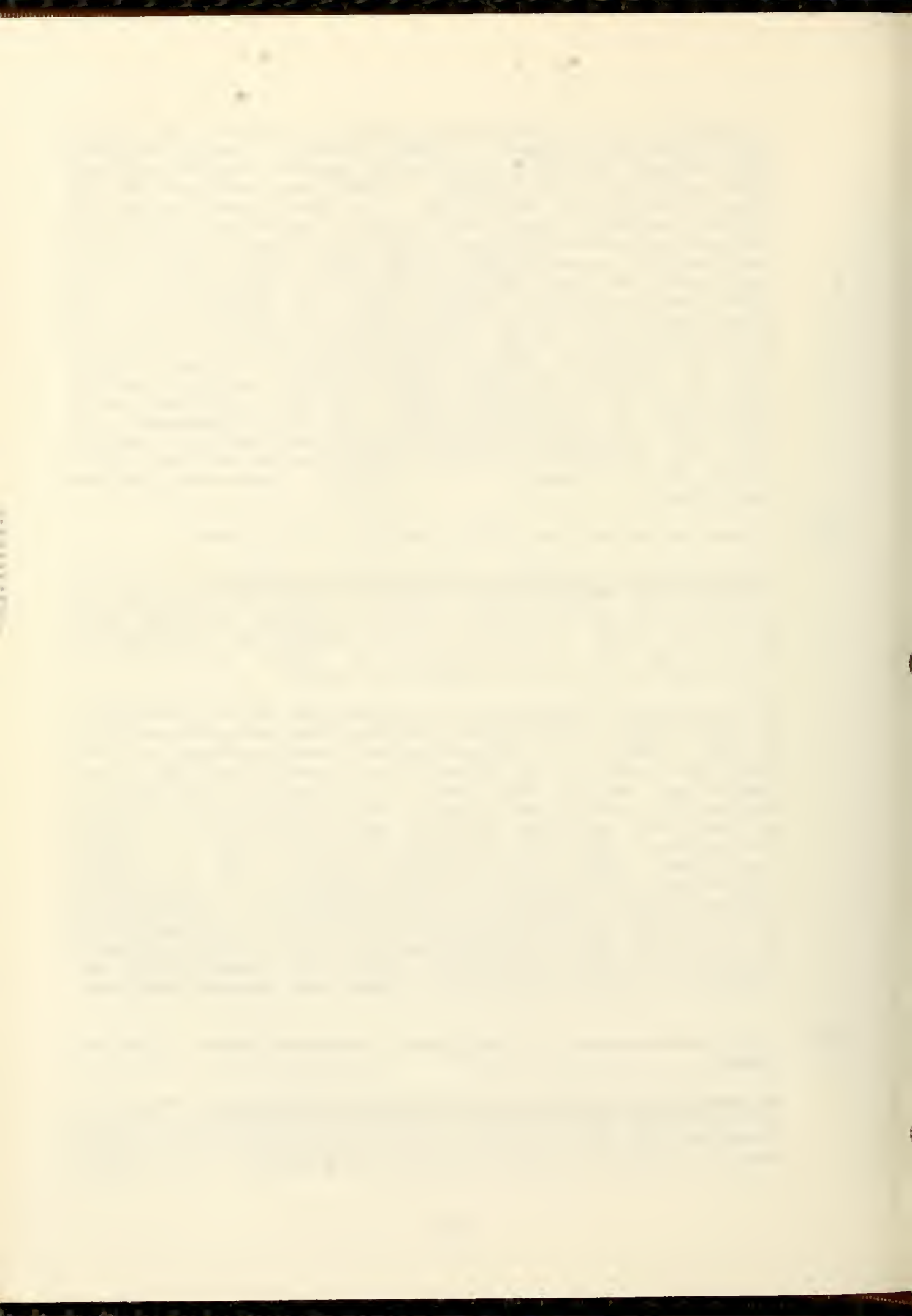
F99 Determine the role of growth factors in oral cancer

Transforming Growth Factor-alpha (TGF- α) has been consistently demonstrated in cancers developed in hamsters as well as in the major form of human squamous cell carcinoma (SCC). A group of NIDR-supported investigators are using the Syrian hamster to study the molecular mechanisms of TGF- α in causing oral tumors.

In preliminary experiments, the objective was to investigate at what stage of the cancer process does aberrant activity of TGF- α begin. The investigators were surprised to find that the activity of TGF- α is not restricted to cancer epithelium, but is also found in the normal epithelium of the mouth. This novel finding has altered the perception of how this cellular peptide can participate in cancer development. The fact that the activity of TGF- α is about 3-4 times higher in cancerous tissue than in normal epithelium may be helpful in developing an understanding of the mechanisms involved in cancer development. Although the work to date has all been done in animals, the results are so convincing that the investigators have recently extended the work to human subjects. They have demonstrated the presence of TGF- α in all of the human oral cancers examined.

F99 The epidemiology of Acquired Immunodeficiency Syndrome (AIDS)

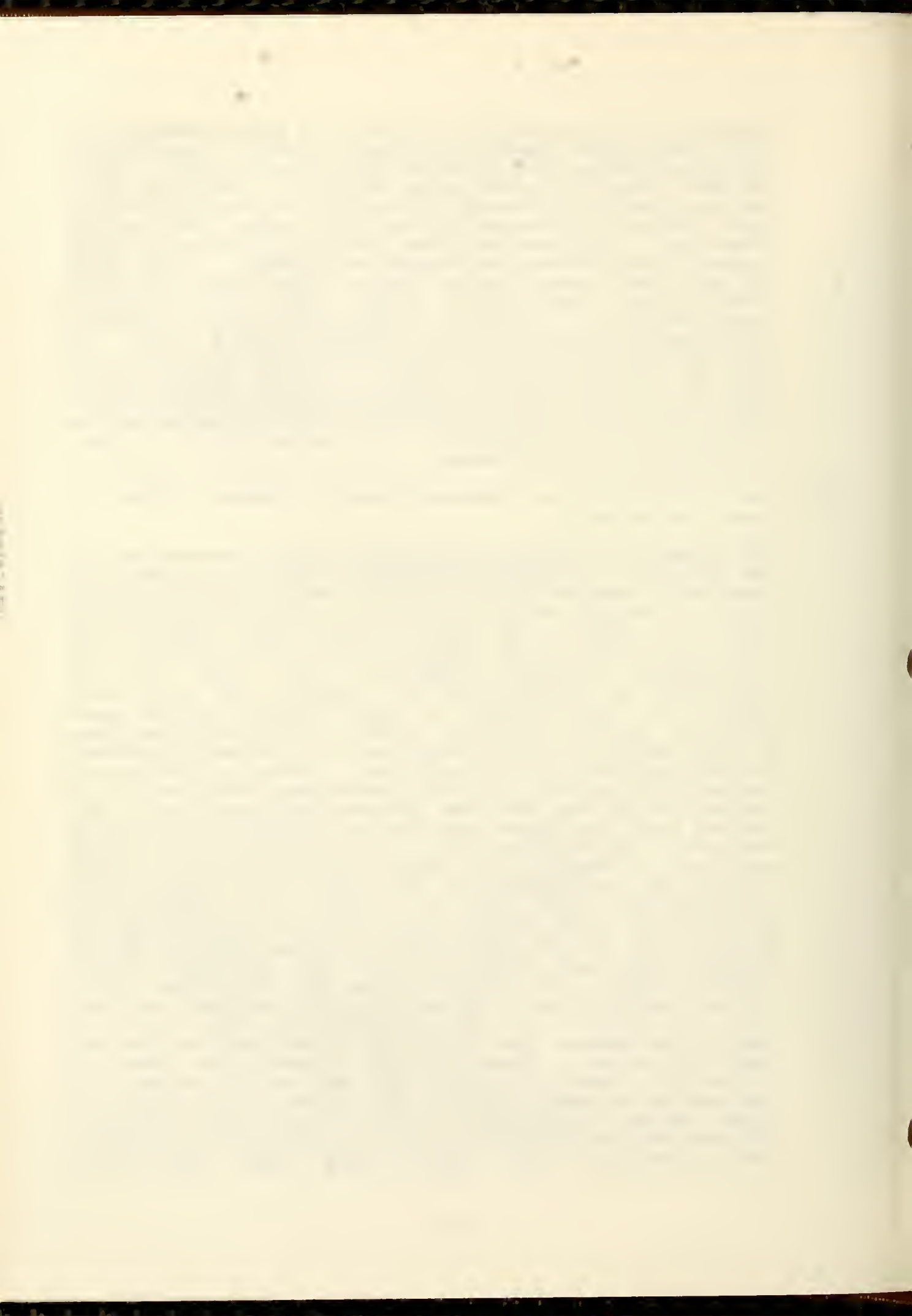
Delayed Tooth Eruption In HIV Infected Offspring: The focus of one investigation at the University of California at San Francisco is to investigate the oral features of children born to mothers at high risk for HIV infection. In a cross



sectional study conducted previously, a high percentage of oral lesions were reported in these children. Longitudinal follow-up studies have produced the following data. Of 52 children enrolled in the study since 1986, two have died, two were lost to follow-up, 8 were HIV+, 10 were HIV- and 10 were less than 10 months and their HIV status could not be determined. Fifteen children born to intravenous drug abuser (IVDA) HIV- mothers served as controls. At one month of age and every three months thereafter complete examinations, including a blood test, a neurological examination and an oral examination, were conducted. Developmental evaluations were conducted every 6 months. No preponderance of oral soft tissue disease was associated with HIV seropositivity; however, a trend toward delayed eruption and an abnormal pattern of eruption were noted. These preliminary data suggest that delayed eruption may be a feature of pediatric HIV infection. Further investigations are warranted.

F99 The Microbiology of Immunodeficiency Syndrome (AIDS) in Humans and Animals

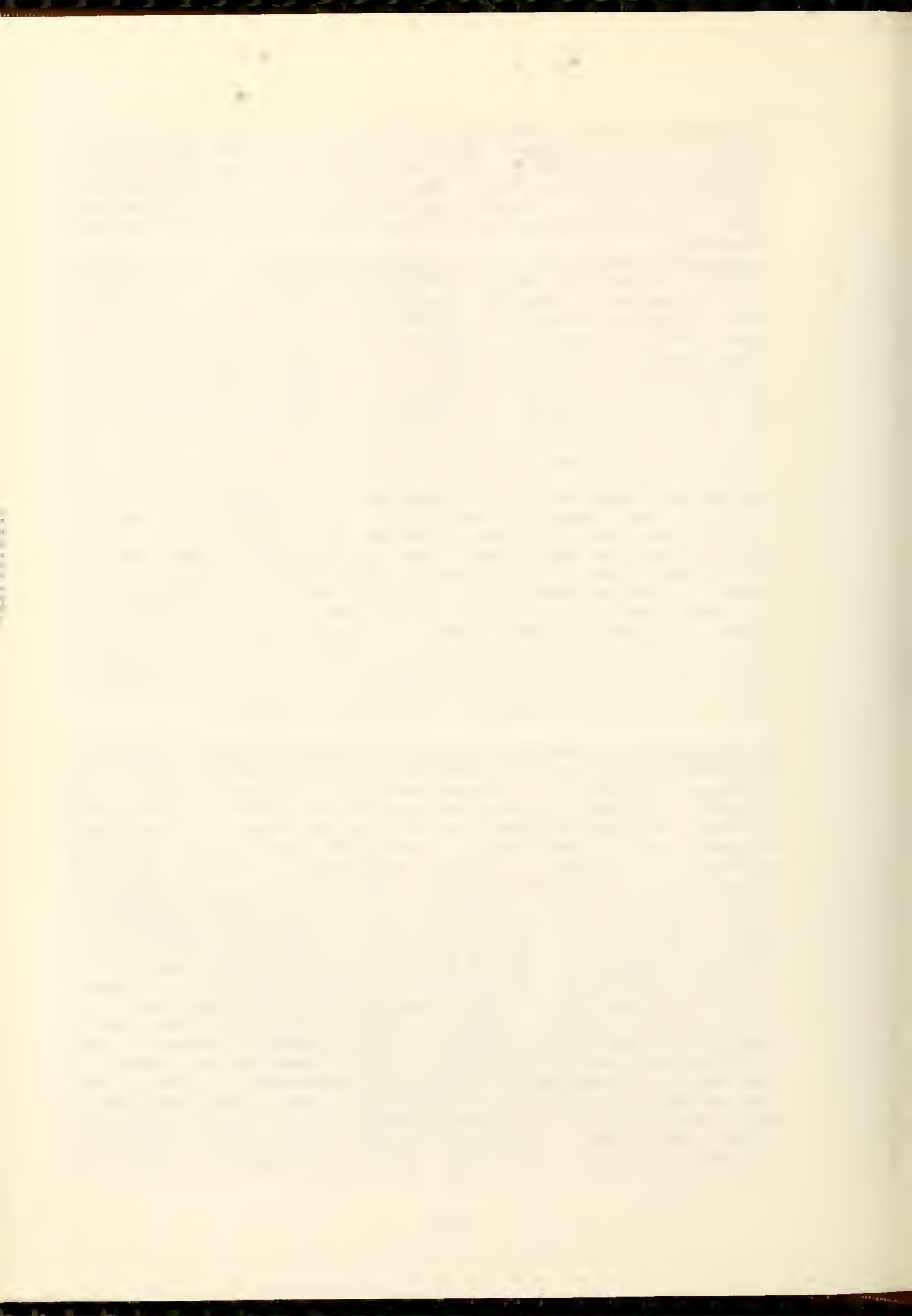
HIV In Human Periodontal Inflammatory Fluid: Researchers at the University of California at San Francisco (UCSF) have previously reported that a high percentage of AIDS patients display a rapidly destructive form of periodontitis and the number is growing. In current studies, the research team found that they could detect actual HIV virus in the inflammatory gingival crevicular fluid (GCF) just as frequently as the standard HIV Western Blot test could detect the HIV antibody in blood samples of the same patients. A total of 112 men at high risk for AIDS had been examined using the Western Blot test and had been classified as HIV-positive or HIV-negative. Using a DNA probe designed for use with GCF, the researchers found that 27 of 30 individuals who had been declared HIV positive by the Western Blot also tested positive for the infecting virus in the GCF. In addition, 43 of 47 individuals who tested negatively by Western Blot method were HIV negative in the GCF by the DNA probe test for the actual virus. The investigators noted that 4 of these individuals were positive for virus at the time of the GCF test, and two months later one of these subjects became positive by the Western Blot test blood test for HIV antibody. These findings suggest that HIV infections may be evident in the GCF well before a serum antibody response develops, and that once the patient is HIV seropositive, there is good correlation between the blood test for antibody and the GCF test for virus. These findings suggest that based on reliability, sample stability and ease of collection, GCF may provide an additional tool for epidemiologic surveys in large patient populations or in third world nations. Although the researchers are unsure how HIV is deposited in the inflammatory fluid, they indicate that HIV-infected



macrophages and T lymphocytes--two of the body's specialized defense cells--probably carry the virus to the periodontal tissues. Once there, HIV may aggravate the periodontal disease and may also enter the oral cavity via the gingival crevicular fluid. Whether or not HIV from the GCF has any role in the transmission of this virus must await further research.

Periodontal Bacteriology in AIDS Risk Groups: Investigators at the University of California-San Francisco in the past have reported a rapidly destructive forms of gingivitis (HIV-G) and periodontitis (HIV-P) in AIDS patients, whereas these lesions were not reported with the same frequency in other geographic areas of the U.S. These reports prompted investigations at SUNY-Buffalo to determine differences in the oral flora between different AIDS risk groups to explain the reported frequency of HIV-P in homosexual patients, but not in other types of AIDS patients. Using an immunofluorescence technique they found that the two periodontopathic microorganisms B. gingivalis and F. nucleatum predominated in homosexual AIDS patients, whereas in I.V. drug abusing individuals, S. sanguis II and L. acidophilus, two cariogenic species were predominant. These results were so consistent that B. gingivalis could not be cultivated from any of the 34 subgingival plaque samples from 11 AIDS patients with a history of IVDA, although this organism was found in 16% of 33 homosexual AIDS patients. These findings suggest that the high prevalence of HIV-associated periodontal disease in homosexual AIDS patients may be due to the specific composition of the dental plaque. Thus, the history of how HIV infections occur may serve as a guide to the most effective therapeutic regime.

Microbiology of Simian Acquired Immunodeficiency Syndrome: Spontaneously occurring simian acquired immune deficiency syndrome (SAIDS) in rhesus monkeys (*Macaca mulatta*) is caused by a type D retrovirus designated (SRV-1). SRV-1 has a broad cellular tropism in vivo and in vitro. Its primary targets are lymphocytes, monocytes and a variety of epithelial tissues. Immune suppression caused by this virus results in opportunistic infections and a spectrum of oral lesions similar to those seen in human AIDS, including oral and esophageal candidiasis and necrotizing gingivitis. These lesions may not only be the result of generalized immune suppression induced by SRV-1, but may also be due to a direct effect of the virus on epithelial cells. Tissues from the oropharynx, cheek pouch, tongue and esophagus of ten animals with terminal SAIDS were studied using immunohistochemistry, double label immunofluorescence and electron microscopy techniques. Viral antigen was found in the oral and esophageal mucosa and submucosa of nine of the ten animals examined. In addition, scattered Langerhans cells were found to be SRV-1 positive and associated with viral particles. Further studies into the mechanism responsible for the oral lesions produced should be



conducted to aid in designing anti-HIV chemicals.



1102



ANNUAL REPORT

CRANIOFACIAL ANOMALIES, PAIN CONTROL

AND BEHAVIORAL RESEARCH BRANCH

EXTRAMURAL PROGRAM, NIDR

One hundred thirty-seven research and training projects received funding during 1989 to study the causes, prevention and treatment of craniofacial defects. Investigations include classical and molecular genetics applied to major anomalies such as cleft lip and palate and the molecular and cell biology of normal and abnormal growth and development of connective tissues in human subjects and animal models. Clinical trials of treatments for the correction of malocclusion in children and adults were initiated. In the area of orofacial pain and behavioral research 67 awards were made. These addressed topics such as the epidemiology and etiology of temporomandibular disorders, behavioral and biological variables associated with the risk of having periodontal diseases and efforts to improve patients' compliance with oral health-promoting behaviors among children and adults.

C03 Characterize the chemical mediators that underlie cell-cell and cell-matrix interactions.

Proper development of the head and face requires that the growth and assembly of the component tissues be integrated and coordinated. Failure to do so results in craniofacial malformations. These processes begin very early in embryogenesis, when the earliest precursors of the brain and oral structures are first established. Many cells, individually or as collectives, migrate from their sites of origin to new locations. Interactions between these cells and neighboring tissues are critical in determining their routes of movement and subsequent differentiation into specific cell types found in nerves, muscle, cartilage, bone and teeth, for example. Investigators at Cornell University use bird embryos because they can be manipulated readily and it is possible to transplant cells from the embryos of one species to another and follow the cells as they migrate within the developing host embryo. Studies have focussed on the movement of neural crest cells, which arise from the dorsal midline of what will become the brain and form the connective tissues of the midface and jaws as well as many peripheral nerves. Contrary to previous reports, the muscles of the jaw were shown to arise in a similar way to that for other voluntary muscles. The migration, elongation and segregation of muscle forming cells into distinct muscles and their attachment to developing jaws has been followed. Muscle precursor cells from any part of the body are able to form anatomically normal craniofacial muscles if



transplanted into the head at an early age. Endothelial cells, which are precursors of blood vessels, are highly invasive and can migrate rapidly in all directions and over great distances. This mode of migratory behavior is unlike that of any other cell type in the embryo. Endothelial cells from any part of the embryo are able to form normal craniofacial and cardiac blood vessels when transplanted into the head region. The differentiation and assembly of these precursor cells into muscles or blood vessels is dependent on interactions with other cell populations and components of the extracellular matrix.

One novel molecular mechanism, which can explain how neural crest cells migrate, is being explored at the University of Texas in Houston. Galactosyltransferase (GT), which is the only enzyme of its class on the surface of neural crest cells, has been shown to bind to laminin components or receptors in the basal lamina. This binding enables the cells to spread and migrate over the basal lamina in the embryo. Factors which inhibit the enzyme activity of GT prevent migration, as monitored by time lapse microphotography. The GT is localized to the lamellipodia or extensions from the cell surface, which are responsible for movement. These studies are being extended to determine whether a similar mechanism is involved in nerve development. At the University of Oregon investigators have shown that, unless they are allowed to migrate, neural crest cells lose their neurogenic potential as they become older. The extent of neurogenic differentiation is also reduced when the neural crest cells are exposed to laminin; the converse is true when the cells are exposed to fibronectin, illustrating the role of the extracellular matrix in neural crest cell differentiation as well as migration.

- D04 Elaborate molecular mechanisms involved in wound healing.
- D05 Study the influence of age.
- D06 Develop mechanisms to enhance wound repair, tissue regeneration, promote tissue grafts and ameliorate scar formation.

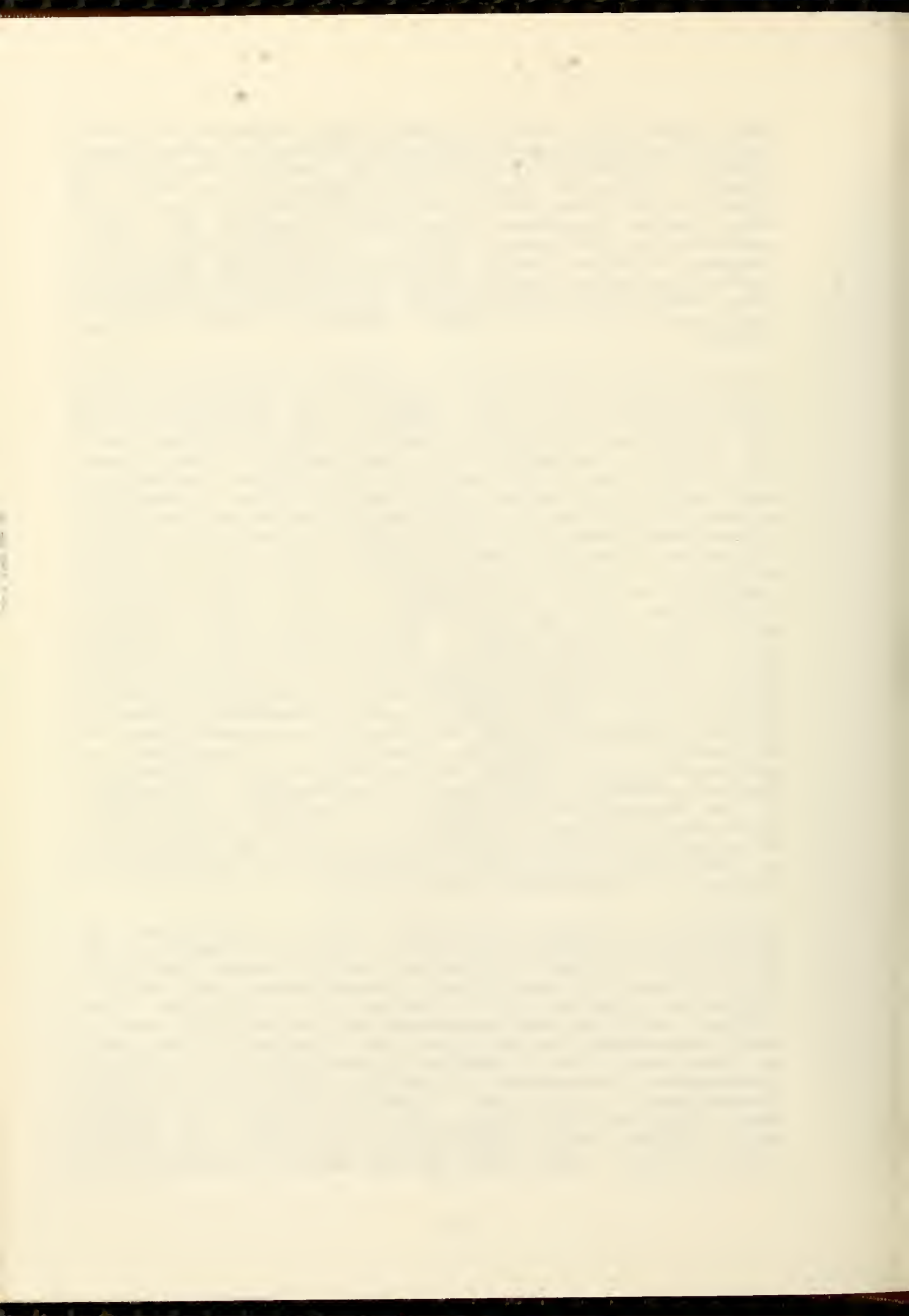
Platelet derived growth factor (PDGF) is the major growth factor present in clotted blood at the site of an injury. Mesenchymal cells such as fibroblasts, smooth muscle and brain glial cells respond to it by increased rates of cell division. Studies at Boston University Medical Center have focussed on its effects on bone cells, which are also derived from the mesenchyme. A human osteosarcoma cell line expressed a gene known to encode PDGF, synthesizing the growth factor. The cells bound PDGF by means of specific receptors but showed no increase in cell division. Another tumor cell line did not synthesize or secrete PDGF but bound it specifically and responded by increasing cell division.



These experiments with bone tumor cells suggested that PDGF may act as paracrine and autocrine factors for normal bone. Paracrine factors are produced by one cell and act on others. Autocrine factors are produced by one cell and stimulate the same cell. Osteoblasts derived from normal human bone expressed the gene encoding for PDGF, synthesizing the growth factor. Cell proliferation was induced in these cells by PDGF, confirming the growth factor's paracrine and autocrine activities for normal bone. Thus PDGF from blood and of local origin may have an important role in the bone remodelling seen in wound healing.

Excessive scar formation is a significant problem following facial surgery to correct congenital or acquired defects resulting from burns, for example. The disfiguring scars may necessitate follow-up surgery. Little is known about the factors responsible for overgrowth of scar tissue. Investigators at the University of California, Los Angeles, have shown that during wound healing there is excessive accumulation of extracellular matrix, which may be due to either overproduction or deficient breakdown of the matrix. Fibroblasts from uninjured and wounded skin differ in their ability to produce extracellular matrix and in their response to the inflammatory factor, interleukin-1. Fibroblasts from skin and mucosa also differ in their ability to produce matrix. This suggests that excessive scar formation may reflect differences in the synthetic properties of fibroblasts from normal individuals and those prone to scarring. Hyaluronidase is a key enzyme in the breakdown of a major class of matrix components, known as glycosaminoglycans, which are present in wounded tissue and elevated in excessive scarring. Breakdown of glycosaminoglycans occurs exclusively within fibroblasts, requiring internalization prior to degradation. Differences in the degradative properties of fibroblasts from various individuals are being correlated with their susceptibility to excessive scarring. These studies offer the possibility of identifying susceptible individuals and modifying the response of their fibroblasts to injury.

Impressive advances are being made in techniques for transplantation and regeneration of skeletal muscles in the treatment of facial injuries and partial facial paralysis. Many of these advances depend on experiments conducted with laboratory animals, at the University of Michigan. Rat muscles, which had been denervated for as long as 22 months, were spontaneously reinnervated when they were transplanted to innervated limbs. Muscles transplanted in young rats regenerated a three-fold greater functional mass than those transplanted to old rats. However, muscles transplanted from young and old animals into young hosts did equally well, whereas those transplanted into old animals did equally poorly. Apparently the regenerative capacity of old



muscles is not different from that of young muscles, but old hosts do not provide a satisfactory environment for regeneration following transplantation. Immediate repair of the vascular connections is essential to prevent degeneration of muscle fibers following transplantation, but even with immediate revascularization functional deficits occur. These deficits are a serious limitation to more widespread use of skeletal transplantation in treating facial injuries. Physiological changes, such as oxidative capacity and substrate utilization are being studied to determine the origin and mechanism of these deficits. Post-operative procedures are being studied to increase the frequency of recruitment of grafted muscles and increase the intensity of their contractions. Training regimens involving running on a treadmill, increasing the loading on a grafted muscle through removal of synergistic muscles or chronic electrical stimulation each proved to be effective in improving functional mass or the metabolic capacity of grafted muscles.

- E05 Expand studies of interceptive treatment aimed at growth modification.
- E06 Study factors affecting the post-treatment stability of bones and teeth.

Nearly fifty percent of children in the US would benefit from orthodontic treatment and about five percent have sufficiently severe malocclusion that they are handicapped in their life adjustment. An increasing number of adults are also receiving treatment both for the correction of physically handicapping malocclusions and also as an aid to psychosocial adjustment. In some orthodontic practices, between thirty and sixty percent of patients in treatment are adults. No clinical trials have been conducted to evaluate the alternative treatment approaches, which are advocated by different clinicians. In order to provide the data to differentiate between alternative treatments with respect to their efficacy, the NIDR has funded several clinical trials. The following two examples illustrate the types of treatment being studied. Class II malocclusion is characterized by protrusion of the upper incisor teeth but is most frequently caused by underdevelopment of the lower jaw. Three possible approaches to treatment will be compared: early modification of growth of the jaws prior to adolescence, followed by a movement of teeth during adolescence; repositioning of the teeth and compensation of jaw discrepancies during adolescence, when the permanent teeth have erupted; or surgical-orthodontic repositioning of the jaws during late adolescence or in young adults. Despite the fact that adults differ from children morphologically, physiologically and psychosocially and have essentially ceased to grow, current treatments for adults are adapted from experience with children. One treatment of



Class II malocclusion in both children and adults involves extraction of the bicuspid teeth. Advocates of extraction claim that this facilitates movement of the remaining teeth, increases the thickness of interproximal bone and reduces lip prominence. Advocates of nonextraction point to reductions in physical and emotional trauma, reductions in root resorption, improved facial contour and improved occlusal function. Each group claims beneficial effects on temporomandibular joint function. One of the clinical trials will compare the outcomes of treatment involving extraction and nonextraction in groups of adult patients.

- J01 Further elucidate the role of hormones, vitamins, peptides and other endogenous factors as regulators or modulators of cellular activities affecting growth, maintenance and repair of mineralized tissues.

