National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1987

U.S. DEPARTMENT OF HEALTH
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Introduction

This fiscal year was a time of great excitement in the dental sciences. Survey findings released in 1987 on the oral health of US adults were testament to the remarkable gains achieved over the past decades. Edentulousness, for example, almost has been eliminated in middle-aged adults; caries is on the decline among young people; and Americans, in general, are enjoying a high level of dental care. This overall picture of continuing improvement in U.S. oral health status is clearly the result of the