Mineralization of hard tissues is carried out by specialized cells or organelles, which deposit mineral ions onto a preformed organic matrix. All mineralizing tissues contain alkaline phosphatase (AP); more than sixty years ago it was proposed that AP plays a key role in mineralization. The enzyme is integrated into the plasma membrane of the mineralizing cell and is capable of hydrolyzing a variety of phosphate esters so that phosphate ions are released and are available for incorporation into mineralized tissues. High concentrations of alkaline phosphatase have been observed in matrix vesicles (MV) budded off from the plasma membrane of chondrocytes. Similar MVs have been identified in most mineralizing tissues, including pre-dentin, cartilage, bone and microorganisms, which form calcified deposits. The MVs are sites of initial mineral deposit and are the focus of attention of several groups investigating the mechanisms of mineralization. Studies at the University of Kansas, using specific antibodies for alkaline phosphatase, showed that the enzyme is bound to the outer surface of the MV membrane by a covalent bond to phosphoinositol. MVs released from rat chondrocytes in culture are trapped in newly formed cartilaginous matrix, where they induce mineral formation. The partial amino acid sequence of AP has been determined and the nucleotide sequence of part of the AP gene has been published. Investigators at the University of Texas, San Antonio, have shown that chondrocytes respond to vitamin D metabolites by increasing cell proliferation and extracellular matrix production. Vitamin D has long been known to be involved in mineralization; vitamin D deficiency causes rickets. The AP activity of MVs derived from chondrocytes is increased following exposure to vitamin D metabolites. The response in chondrocyte-MV levels of AP differs according to the site of origin of the parent chondrocytes - whether from resting zones or actively growing zones of bone - and the metabolite of Vitamin D used. Other factors such as parathyroid hormone and transforming growth factor, which are known to influence bone formation, also

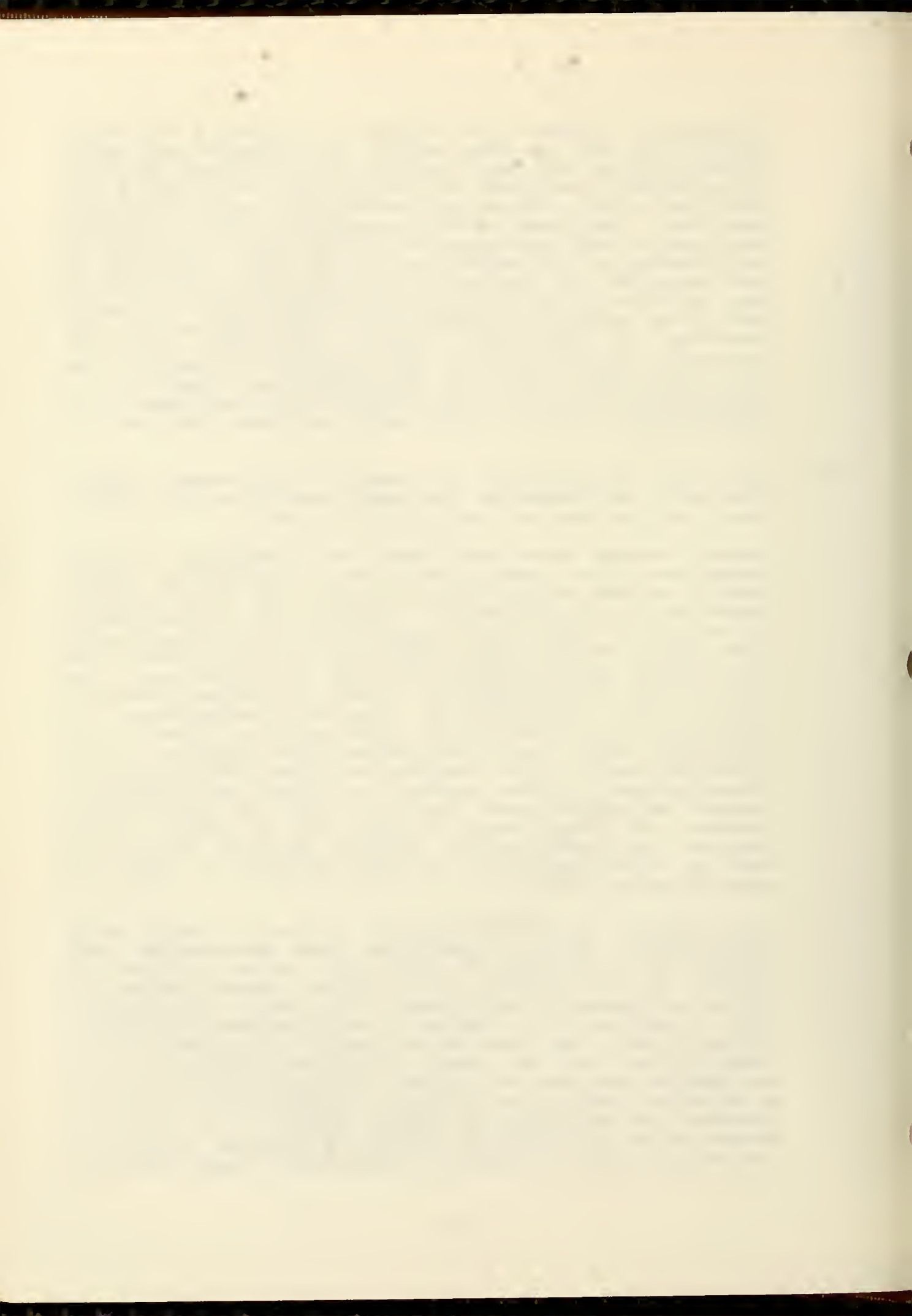


stimulate AP levels. The effects of vitamin D and other factors on MV AP may provide a mechanism by which mineralization of the matrix is modulated locally. Investigators from Tufts University have developed a rat model to study effects of parathyroid hormone on bone formation. Low doses of the hormone given daily increase bone calcium and hydroxyproline content. Hydroxyproline is an indicator of the organic content of bone. Increases in bone mass are not the result of increased bone turnover; they are seen in both newly forming and hard cortical bone. Bone loss normally observed following ovariectomy was prevented by parathyroid hormone, suggesting potential therapeutic application of this research. There are no truly effective methods for preventing and reversing the generalized bone loss associated with osteoporosis in postmenopausal women or the localized bone loss due to periodontitis.

- J09 Study the interactions of mineral with enamel matrix components and determine how each contributes to crystal growth and the physical properties of enamel.

Several awards have been made to outstanding foreign scientists; these extend the research capability, often involve collaborations and complement the investigations conducted by US scientists. Scientists at the Hebrew University in Israel are investigating the mechanisms by which enamel develops and mineralizes in human teeth. The crystallinity of the enamel increases as it matures. The only mineral phase detectable in developing enamel, using a battery of sophisticated physical techniques, is hydroxyapatite. Fluoride is preferentially taken up by developing enamel and incorporated into apatite. The distribution of fluoride observed in mature teeth, with high concentrations at the surface and lower concentrations towards the dentin-enamel junction, is already appearing in incisors and first molars at the time of birth. This suggests the importance of supplying fluoride around the time of birth and prior to tooth eruption in order to prevent future caries.

Two classes of proteins with different amino acid compositions, known as enamelin and amelogenin, are secreted by ameloblasts in developing enamel. These are thought to be involved in initiating mineralization by acting as nucleators for crystal development. The specific role of each class of proteins is being explored in several laboratories. The first enamel matrix to be secreted is enamelin-rich and, as enamel formation progresses, more amelogenins are produced. Later, as enamel matures there is a selective loss of amelogenins, resulting in a relative increase in enamelin in mature tissue. In order to determine how the different classes of proteins are formed, the Israeli group and several others have cloned genes for



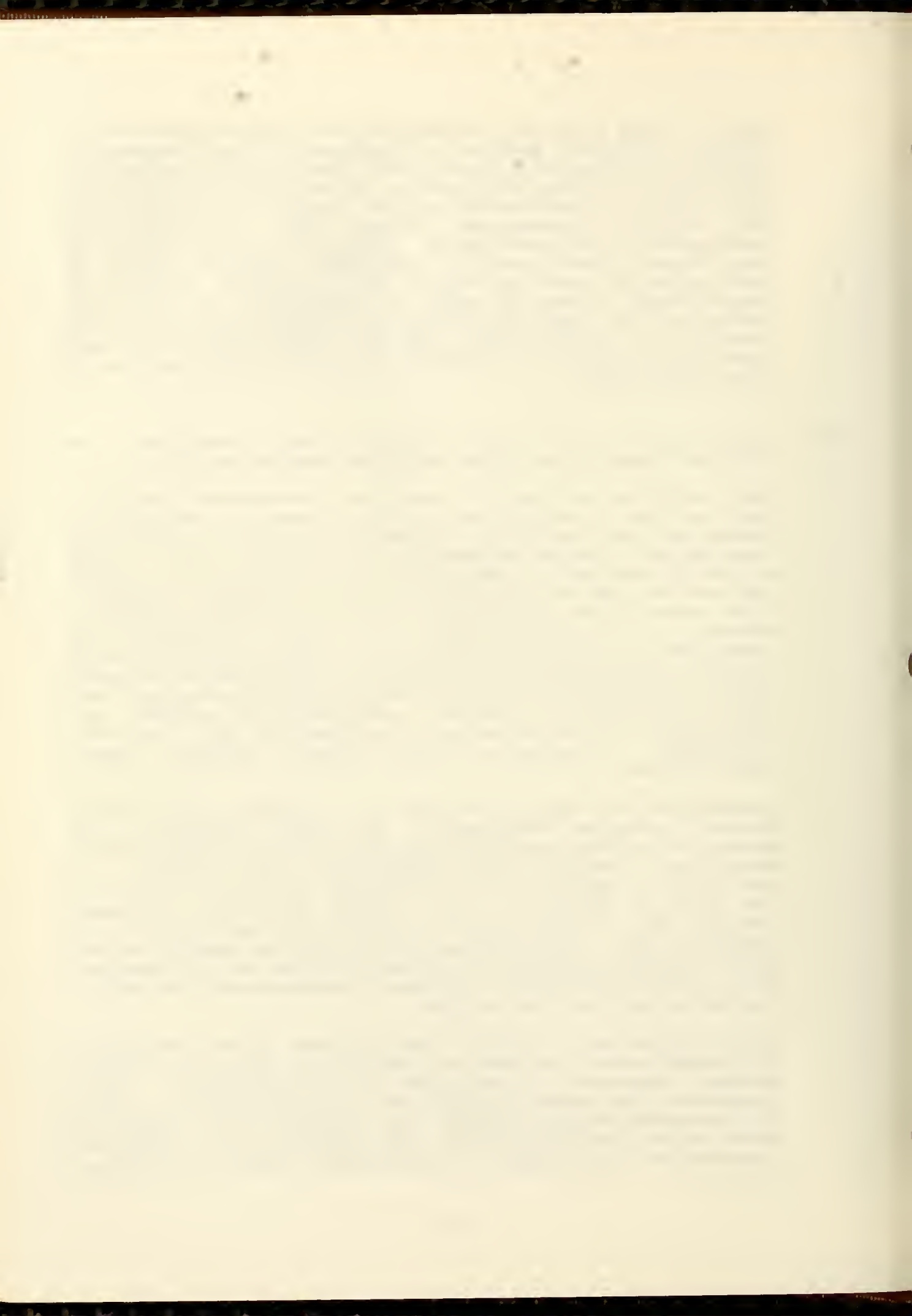
some of these proteins. Different genes may be expressed to produce different species of amelogenins and enamelin. However, the Israelis found that there are different but structurally related mRNA species in ameloblasts, indicating that different amelogenins may be produced by alternative RNA splicing mechanisms rather than reflecting the expression of different genes. Other groups are working on the theory that various amelogenin species arise by degradation of parent proteins. The origin and structure of these enamel proteins must be determined in order to understand how they are involved in mineralization. This is essential to understanding how the teeth are formed, why some are susceptible to disease and how inherited defects of human teeth occur.

- G09 Conduct basic and clinical studies of pain associated with the temporomandibular joint and other myofascial pain.

Many patients are seeking care for jaw-related problems, such as jaw "locking" or clicking, chewing problems, or persistent pain or aching in the jaw joint or surrounding musculature. Recent epidemiological findings from a study at the University of Washington indicate that at least 12 per cent of the adult population suffers frequent jaw pain, with even more suffering other jaw-related problems. However, our understanding of these conditions is incomplete. Diagnostic measures often fail to differentiate between jaw-related dysfunction/pain conditions which would resolve spontaneously, or respond to simple therapies, and those destined to become progressively painful and disabling. NIDR-supported investigators are, however, beginning to make strides toward reducing these uncertainties.

Researchers at the University of Rochester, the Oregon Health Sciences University, and the University of Pennsylvania have studied characteristics of tissues within normal and abnormal temporomandibular joints (TMJs). A particularly important and vulnerable structure within the jaw joint is the disk, a small fibrous structure interposed between the condyle and the articulating portion of the temporal bone. Its position relative to the condyle varies as jaw opening and closure occurs. Within this often-used joint, disk dislocations (internal derangements) can occur, producing pain and dysfunction.

NIDR-supported clinical scientists have completed histopathological analyses of disk tissues from cadaver TMJs showing anatomical evidence of disk displacement. They discovered that normally positioned disks showed low indices of histopathological change. TMJ specimens with displaced disks showed abnormally high concentrations of calcium and phosphorous, and evidence of metaplastic hyaline cartilage,



hyalinization, and abnormal collagen patterns. Some tissues also showed calcification in disk tissue and ligament. They also found that magnetic resonance imaging (MRI) correctly detected the presence of displacements in 83 percent of the joints with disk displacement. Most of the 17 percent not correctly classified showed a type of displacement ("rotational" displacement) involving medial or lateral, as well as anterior, displacement of the disk. This type of displacement had not previously been well documented and was very difficult to see through imaging techniques.

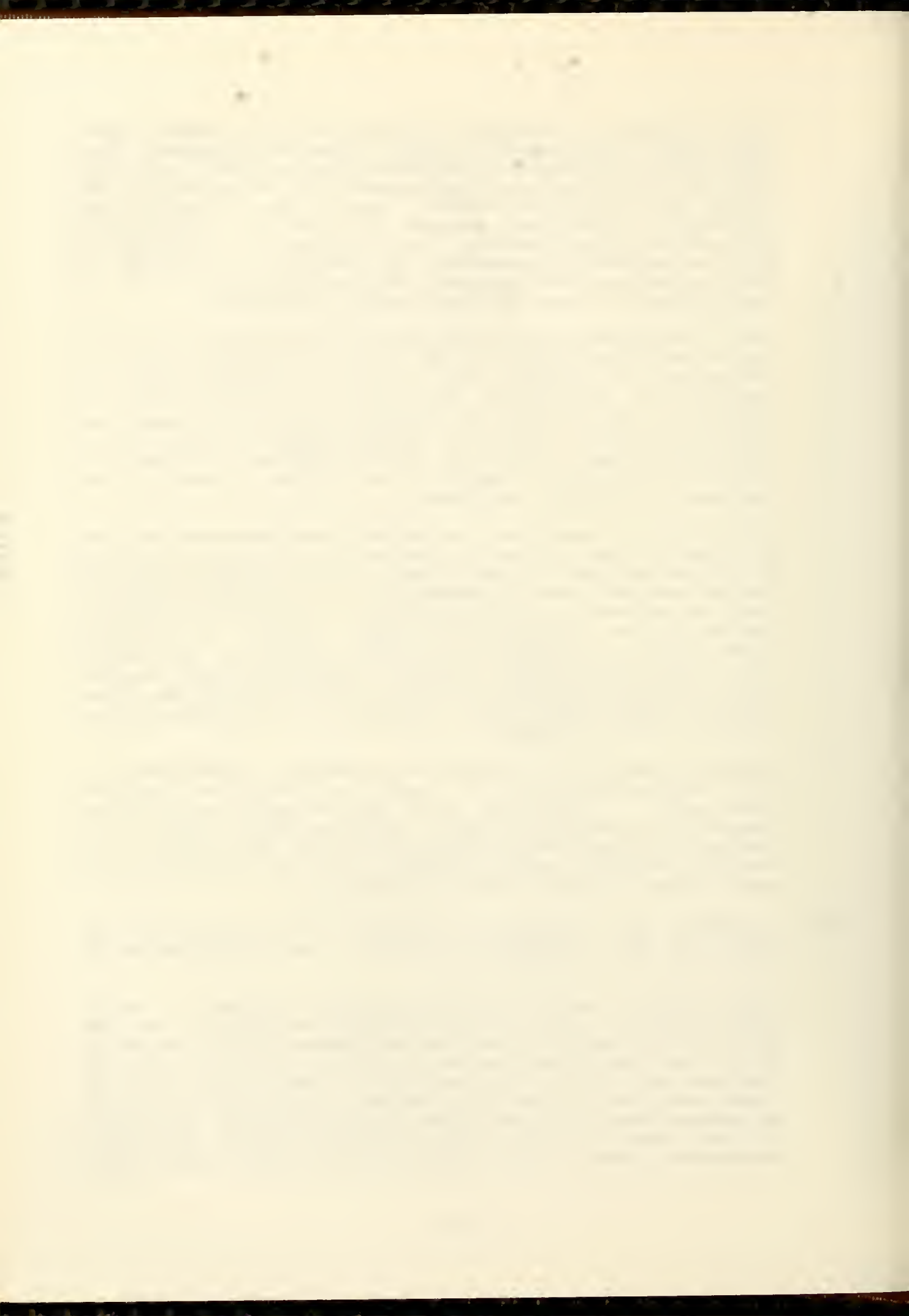
These investigators also developed modified MRI procedures for better visualization of TMJ tissues. Applying these new procedures to clinical TMJ patients who had agreed to undergo both arthrography and MRI, they found levels of diagnostic accuracy for MRI approaching (and in some case equalling) those found for arthrography. This is of interest because arthrography is an accurate, but invasive, diagnostic procedure resulting in patient discomfort and exposure to ionizing radiation.

University of Pennsylvania scientists have demonstrated that MRI signals from different parts of the disk vary according to the orientation of the tissues to the magnetic axis of the MR scanner. When characteristics of the MR signal from the disk exceeded values predicted by orientation, elevation indicated the presence of extra amounts of sulfated proteoglycans, a substance frequently found in association with hyaline cartilage. Their findings strongly suggest that position-corrected MRI signal elevations may be a new, and especially sensitive, indicator of histopathological changes in disk tissues.

These new diagnostic findings are expanding understanding of the characteristics of various jaw-joint related pathologies. They also hold considerable promise for clearly differentiating joint pathology which may benefit from surgical intervention from other more common sources of chronic orofacial pain disorders, such as stress-related muscle hyperactivity or muscle pathologies.

M01 Determine how behavioral, social, and cultural factors relate to the incidence, prevalence, and distribution of oral diseases and conditions.

NIDR-supported dental epidemiologists studying a large, random sample of black and white North Carolinians over the age of 65 discovered that the periodontal status of older blacks was considerably worse than that found in whites of the same age. Fifty-five per cent of the blacks showed at least one tooth site with severe (7mm+) loss of tooth attachment from surrounding periodontal tissues, while only 29 per cent of the white seniors showed comparable attachment losses at one or more sites. Blacks were 5 times



more likely than whites to show average attachment losses across all tooth sites of 5.5 mm or more. Significant differences between the periodontal health of blacks and whites persisted even when statistical corrections were made for differences in socioeconomic status, educational level, and interval since last dental visit.

These results indicated considerably higher levels of periodontal diseases in blacks than had been indicated in studies of elderly whites in Iowa, or in a recent NIDR national survey evaluating the oral health of employed adults and white or black seniors attending senior centers.

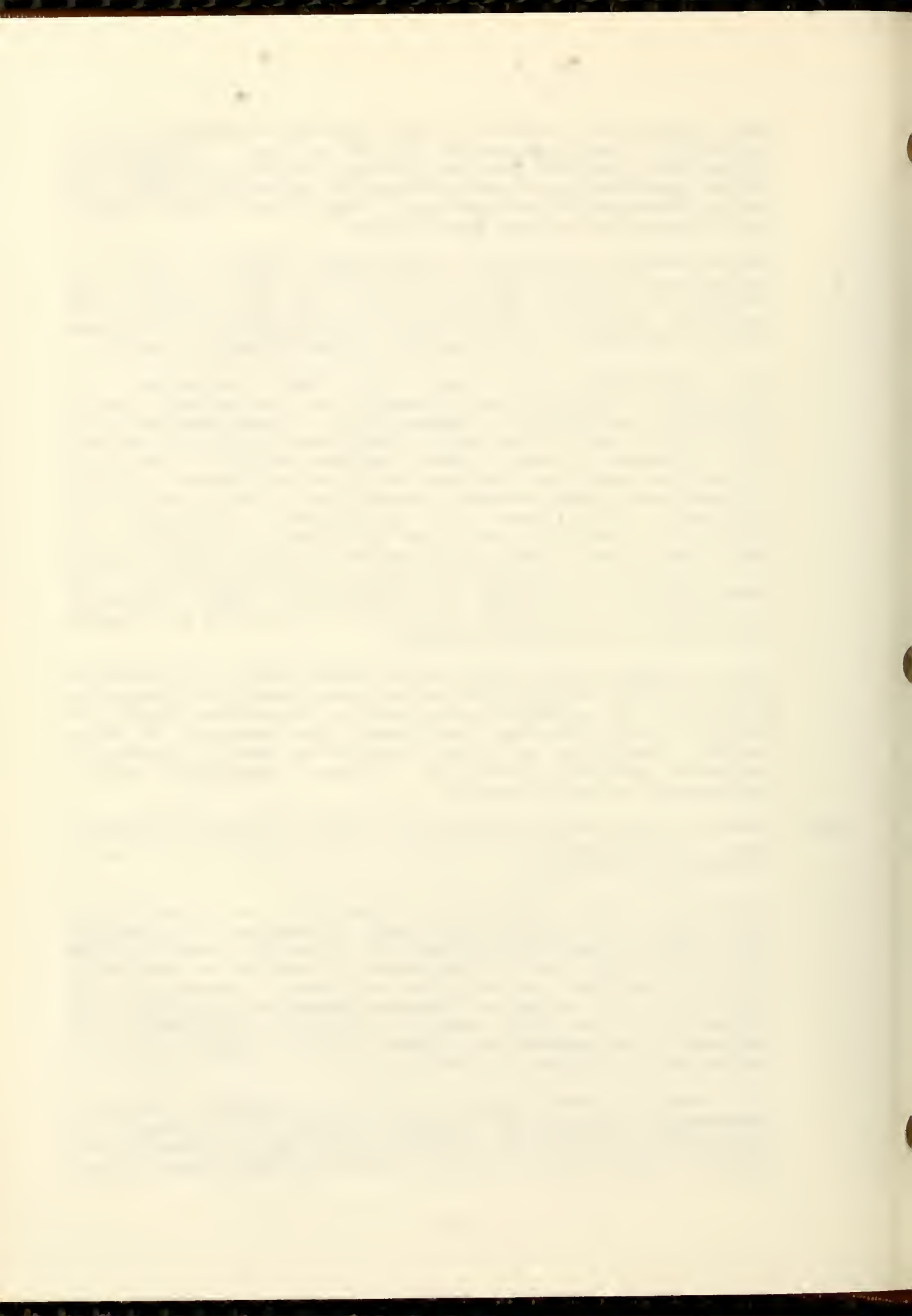
The researchers also found that both behavioral and biological variables were needed in statistical models predicting periodontal disease risk. Characteristics of both blacks and whites which predicted having an average tooth attachment loss of more than 5mm included: last visit to dentist more than one year ago; use of tobacco; history of moderately heavy alcohol consumption; presence of an oral pathogen associated with periodontal diseases (*B. gingivalis*); having less than 20 teeth; perception that their mouth health was poor; and having less than 12 years of education. Using the above characteristics, the investigators developed a statistical model correctly classifying 90 per cent of the seniors having an average attachment loss greater than 5mm.

Studies identifying risk factors associated with specific oral diseases can provide an improved basis for targeting prevention or screening programs to those most likely to benefit. Such findings also reveal how important it is to include minorities in study populations and to intensify research efforts identifying factors underlying racial differences in health status.

M05 Identify the factors determining whether and how individual oral health-promoting behaviors are learned from early childhood to old age.

Adults with mild gingivitis participated in a behavioral intervention to increase long-term adherence to a thorough home care regimen of brushing and flossing. Results from this study conducted at the State University of New York-Buffalo provide encouraging evidence concerning the feasibility of changing disease-preventing behaviors in adults over the long term--a critical requirement for successful implementation of NIDR plans to improve the oral health of U.S. adults and seniors.

All study participants received baseline clinical assessments (plaque and gingival indices and a measure of gum bleeding) during two consecutive months, to assess usual gingival status and individual rates of plaque accumulation.



Subjects were then randomly assigned to control or experimental groups, with control subjects receiving standard oral hygiene and home care instructions. Experimental subjects received identical instructions, but were also asked to view slides showing their own oral pathogens on a videotape monitor, while the hygienist related these visual materials to requirements of the patient's home care program. At two additional visits, experimental subjects viewed and rated changes shown on the videomonitor in their oral pathogens. Control subjects received monthly feedback and encouragement from the same hygienist. Both control and experimental subjects had a total of three "treatment" visits.

When the clinical indices obtained during these three visits were compared, both groups showed significant, but comparable, improvements. However, after the intervention ceased, experimental subjects were significantly more likely to maintain their new oral self-care habits; on some indices they even showed further improvement. In contrast, control subjects reverted toward earlier levels of plaque buildup and of gingival inflammation. Each of the clinical indices showed statistically significant differences in favor of the experimental group.

Interviews indicated similar trends. Fifty-three per cent of the control subjects, for example, reported not having flossed in the past three days, compared with only 22 per cent of experimental subjects.

Interestingly, these differences were not evident until the supportive effects of regular visits and checkups were removed. These findings underline the importance of evaluating response to health promotion interventions over longer periods. They also suggest a practical, brief intervention which may merit further evaluation for dissemination and/or refinement within multi-agency efforts to improve the oral health of America's adults.

M08 Identify aspects of dentist-patient (auxiliary-patient) interactions which influence patient satisfaction, continuity of care, and patient response to dental treatment.

Patient noncompliance during orthodontic treatment is a pervasive, expensive, and frustrating problem for dentistry. It often leads to additional time/money costs and may even jeopardize treatment outcomes. A psychologist-dentist team at the University of Mississippi has recently developed a series of behavioral interventions to reduce compliance problems in young orthodontic patients.

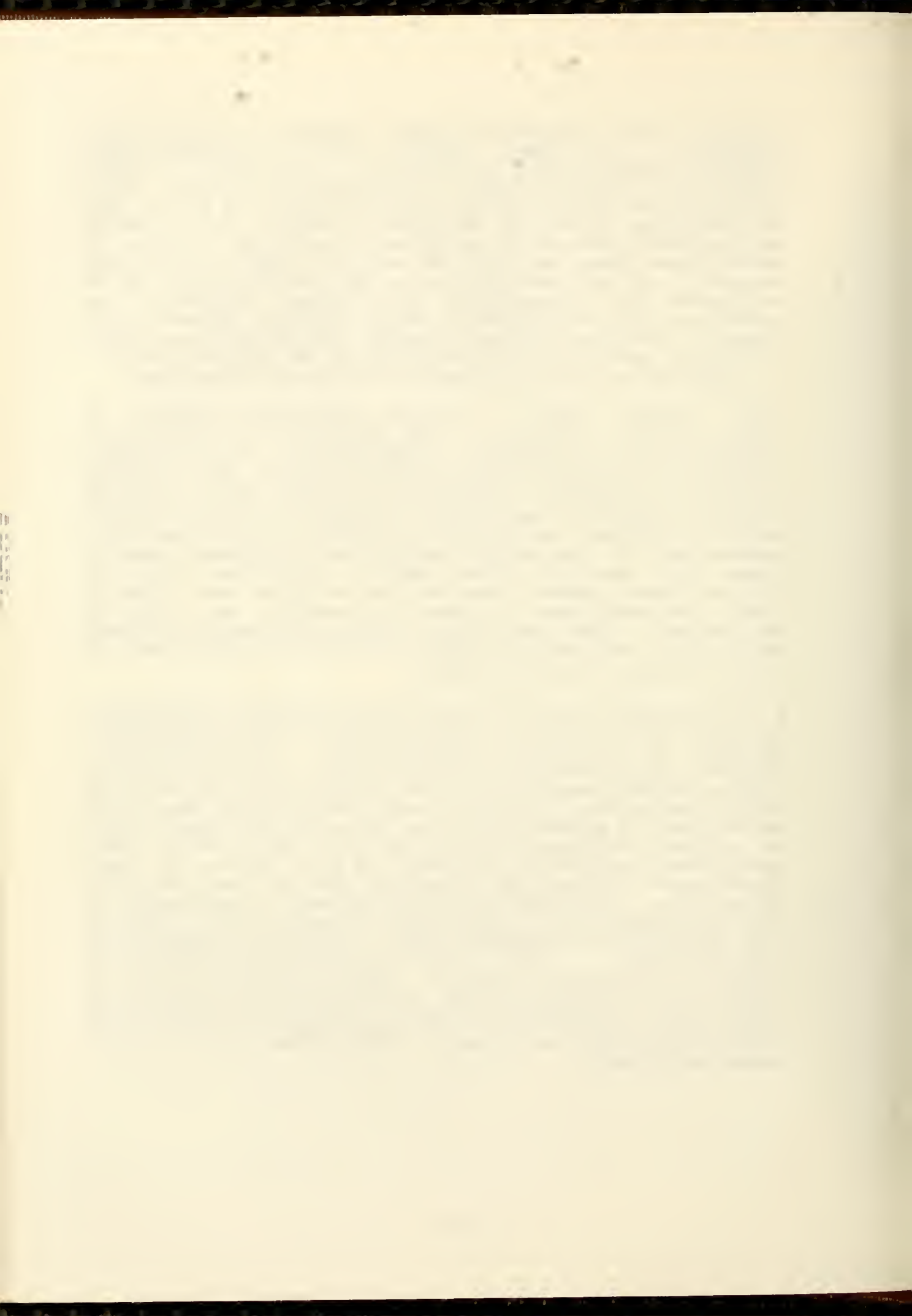
One study attempted to improve oral hygiene practices in



children whose orthodontists were considering discontinuing treatment. All these children showed inadequate oral hygiene, wore orthodontic wires or brackets, and were beginning to develop dental or periodontal problems. After monitoring baseline levels of tooth-brushing and plaque, the investigators taught the children's parents how to design and implement behavioral programs encouraging brushing at least three times per day, as well as other oral hygiene changes. At each dental visit a plaque-disclosing tablet was used and photographs were taken indicating the percentage of the child's tooth surfaces covered with plaque. The interventions produced significant reductions in plaque levels and improved ratings of gingival health. All subjects were able to continue orthodontic treatment.

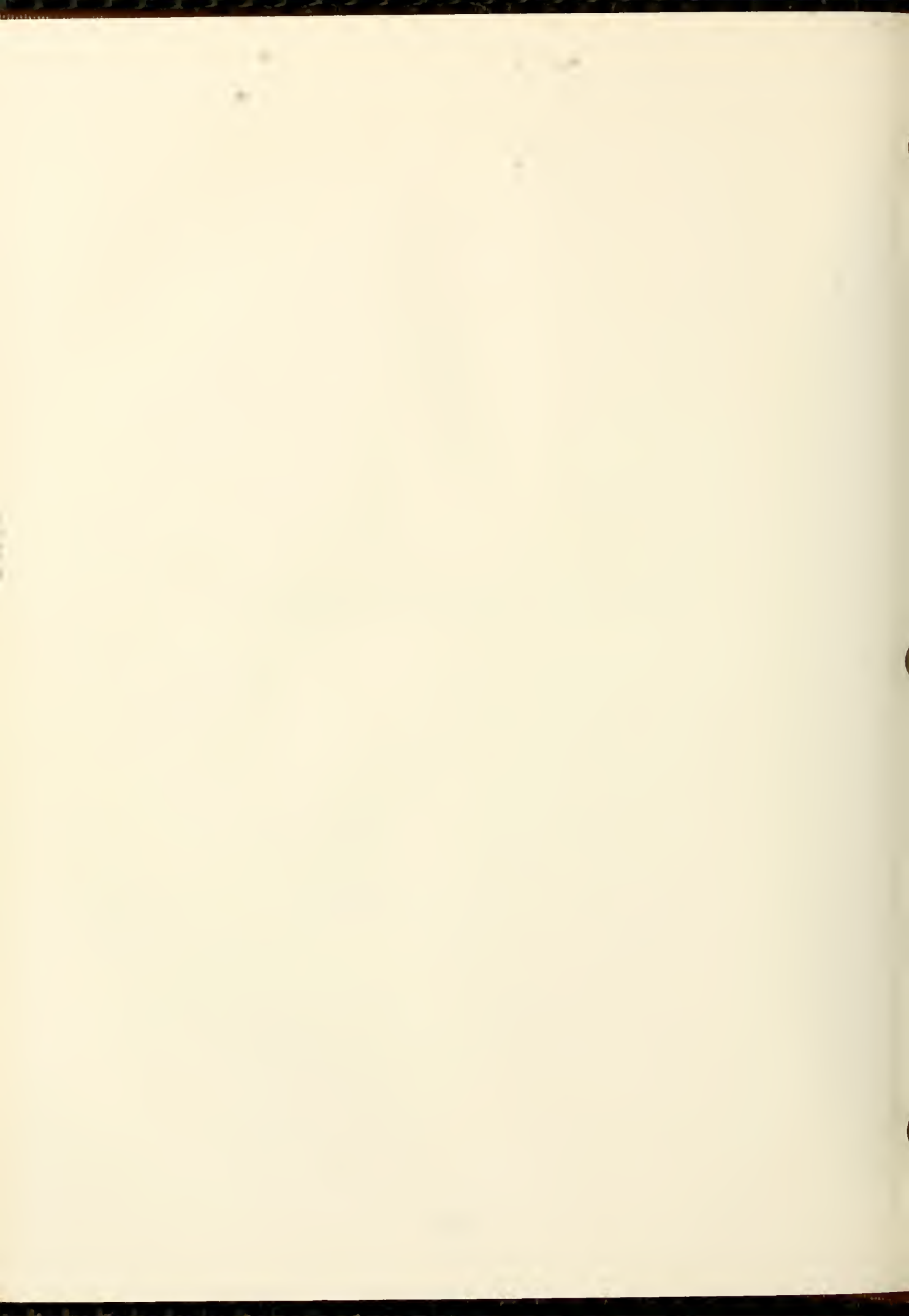
Other studies aimed to increase appropriate wearing of headgear (e.g., retainers, oral elastics) and to improve keeping scheduled orthodontic appointments. In the headgear intervention, parents were shown how to plan and implement specific contingency management procedures which involved providing specific rewards when acceptable, or increasing, levels of headgear use occurred. Control subjects and their parents saw the dentist and dental staff the same number of times, but were not given specific behavioral training. Children whose parents received the training were rated by their orthodontists as more cooperative at follow-up evaluations. They also showed more regular use of headgear devices and were rated overall, by their orthodontists, as showing better clinical outcomes.

A clinic-based behavioral intervention was also implemented to reduce "no-shows", last minute cancellations, and delayed arrivals for orthodontic appointments. The investigators obtained eleven weeks of baseline data on how frequently clinic patients appeared on time, arrived late, or cancelled at the last minute. Patients were then told that they could participate in a monthly drawing for a \$15 gift certificate at a local shopping center. Children could enter their appointment card in the "drawing" if they arrived for their appointments on the day scheduled and no more than one minute late. Implementing this intervention produced a 57 per cent decrease in last minute cancellations, a 75 per cent reduction in "no-shows", and a 15 per cent reduction in late arrivals. This simple intervention provided an effective, economical means to improve appointment-related behaviors. (Parenthetically, it was so well accepted by clinic staff, patients, and their parents that the clinic staff maintained the program, even after the University researchers left).



EPIDEMIOLOGY AND ORAL DISEASES PREVENTION PROGRAM



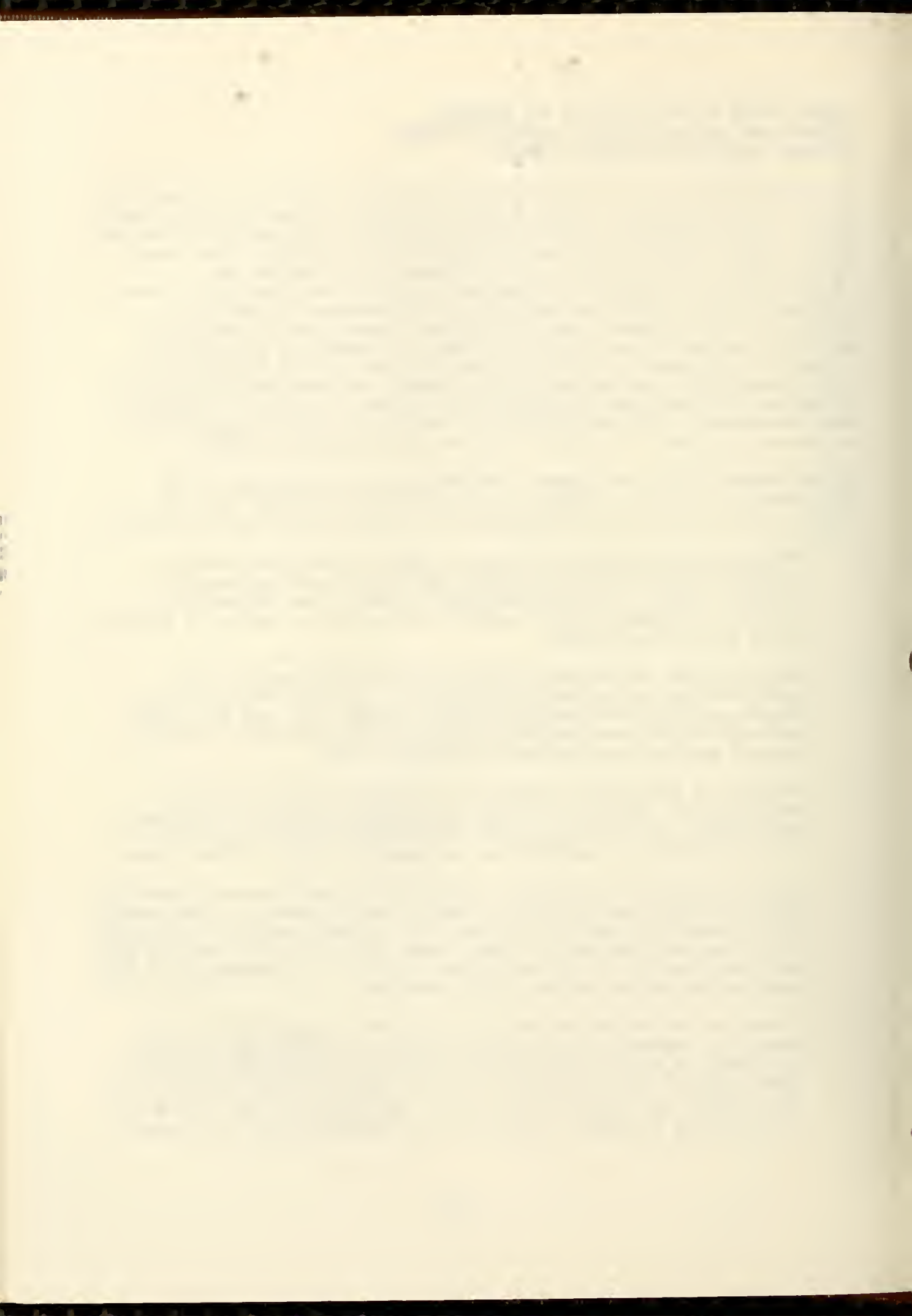


**ANNUAL REPORT OF THE OFFICE OF THE DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH**

The changing needs for oral epidemiology and science transfer in the upcoming decade is the basic horizon against which the recent achievements and current plans of the Epidemiology and Oral Disease Prevention Program (EODPP) are most aptly viewed. Spurred on by Institute-wide efforts to define a long-range research plan for the nineties, and challenged by the findings and recommendations of numerous ancillary meetings which have dealt with a broad spectrum of issues--fluorides and health, risk assessment in dentistry, collaboration with industry, minority health, international collaboration, nutrition, and soft tissue lesions--challenged by these and other related scientific developments, staff have staked out areas of the new frontiers of oral epidemiologic research and science transfer, and have developed bold new initiatives and approaches designed to advance those frontiers. During this same time period, staff have continued to settle-in to the expanded mission and new organizational structure of the Program implemented in 1988.

In this report, and in the reports from the three major Branches of the Program which follow, these themes are exemplified in numerous ways, and underlie the following key achievements of the Program during this past year.

- o Population-based studies of the oral health of Americans, based on information obtained through NIDR's periodic national surveys of children, adults, and older Americans, as well as on national data resources developed by other agencies, have been carried out by staff in every area of the Program.
- o Major national initiatives in data collection have begun: (1) a comprehensive oral examination component in the 1988-1994 National Health and Nutrition Examination Survey (NHANES III), and (2) a 1989 National Health Interview Survey supplement on oral health, including several questions on symptoms of oral-facial pain.
- o Analyses of longitudinal measures of periodontal disease, based on studies of Sri Lankan tea laborers and Norwegian males, are developing new insights into the progression of periodontal disease, and the role of bacteriological, immunological, and genetic factors in this disease.
- o An NIDR dental clinic has been established in a newly renovated ward of the U.S. Walter Reed Army Medical Center, and is becoming a fine example of the research potential of a fixed-site research setting. This dental clinic has been designed for NIDR's study of the natural history of the oral manifestations of HIV-infection, as part of the broader Walter Reed study of the natural history of HIV-infection.
- o Efforts to further define and institutionalize a research and action program to improve the oral health of adults and older Americans have continued. A broad spectrum of analytic efforts within the Program has been guided by the principles of this initiative, including analyses of the patterns and sources of tooth loss, and analyses of the feasibility of developing a computer model which will generate condition forecasts

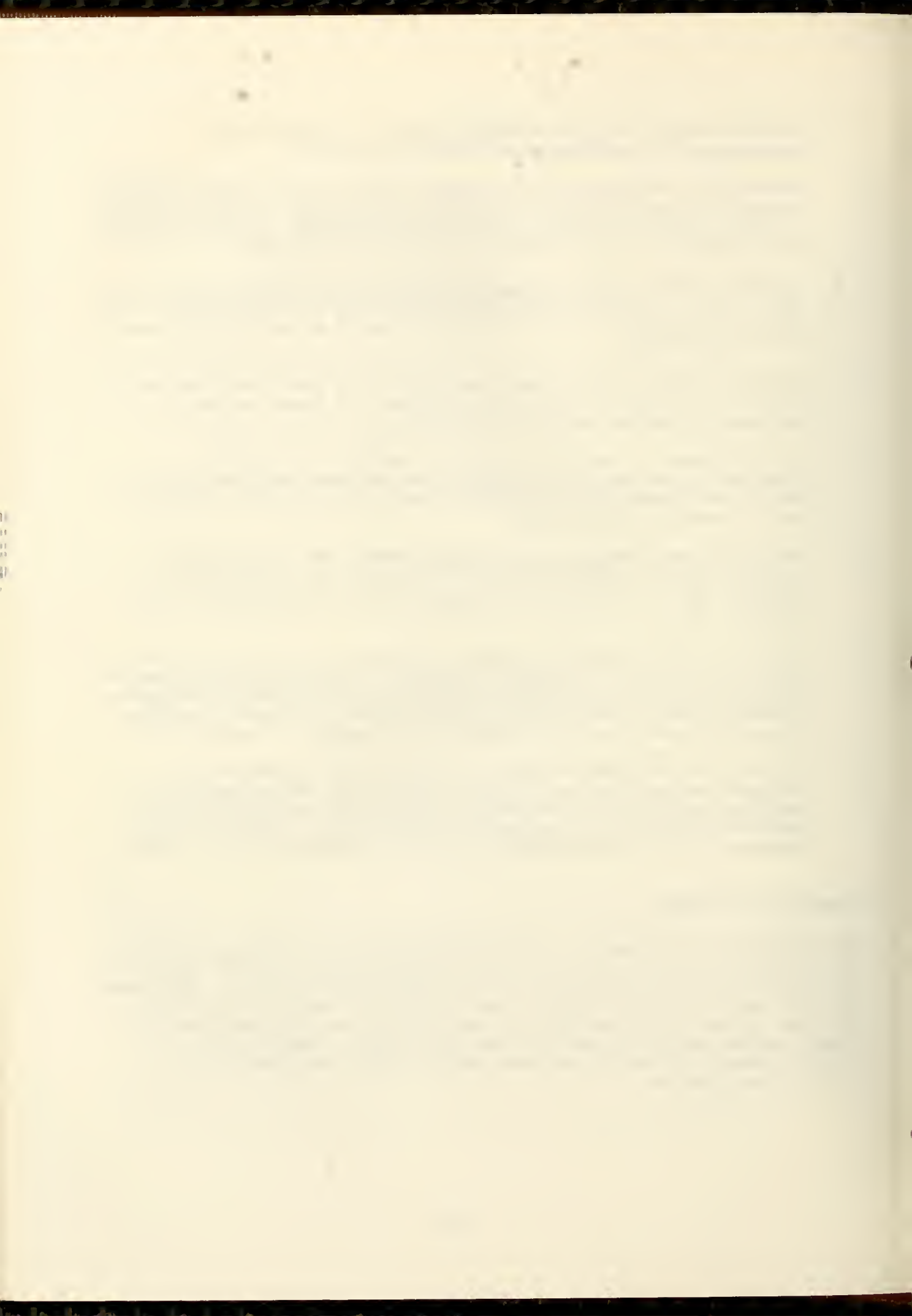


of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S.

- o Primary data collection has also been initiated in the context of the VA Longitudinal Dental Study, in an effort to enrich the clinical findings from this ongoing longitudinal study with information on the use of, and actual charges for, dental services over the past decade.
- o Ongoing clinical trials of therapeutic approaches to the prevention of dental caries have entered into their final data collection phases, and ideas for new clinical trials have been proposed and actively discussed within the Program.
- o Important clinical studies have continued in the development and uses of intra-oral releasing devices in the treatment of dental caries, periodontal disease, and oral candidiasis.
- o The development of sensitive statistical methods for analyzing longitudinal periodontal measurements has continued, as have efforts to develop new approaches for resolving issues which presently confound caries prediction methodologies.
- o Coding systems, standards, and computer methods for international communication of microbial and cell line clone data have been expanded, and their use in interlaboratory comparisons of such data have been demonstrated.
- o Systematic efforts have been made to evaluate the current status and future directions of the Program's efforts in the epidemiologic study of oral-facial injuries, craniofacial defects, and soft tissue pathologies through reviews of the literature, consultation with experts, analyses of available data, and the development of diagnostic criteria.
- o Efforts have continued in the area of chronic pain epidemiology, including critical review of existing theoretical, methodological, and empirical perspectives; and the clarification of concept-measurement problems in the survey measurement of chronic pain. Efforts are also underway to clarify epidemiologic aspects of oral-facial pain syndromes.

Organization of Report

The remainder of this report is organized into three main sections. After a brief description of the overall mission of the Program, the first section describes three special initiatives of the Office of the Director. The second and third sections provide detailed reports for those sections of the Program which are located within the Office of the Director; namely, the Office of Program Support and the Microbial Systematics Section. More detailed, separate reports for each of the three Branches follow this report of the Office of the Director.



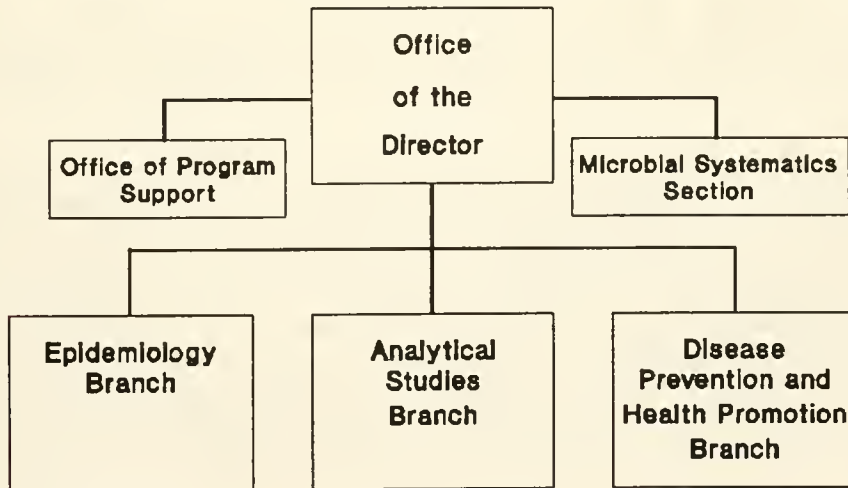
OFFICE OF THE DIRECTOR

The overall mission of the Epidemiology and Oral Disease Prevention Program includes the following key interrelated objectives:

- o To plan, develop, direct, and apply epidemiologic methodologies to investigation of oral diseases and disorders.
- o To plan and conduct research analyzing the effect of changing oral disease patterns on dental research, education, and delivery systems.
- o To plan and conduct clinical trials, field, demonstration, and related studies in the prevention of oral diseases.
- o To plan and coordinate activities related to the prevention of oral diseases and disorders, targeted to the general public, health professionals, and the scientific community.
- o To design, develop, and coordinate programs which facilitate or implement the transfer of research findings to application.

EODPP's activities in pursuit of these objectives are organized according to the schematic shown below. Further details on the functions, internal structure, and staffing patterns of these Program components are provided in the narrative which follows or in the separate Branch reports.

EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM



The Office of the Director is responsible for the overall scientific direction and administrative management of the Program. Dr. Harald Loe is Acting Director, Epidemiology and Oral Disease Prevention Program. Dr. Thomas F. Drury is Deputy Director; and Ms. Phoebe Edwards is lead secretary for the Program. Because most of the Program's scientific activities are described in



the reports from the three Branches, the remainder of this section of the Report of the Office of the Director focuses on three special initiatives which have their organizational locus in the immediate Office of the Director.

Collaboration with Dental Public Health Organizations

On December 5, 1988, the EODPP hosted a meeting with representatives of selected dental public health organizations, including the Association of State and Territorial Dental Directors (ASTDD); the American Association of Public Health Dentistry (AAPHD); the American Board of Dental Public Health (ABDPH); the Association of Community Dental Programs (ACDP); the American Dental Association (ADA) Council on Community Health, Hospital, Institutional and Medical Affairs; the Dental Health Section of the American Public Health Association (APHA), the American Association of Dental Schools (ASDS), and the American Dental Hygienists' Association (ADHA).

The purpose of the meeting was manifold: to exchange notes on work in progress, to discuss the implications of what is happening in the community for programs of oral epidemiologic research, to examine the implications of oral epidemiologic research for community-based programs, and to explore areas of possible collaboration.

The agenda for the meeting was divided into two main parts. After brief introductions and a statement of the overall purposes of the meeting, the first part of the meeting included the following brief presentations: (1) An Overview of the EODPP, (2) Prevalence of Dental Caries in U.S. School Children, and (3) Research and Action Program for Improving the Oral Health Of Adults and Older Americans. Each presentation was followed by a question and answer period and discussion.

The second part, which was more unstructured and flowed naturally from the earlier part of the meeting, provided an opportunity for frank and open discussion on other, sometimes broader, issues, which frequently went well beyond the mission of the EODPP.

Overall, this meeting of EODPP staff and representatives from major dental public health organizations provided a unique opportunity to identify issues which need to be systematically addressed, and to identify areas in which collaborative efforts, involving not only epidemiologists and community health professionals, but also basic researchers, practicing clinicians, and dental educators are necessary. One specific outcome of this meeting was that EODPP agreed that prior to general release to the public, future publications would be reviewed from a public health perspective--a promise which was implemented for the first time this year by having five experts in dental public health review the main text, tables and charts from the draft monograph, "Dental Caries in United States Children, 1986-87," currently in press.

The Oral Health of Minorities

Although efforts have been made in recent years to develop health profiles of minority populations in the United States, the epidemiology of the oral health of minorities remains to be systematically and comprehensively explored. To



meet this need, EODPP has embarked on a project entitled, "The Oral Health of Racial and Ethnic Minorities in the United States." This project has three major objectives: (1) to critically evaluate what is currently known about the oral health of racial and ethnic minorities in the U.S. based on existing literature; (2) to derive from analyses of existing data comprehensive and systematic information on the oral health of these minority populations; and (3) to identify new research and action initiatives to improve the oral health of these minorities. The initial planning and design of this project were begun this year.

On May 22, 1989, EODPP staff participated in a special NIDR Meeting on "Dental Research and Minority Group Issues" by presenting a series of brief statistical vignettes on the oral health of Blacks and Hispanics. These presentations were designed to stimulate discussion of several broad questions about dental research in the context of minority group oral health concerns. The primary objective of the staff presentations was to provide preliminary estimates of certain oral health characteristics, and to identify pertinent NIDR educational and health promotion initiatives.

Staff presented preliminary findings relating to minority oral health status in the following areas: tooth loss and edentulism; dental caries; periodontal diseases; soft tissue lesions and conditions; oral-facial injuries, craniofacial anomalies, and malocclusion; utilization of dental and oral health services; expenditures and insurance coverage; and NIDR health education and health promotion activities for minorities. The presentations were based on preliminary analyses of NIDR surveys of children, adults, and older Americans, as well as upon national survey data collected by the National Center for Health Statistics and the National Center for Health Services Research. Formal and systematic analyses of these data bases will continue in the coming year.

Pain Epidemiology Initiatives

The Office of the Director is also providing scientific leadership for broad initiatives in the general area of chronic pain epidemiology and in the specific area of the epidemiology of oral-facial pain syndromes.

Chronic Pain Epidemiology--The broad initiative extends work begun several years ago at the National Center for Health Statistics (NCHS) by the Deputy Director, and is continuing in collaboration with NCHS staff. These ongoing collaborative projects involve the development of an inventory of currently available population-based survey data on chronic pain; a critical review of existing theoretical, methodological, and empirical perspectives; the clarification of concept-measurement problems in the survey measurement of chronic pain; and the codification of methodological principles involved in writing questions, designing questionnaires, and conducting interviews about chronic pain in general population surveys.

Epidemiology of Oral-Facial Pain--Efforts are also underway to clarify epidemiologic aspects of oral-facial pain syndromes. Efforts were begun this past year to update current understandings of problems of case definition and case ascertainment in epidemiologic studies of chronic oral-facial pain by reviewing the literature, by attending major meetings addressing key issues,



and by participating in the epidemiology workgroup of NIDR's Workshop, "New Approaches to the Differential Diagnosis of Chronic Orofacial Pain."

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Drury TF. Explorations in the oral health of racial and ethnic minorities: summary and implications. Presentation at the NIDR Meeting on Dental Research and Minority Group Issues, May 22, 1989.



OFFICE OF PROGRAM SUPPORT

The Office of Program Support conducts and directs a broad spectrum of scientific, technical, and administrative activities in support of Program needs. These activities are carried out within three functional workgroups: (1) a Biometry Unit, (2) a Data Services Unit, and (3) a Contract and Reports Unit. Dr. Drury is Acting Chief of the Office of Program Support, which also includes Drs. Kingman and Li; Mss. Smith, Bock, Rodgers, Gregg, Davis, and Webb; Mr. Lee, and Drs. Miller-Chisholm and Mirth.

Biometry Unit

The Biometry Unit within the Office of Program Support is involved in several research and consulting activities. The primary activities of this unit continue to be conducting research in methodological areas. The application of known methods, as well as the derivation and modification of new statistical methods, for analyzing dental epidemiologic data is in constant demand and involves a considerable amount of staff time. Specific examples would include the review and development of sensitive statistical methods that can be used in analyzing longitudinal periodontal measurements, specific types of caries prediction methodologies (including the estimation of average DMFS or DMFT scores for a community based only on the knowledge of age-specific caries-free percentages, as well as prediction models useful for explaining the relationship among the most important caries prediction variables, and logistic regression models useful for predicting subjects who are at "higher" risk of developing dental caries), and growth curve models useful for describing growth rates of specific clinical phenomena (such as microorganism levels) over time.

Consulting activities also continue to be needed, and current activities include the following studies:

- o Epidemiologic periodontal study in American Indians
- o Gingivitis clinical trial comparing two specific disease prevention strategies (bleeding versus plaque control)
- o Demonstration of sealant effectiveness in combination with fluoride modalities
- o Comparison of fluoride rinses and fluoride tablets in preventing dental caries
- o Development of caries prediction models
- o Evaluation of microbial assays for Mutans streptococci and Lactobacilli in identifying subjects likely to develop high caries levels
- o Epidemiologic measles study in Philippines

Consulting activities outside the NIDR also continue, and include working with the ADA Council on Dental Therapeutics; lecturing at the Bethesda Naval Dental School; and collaborations with faculty at Columbia University and the University of Göteborg, Sweden.



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Berude J, Hicks ML, Li SH. Resorption after physiologic and rigid splinting of replanted permanent incisors in monkeys, *J Endodont* 1988; 14:592-600.

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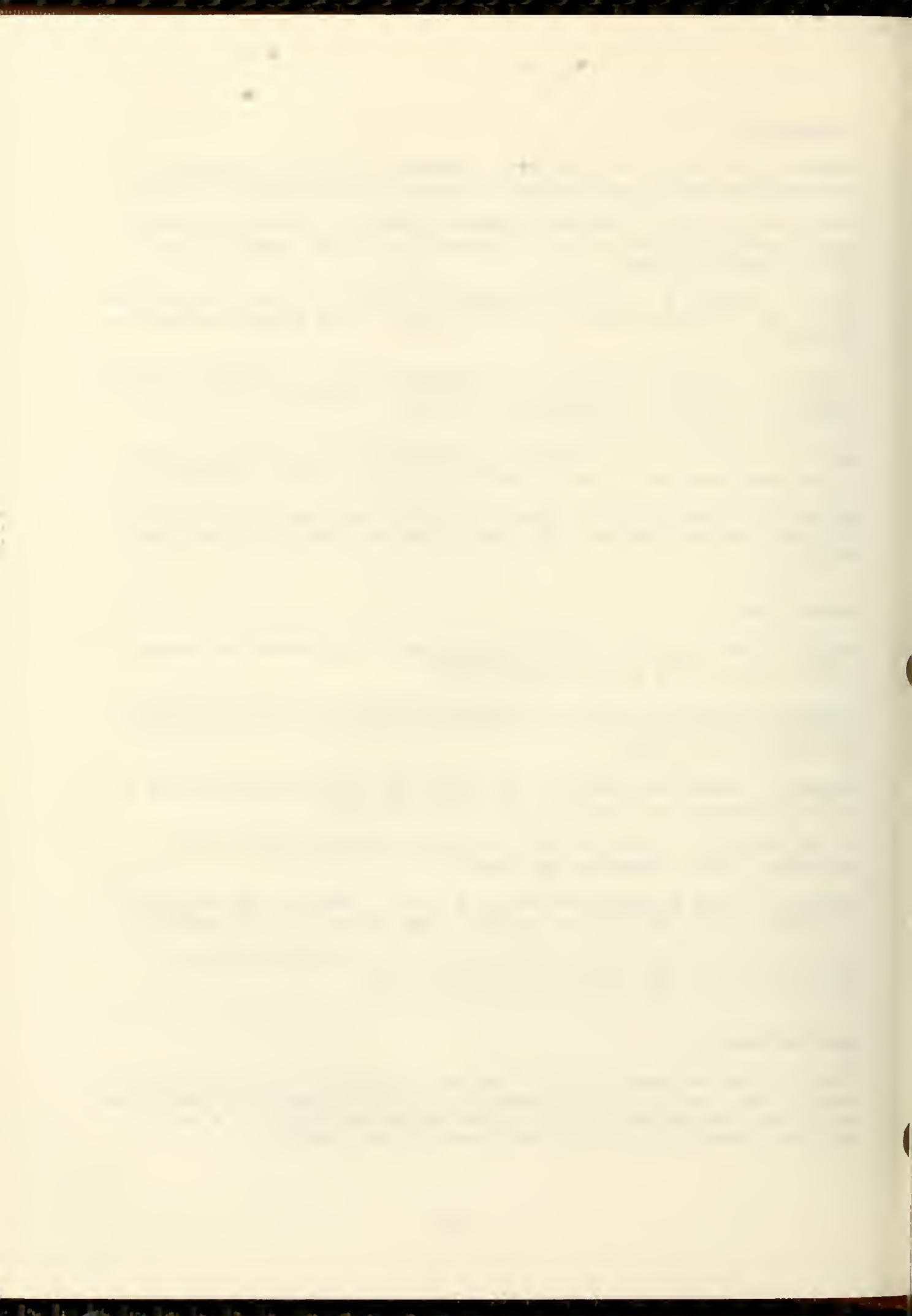
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Shern R, Li SH. A new method of measuring the flow of saliva from minor salivary glands. 1989; *J Dent Res*, 68: Abstr 1072.

Data Services Unit

A major effort was made by staff of the Data Services Unit this past year in carrying out a variety of data processing activities in preparing camera-ready copy of detailed statistical tables, text tables, and charts for a survey monograph, "Dental Caries in United States Children, 1986-87."



Staff also played a key role in the development of data which was used by staff of the Biometry Unit to calibrate the dental examiners in the 1988-1994 National Health and Nutrition Examination Survey (NHANES III).

Staff prepared the school rosters and data collection forms for another wave of examinations in Springfield, Ohio, and in York County, Virginia. In early May, three staff members also traveled to Springfield, Ohio, to help with the recording of the data obtained through the oral examinations, as well as with the training of one new examiner.

Routine data processing--including code development, coding, data entry, data editing, and tabulation of basic statistical estimates for selected sociodemographic categories--also continued for the following studies:

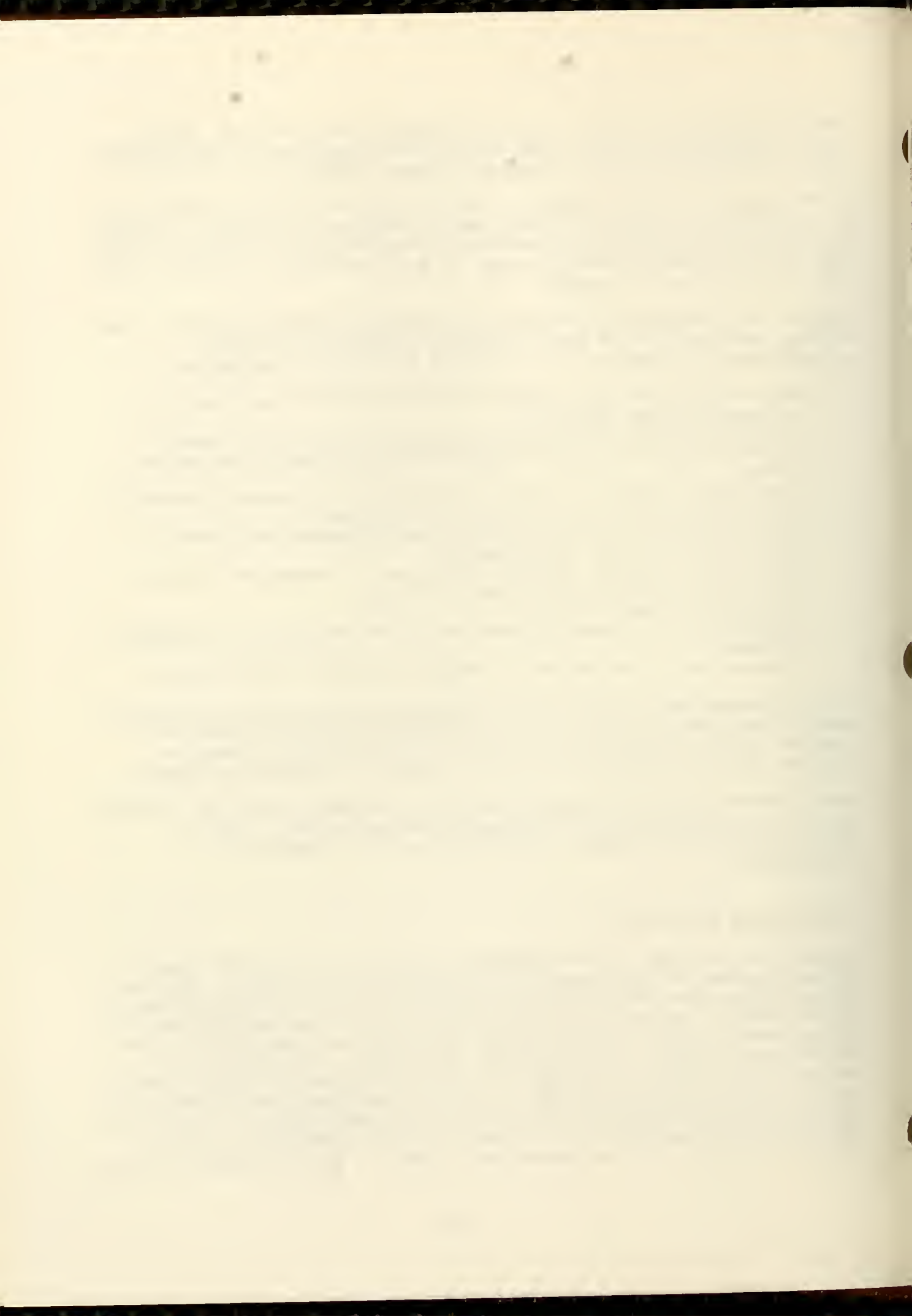
- o Nelson County, Virginia, study of dental sealants in combination with selected fluoride procedures
- o Springfield, Ohio, study comparing the combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone
- o York County, Virginia, study of the elimination of gingival bleeding as a motivational tool for long term oral maintenance
- o China study of dental caries and periodontal disease indicators
- o West Virginia study of dental caries
- o Perry Point, MD study including collection of information on dental caries and indicators of periodontal diseases
- o Guam study of dental caries
- o Philadelphia, PA, study of dental caries and indicators of periodontal diseases
- o Eastman study of the effects of prenatal fluoride on dental caries

One staff member made a major effort to develop SAS data sets for the 20-year study of periodontal disease in Norwegian males, and developed extensive computer analyses of these data, as well as the data for Sri Lankan tea laborers, for the Periodontal Diseases Section of the Epidemiology Branch.

Special efforts were also begun this past year to develop public use microdata tapes for the 1985-86 Survey of Employed Adults and Seniors. Work is continuing on the development of these tapes and their appropriate documentation.

Contracts and Reports Unit

Research has continued on the development of intraoral controlled-release delivery systems for the prevention and treatment of oral disease. Four new holding systems for retaining and protecting the Intraoral Fluoride Releasing Device (IFRD) in the mouth have been developed under a contract with the Eastman Dental Center. The combination of holding system and IFRD is called the Intraoral Fluoride Releasing System (IFR System). The IFR System is designed to provide continual topical fluoride for periods of up to six months and is being developed by the NIDR in order to investigate the efficacy of continual topical fluoride for the prevention of dental caries. The four new IFR Systems were subjected to a six month clinical evaluation in children, aged 12 to 15 years, at the Eastman Dental Center. The IFR Systems were well



tolerated by the children and significantly elevated salivary fluoride concentrations. Analysis of the data from this study should provide guidelines for the production of an IFR System suitable for evaluating the efficacy of this fluoride delivery system for the prevention of dental caries. In addition, the holding system should be suitable for retaining controlled-release pellets for other therapeutic agents and, therefore, could be utilized for the treatment of other oral diseases.

A new contract research project has been initiated with the University of Kansas to investigate the feasibility of developing a sustained-release bioadhesive delivery system for an antifungal drug that would be suitable for the prevention and/or treatment of oral candidiasis. It is anticipated that at the completion of this three year project, sufficient data will have been collected from both laboratory and animal studies to determine if human testing of the delivery systems developed under this project is warranted.

The collaborative project initiated in 1988 with the Diagnostic Systems Branch, NIDR, to investigate the effect of flurbiprofen, a non-steroidal anti-inflammatory agent, on the rate of alveolar bone loss in progressive periodontitis has entered the treatment phase. Thirty subjects have been enrolled in this double-blind, placebo-controlled trial which is scheduled to run for 12 months.

Contractors at the University of Texas Health Science Center at San Antonio and the State University of New York at Buffalo completed the development of manuals for clinical and laboratory procedures for the study of periodontal diseases in older adults. The data obtained from the application of these procedures in small populations will be summarized during the coming year and should be useful in the design of larger investigations of periodontal diseases in older adults.

Contractors at the University of Colorado and the University of Iowa completed a review and analysis of the literature from 1970 to 1989 on oral health care variables affecting institutionalized and homebound individuals. This report will be utilized in the development of research goals for studying the oral health care needs of this population.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00282-10 EODP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Refinement of the Intraoral Fluoride Releasing Device

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

Mirth, Dale B.

Research Chemist

NIDR, OPS

COOPERATING UNITS (if any)

Eastman Dental Center, Rochester, New York 14620

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of Program Support

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS.

PROFESSIONAL:

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

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

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

The Intraoral Fluoride Releasing Device (IFRD) is an intraoral controlled-release therapeutic system being developed by the National Institute of Dental Research in order to investigate the efficacy of continual topical fluoride for the prevention of dental caries. IFRD is designed to provide continual topical for periods of up to six months. IFRD has reduced the incidence of experimental dental caries in rats by more than 50% and has been used in humans for periods of up to six months without producing adverse effects.

The present objective of this project is to develop and clinically evaluate new methods of retaining and protecting IFRD in the mouth in order to make IFRD more durable and easier to use in humans.

Four new methods for retaining and protecting IFRD have been developed. The combination of retention mechanism and IFRD is called an Intraoral Fluoride Releasing System (IFR System). The four new IFR Systems were evaluated clinically for six months in adolescents, aged 12 to 15 years, at the Eastman Dental Center. Analysis of the data from this study should provide guidelines for the production of an Intraoral Fluoride Releasing System suitable for evaluating the efficacy of continual topical fluoride for the prevention of dental caries.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00417-04 EODP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Intraoral therapeutic systems for periodontitis and AIDS-related infections

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

Mirth, Dale B.

Research Chemist

NIDR, OPS

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of Program Support

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

0.1

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this project is develop intraoral controlled-release therapeutic systems for antibiotics/antimicrobials and antifungal agents for the treatment of periodontal disease, AIDS-related oral diseases and other opportunistic oral mycotic infections.

Biocompatible copolymers of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were used to produce a membrane-controlled delivery system for tetracycline that should be suitable for short-term intraoral treatment of periodontal disease. Results with this system have shown that the hydrogel copolymers used in the clinically tested Intraoral Fluoride Releasing Device also can be used for the delivery of large organic molecules in vivo. In a study in monkeys, ten days of treatment with tetracycline controlled-release pellets releasing 0.4 to 1.0 mg of tetracycline per day produced significant decreases in crevicular fluid flow, number of bleeding sites on probing, and bacterial morphotypes associated with periodontal disease in subgingival plaque samples.

Prototype controlled-release pellets for chlorhexidine were fabricated using the same copolymers used in the tetracycline and fluoride systems. These pellets released biologically active chlorhexidine for 30 days.

It should be possible to use this technology to develop intraoral controlled-release delivery systems for the lower molecular weight antifungal agents. Such systems could be beneficial in immunocompromised patients.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00418-04
EODP

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Methods for Analyzing Longitudinal Periodontal Data

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

Kingman, Albert Statistician (Health) OPS, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Office of Program Support

SECTION
Biometry Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS. .2	PROFESSIONAL: .2	OTHER: .0
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CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

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

Statistical methods are presented for analyzing longitudinal periodontal loss of attachment data using subject based summary measures. The methods are illustrated by using data from a 2-year clinical study in which a conservative periodontal therapy was evaluated. The 2-year study period was divided into the 1st 6-month period (treatment period) and the 2nd 18-months (maintenance period). Individual sites within patients were classified by their initial probing packet depth values: shallow, moderate or deep. Treatment and maintenance effects were assessed by using multivariate statistical methods (Hotelling T-square tests) jointly, and for each class of sites, separately.

For this data set, it was shown that this conservative therapy produced significant improvement for deep sites, minor improvement for moderate sites, and significant deterioration for shallow sites.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
EODP
Z01 DE 00461-02

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Methods for Caries Prediction and Identifying Subjects at High Risk

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

Kingman, Albert Statistician (Health) OPS, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Program Support

SECTION

Biometry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS.

.3

PROFESSIONAL

.3

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

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

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

Data from a recently conducted longitudinal caries study were used to assess the predictive value of specific groups of variables in predicting the level of dental caries incidence in adolescent subjects. The data available included demographic information, use of topical and systemic fluoride, initial caries scores, microbiologic levels of Mutans streptococci and Lactoobacilli on three occasions, and multiple 24-hour recall dietary intake information.

These data were analyzed from two perspectives: 1) to assess the predictive value of predicting caries incidence over the whole range of caries experiences on the log scale; and 2) evaluate the predictive value of selected variables to identify subjects likely to develop high caries incidences.

The initial caries scores together with the microbiologic counts were the best predictors of caries incidence for the group. The initial caries scores, the microbiologic counts and the use of fluoride prophylaxes were the best fitting variables in the logistic models.

The first part of the report deals with the general situation of the country and the progress of the work during the year. It is followed by a detailed account of the various expeditions and the results obtained. The second part of the report is devoted to the study of the flora and fauna of the country, and the third part to the study of the geology and the physical features of the country.

The first expedition was made in the month of January, and was led by Mr. A. B. C. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The second expedition was made in the month of February, and was led by Mr. D. E. F. It was also very successful, and resulted in the discovery of several new species of plants and animals.

The third expedition was made in the month of March, and was led by Mr. G. H. I. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The fourth expedition was made in the month of April, and was led by Mr. J. K. L. It was also very successful, and resulted in the discovery of several new species of plants and animals.

The fifth expedition was made in the month of May, and was led by Mr. M. N. O. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The sixth expedition was made in the month of June, and was led by Mr. P. Q. R. It was also very successful, and resulted in the discovery of several new species of plants and animals.

The seventh expedition was made in the month of July, and was led by Mr. S. T. U. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The eighth expedition was made in the month of August, and was led by Mr. V. W. X. It was also very successful, and resulted in the discovery of several new species of plants and animals.

The ninth expedition was made in the month of September, and was led by Mr. Y. Z. A. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The tenth expedition was made in the month of October, and was led by Mr. B. C. D. It was also very successful, and resulted in the discovery of several new species of plants and animals.

The eleventh expedition was made in the month of November, and was led by Mr. E. F. G. It was a very successful one, and resulted in the discovery of several new species of plants and animals. The twelfth expedition was made in the month of December, and was led by Mr. H. I. J. It was also very successful, and resulted in the discovery of several new species of plants and animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

EODP
Z01 DE 00462-02

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Methods for Estimating Age-Specific DMFS and DMFT Scores in the U. S.

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

Li, Shou-Hua	Statistician (Health)	OPS, EODPP, NIDR
Kingman, Albert	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Office of Program Support

SECTION

Biometry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

0.5

PROFESSIONAL

.5

OTHER

.0

CHECK APPROPRIATE BOX(ES)

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

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

Typically the prevalence of dental caries is determined by a full mouth examination for each subject. However, limitations of manpower or time may often preclude such an approach. Knutson showed that a screening examination using only the presence or absence of caries could be used to predict DMFT scores for children. In this study we investigate how well DMFS scores for subjects under 35 years of age can be estimated by specific models based on age-specific prevalences, using data from the NIDR prevalence surveys (1987 children's survey and the 1985 adult survey). Data for adult older than 35 were not used, since the DMFS is not caries specific for adults in the survey.

Knutson original model was the relationship between the age-specific mean DMFT and the age-specific caries prevalence. We considered two types of models. One is Knutson's model with DMFS replacing DMFT. The second one is the linear regression model. The assumption of linear regression model is that the prediction of DMFS can be expressed as a linear function of log of proportion of caries-free individual and age.

Both Knutson formula and regression model can be used in both children and young adult separately to describe the relationship between caries severity (DMFS) and caries prevalence. The regression models are easier to interpret and estimate than the nonlinear Knutson formula. The regression model also emphasize the need to adjust for age.



MICROBIAL SYSTEMATICS SECTION

The Microbial Systematics Section (MSS) has three main areas of activity: (1) international communication of microbial and cell line clone data; (2) development of methods for the computer management and analysis of such data; and (3) studies of microbial systematics and ecology. The Section has been active in all three areas this year. Dr. Krichevsky is Chief of the Microbial Systematics Section which also includes Mss. Walczak, McManus, Mercer, and Chiu, and Mr. Kennedy.

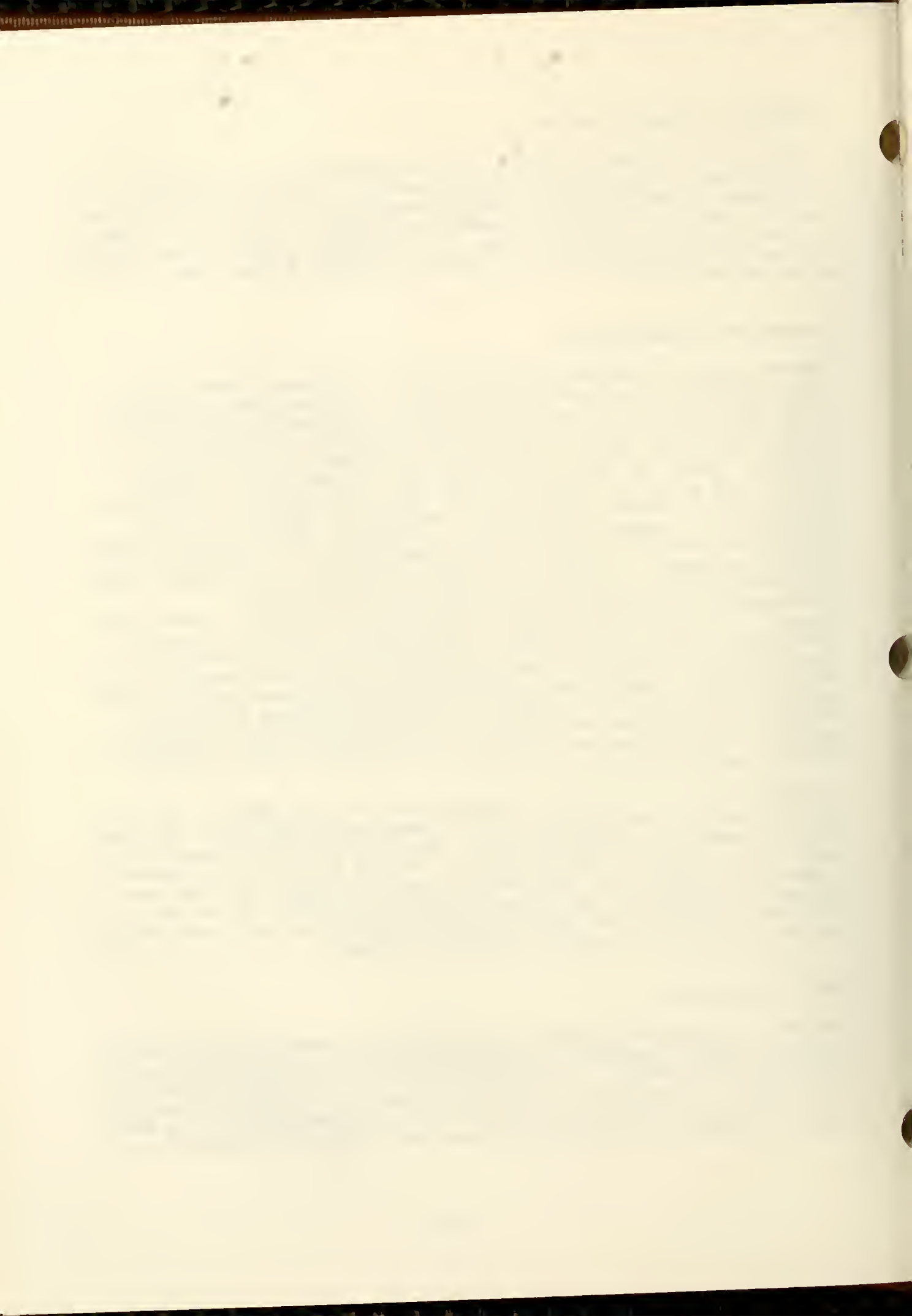
International Communication

Various members of the MSS have participated in the establishment of the Microbial Strain Data Network (MSDN) which is a collaborative initiative of three components of the International Council of Scientific Unions (ICSU): Committee on Data for Science and Technology (CODATA), World Federation for Culture Collections (WFCC), and International Union of Microbiological Societies (IUMS). The Chief, MSS, chairs the MSDN Committee of Management which is the policy and design oversight body. MSS staff are active on the Technical (i.e., Network operations and software development) and Central Directory (i.e., Network main database) Committees. The Information Officer, MSDN, works with the MSS Staff to design and implement the electronic mail, database management, and computer conferencing facilities of the MSDN. These facilities are now operational worldwide on the international telecommunications networks (PSS). They have been used by the Epidemiology Branch in aiding communication during the design of a multisite study of periodontal disease in older adults. MSS staff collaborated in design and as faculty members for MSDN training courses on the use of microcomputers in interlaboratory communication, culture collection management, and strain data analysis. These courses are sponsored by the United Nations Environment Programme. Courses have been held in Czechoslovakia and Brazil. Venues for courses in the upcoming two years are the USA, Egypt, the USSR, Guatemala, the People's Republic of China, and India.

The MSS actively participates in the Hybridoma Data Bank (HDB), an initiative of CODATA and the International Union of Immunological Societies. The Chief, MSS, is a member of the HDB Task Group. Staff of the MSS are responsible for much of the software and database design. The HDB also uses the electronic communication network described above to communicate among its three nodes, located at the American Type Culture Collection, Rockville, MD, at the Institute for Chemical and Physical Research (RIKEN), Saitama, Japan, and at the Medical School, University of Nice, Nice, France.

Method Development

The MSS, in collaboration with many microbiologists around the world, has an ongoing project of developing a unified coding system for computer management of microbial information. The current version of this system is published in book form and is installed as a reference database on the aforementioned MSDN system. As such, the database forms the controlled vocabulary for the MSDN Central Directory of Collections of Strain Data. Further, the coding system is



being accepted as an international standard for communicating strain data among microbiologists.

Design and programming of a comprehensive suite of computer programs--the Microbial Information System (MICRO-IS)--is a long term, ongoing project of the MSS. A main-frame version of the MICRO-IS is currently used extensively by the MSS for management of strain data. Additionally, the FDA and EPA use the system for managing and analyzing microbial data in their regulatory roles. The latest thrust of these methodological efforts is development of a portable version of the MICRO-IS for installation on a wide size range of computers including personal computers, mini-computers, and main-frames.

A program for conversion of controlled vocabulary information in text records of the HDB into the highly compressed, table oriented MICRO-IS format has been implemented by the MSS for the HDB. The conversion process also has extensive spelling, syntax, and format error checking capabilities. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required if we are to have any realistic chance of computerizing valuable archival records of phenotypic strain data.

Microbial Systematics and Ecology

The MSS continued the collaboration with the International Working Group on Mycobacterial Taxonomy to elucidate the taxonomic relationships within this genus of pathogens and saprophytes by providing computer analysis of the phenotypic data submitted by the cooperating reference laboratories. The latest findings are yielding new criteria for the separation of the AIDS patient infective M. avium from the closely related non-infective M. intracellulare.

The MSS is continuing collaboration with the Center for Veterinary Medicine, FDA, on data concerning the results of use of antibiotics as growth promotants for commercial meat animals.

With staff of the EPA and ATCC, the MSS is establishing resources for use in risk assessment of release of genetically engineered organisms in the environment. These resources include accession and analysis of databases of phenotypic characteristics of microorganisms known to be used in genetic manipulation and biotechnological processes, analysis of problems in accession and standardization of such databases from diverse sources, and redefinition of taxonomic boundaries of such organisms. The redefinition is critical to the design of the computer registry for such organisms under the Toxic Substances Control Act.

Staff of the MSS are continuing analysis of phenotypic data on oral microbiota to improve taxonomy and identification criteria for these organisms.

Publications

Krichevsky MI, Walczak CA. Establishing a meaningful relationship with your computer. In: Microbial technology in the developing world. Oxford University Press, in press.



Krichevsky, MI, Sugawara H, Fabricius BO. Culture collections as information resources for biotechnology. In: Hawksworth DL, Kirsop BE, eds., *Filamentous fungi: (Living resources for biotechnology)*. Cambridge: Cambridge University Press, 1988.

Walczak, CA, Krichevsky MI. An opinionated overview of information needs in biotechnology. In: Allison, Olson, eds., *Piecing the puzzle together: a conference on integrating data for decision making*, 60-66, 1988.

Jong SC, Holloway L, McManus C, Krichevsky MI, Rogosa M. Coding of strain features for computer-aided identification of yeasts, *Mycotaxon* 1988; 31:207-19.

Molitoris E, Marii MA, Joseph SW, Krichevsky MI, Fanning GR, Last G, El-Mishad AM, El Batwani YA. Numerical taxonomy and deoxyribonucleic acid relatedness of environmental and clinical vibrio species isolated in Indonesia. *Int J Syst Bacteriol*. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00044-19
EODP

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Handling of Microbial Strain Information by Computers

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

McManus, Candace
Krichevsky, Micah I.

Microbiologist
Research Chemist

MSS, EODPP, NIDR
MSS, EODPP, NIDR

COOPERATING UNITS (if any)

See Attachment

LAB/BRANCH

EODPP

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

2.56

PROFESSIONAL

1.25

OTHER

1.31

CHECK APPROPRIATE BOX(ES)

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

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

The MSS is developing a unified computer coding system for microbial information which is becoming an international standard for communicating strain data. The original bacterial system now includes the algae, yeasts, molds, protozoa, and hybridomas.

Strain data are being entered into a data bank to provide: data on specific organisms, identification of unknown isolates, definition of parameters of taxa, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Files of primary data on microorganisms found in the oral cavity and related types provide a resource for asking both ecological and epidemiological dental research. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

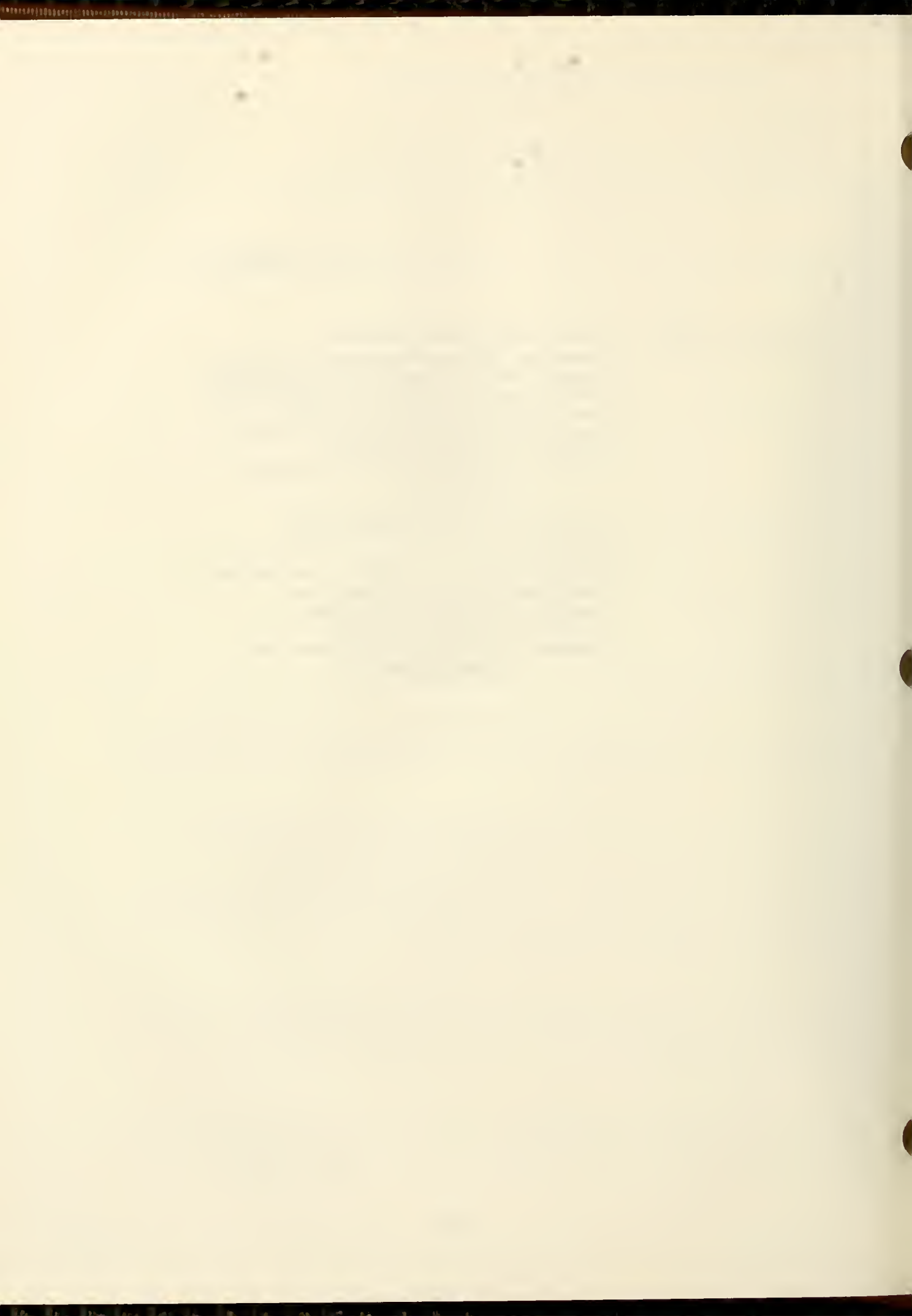
The MSS analyzes the phenotypic data submitted by the cooperating reference laboratories to the International Working Group on Mycobacterial Taxonomy to elucidate the taxonomic relationships within this genus. The latest analysis demonstrated at least one new distinct group of clinically important mycobacteria.

With EPA and ATCC staff, the MSS is building databases to aid risk assessment of release of genetically engineered organisms in the environment including features of microorganisms used in genetic manipulation and biotechnological processes and redefinition of taxonomic boundaries of such organisms.



Z01-DE-00044-19

COOPERATING UNITS: E. Baron, Wadsworth VA Hospital
F. Benedict, Food and Drug Administration
R. Gherna, American Type Culture Collection
L. Blaine, American Type Culture Collection
R. Good, Centers for Disease Control
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture
Collections
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
M. Newman, UCLA
S. Holt, University of Texas at San Antonio
V. Levy-Frebault, Pasteur Institute
D. Swartz, University of Maryland
A. Bussard, University of Nice
H. Sugawara, Institute for Physical and
Chemical Research



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00250-12
EODP

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Algorithms for Microbial Systematics

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

Walczak, Cynthia A.	Computer Scientist	MSS, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR
Mercer, Paula	Computer Programmer	MSS, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, EODPP, NIDR

COOPERATING UNITS (if any)

See Attachment

LAB/BRANCH

EODPP

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

2.68

PROFESSIONAL

2.37

OTHER

.31

CHECK APPROPRIATE BOX(ES)

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

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

The Microbial Information System (MICRO-IS), is an ongoing project to enter, retrieve, and analyze microbiological data for epidemiological, diagnostic, taxonomic, ecological, and regulatory uses. The long term goal is to establish a world-wide data network at a series of cooperating centers. A main-frame version of the MICRO-IS is currently used extensively by the MSS for management of strain data. The FDA and EPA use the system for analyzing microbial data in their regulatory roles. The latest thrust of this effort is development of a portable version of the MICRO-IS for installation on a wide size range of computers including personal computers, mini-computers, and main-frames.

The programs for conversion of controlled vocabulary information in text records of the Hybridoma Data Bank into the highly compressed, table oriented MICRO-IS format were enhanced. The conversion process performs extensive spelling, syntax, and format error checking. A related project is the development of algorithms for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. Such facilities are required if we are to have any realistic chance of computerizing valuable archival records of phenotypic strain data.

A new program to display a numerical taxonomy similarity triangle in four distinct formats simultaneously is functional. The program allows the user to inspect the four views and choose any or all for full scale output. The program makes use of both color and three dimensional graphics to enhance the user's perception of groupings and taxonomic relationships.



Z01 DE-00250-12

COOPERATING UNITS: F. Benedict, Food and Drug Administration
E. Baron, Wadsworth VA Hospital
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture
Collections
S. Socransky, Forsyth Dental Center
V. Levy-Frebault, Pasteur Institute
D. Swartz, University of Maryland
H. Sugawara, Institute for Physical and
Chemical Research

**ANNUAL REPORT OF THE EPIDEMIOLOGY BRANCH
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH**

The Epidemiology Branch plans, conducts, and directs a coordinated program of epidemiologic research on oral diseases and conditions, craniofacial diseases and disorders, and pain syndromes. This program includes studies designed to monitor the incidence and prevalence of oral diseases and conditions, as well as studies designed to explore the role and relative importance of major risk factors, and the natural history of oral diseases and conditions.

The Branch is formally organized into three sections: (1) a Caries Section, (2) a Periodontal Diseases Section, and (3) a Soft Tissue, Craniofacial Defects, and Pain Section. Dr. Carlos is Chief, Epidemiology Branch, and Acting Chief of the Caries Section, which also includes Ms. Brunelle, Ms. Wolfe, and Ms. Witt. Dr. Loe is Acting Chief, Periodontal Diseases Section, which, this past year, has also included Dr. Age Anerud as a Visiting Scientist. Dr. Kleinman is Chief, Soft Tissue, Craniofacial Defects, and Pain Section, which also includes Dr. Swango, Dr. Bhat, Ms. Bock, Dr. Gloriana Lopez, and Ms. Lettice. Ms. Baxter is the Branch secretary.

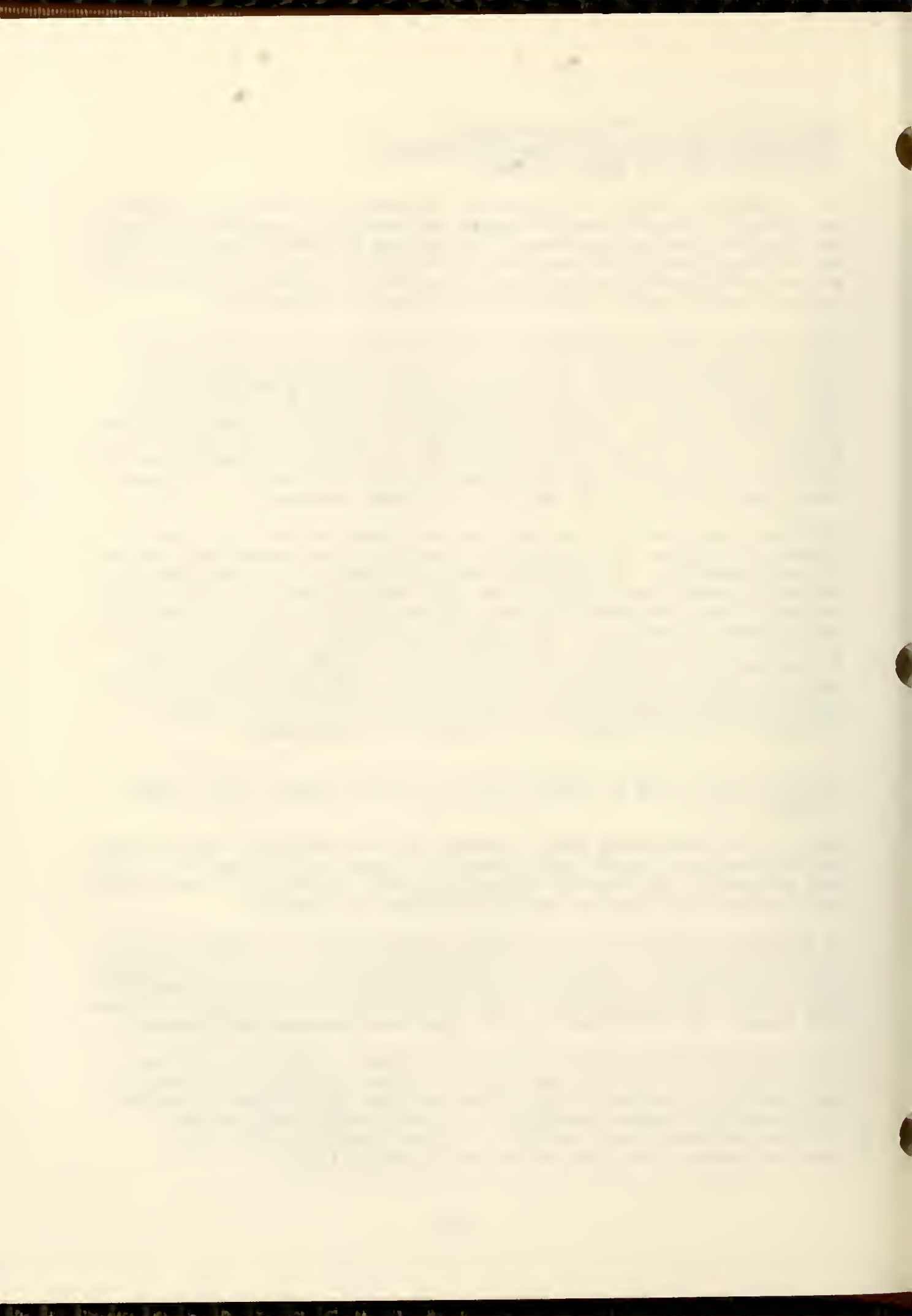
The Branch benefited this past year from the expert advice of Dr. Jens J. Pindborg in the area of HIV infection as well as in the area of oral mucosal tissue epidemiology. Dr. Glorianna Lopez has joined us as a part-time resident in Dental Public Health. Ms. Ruth Nowjack-Raymer, RDH, MPH, joined the Soft Tissue, Craniofacial Defects and Pain Section in August, 1989 as a Health Research Specialist. Ms. Nowjack-Raymer spent the past three years as a clinical trials specialist with the Disease Prevention Section of the Disease Prevention and Health Promotion Branch. Continued consultation for the oral examination component of NHANES III has been provided by Dr. Todd Beckerman and Dr. Edith Morrison. Ms. Kimberly Musial and Mr. Kenneth Livingston were COSTEP members of the Branch this past summer.

Analysis of Data from the 1986-87 Survey of the Oral Health of U.S. School Children

Staff of the Epidemiology Branch completed detailed analyses on the prevalence and distribution of dental caries among U.S. school children ages 5-17, using data gathered in the 1986-1987 nationwide survey. A monograph reporting these data, including national and regional estimates, was prepared.

In a separate analysis of the same data, it was shown that children who were lifelong residents in optimally fluoridated communities had levels of dental caries approximately 18 percent lower than those never exposed to fluoridated water. When exposure to topical fluorides and fluoride supplements was taken into account, the difference in caries experience increased to 25 percent.

Preliminary analyses of examination and self-report findings of oral mucosal tissue lesions in a national sample of U.S. school children were conducted. Approximately 4.1 percent of U.S. school children ages 5-17 were estimated to be affected with mucosal pathologies. The most common conditions were recurrent aphthous ulcerations (RAU), recurrent herpes labialis (RHL), smokeless-tobacco associated lesions and geographic tongue.



Branch staff analyzed data on gingival bleeding, calculus and periodontal attachment loss from the National Survey of Oral Health of U.S. School Children, 1986-87. These analyses showed that the periodontal health of adolescents is quite good, with mean attachment loss being less than half a millimeter (0.26 mm for buccal and 0.40 mm for mesial sites). There was very little variation by age, sex or ethnicity. On the other hand, the prevalence of gingival bleeding was rather high, with 59 percent of the children exhibiting at least one site with gingival bleeding. The mean proportion of sites with bleeding, however, was less than 6 percent of the examined sites per child.

Analyses of interview data from the National Survey of Oral Health in U.S. School Children on tobacco and alcohol use were initiated. About 16 percent of children in grades six through 12 reported past or present use of any form of tobacco. Product-specific use was 9.6 percent for cigarettes, 4.6 percent for snuff, 5.2 percent for chewing tobacco, 0.5 percent for cigars and 0.2 percent for pipes. With regard to alcohol use, about 58 percent reported having used alcoholic beverages. Eighty-two percent of high school seniors reported some use of alcohol, as contrasted with 30.6 percent of 6th graders. Five percent of the adolescents reported use on 52 or more days during the year.

The Natural History of Periodontal Disease in Man

The study of the natural history of periodontal disease in Sri Lankan tea laborers and Norwegian males has continued. During this past year, work has concentrated on the following areas:

(1) The longitudinal study of calculus in populations with and without dental care. In the Sri Lankan population, teeth with subgingival calculus had a significantly higher rate of loss of attachment than surfaces that remained calculus-free over 15 years. In the Norwegian population, subgingival calculus had no impact on loss of attachment over 20 years.

(2) Longitudinal study of caries and dental restorations in gingival location and their relationship to the initiation and progression of periodontal disease. Surfaces without subgingival restorations and caries over 20 years had less loss of attachment than teeth that had subgingival restorations and caries at all examinations over 20 years.

(3) Longitudinal studies of gingival recession. Gingival recession was rarely seen in both populations before 20 years of age. There is no difference in the prevalence of recession in a population that practiced daily oral hygiene and in a population where oral hygiene was nonexistent.

Bacteriological, immunological, and genetic studies of the two populations are underway. Analysis in the coming year will also focus on the conversion of gingivitis to periodontitis in the two countries, and on problems of reproducibility and method error in epidemiological studies.



Study of the Natural History of the Oral Manifestations of HIV-Infection Launched

The NIDR oral health research facility located in a newly renovated ward at the U.S. Walter Reed Army Medical Center was officially opened on May 30, 1989. The facility will be used for the study of oral manifestations of HIV infection, and is part of a broader natural history study conducted by the Walter Reed Army Institute of Research. The objective is the documentation of the prevalence and incidence of oral diseases and conditions and their relation to the progression of HIV infection as well as to systemic diseases. Specific areas of investigation include oral candidiasis, periodontal diseases and compositional changes in saliva. To date, approximately 100 subjects have been examined. It is anticipated that about 1,000 individuals will be re-examined every six months during the course of their disease.

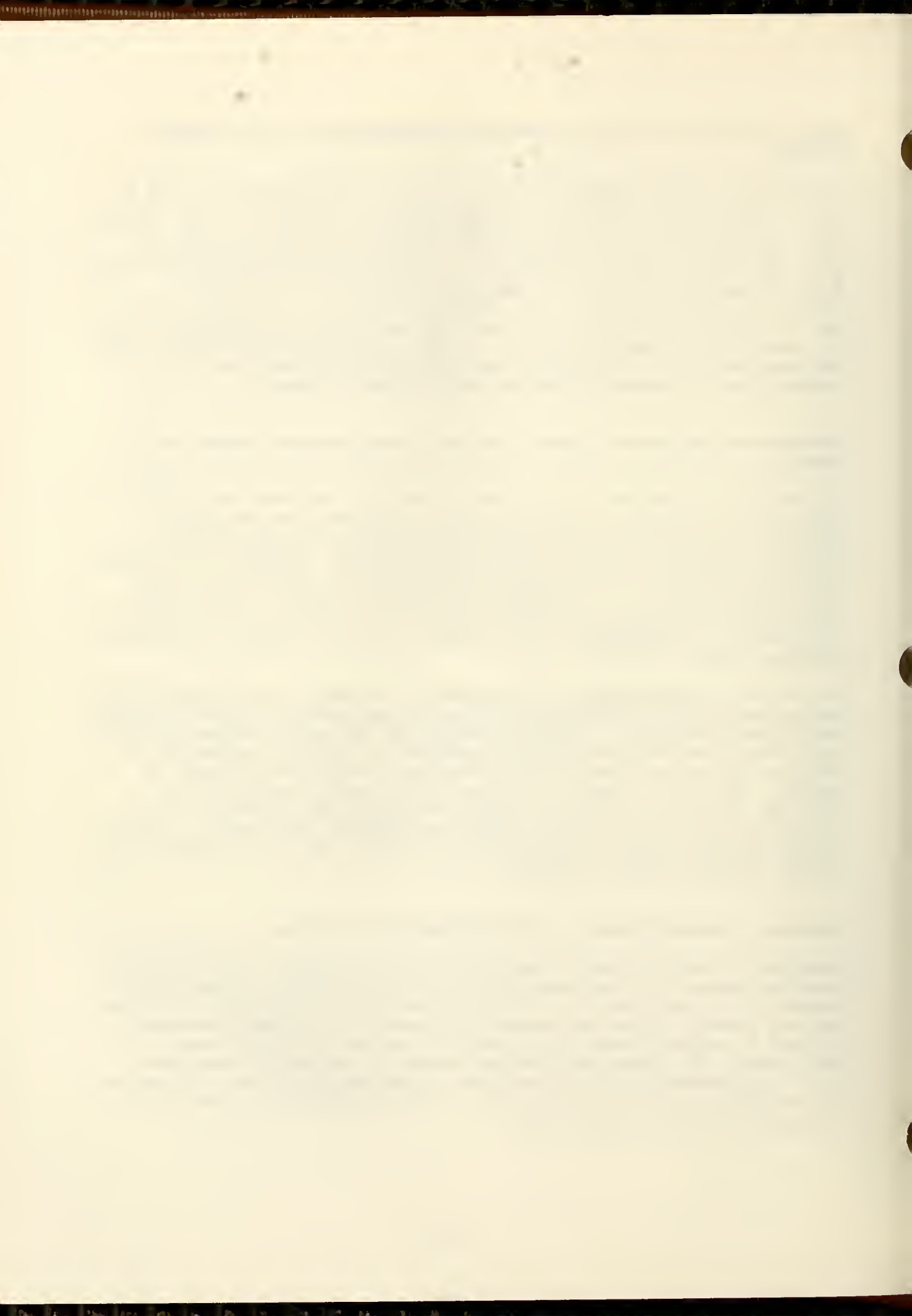
Evaluation of Epidemiologic Uses of National Data Resources on Oral-Facial Injuries

Research has included the continuation of studies on injuries to the teeth, face and skull, and work on enamel defects in children with neurological handicaps. Product-related hospital-treated injuries to teeth from the National Electronic Injury Surveillance System (NEISS) database for the years 1979-87 were analyzed. This analysis showed that over 75 percent of such injury episodes occurred in persons under 15 years of age. The most common categories of products and activities associated with tooth injuries were sports and play, followed by falls on floors, stairs or showers, and bicycles and other wheeled vehicles.

Data on first-listed diagnosis of fractures of the skull and face bones from the National Hospital Discharge Survey were re-analyzed with inclusion of data for the years 1980-1987. Findings show that for the period studied, skull and facial fractures (ICD Codes 800-803) accounted for nearly 12 percent of all fractures. Males had almost twice as many skull and facial fractures as females. There was a seasonal variation by the month of admission for treatment of skull and facial fractures, with the months of June and July showing the highest percentages; January and February, the lowest. There was a declining trend in the estimated numbers of skull and facial fractures from 158,000 in 1981 to 109,000 in 1987.

Advances in Computer Methods for Oral Epidemiologic Studies

Detection of loss of alveolar bone from dental radiographs is an accepted method of diagnosis of periodontal disease in epidemiologic studies, but the observer error associated with this technique can be substantial. Extensive work was directed at the development of a computer-based image-enhancement protocol to improve diagnostic accuracy of bone loss, using bite-wing radiographs. The system which evolved reduces intra-examiner measurement error to approximately 0.3 mm, even with inexperienced examiners. The method has now been used to diagnose bone loss in two longitudinal studies of periodontal disease in adolescents.



The National Center for Health Statistics' NHANES III study began field operation in October, 1988. Branch staff have participated in training of dental examiners and overall monitoring of the quality of data collection for the oral health component. A major initiative has been the development of a direct data entry system which is now in field use.

Enhancing Epidemiologic Research Through International Collaboration

Branch staff have continued to participate in international activities related to epidemiology and surveillance activities of oral manifestations and HIV infection. The staff were responsible for organizing and participating in a workshop which established minimum essential data to be collected in studies of HIV infection so that comparisons can be made across studies and among countries.

Technical Consultation

Staff of the Branch provided extensive consultation and assistance to other organizations and agencies including the World Health Organization, American Dental Association, American Public Health Association, National Center for Health Statistics, Republic of Ireland, and the University of Hong Kong.

Publications

Bhat M, Nelson KB, Swango PA. Lack of stability in enamel defects in primary teeth of children with cerebral palsy or mental retardation, *Pediat Dent* 1989; 11(2):118-120.

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Brunelle JA, Miller-Chisholm AJ, Loe H. Oral health of U.S. adults - regional findings. NIH Publication No. 88-2869, May 1988.

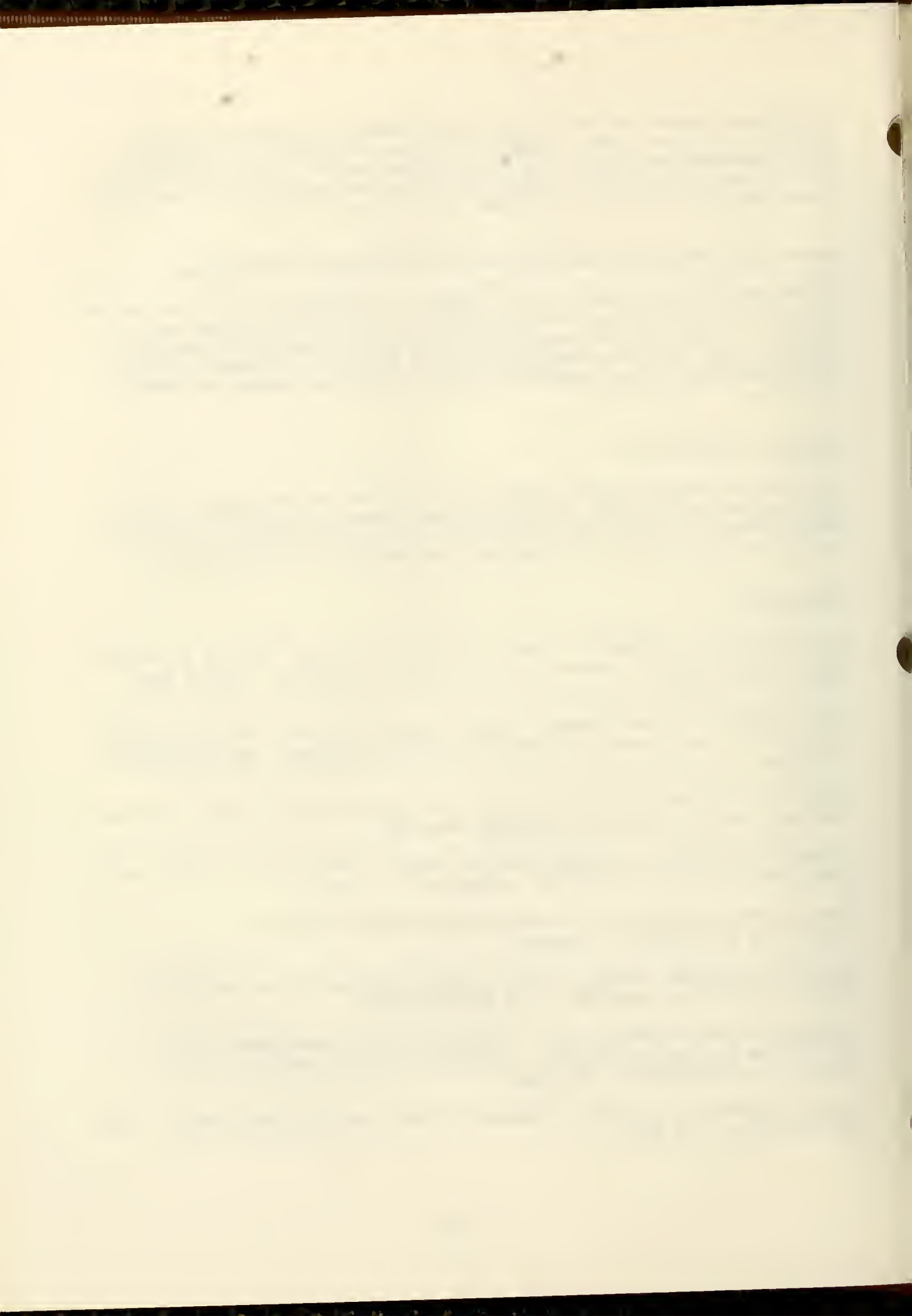
Brunelle JA, Carlos JP. Recent trends in dental caries in U.S. children and the effect of water fluoridation, *J Dent Res*. In press.

Brunelle JA. Dental caries in United States children, 1986-87. NIH Publication No. 89-2247, in press.

Carlos JP, Wolfe MD. Dental caries: historic and current perspectives, *Compend Contin Educ Dent* 1988; Suppl No 11:S356-S364.

Kleinman DV, Horowitz AM, Mirth D. Dental technology assessment in the U.S.: an overview for the practitioner. In: Clark's Clinical Dentistry, JB Lippincott, Philadelphia. In press.

Louie R, Brunelle JA. Caries prevalence in Head Start children 1986-87, *Comm Dent Oral Epid*. In press.



Sterritt GR, Freu RA, Rozier RG, Brunelle JA. Evaluation of a school-based fluoride mouthrinsing and clinic-based sealant program at Non-F island. *Comm Dent Oral Epid*. In press.

Wilentz J, Kleinman DV, Van Ostenberg P. Creating a national agenda for oral research in special patients. *J Spec Care in Dent*. In press.

Abstracts

Bhat M, Swango PA, Mussleman RJ, and Schneider PE. A new approach for determining prevalence and risk factors for traumatized anterior teeth, *J Pub Health Dent* 1989; 49(2):105.

Bhat M, Drury TF. Indicators of utilization and perceived barriers to orthodontic care by 12- to 17-year-olds in U.S. school children. Program and Abstract, American Public Health Association, Oct 1988.

Bhat M, Brunelle J. Gingival status of 14-17-year-old school children. Abs. No. 704, *J Dent Res* 1989; 68:955.

Brunelle JA. The prevalence of dental sealants in U.S. school children. Abs. No. 12, *J Dent Res* 1989; 68:183.

Brunelle JA. The prevalence of dental fluorosis in U.S. children, 1987. Abs. No. 1029, *J Dent Res* 1989; 68:995.

Cassingham RJ, Musselman RJ, Schneider P, Bhat M. Attachment loss in Bogalusa, LA, adolescents. Abs. No. 302, *J Dent Res* 1989; 68:904.

Feldman CA, Feldman RS, Esenauer AE, Brunelle JA, Cohen DW. Abs. No. 154, *J Dent Res* 1989; 68:886.

Kleinman DV, Swango PA. Prevalence of oral mucosal pathologies in U.S. school children, 1986-87. Abs. No. 1479, *J Dent Res* 1989; Vol 68, Special Issue.

Kleinman DV, Swango PA. Self-reported history of tobacco use by U.S. school children, grades 6-12. (Accepted for presentation at the APHA Annual Meeting, October 1989).

Niessen L, Swango PA, Swisher L, Brunelle J, Levinson P. Caries prevalence in institutionalized veterans. Abs. No. 450, *J Dent Res* 1989; 68:237.

Swango PA, Kleinman DV. History of aphthous ulcers and herpes labialis in U.S. school children. Abs. No. 1480, *J Dent Res* 1989; Vol 68, Special Issue.

Swango PA, Brunelle JA. Surface-specific caries patterns from a national survey of school children: implications for sealant use. (Accepted for presentation at the AAPHD Annual Meeting, November, 1989)



Presentations

Bhat M. Periodontal disease - a dental public health problem. Lecture given to Howard University Dental Students, September 29, 1988.

Bhat M. Oral-facial injuries, craniofacial anomalies, and malocclusion. Presented at NIDR meeting on Dental Research and Minority Group Oral Health Issues, May 22, 1989.

Bhat M. Periodontal diseases. Presented at NIDR meeting on Dental Research and Minority Group Oral Health Issues, May 22, 1989.

Brunelle JA. Dental caries in racial and ethnic minorities. Presented at NIDR meeting on Dental Research and Minority Group Oral Health Issues, May 22, 1989.

Carlos JP. Modelling risk-factors for periodontal disease. Epidemiology Seminar, July, 1989.

Cummins SK, Grether JK, Bhat M, Nelson KB. Defects in primary teeth enamel: a clue to timing of insult in cerebral palsy? Annual Meeting of the American Academy of Cerebral Palsy.

Kleinman DV. Personnel for health needs of the elderly through the year 2020: an overview. Presented at the American Association of Dental Schools Special Meeting on Geriatrics. San Francisco, March 1989.

Kleinman DV. Perspectives on dental public health. Presented at the 25th Anniversary of the Henry M. Goldman School of Dentistry. Boston, November 1988.

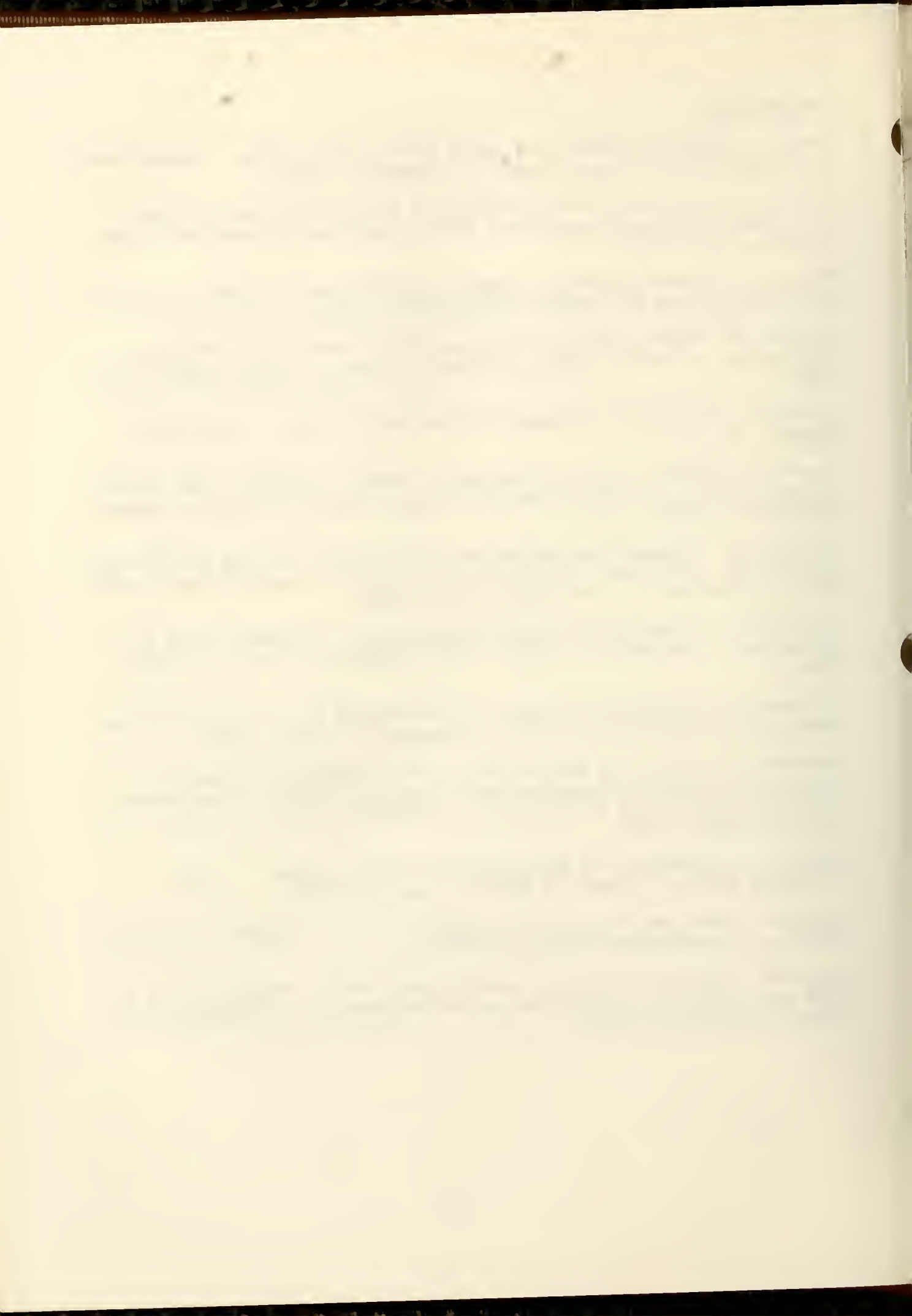
Kleinman DV. International aspects of HIV infection and oral manifestations. Presented at Georgetown University, Washington, DC, February 1989.

Kleinman DV. The role of dental public health in the global epidemic. Organized and moderated symposium held at the joint meeting of the Federation Dentaire Internationale and the American Association of Public Health Dentistry, August, 1989.

Swango PA. Epidemiology of dental caology of dental caries. Lecture, Georgetown University School of Dentistry, April 6, 1989.

Wolfe MD. Inter- and intra-examiner reproducibility in diagnosis of alveolar bone loss. Epidemiology Seminar, July 1989.

Wolfe MD. Nutritional issues in assessing oral health. Presentation at the NIDR meeting on Diet and Nutrition and the Maintenance of Oral Health in the Elderly, September 21, 1989.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00399-05 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Periodontal Diseases in Adolescents

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

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any)

Department of Oral Biology, SUNY at Buffalo, Buffalo, NY

LAB/BRANCH

Epidemiology Branch

SECTION

Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER:

.30

.20

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this continuing study is to determine the prevalence and progression of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in the U.S.

The original cross-sectional study population consisted of approximately 600 Navajos, ages 14-19. Twenty-four posterior interproximal sites were examined on each subject. Gingivitis was assessed using a modification of the G.I. Loss of attachment was assessed using Ramfjord's technique. Bone loss was diagnosed from standardized bite-wing radiographs using a microfiche reader. Analyses indicated a high prevalence of disease: gingivitis (71%); attachment loss (89%); and bone loss (89%). The average number of sites in the mouth affected with the more advanced form of disease (attachment loss and bone loss) was also high: 32% of the sites had attachment loss, and 22% had bone loss.

A longitudinal study is in progress of the youngest subjects to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease. Two hundred twenty-six subjects were examined in February 1986, 1987 and 1988 using the same clinical and radiographic techniques. Subgingival plaque samples were obtained from all first molars and analyzed for A. actinomycetemcomitans, B. gingivalis and B. intermedius. Analyses of first year data indicate that the combination of calculus, gingival bleeding and B. intermedius gave the most parsimonious explanation of the presence of attachment loss. Bite-wing radiographs from all three years have now been read for evidence and quantification of alveolar bone loss using image enhancement software (JAVA). Data sets will soon be assembled for multiple logistic regression analyses.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00410-05 EE

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

The Natural History of Periodontal Disease in Man

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

Løe,, Harald	Director, NIDR	PDS, EODPP, NIDR
Anerud, Age	Visiting Scientist	EB, EODPP, NIDR
Kingman, Albert	Statistician	OPS, EODPP, NIDR
Smith, Jacqueline	Statistician (Health)	OPS, EODPP, NIDR

COOPERATING UNITS (if any)

University of Texas Dental School, San Antonio, Texas
Department of Oral Biology, University of Buffalo, New York

LAB/BRANCH

Epidemiology Branch

SECTION

Periodontal Diseases Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, Maryland

TOTAL MAN-YEARS.

1.05

PROFESSIONAL.

1.05

OTHER.

0

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The study of the natural history of periodontal disease in Sri Lankan tea laborers and Norwegian males is continuing. The work has concentrated on: 1) The longitudinal study of calculus in populations with and without dental care. In the Sri Lankan population, teeth with subgingival calculus had a significantly higher rate of loss of attachment than surfaces that remained calculus free over 15 years. In the Norwegian population, subgingival calculus had no impact on loss of attachment over 20 years. 2) Longitudinal study of caries and dental restorations in gingival location and their relationship to the initiation and progress of periodontal disease. Surfaces without subgingival restorations and caries over 20 years had less loss of attachment than teeth that had subgingival restorations and caries at all examinations over 20 years. 3) Longitudinal studies of gingival recession. Gingival recession was rarely seen in both populations before 20 years of age. There is no difference in the prevalence of recession in a population that practiced daily oral hygiene and in a population where oral hygiene was nonexistent. 4) Conversion of gingivitis to periodontitis in the two countries; and 5) Problems of reproducibility and method error in epidemiological studies. Bacteriological, immunological, and genetic studies of the two populations are underway. The specific aims of these studies are to compare the presence and levels of selected putative periodontal pathogens, peripheral blood antibody titers and to evaluate genetic determinants according to severity of periodontal destruction in the Norwegian and Sri Lankan participants. Building of two master data sets in SAS for the longitudinal Norwegian surveys 1968-1988 was completed. Data bases are: LOE.NOR.AVO (All Valid Observations) and LOE.NOR.IAS (In All Surveys). The observations have been assimilated into the previous data bases.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00420-04 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Design and Analysis of National Survey of Oral Health in School Children

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

Brunelle, Janet A. Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

Dental Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

.34

PROFESSIONAL

.30

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

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

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

A national survey of the Oral Health of School Children was conducted during the 1986-87 school year. Dental exams were conducted on approximately 41,000 children by 13 trained and calibrated dental teams. A questionnaire on use of smokeless tobacco smoking and alcohol was administered and a salivary sample was collected. Overall response rate was 78%. Data was processed, edited and final records weighted.

Analysis of DMFS indicated a 37% change in mean estimates compared to a similar study conducted in 1979-80. There was a lower level of disease at every age. Mean DMFS was also lower in all regions of the country; however, there were still regional variations as before. Approximately 50% of the children aged 5-17 were caries free in their permanent dentition. A monograph "Oral Health of U.S. Children, 1986-87" on caries levels was prepared.

Analysis of sealant observations was done. Only 7.6% of the children aged 5-17 had sealants present. More females than males had sealants. 11% of the 8-, 9- and 10-year-old children had sealants. The average number of sealants in children with sealants was 4.2 per child. There were regional differences in the prevalence of sealants.

An estimate of the prevalence of dental fluorosis was made using Dean's Index on 2nd through 12th graders. 22% of children showed definite signs of fluorosis, 17% very mild, 4% mild, 1% moderate and 0.3% severe. Prevalence ranged from 18.5% of 17-year-olds to 25.8% of 9-year-olds. Regional distributions showed large variations.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-00430-03 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Product - Related Injuries to Teeth, Mouth and Face

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

Mohandas Bhat Visiting Scientist, EB, EODPP, NIDR
Shou-Hua Li Statistician (Health), EB, EODPP, NIDR

COOPERATING UNITS (if any)

Consumer Product Safety Commission, Bethesda, Maryland

LAB/BRANCH

EB, EODPP, NIDR

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided)

The objective of this study is to do a trend analysis of product-related, hospital emergency room-treated injuries to the teeth, mouth and face, including an analysis of the frequencies of such injuries related to specific consumer products, on data compiled by the National Electronic Injury Surveillance System (NEISS) maintained by the Consumer Product Safety Commission.

The NEISS database has separate data on injuries to the teeth, mouth and face. The injuries to the teeth form a subset of mouth injuries. During this reporting period the subset of injuries to teeth were further analyzed, and a report was prepared. Results showed a slight but consistent increase in episodes of product-related tooth injuries during the nine-year period studied. Over 75 percent of such injury episodes occurred in persons under 15 years of age. Over sixty percent of the tooth injuries in such episodes could be crudely classified into the following types by descending rank order: avulsed teeth, broken teeth, loosened teeth, chipped teeth, intruded teeth and soft tissue injuries.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00432-03 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

The Relationship of Bone Loss in Adolescents to Periodontal Status in Adults

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

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any)

University of Umeå
Umeå, Sweden

LAB/BRANCH

Epidemiology Branch

SECTION

Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

.15

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The purpose of this investigation was to determine whether periodontal destruction, diagnosed as an adult, can be predicted by examining bite-wing radiographs taken in adolescence.

This retrospective investigation was conducted in Sweden where a unique system of lifetime address registry and public dental care records are available for cross-referencing and locating subjects. The University of Umeå identified 250 subjects, approximately 30 years of age, for whom bite-wing radiographs taken at age 15 were still available, and who resided within a short distance from Umeå. Dental examinations were conducted on 250 subjects at four dental clinics in the surrounding county of Vasterbotten. Clinical components consisted of exam for evidence of gingival bleeding, calculus, pocket depth, and attachment loss at the buccal and mesio-buccal aspects of all teeth excluding third molars. Bite-wing radiographs were taken using an Eggen film standardizing device.

The radiographs taken at age 15 vary enormously in quality and angulation. Several computer assisted imaging protocols are being explored to determine how the images can be improved to provide a suitable baseline for diagnosis of bone loss.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-00443-03 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

The Prevalence of Oral Soft Tissue Lesions in Patients in a Long-Term Care Facility

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

Swango, Philip A. EODPP, NIDR
Kleinman, Dushanka V. EODPP, NIDR

COOPERATING UNITS (if any)

Perry Point VA Medical Center
Perry Point, Maryland

LAB/BRANCH

EB, EODPP, NIDR

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

.2

PROFESSIONAL

.2

OTHER

CHECK APPROPRIATE BOX(ES)

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

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

The study is a cross-sectional survey to document oral soft tissue pathologies occurring in patients presenting to the Perry Point VA Medical Center during a 24-month period. It is expected that about 900 patients will be examined during the study. Pathologies that cannot be definitively diagnosed by the examining dentist are referred to a consultant oral pathologist. The objectives of the study are to estimate the prevalence of oral soft tissue lesions in this patient population, to characterize the range and severity of the pathologies noted and to assess treatment needs resulting from these conditions. Information is also collected from existing records regarding risk factors such as dental prostheses, medical condition, medications, and use of tobacco and alcohol.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-00464-02 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Natural History of Oral Manifestations of HIV Infection in a U.S. Military Population

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

Philip A. Swango	EODPP, NIDR
Dushanka V. Kleinman	EODPP, NIDR
Philip Fox	CIPCB, IRP, NIDR
Carolyn Tylenda	CIPCB, IRP, NIDR
Joseph Zambon	SUNY at Buffalo
Edmond Tramont	Walter Reed Army Medical Center
Charles Oster	Walter Reed Army Medical Center

COOPERATING UNITS (if any)

Walter Reed Army Institute of Research

LAB/BRANCH

EB, EODPP, NIDR

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

.4

PROFESSIONAL

.4

OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

United States Army Personnel who have been tested as seropositive for the Human Immunodeficiency Virus (HIV) will be given medical examinations and treatment at Walter Reed Army Medical Center (WRAMC). These subjects will also be given the opportunity to participate in a medical research protocol to study the natural history of HIV infection, administered by the Walter Reed Army Institute of Research (WRAIR). An oral component developed by staff of the National Institute of Dental Research (NIDR) in collaboration with U.S. Army medical and dental staff at WRAMC and WRAIR is part of this natural history study.

The oral component permits documentation of the prevalence and incidence of the full range of oral conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions will also be characterized, and the role of oral manifestations as early predictors or markers of disease progression will be elucidated. Finally, the role of saliva in the transmission of the virus will be investigated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DE00468-02 EB

PERIOD COVERED
October 1, 1988 - September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Dental Caries in U.S. Children on Fluoridated/Non-fluoridated Water Supplies

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

Brunelle, Janet A.	Statistician (Health)	EB, EODPP, NIDR
Carlos, James P.	Chief	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology

SECTION
Dental Caries

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.47	.47	.02

CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type Do not exceed the space provided.)

Residential histories collected during a national survey of oral health of U.S. schoolchildren conducted in 1986-1987 were used to establish two groups: those children who always lived on a public water supply in a fluoridated community (N=8,165) and those who never lived in a fluoridated community (N=8,233).

Comparisons of the mean levels of Decayed, Missing and Filled Surfaces were made by age, sex and region of the country. Mean DMFS for children with lifelong exposure to water fluoridation was 2.8 compared to 3.4 mean DMFS for children who had never lived in areas with fluoridated water. Mean DMFS for mesial-distal surfaces was about one-third higher in children without water fluoridation. Both groups of children reported high use of supplemental and/or topical fluorides. Regional differences between groups varied greatly from 61% difference in Region VII to 6% difference in Region III. Material presented at workshop "Mechanisms of Fluoride" and will be published in Journal of Dental Research.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00469-02 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Distribution of Root Caries in U.S. Adults

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

Brunelle, Janet A. Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology

SECTION

Dental Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

.12

PROFESSIONAL

.12

OTHER

CHECK APPROPRIATE BOX(ES)

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

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

In 1985 the NIDR conducted a national survey of the oral health status of employed adults and seniors, in which measurements of root caries were made for the first time on a national sample of the U.S. population. Examinations were made on 15,132 employed adults representing 99.6 million persons. Previous reports of the distribution of root caries vary widely, which may be related to how root caries was defined or measured. For the survey, visual tactile examination were made for decayed and filled root surfaces. Each tooth was considered to have four root surfaces. No x-rays were taken.

Approximately 21% of the employed dentate population aged 18-64+ years had at least one decayed (D) or filled (F) root surfaces (S) with more males than females affected. The mean number of DFS was less than one for the employed dentate population. Less than half (47%) of the DFS were filled.

Distribution of the disease by tooth and surface type was analyzed. Buccal surfaces were the most frequently affected surfaces with decay or fillings occurring four times as often here as on any other surface. The distal and mesial surfaces are the next most frequent sites of root caries and the lingual surface is the least affected. However, the differences among these last three surface types is minimal. Root caries was most common in mandibular pre-molars, molars and maxillary cuspids. Lower incisors were rarely involved (<1% of the DFS occurred in these teeth). Analysis is continuing using multiple and logistic regressions to ascertain relationship of root caries to other variables measured in the survey. Manuscript on surface distribution is in progress.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00470-0 2 EB

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Demographic and Environmental Correlates of High and Low Caries Experience in Children

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

Carlos, James P.	Chief	EB, EODPP, NIDR
Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology Branch

SECTION

Caries

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

1

PROFESSIONAL

.50

OTHER

.50

CHECK APPROPRIATE BOX(IES)

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

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

One of the striking findings of the 1986-1987 NIDR Survey of Dental Caries of U.S. School Children was that approximately half the children ages 5-17 had never experienced caries in their permanent teeth. This study is exploring the relationship between a caries-free status and such variables as sex, ethnicity, geographic region and urban-rural residence. Controls are the approximately 1400 children of the same ages whose cumulative caries experience exceeds two standard deviations of the mean (mean DMFS) for that age group.

Preliminary analyses indicate highly significant differences among the two groups with respect to geographic region of residence. Therefore, the study was expanded to include data on ten trace metals obtained from drinking water samples from the schools attended by the subjects.

Because the probability of being in the high caries group was strongly correlated with age, separate logistical regression models were fit for each year of age. Phosphorus, silicon, strontium and molybdenum were significantly ($p < 0.10$) but weakly associated with caries status, though the relationship was inconsistent. Further models, testing interactions among the independent variables, are being examined.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-00486-01-EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Periodontal Health of 14-17 Year Old School Children - U.S. 1986-87

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

Mohandas Bhat Visiting Scientist, EB, EODPP, NIDR
Carla Bock Computer Programmer, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

EB, EODPP, NIDR

SECTION

Soft Tissue, Craniofacial Defects and Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

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

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

Data on gingival bleeding, calculus, and periodontal attachment loss from the 1986-87 national survey of oral health in U.S. school children were analyzed. These data were obtained from a subset of children in the sample studying in the 9th through to 12th grades. The analysis was confined to 11,111 children in the sample, aged 14-17 years, who represent an estimated 13 million U.S. school children in this age group.

The national estimate for prevalence of gingival bleeding assessed by the presence of at least one bleeding site was approximately 5% for children in this age group. The percent of bleeding sites per child, however, was less than 6%. There was an overall age associated decline in prevalence of gingival bleeding. Males, in general, and nonwhite children showed higher prevalence of gingival bleeding.

The prevalence of calculus (percent of children with at least one site with calculus was nearly 34% for supragingival calculus alone and approximately 23% for subgingival calculus with or without supragingival calculus. The percent of sites with calculus per child was approximately 8% for the former and 4% for the latter type of calculus. There was a slight age-associated increase in the prevalence of latter type of calculus. Males and nonwhite children showed higher prevalence of both types of calculus.

Mean attachment loss, determined by probing with a #2-12 probe, was less than half a millimeter (0.26 mm for buccal and 0.4 mm for mesial sites) and varied very little by age, sex or ethnicity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER EB
Z01-DE-00489-01

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Oral Soft Tissue Pathologies and Risk Factors in U.S. Schoolchildren

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

Kleinman, Dushanka V. EODPP, NIDR
Swango, Philip A. EODPP, NIDR

COOPERATING UNITS (if any)

Epidemiology Branch

LAB/BRANCH

Soft Tissue, Craniofacial Defects and Pain Section

SECTION

NIDR, NIH, Bethesda, Maryland

INSTITUTE AND LOCATION

TOTAL MAN-YEARS

.6

PROFESSIONAL

.4

OTHER

.2

CHECK APPROPRIATE BOX(ES)

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

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

As a component of the National Survey of Oral Health of School Children conducted in 1986-87 (Z01-DE-00420), a sample of approximately 41,000 children in grades K-12 were examined for the presence of oral soft tissue lesions and questioned about their history of recurrent aphthous ulcers and herpes labialis. Children in grades 6-12 were interviewed to determine their use of tobacco and alcohol.

Preliminary analysis showed that about 4.1 percent of the children had an oral soft tissue lesion or condition. The conditions most frequently observed were aphthous ulcers, herpes labialis, smokeless tobacco-associated lesions, and geographic tongue. The prevalence of a positive history of aphthous ulcers and herpes labialis was 36.8 percent, 32.9 percent, respectively. About 16 percent of the children reported being affected by both conditions.

About 16 percent of children in grades 6-12 reported past or present use of tobacco in any form. Prevalence of use was 9.6 percent for cigarettes, 4.6 percent for snuff and 5.2 percent for chewing tobacco. Cigarette use showed no difference by gender, whereas for smokeless tobacco products males reported use almost 20 times more frequently than females. About 58 percent of adolescents reported ever having used alcohol, with prevalence increasing from 31 percent in sixth graders to 82 percent in high school seniors. Five percent of the sample reported alcohol use on 52 or more days per years.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00490-01 EB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Radiographic Image-enhancement for Diagnosis of Bone Loss

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

Wolfe, Mary D.	Epidemiologist	EB, EODPP, NIDR
Carlos, James P.	Chief	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Epidemiology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS

1.05

PROFESSIONAL

1.05

OTHER:

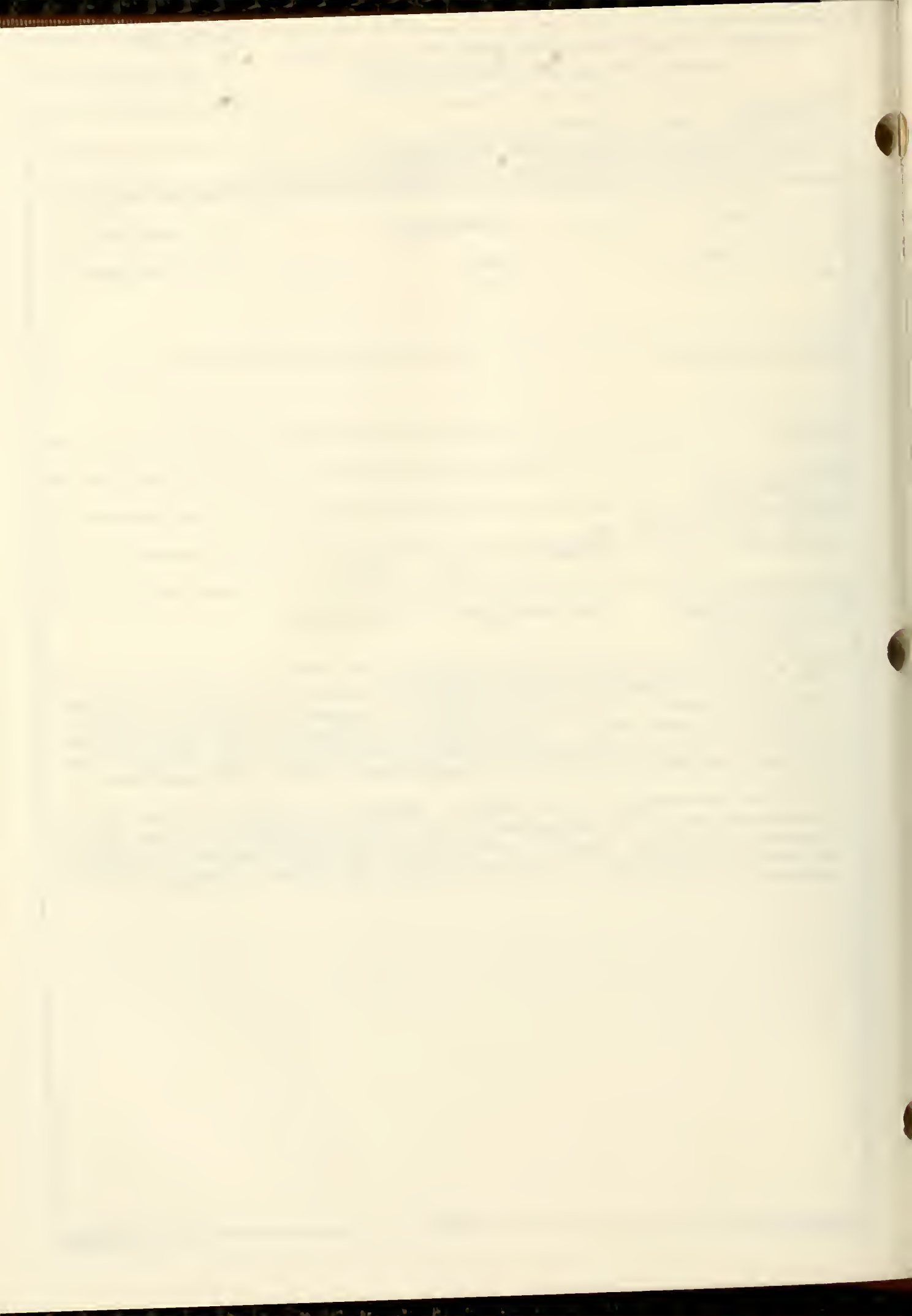
CHECK APPROPRIATE BOX(ES)

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

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

This study developed and tested a series of protocols for computer-assisted image-enhancement of bite-wing radiographs to improve consistency of diagnosis of alveolar bone loss. Video images of radiographs were digitized and manipulated by edge-enhancement and contrast-enhancement filters. Bone loss was measured from the CE junction to the alveolar crest, in mm, at 20x magnification.

Replicate measurements by the examiners showed the standard deviation of the measurement error to be approximately 0.3 - 0.4 mm for both intra- and inter-examiner comparisons. However, this was not substantially better than could be achieved with unenhanced, digitized images. The latter technique was also considerably faster.



**ANNUAL REPORT OF THE ANALYTICAL STUDIES BRANCH
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH**

The Analytical Studies Branch conducts research analyzing the relationships among patterns of oral diseases and disorders, the need for, and utilization of, dental care, dental education, dental research, and dental delivery systems. These functions are carried out by: (1) investigating the interrelationships among economic, social and personal characteristics, the distribution of various oral diseases, and dental treatment needed and utilized; (2) developing new models and theories which integrate social and epidemiological factors into models which explain the relations between oral disease and the social, economic, and personal characteristics of individuals; (3) conducting cost-effectiveness and cost-benefit analyses of various preventive and treatment methods; and (4) providing consultation and technical assistance in these subject areas to other Institute components, the NIH, PHS, and other Federal and nonfederal scientists and organizations.

Dr. Brown is Chief, Analytical Studies Branch, which also includes Mr. Oldakowski and included, during the past year, Dr. Warren and Ms. Offutt. Carrie Yang was with the Branch as a summer student.

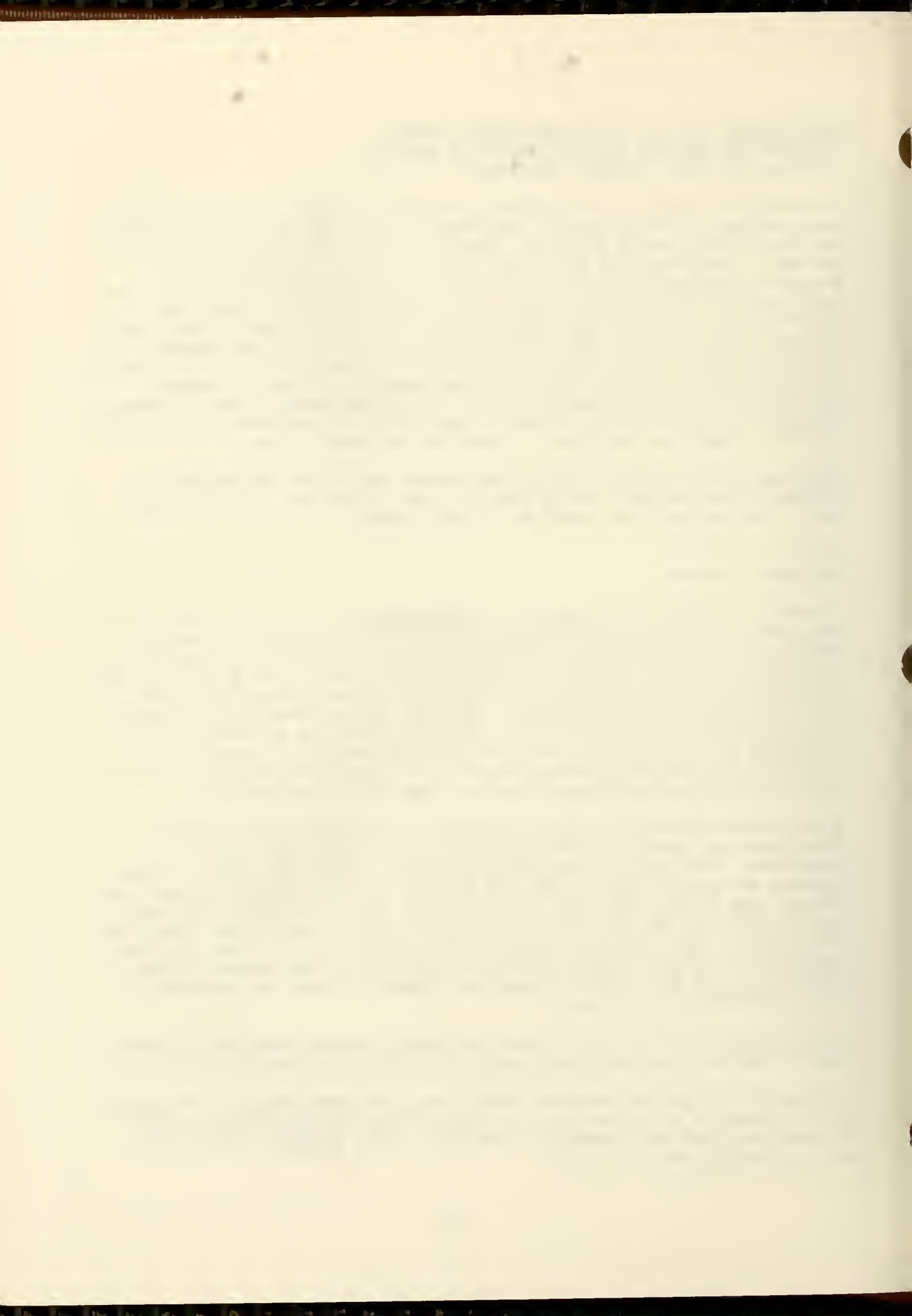
Analytical Studies

A variety of analytical research was conducted by Branch staff during fiscal year 1989. Several of the studies involved collaboration with university researchers. The periodontal health of employed U.S. adults was studied in collaboration with Dr. Richard C. Oliver of the University of Minnesota. The overall description of periodontal conditions is nearing completion; the association between sociodemographic variables and periodontal status will be the next focus. Branch staff worked with Drs. Beazoglou and Heffley of the University of Connecticut to study the estimated savings in dental expenditures which resulted from prevention of dental disease. Dr. Lawrence Meskin collaborated with Branch staff to study tooth loss patterns.

Branch staff conducted a study of variations in the periodontal health of older Americans among different sociodemographic categories of this population. The status of the dentition in U.S. adults was studied, using measures of need for care in addition to usual measures of oral disease. Two studies used longitudinal data to investigate: (1) the association between dental status and the utilization of dental services, and (2) the transition of individual teeth from one dental state to another (e.g., sound to carious, present to missing). Branch staff participated with other members of the Epidemiology and Oral Disease Prevention Program to study the epidemiology of the oral health of minorities.

Partly because of their significance, and partly because they involve primary data collection, two analytical studies are discussed in more detail below.

A Study of Utilization, Treatment Needs, Cost, and Dental Disease--The purpose of this study is to collect data to study the relationships between dental disease and other oral conditions, treatment needs, economic factors, and utilization of dental services.



In 1963, the Veterans Administration initiated an interdisciplinary and longitudinal investigation of the normal aging process. Participants consisted of 2,400 men with stable living and work conditions in the Boston, Massachusetts area. From this panel, 1,221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The examinations included a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene.

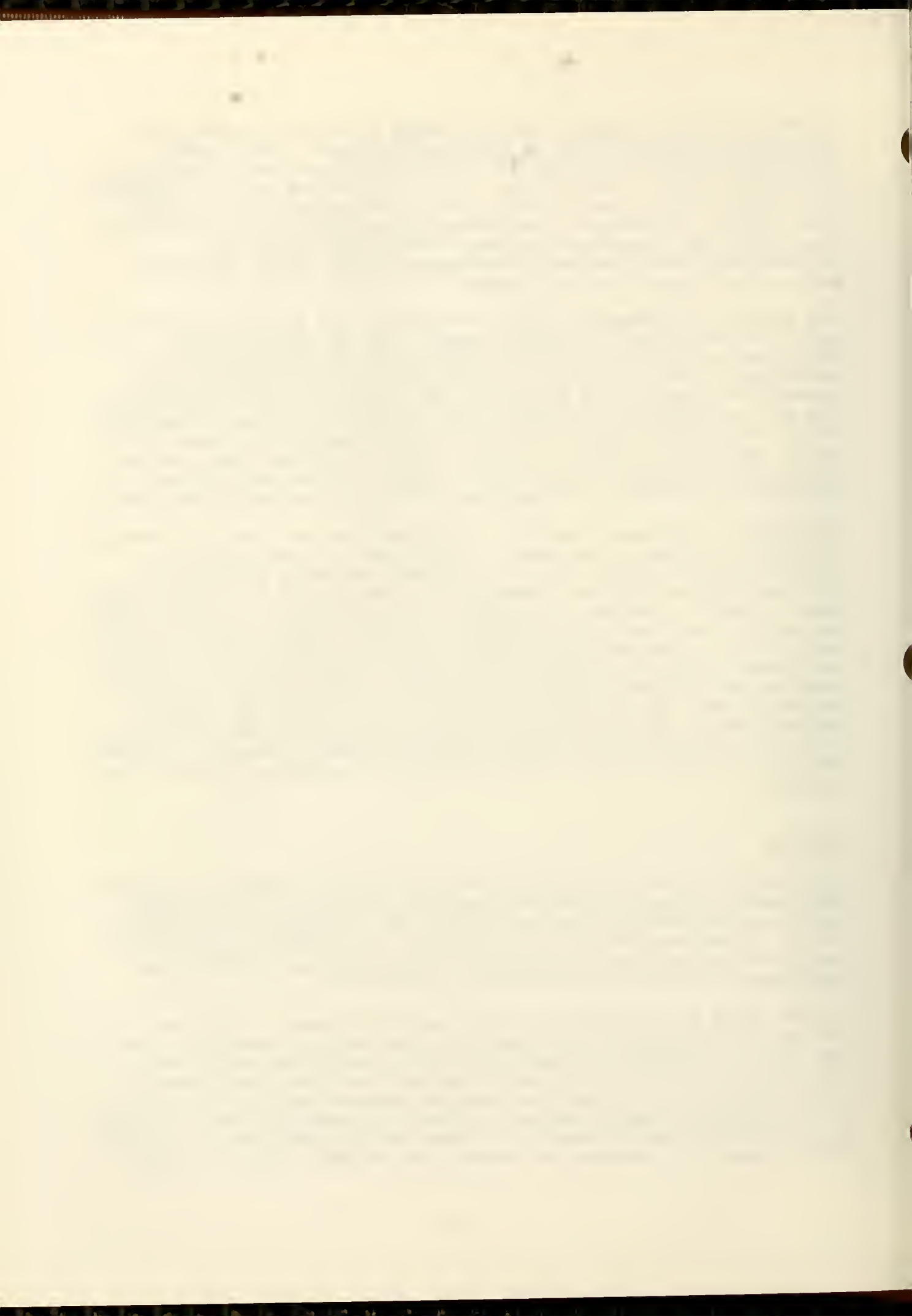
This project is supplementing these clinical data with detailed data from the dental offices attended by the panel members over the past ten years. Utilization and cost data are being collected for approximately 728 panel participants. Data collection is approximately one-third complete and is scheduled for completion in December of 1990. Once these data are collected they will be integrated with the clinical data from the VA Dental Longitudinal Study. They will represent one of the richest databases ever assembled to analyze the relation between oral health measures, treatment needs, and the utilization of specific dental services. Analysis of the data will be undertaken by the NIDR in collaboration with VA staff and expert consultants.

Determinants of Permanent Tooth Loss in the United States--Tooth loss remains an important problem in this country. Understanding its determinants is essential for the identification of individuals and groups at high risk for tooth loss and for effective interventions to reduce tooth loss among persons where it remains a problem. The objective of this project is to measure tooth loss and its determinants in the natural settings of dental offices and larger public clinics. The initial phase of the project is a pilot study to field test and refine a protocol for the full study. Information will be collected regarding the influence of disease/clinical conditions, economic variables, patient/provider characteristics, and attitudes on decisions to extract permanent teeth. The collected data will be used to assess the significance of factors contributing to the loss of permanent teeth in the U.S. With these data, a more complete model of tooth loss than is currently available can be estimated.

Modelling

Branch staff have been investigating the possibility of developing a computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. These forecasts will be developed in considerable sociodemographic detail. An initial study of alternative modelling methods indicates that development of a model is both feasible and desirable.

The next phase of the project will begin model development. Microsimulation will be the approach used. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. It will take approximately two years to develop an operational model. Policy experiments with the full model will be done both for past times and also for future times. As a framework for synthesizing research findings, the NIDR



micro model would provide a vehicle for carrying out experiments in which the latest dental research could be applied consistently and systematically to key dental policy issues.

Other Activities

Branch staff participated in review and training of the dental examiners for the NHANES III study. Training sessions were held for prostheses assessment on December 5, 1988 and on February 28, 1989, and for tooth conditions on March 1, 1989. In addition, the review, training, and calibration of a newly hired dental examiner was held on May 16, 23 and 24. Branch staff participated in the Workshop on Cost-Effectiveness of Caries Preventive Procedures, held May 17-18, 1989 at the University of Michigan.

Several presentations were made at major dental meetings throughout the country. Two papers were read at the annual meeting of the American Association for Dental Research. Papers were also given at the annual meeting of the American Public Health Association in Boston in November, the annual meeting of the Eastern Economic Association in Baltimore, Maryland, and the annual conference of the American Association of Dental School Deans.

Publications

Brown LJ, Meskin LH. Sociodemographic differences in tooth loss patterns in U.S. employed adults and seniors, 1985-1986, *Gerodontology* 1988; 4:345-362.

Brown LJ. The long-run cost characteristics of dental practices in the U.S.A., *Soc Sci and Med* 1989; 29(6):695-703.

Meskin LH, Brown, LJ. Prevalence and patterns of tooth loss in U.S. adult and senior populations, 1985-86, *J Dent Educ* 1989; 52:686-691.

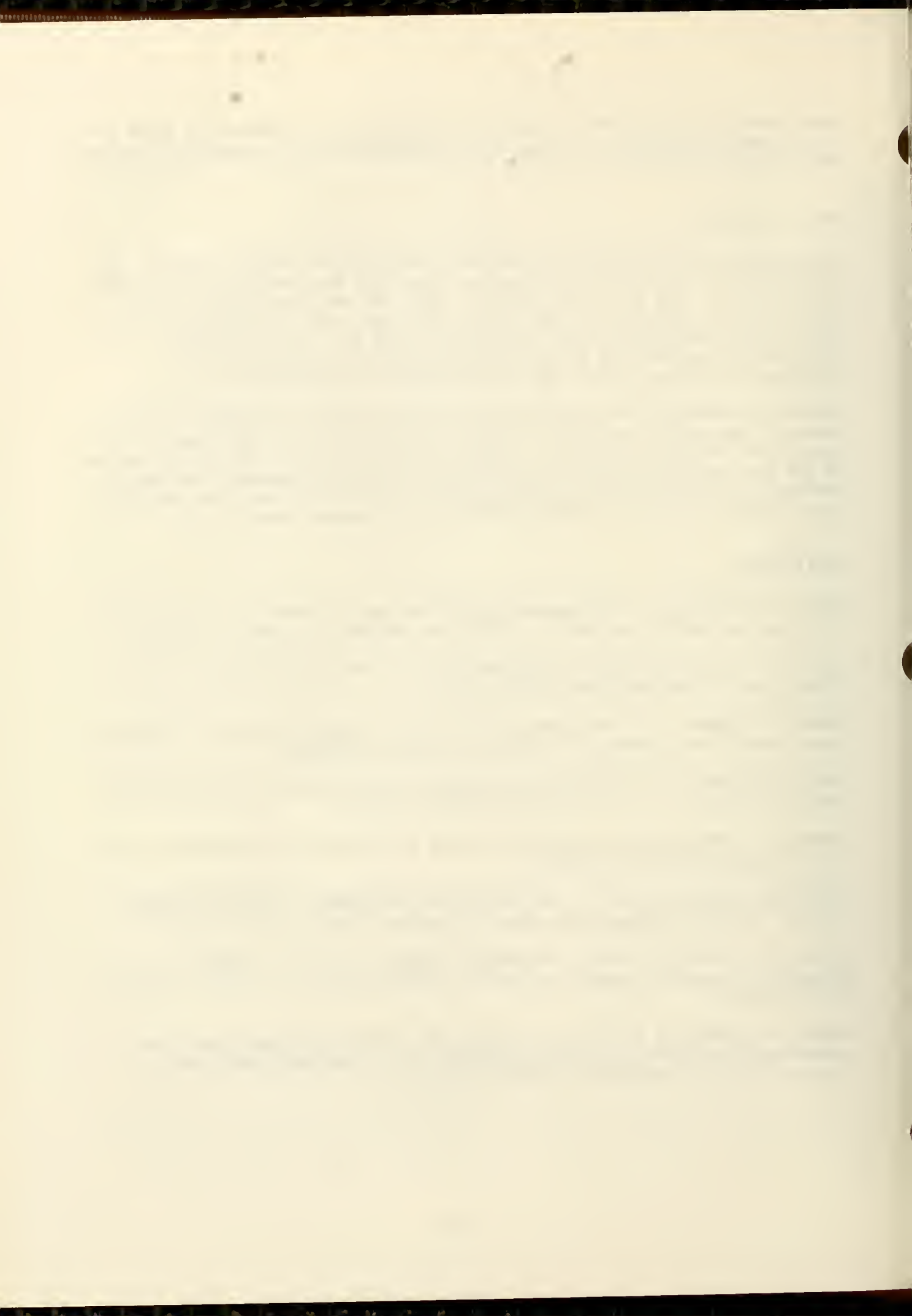
Meskin LH, Brown J. Prevalence and patterns of tooth loss in U.S. adult and senior populations, *Int J Oral Implant* 1988; 5:59-60.

Brown LJ. Contrasting the economic outlook for dentistry and medicine, *Med Pract Management* 1989; 5(1):8-17.

Oliver RC, Brown LJ, Loe, H. An estimate of periodontal treatment needs in the U.S. based on epidemiologic data, *J Periodont* 1989; 60(7):371-380.

Brown LJ, Oliver RC, Loe H. Periodontal diseases in U.S. in 1981: prevalence, severity, extent, and role in tooth mortality, *J Periodont* 1989; 60(7):363-370.

Meskin LH, Brown LJ, Brunelle JA, Warren GB. Patterns of tooth loss and accumulated prosthetic treatment potential in U.S. employed adults and seniors, 1985-86, *Gerodontology* 1988; 4:126-135.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00473-02 ASB

PERIOD COVERED

January 12, 1987 - March 14, 1988

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

Restorative and Treatment Needs in the U.S. in 1981

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

Brown, L. Jackson	Chief	EODPP NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

.20

PROFESSIONAL

.20

OTHER

CHECK APPROPRIATE BOX(ES):

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

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

Treatment needs based on the existence of dental clinical conditions are important determinants along with socioeconomic variables of the demand for dental services and dental manpower. Little information is currently available on the extent of needs in the U.S. This study reports the types and amount of treatment needed by the U.S. population in 1981 and provides an estimate (in 1985 dollars using ADA fees) of the expenditures required to treat those needs. From a household probability survey of 7700 persons in the U.S., information was collected on the condition (sound, filled, missing, etc.) and the needed treatment (filling, crown, extraction, etc.) of each permanent tooth. Information was also collected on type of prostheses (full, partial, fixed) present and needed for each permanent tooth. Selected findings follow: 43 percent of the U.S. population needed 1 or more restorations at the time of examination; 37 percent needed a posterior restoration while only 18 percent needed an anterior restoration; 9 percent needed an extraction; 2 percent needed endodontics; 4 percent needed an anterior crown; and 11 percent needed a posterior crown. The total cost in 1985 dollars to treat these conditions is estimated at slightly over 100 billion dollars. Close to 70 percent of the total is for the placement of new prostheses where none existed at the time of exam. Persons 55 years and older accounted for 30 percent of the cost while persons 35 to 54 accounted for 40 percent.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 DE00475-02 ASB
PERIOD COVERED October 1, 1988 - September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Study of Utilization, Treatment Needs, Cost, and Dental Disease in Veterans		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Brown, L. Jackson	Chief	EODPP, NIDR
Chauncey, Howard M.	Associate chief of staff for R & D	VA
COOPERATING UNITS (# any) Veterans Administration Outpatient Clinic, Boston, Massachusetts		
LAB/BRANCH Analytical Studies Branch		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.1	PROFESSIONAL 0.1	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 (Use standard un-reduced type. Do not exceed the space provided.)		
<p>The Veterans Administration in 1963 initiated an inter-disciplinary and longitudinal investigation of the normal aging process. Participant consisted of 2,400 men with stable living and work conditions in the Boston, Mass. area. From this panel, 1221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The triennial examinations include a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene.</p> <p>This project is supplementing these clinical data with detailed utilization data from the dental offices attended by the panel members over the past ten years. The data collection is about one-third complete. Once these data are collected they will be integrated with the clinical data. The full dataset will be used to analyze the relation between oral health measure, treatment need as derived from those measures and the utilization of specific dental services.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00477-02 ASB

PERIOD COVERED

October 1, 1989 - May 11, 1989

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

Condition of the Dentition in U.S. Adults - 1981

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

Brown, L. Jackson	Chief	EODPP, NIDR
Warren, Galen B.	Dental Research Analyst	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.65

PROFESSIONAL

0.55

OTHER

0.1

CHECK APPROPRIATE BOX(ES)

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

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

In 1981, the United States Public Health Service funded a survey, A Study of Dental Health Outcomes Related to Prepayment. Data were collected on a national probability sample of the U.S. civilian population. Calibrated dental examiners assessed the condition of each of 28 tooth spaces in the permanent dentition in individuals receiving an oral examination utilizing standardized criteria. The percent of all teeth that were sound ranged from 17% in the oldest age category to 67% in the youngest. Molars were least likely to be sound, and most likely to be satisfactorily filled, to need fillings, and to be missing than premolars or the anteriors. The percent of teeth needing a filling varied from three percent to seven percent. The percent of missing teeth increased with age from four percent in the youngest age group to 65% in persons 65 and older. The percent of sound teeth did not vary substantially with education; however, the percent of teeth needing fillings and the percent of missing teeth were negatively correlated with education. Biological factors are critical in the initiating stage of caries while sociodemographic factors may be more important in the treatment that carious teeth receive or that they do not receive. The intent of this project is to prepare a paper for publication.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00491-01 ASB

PERIOD COVERED

October 1, 1988 - September 30, 1989

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

Estimated Savings in Dental Expenditures from Prevention of Disease

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

Brown, L. Jackson	Chief	EODPP, NIDR
Beazoglou, Tryfon	Assistant Professor	Univ. of Conn.-School of Dentistry
Heffley, Dennis	Associate Professor	Univ. of Conn.-Dept. of Economics

COOPERATING UNITS (if any)

University of Connecticut
School of Dental Medicine and Department of Economics

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.4

PROFESSIONAL:

0.4

OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

Real expenditures for dental services have increased very little since 1978. Most traditional demand factors were moving in a direction to increase expenditures during this period. Moreover, the supply of dentists was increasing. To test whether a non-economic change occurred which influenced aggregate dental expenditures, an aggregate demand function was estimated using real per capita expenditures as the dependent variable. The relative price of dental services, real per capita income, percent of the population covered by dental insurance were explanatory variables. Analysis indicates a change in the demand for dental services appears to have occurred in the late 1970s or early 1980s. Expenditures are less than they would have been under similar economic conditions if this structural change had not occurred. Estimated annual savings in expenditures are \$4.7 billion in 1986. Estimated cumulative savings from 1980 to 1986 are \$23.3 billion in 1986 dollars.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 DE00492-01 ASB
PERIOD COVERED October 1, 1988 - September 30, 1989		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) Periodontal Health in Older Americans - A Summary of Sociodemographic Findings		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Brown, L. Jackson	Chief	EODPP, NIDR
Miller-Chisholm, Ann	Epidemiologist	EODPP, NIDR (detailed to NH LBI)
Oldakowski, Richard	Computer Programmer	EODPP, NIDR
COOPERATING UNITS (# any) 		
LAB/BRANCH Analytical Studies Branch		
SECTION 		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS. 0.5	PROFESSIONAL: 0.4	OTHER. 0.1
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 (Use standard unreduced type Do not exceed the space provided.) <p>The study is using data from the 1985 National Survey of Oral Health in U.S. Employed Adults and Seniors conducted by the NIDR. Associations between periodontal health status and selected sociodemographic characteristics among U.S. seniors are being analyzed. Periodontal conditions included in the study are gingival bleeding, gingival recession, loss of attachment and pocket depth. Sociodemographic characteristics are age, gender, race, years of education, household income, dental insurance coverage, and the interval since the person's last visit to a dentist. Preliminary findings are consistent with other regional and national studies of the U.S. and other countries. Generally periodontal conditions are more prevalent and extensive in males than females. Persons who had not visited a dentist in the last 5 years had the most pronounced periodontal destruction.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE00493-01 ASB

PERIOD COVERED

December 1, 1988 - April 1990

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

Periodontal Health of Employed U.S. Adults in 1985

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

Brown, L. Jackson	Chief	EODPP
Oldakowski, Richard	Computer Programmer	EODPP
Oliver, Richard C.	Professor	University of Minn.

COOPERATING UNITS (if any)

University of Minnesota, School of Dentistry

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.4

PROFESSIONAL:

0.35

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

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

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

The study is using data from the 1985 National Survey of Oral Health in U.S. It will describe the prevalence, severity, and extent of the periodontal disease in the employed population between the ages of 18 and 65 years old. Within the limits imposed by different methods, these results will be compared with other contemporary national surveys. Preliminary results indicate that loss of periodontal attachment is frequently found in even the youngest employed person (those between 18 and 24 years of age). Loss of attachment increase in prevalence, severity and extent with age. Periodontal pockets were found in only 14% of the sample usually affecting one or two teeth. Deep periodontal pockets (>6 mm) were found in only 0.5% of the sample. Recession partially accounted for the differences in prevalence, severity and extent of loss of attachment and pockets. Only 6% of the sample had four or more periodontal pockets indicating that they were at high risk for the disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE00494-01 ASB

PERIOD COVERED

March 1, 1989 - July 1, 1990

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

Longitudinal Analysis of Tooth Loss and Other Tooth Conditions

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

Brown, L. Jackson	Chief	EODPP
Oldakowski, Richard	Computer Programmer	EODPP

COOPERATING UNITS (# any)

University of Colorado

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.15

PROFESSIONAL:

0.15

OTHER:

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK (Use standard un-reduced type Do not exceed the space provided.)

Longitudinal data from a panel of male veterans who have been examined every 3 years since 1968 and data from an experimental study of health insurance are being used to analyze the progression of tooth loss and the conditional probability of change in tooth conditions over time. Both data designate each tooth as missing or present. If present, a tooth is designated as sound, carious, or filled. If missing, the space is designated as replaced or not replaced. If replaced, the type of prosthetic replacement is recorded. These data will be analyzed to develop conditional probabilities of a tooth being lost over time. These probabilities will be conditioned on the status of the tooth at the previous examination and other putatively important explanatory variables. The panel of male veterans will allow several longitudinal examinations to be analyzed. The experimental data contains a wide array of potentially important explanatory variables which will allow the impact of economic and social variables on the probabilities to be assessed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00495-01 ASB

PERIOD COVERED

March 1, 1989 - September 30, 1990

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

Association Between Dental Conditions and Utilization of Dental Services

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

Brown, L. Jackson	Chief	EODPP
Oldakowski, Richard	Computer Programmer	EODPP

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS.

0.3

PROFESSIONAL:

0.3

OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

It is now documented that dental caries in persons aged 5-17 years old are declining. These reductions have occurred for a sufficient number of years so several age cohorts of children who experienced reductions in caries are now adults. Some evidence suggests that young adults maintain these caries reductions. Less evidence is available regarding whether or not these caries reductions are maintained throughout life. Reductions in tooth loss also occurred in U.S. adults between 1974 and 1985. This continues a trend which was apparent in the 1960s. Reductions in the percentage of the population experiencing total tooth loss (edentulism) is especially pronounced.

This study is analyzing the impact of these changes in the extent of dental caries on the utilization of dental services. Longitudinal data from the Rand Corporation Health Insurance Experiment will be used to estimate the probability of subjects enrolled in the experiment attending a dental office over different time periods. For those subjects who attended a dental office, a demand function will be estimated to predict the amount of dental expenditures over these same time periods. The prevalence and extent of dental and periodontal conditions will be controlled.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 DE00496-01 ASB

PERIOD COVERED
 May 1, 1989 - September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Determinants of Permanent Tooth Loss Using Connecticut Dental Practice

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

Brown, L. Jackson	Chief	EODPP, NIDR
Beazoglou, Tryfon	Assistant Professor	University of Conn.
Crall, James	Assistant Professor	Universiyt of Conn.

COOPERATING UNITS (if any)
 University of Connecticut

LAB/BRANCH
 Analytical Studies Branch

SECTION

INSTITUTE AND LOCATION
 NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

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 (Use standard un-reduced type. Do not exceed the space provided.)

Notwithstanding significant reduction since the 1960s, tooth loss remains an important problem in the U.S. Its uneven distribution among adults makes this problem more severe for some individuals than the mean values of tooth loss indicate. While the role of disease in tooth loss is important, focusing on which disease-causes the most tooth loss may obscure the complexity of the issue and underemphasize the influence of other factors. The overall objective of this Project is to measure tooth loss and its determinants in the natural settings of operating private dental offices and larger public clinics. The initial phase of the project will be a pilot study which will field test a protocol in a selected number of practices in Connecticut. The information from the pilot study will be used to refine and finalize the data collection procedures and the conceptual model of tooth loss for the full-scale study. Once the final protocol is set, the data to be collected will be used to estimate a more complete model of tooth loss than has been available to date and assess the relative significance of factors contributing to the loss of permanent teeth in the U.S. More specifically, the investigation will provide information regarding the relative influence of disease/clinical conditions, economic variables, and patient/provider characteristics and attitudes on decisions to extract permanent teeth.





**ANNUAL REPORT OF THE DISEASE PREVENTION AND HEALTH PROMOTION BRANCH
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH**

The Disease Prevention and Health Promotion Branch (DPHPB) consists of two sections. Dr. Driscoll serves as Chief, Disease Prevention Section and as Acting Branch Chief. Staff of the Disease Prevention Section included Ms. Nowjack-Raymer (who joined the staff of the Epidemiology Branch in August, 1989) and still includes Ms. Furnia and Mr. Small. Dr. Gift is Section Chief, Health Promotion Section. Staff include Ms. Horowitz, Mr. Zindel, and an open position. The Branch also includes a detail from the Health Resources and Services Administration (HRSA), Dr. Selwitz; and from the Centers for Disease Control, Dr. Corbin.

The Disease Prevention Section (1) plans and directs clinical trials, field studies and demonstrations of the effectiveness, feasibility and cost-effectiveness of accepted or new agents and procedures for the prevention of oral diseases; (2) provides consultation, technical assistance, scientific issue resolution and other assistance to communities, organizations, institutions and individuals with regard to the efficacy, implementation and continuation of various oral disease prevention procedures and programs; (3) and participates in collaborative research efforts with other Institute staff members.

The Health Promotion Section (1) promotes the use of established effective preventive and therapeutic procedures by both the public and health professionals; (2) promotes the adoption of behaviors that are conducive to the improvement of oral health; (3) conducts research to identify and evaluate new materials and strategies for promoting oral health and to evaluate and improve existing materials and strategies; (4) and participates in collaborative research and promotional efforts with other Institute staff members.

Disease Prevention Section

Community water fluoridation remains the most efficient and cost-effective method for preventing dental caries. The Section has continued to provide technical information support of fluoridation initiatives and to coordinate the resolution of issues about fluorides and health that are based on technical or judicial questions. Staff have reviewed scientific and administrative documents concerning fluorides and health as requested by several Federal or state agencies and professional organizations and publishers. National progress was highlighted by the passage of mandatory fluoridation by the Pennsylvania House (Senate still to vote), an overall majority of "yes" results in the 1988 referenda on fluoridation, and significant increases in coverage of persons on public water supplies (Florida topped 64%; Georgia more than 90%; Texas, Arizona, Massachusetts, Wisconsin moved up significantly).

Research continues in communities which lack the benefits of optimal fluoride concentrations in the water system. One such study, initiated in 1983 in Nelson County, Virginia, investigated the use of certain combinations of fluoride procedures in conjunction with dental sealant therapy; fluorides are

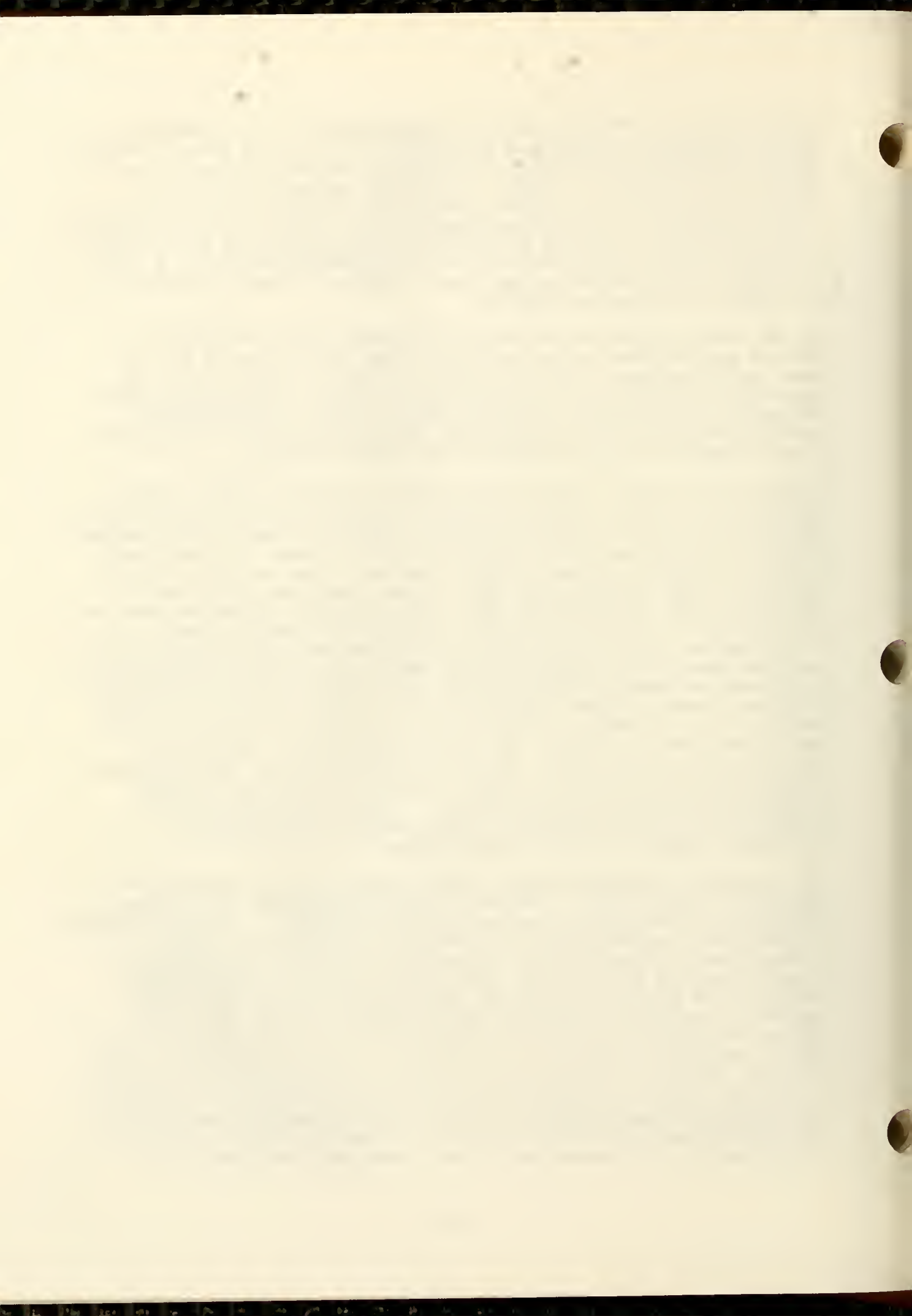


most effective in inhibiting decay in smooth surfaces of teeth, whereas sealants have proven effective in preventing pit and fissure decay. The most immediate challenge is to improve the methods of delivery of fluoride and sealants in ways that will enhance effectiveness and permit more cost-effective application to a greater proportion of the population at risk. The fluoride procedures, which had been ongoing for 11 years, consisted of weekly rinsing in school with a 0.2% NaF solution, daily ingestion in school of a 1 mg fluoride chewable tablet and home use of a fluoride dentifrice. A final evaluation of these procedures had shown a reduction in caries prevalence of 65% compared with baseline findings.

For the sealant procedure, newly erupted permanent teeth of children of selected ages received sealant therapy each year for a period of four years. Final results obtained in the fall of 1987, along with interim data, are currently being analyzed. In addition to caries data, information has been obtained on a variety of factors that influence cost, including sealant retention, number of teeth treated, time required to carry out the procedure, salary of dental health professionals, and cost of materials.

Although the anticaries effects of the combined fluoride regimen in Nelson County had been greater than those usually reported for any of the individual components, no certain claims of additive effectiveness could be made because all children in Nelson County received the entire regimen. To specifically compare the combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone, a long-term clinical trial was initiated in 1981 in Springfield, Ohio. Study subjects at baseline comprised 1,640 subjects in the first and second grades of 20 elementary schools. Interim two-year findings showed that the combined procedure produced a 33% lower increment in dmfs (primary teeth), and five-year interim findings showed a 31% lower increment in DMFS (permanent teeth) compared with the fluoride mouthrinse procedure alone, which may itself have had an effect in lowering dental caries. The tablet procedure alone also showed a lower caries score than the mouthrinse procedure, but the difference was not statistically significant. Interim results, therefore, indicate that the combined fluoride treatment does provide additive benefit beyond that which may have been derived from fluoride mouthrinsing alone. Results from final treatment examinations conducted in May 1989, should provide more definitive relationships among the three treatment groups.

The reduction of gingivitis remains the best approach to the promotion of periodontal health to date. The use of effective mechanical oral hygiene measures can achieve the greatest reduction in gingivitis. However, a number of studies have indicated that it is difficult to motivate children to maintain good oral hygiene over long periods of time when the emphasis is placed on removing dental plaque. Additional research has suggested that elimination of gingival bleeding may produce better results than plaque control as a motivational tool for long term maintenance of optimal oral hygiene. To test this hypothesis, a two-year clinical trial was initiated in 1987 in York County, Virginia, in two groups of ninth and tenth grade school children. Children in one group received instruction for the self-assessment of gingival bleeding, whereas those in another group received the traditional instructions in plaque control. Children in both groups were supplied with soft toothbrushes, wooden toothsticks for interdental cleaning, and detailed instructions in their proper use. An oral prophylaxis was given to all



available participants at the end of the first year. Follow-up examinations will determine if any differences can be shown between the groups in their periodontal health. If the expected benefit in favor of the bleeding approach can be verified and sustained, the results have implications nationally for oral health education programs. Results are being analyzed.

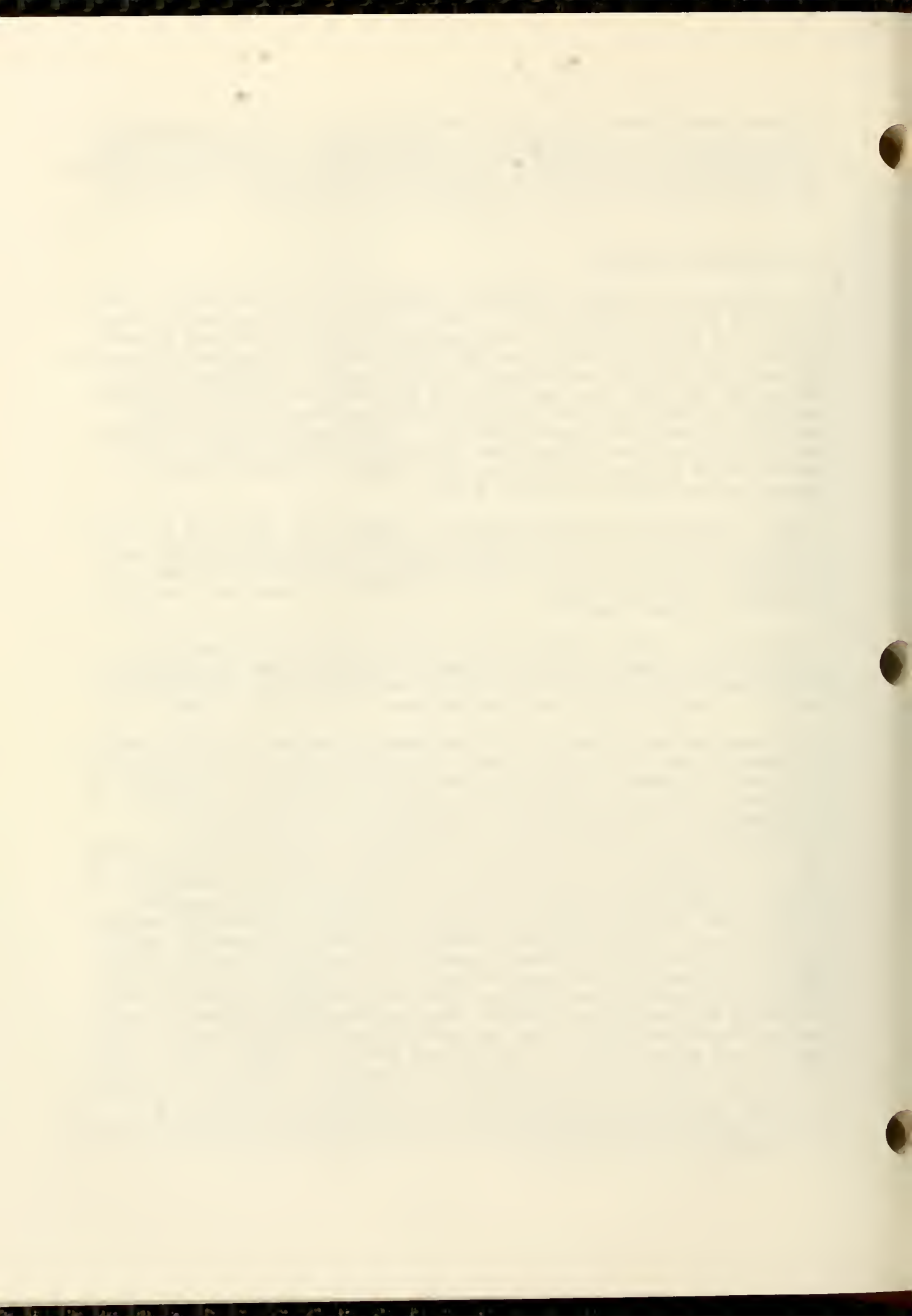
Health Promotion Section

As a follow-up to a study initiated to inventory all existing national oral health and dental databases and to assess their analytical potential, several professional services contracts were awarded to develop models and conduct analyses. Topics of contracts include the following: application of secondary data analyses to policy and research, synthetic cohort approaches for the forecasting of tooth loss, description of behavioral and biological factors important to tooth loss, and lifestyle and socioeconomic factors in oral health status and routine use of services. Analytical models and secondary data analyses have been accomplished as a result of these projects. The administration of these projects was split between the Health Promotion Section and the Analytical Studies Branch.

A study initiated and supported through a purchase order mechanism to inventory existing oral health education and promotion activities for adults was completed, and evaluation of the material and activities has been performed. A summary of the oral health programs and educational materials for adults has been prepared.

Staff have been active in monitoring the field phase of the dental and orofacial pain components of the household interview of the 1989 National Health Interview Survey as well as that of the International Collaborative Study, II. Analysis plans are being developed as activities in 1990.

Staff have continued to be active in developing a research and action program for improving the oral health of adults and older Americans. The Section staff were instrumental in preparing the report to Congress. Using existing data sources, major oral health issues and key target groups have been developed based on observed needs. Individual, professional, and environmental interventions have been identified. Staff have been analyzing national data bases to describe dentally related attitudes and behaviors, as well as sociodemographic variables, in relation to oral health measures in an attempt to improve the targeting potential of the oral health promotion program. The specific emphasis continues to be the acceleration of reduction in tooth loss. NIDR has initiated meetings with other governmental agencies and private sector organizations to expedite the implementation of the plan. Meetings have been held with agencies such as the Centers for Disease Control (CDC), Health Resources and Services Administration (HRSA), National Center for Health Services Research (NCHSR), American Association of Dental Schools, American Dental Association, and the American Fund for Dental Health. Other agencies are being contacted. Both CDC and HRSA have assigned staff to work actively with NIDR in the initiation of this program. Efforts are proceeding to make this a PHS-wide activity involving a chartered interagency task force. Also, the effort has been discussed at national meetings, including the American Association for Dental Research and the American Association of State and Territorial Dental Directors.



As part of NIDR's contributions to the National Institute on Aging for the support of the Institute of Medicine's (IOM) "Health Promotion in the Second Fifty" project, staff have prepared a draft of the chapter on oral dysfunction and participated actively in three committee meetings.

One staff member is the lead NIH coordinator for developing the oral health objectives for the Year 2000, and other staff have been active in this process. A working group met, and identified a draft set of objectives and supporting evidence. The drafts of these objectives and supporting documentation were reviewed by the working group and nominated panel of expert reviewers. In total, over 50 reviewers commented on the objectives and the justifications. The revised document was submitted to NIH, CDC and the Office of the Assistant Secretary. The objectives from all of the areas are being made available for public comment. The American Association of Public Health Dentistry has agreed to publish the oral health document.

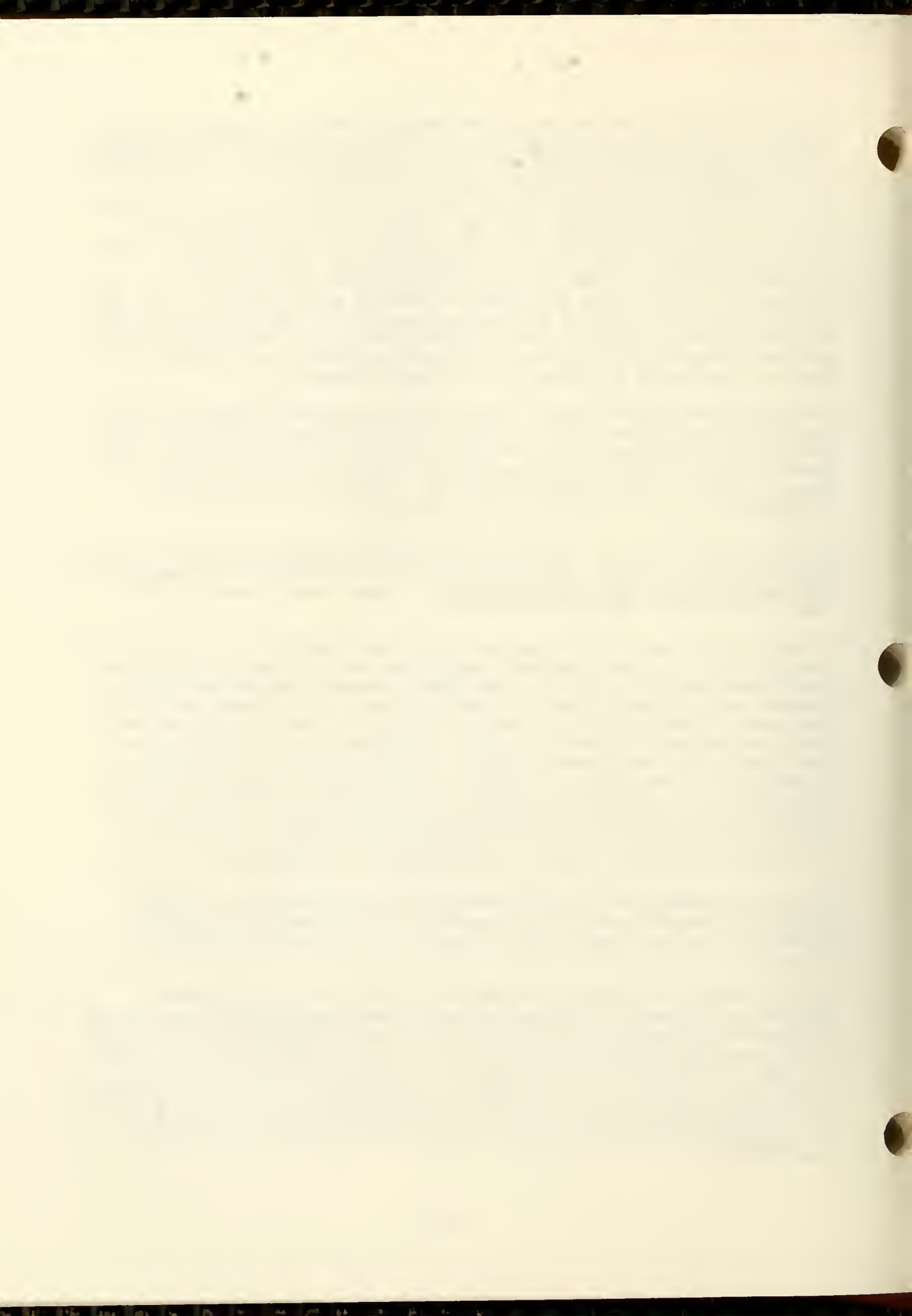
Staff prepared a paper "Social, Economic and Professional Dimensions of the Oral Health Care Delivery System" which was one of five contributions at the Third National Conference on Behavioral Dentistry held in San Antonio in May. Staff also participated in this conference to identify future research opportunities. It is anticipated that the issue papers and conference recommendations will be published in a monograph.

Staff attended a workshop sponsored by the American Dental Society of Anesthesiology as part of the preparation for the publication of Management of Pain and Anxiety in Dental Practice which includes a contributed chapter "Rationale for Pain and Anxiety Control".

Science transfer activity is ongoing. Staff participated in a health fair for the Senate staff. Staff provided consultation and assistance to health and educational agencies in the U.S. and abroad concerning prevention of oral diseases and oral health education and promotion. In collaboration with the Department of Veterans Affairs, Centers for Disease Control, Food and Drug Administration and the American Dental Association, staff assisted in the development of an educational package, "Infection Control in the Dental Environment." The program is self-paced and consists of three units using videotapes with accompanying workbooks, self-assessment checklists, lists of resources and a post-test for continuing education credit. Titles of the three units are: (1) Principles and Fundamentals of Infection Control; (2) Clinical Procedures; and (3) Sterilization and Disinfection.

The NIDR is sending one of these packages to each dental school, dental hygiene program and dental assisting program. Other cosponsors of this project are sending single copies to dental societies and state dental directors.

A staff member worked through the American Association of Public Health Dentistry's Oral Health Committee to develop recommendations for teaching how to prescribe dietary fluoride supplements. Staff, in collaboration with OPEC staff, developed clearance materials for a new educational leaflet on dental sealants. The leaflet will be prepared in English and Spanish. Staff continues to supervise the distribution of the program's educational exhibits, films and printed materials. The educational materials are used in school and other community-based activities by state and local health and education departments.



The film, Prescription for Periodontal Health, has been updated.

Other DPHPB Activities

Staff prepared two oral health issue papers, "Factors Contributing to Maternal and Child Oral Health", and "Oral Health Education and Promotion in Maternal and Child Health" for the National Workshop on Maternal and Child Oral Health. It is anticipated that these papers and others commissioned for the workshop will be published in a monograph.

Staff have been active in numerous NIDR committees and working groups. One staff person is a member of the advisory group of the NIH/NIDR contract (86-3309), "Assessment of NIH, Industry, and Academic Relationships in Restorative Materials Research", providing expertise in survey instrument design and data analysis. One staff person served as co-chair of the "Panel on Epidemiology, Science Transfer, Oral Health Promotion/Disease Prevention" as part of the Long Range Planning activities, and assisted in the preparation of the Panel's report, while other staff actively participated in this effort.

Staff have also been active in working with other Institutes and Agencies, providing health promotion and research expertise. A staff member continued to serve on, and was reappointed to, the ADA's National Advisory Committee on Fluoridation. Staff are working with the National Institute on Aging (NIA) on an Institute of Medicine project "Health Promotion and the Second Fifty". One staff member is participating as a member of a planning group for a 1989 meeting on oral health initiatives in maternal and child health, a HRSA Maternal and Child Health program; serving on an NCI working group on tobacco cessation strategies; and working on organizing evaluation of scientific programs and membership services for the Federation Dentaire Internationale (FDI). Also, staff serve on several committees of the FDI: Oral Health Promotion Working Group, Marketing Working Group, Health, Scientific Program Committee, and the Periodontal Health Services Working Groups. Several staff have consulted with universities and health departments regarding education, promotion and preventive activities. Several staff members participated in training for computer use and statistical applications. Staff have served as reviewers for manuscripts submitted for publication to numerous journals, including the Journal of Dental Research, Social Science and Medicine, Health Education Quarterly, Journal of Public Health Dentistry, Community Dentistry and Oral Epidemiology, and Advances in Dental Research.

Staff also are active in the broader NIH community. One staff member is NIDR's representative to the NIH Women's Health Committee, the BID Prevention Coordinator's Committee, an NIH committee to plan the Christopher Columbus Quincentennial Conference on Aging, and is President of the R&W. Other staff members serve as NIDR's representatives on such bodies as the Coordinating Committee on Assessment and Transfer of Technology (CCATT); National Center for Nursing Research, and Task Force on Nursing Research.



Publications

Driscoll WS, Nowjack-Raymer R, Heifetz SB, Li SH, Selwitz RH. Evaluation of the comparative effectiveness of fluoride mouthrinsing, fluoride tablets and both procedures in combination: interim findings after five years, J Pub Health Dent. In press.

Horowitz AM, Frazier PJ. Oral health education and promotion in maternal and child health, Background Issue Paper for the 1989 National Maternal and Child Oral Health Workshop. September 1989.

Kleinman DV, Horowitz AM, Mirth DB. Dental technology assessment in the U.S.: an overview for the practitioner. In: Hardin, JF ed. Clark's Clinical Dentistry. Philadelphia: JB Lippincott, in press.

Nowjack-Raymer R, Gift HC. Factors contributing to maternal and child oral health, Background Issue Paper for the 1989 National Maternal and Child Oral Health Workshop. September 1989.

Russell BA, Horowitz AM, Frazier PJ. School-based preventive regimens and oral health knowledge and practices of sixth graders, J Pub Health Dent 1989; 49:Fall, in press.

Verrusio, A.C., Neidle, E.A., Nash KD, Silverman S, Horowitz AM, Wagner KS. The dentist and infectious diseases: a national survey of attitudes and behavior. J Am Dent Assoc 1989;118:553-562.

Presentations

Gift HC. "The application of the model of determinants of oral health status in oral health promotion programs," Paper presented at the 116th Annual Meeting of the American Public Health Association, November 1988.

Gift HC. The National Institutes of Health health promotion models: application to oral health. Paper presented at the Association of State and Territorial Dental Directors Meeting, March, 1989.

Gift HC. Social, economic and professional dimensions of the oral health care delivery system. Paper presented at the Third National Conference of Behavioral Dentistry, May, 1989.

Gift HC. Oral health promotion in the elderly. Presentation for Lunch and Learn, AADR, March, 1989.

Horowitz AM. Oral health education: policies for the future. First Jerusalem Health Exposition, Jerusalem, Israel, October, 1988.

Frazier PJ, Horowitz AM. Seminar on maximizing consensus development conferences: dental sealants, CCATT meeting, November 18, 1988.

Horowitz AM. Current concepts of preventing dental caries. Seminar for residents and faculty, Department of Pediatric Dentistry, Children's Hospital, Washington, D.C., February 3, 1989.



Horowitz AM. Strategies for effective caries prevention: the role of the dental hygienist. IDHA 60th Annual Mid-Winter Meeting, Chicago, Ill. February 19, 1989.

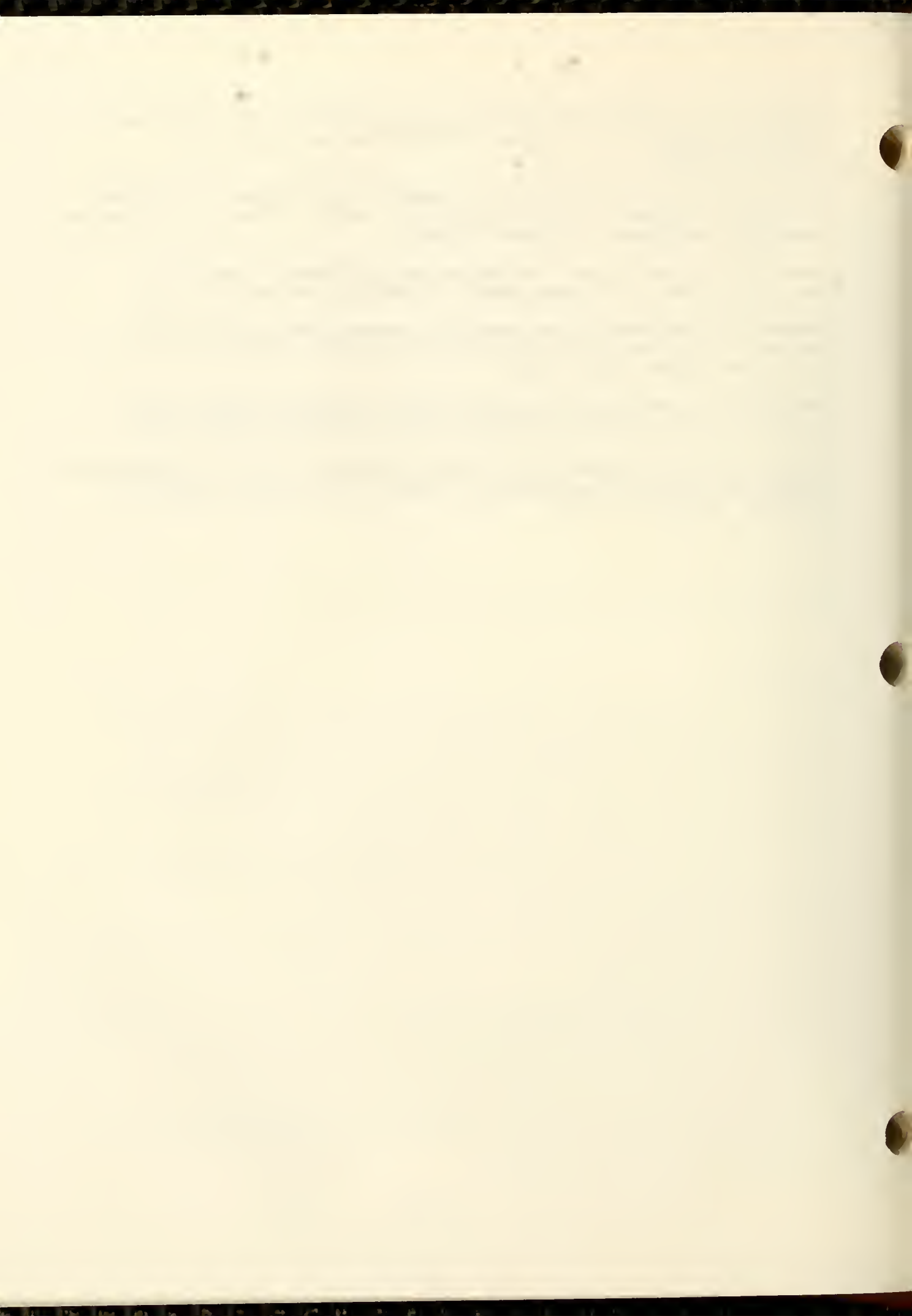
Horowitz AM. The use of fluorides in community-based programs. Georgetown University, Department of Operative Dentistry, School of Dentistry, Georgetown University, Washington, D.C. March 1, 1989.

Horowitz AM. School-based fluoride programs. Department of Community Dentistry. University of Pennsylvania, Philadelphia, PA, March 2, 1989.

Horowitz AM. Implementing and maintaining community-based oral health programs. Department of Preventive Sciences, School of Dentistry, University of Michigan. March 6 and 8, 1989.

Horowitz AM. Community-based sealant programs. Statewide Conference for Pennsylvania's School Dental Hygienists. State College, PA. May 3, 1989.

Horowitz AM. Faculty member of a continuing education course on epidemiologic studies and field trials. Intercountry Center for Oral Health, Chiang Mai, Thailand, August 7-19, 1989.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00070-17
DPHP

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)
Combined self-applied fluorides and sealants for caries prevention

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

Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Selwitz, Robert	Disease Prevention Research Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Disease Prevention and Health Promotion Branch

SECTION
Disease Prevention Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS .69	PROFESSIONAL: .63	OTHER: .06
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CHECK APPROPRIATE BOX(ES)

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

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

A self-administered dental health program was initiated in Nelson County, Va., a fluoride-deficient community in October 1972. Children in the County's schools, under teacher supervision, chewed and ingested daily a 1 mg F tablet and rinsed weekly with a 0.2% NaF solution. A fluoride dentifrice was provided for ad libitum use at home. Baseline DMFS examinations were made of 2,138 children in the County's elementary (grades 1-6), junior (grades 7 and 8), and senior (grades 9-12) high schools. Follow-up DMFS examinations were conducted at two-to-three year intervals until 1983 when the full effectiveness of the program was achieved.

In the fall of 1983, a sealant program was added to the ongoing fluoride program. Children who were 6, 7, 12, and 13 were eligible to have pit-and-fissure sealants applied. An initial screening to identify those tooth surfaces to be sealed was made in December 1983. Caries data (DMFS) from the September 1983 dental examination will serve as a baseline for those children who participate in the sealant phase of the study. In succeeding years, new groups of 6 and 12 year olds have been enrolled. Treatments have continued for four years. Interim dental examinations took place at the start of the third year of the study (September 1985) and final examinations were made in September 1987. Following data analysis final reports will be prepared.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 DE00310-02 DPHP
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institution)		
Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Selwitz, Robert	Disease Prevention Research Specialist	EODPP, NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Disease Prevention and Health Promotion Branch		
SECTION Disease Prevention Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.01	PROFESSIONAL .73	OTHER .28
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 (Use standard unreduced type. Do not exceed the space provided.)		
<p>In September 1981, a study designed to compare a combined regimen of weekly fluoride rinsing and daily fluoride tablets with each procedure used alone was begun in Springfield, Ohio, a fluoride-deficient community. The approximately 1700 children attending 20 public and non-public elementary schools were randomly assigned to one of three treatment groups. The children in Group I dissolved and ingested daily a 1 mg F tablet; the children in Group III rinsed weekly with a 0.02% NaF solution; and Group II carried out both procedures. The assigned treatments were self-administered under the supervision of teachers who received in-service training.</p> <p>Before the procedures were started, baseline examinations were conducted. First and second follow-up dental examinations were conducted in October 1983 and November 1986 respectively. Final treatment examinations were conducted in May 1989, data from this exam are being analyzed and reports prepared.</p> <p>Three to four years following the cessation of treatment, a post-treatment examination will be conducted in high school to determine the extent of continued protection.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00439-03
DHP

PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) An evaluation of different approaches to prevent gingivitis in teenage children

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

Nowjack-Raymer, Ruth E.	Clinical Trials Specialist	EODPP, NIDR
Driscoll, William S.	Acting Chief, (DHPB)	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.60

PROFESSIONAL:

.40

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

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

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

A study to evaluate two approaches to the prevention of gingivitis in teenage children was begun in York County, Virginia in April 1987. Baseline examinations for periodontal health, DMFS and gingival recession were conducted on 500 ninth and tenth graders. Questionnaires regarding oral hygiene methods and professional care practices were completed. Following the examinations, subjects were randomly assigned by grade to either a positive control or test group.

The control group received a manual for the self-assessment and control of plaque and the test group received a manual for the self-assessment and control of gingival bleeding. Small group and individual sessions for instruction in the self-assessment procedures were held to ensure that all procedures were understood.

Examinations of periodontal health were conducted in October and April of FY'88 and FY'89. In May 1988 each participant was given the opportunity to have an oral prophylaxis and all participants received individual instruction to reinforce the appropriate group methods. DMFS and gingival recession examination was repeated at the final examination in April 1989. Following data analysis, final reports will be prepared.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00444-03
DHP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Oral Health Attitudes and Dentally Related Behaviors

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

Gift, Helen Sociologist EODPP, NIDR

Oldakowski, Richard Computer Programmer ASB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda,

TOTAL MAN-YEARS.

.11

PROFESSIONAL

.10

OTHER.

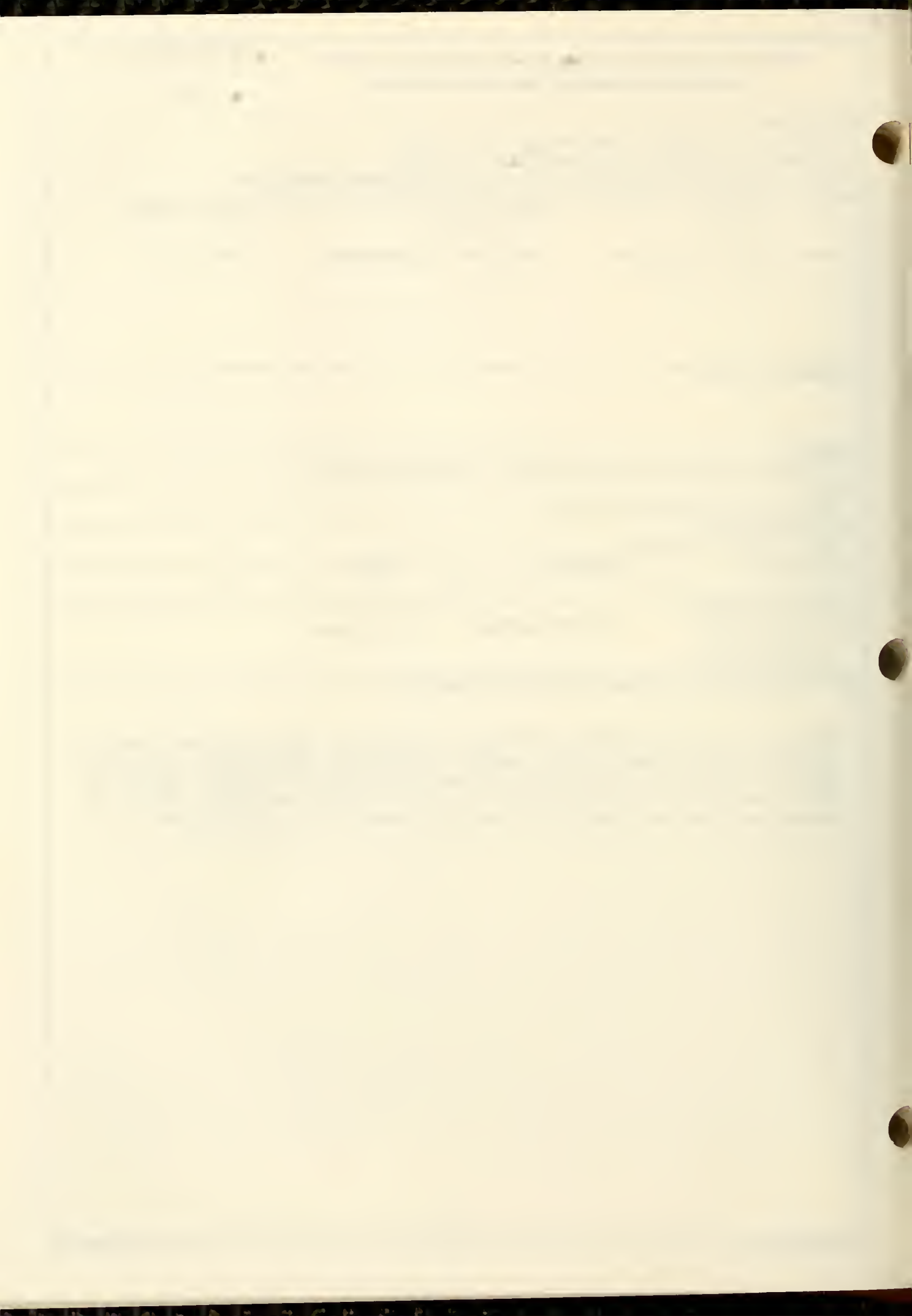
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CHECK APPROPRIATE BOX(ES)

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

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

The study uses existing survey and interview data to describe and analyze the associations among attitudes, behaviors and oral health. The data analyses are used in the oral health promotion plan, papers and publications in preparation and will assist in understanding and improving the oral health of individuals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00453-03
DHPH

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of Existing Oral Health Data on National Surveys

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

Gift, Helen	Sociologist	EODPP, NIDR
Oldakowski, R.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Disease Promotion and Health Promotion Branch, EODPP

SECTION
Health Promotion Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS .11	PROFESSIONAL .10	OTHER: .01
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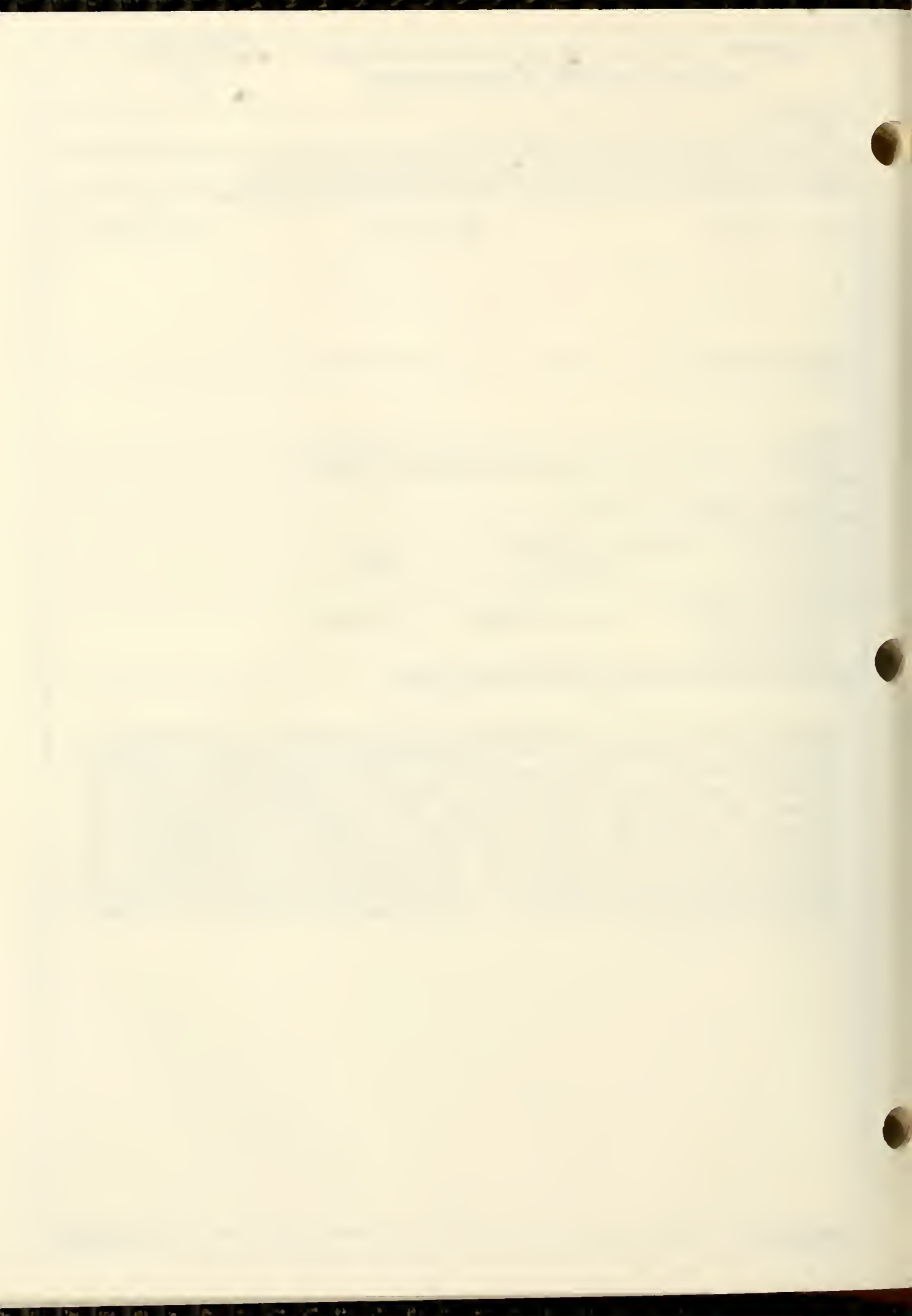
CHECK APPROPRIATE BOX(ES)

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

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

The potential value of existing data sources for further analyses has been investigated utilizing a purchase order mechanism. National dental survey and oral clinical exams conducted over the past 30 years were identified and contacts were made with sponsors and principal investigators to determine the status of data files. Reviews of publications and other mechanisms were used to determine the appropriateness of additional work, with emphasis on possible trend analysis and interpretation for public policy. Professional services contracts have been issued to several scientists to develop analytic frameworks or collaborate in data analyses and publications.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-DE-00487-01
DPHP

PERIOD COVERED

October 1, 1988 to September 30, 1989

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

Oral Problems for the Second Fifty

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

Gift, Helen

Sociologist

EODPP, NIDR

COOPERATING UNITS (if any)

Institute of Medicine

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

.10

PROFESSIONAL:

.10

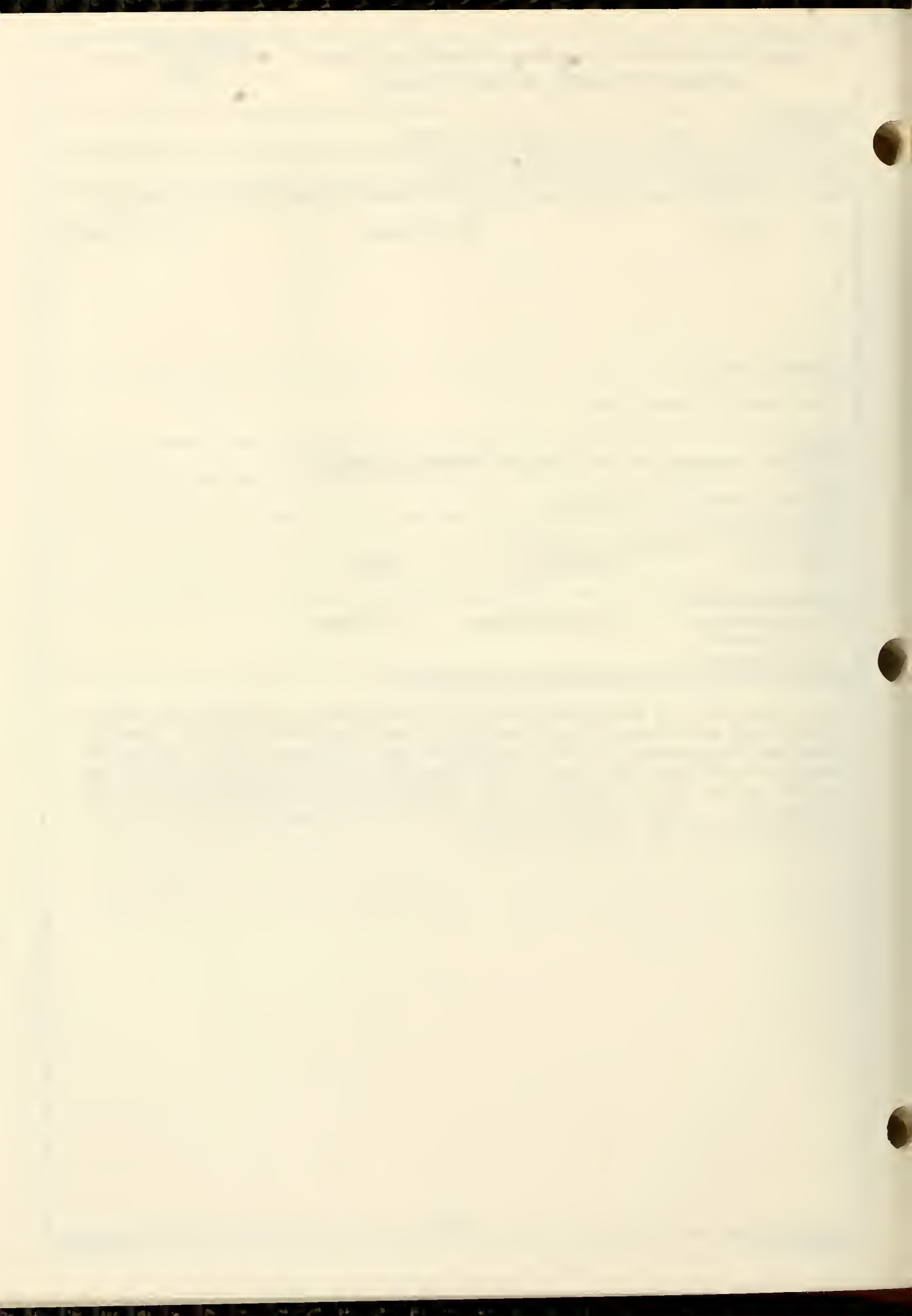
OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The Institute of Medicine (IOM) has undertaken a project on Health Promotion and Disability Prevention for the Second Fifty. One of the ten components is oral problems for this age group. NIDR has contributed funds to support the committee activities and provided staff to do data analyses and prepare the issue paper for the committee. The review paper has been prepared and is being considered by the IOM committee.



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INDEXES

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76158
32

4





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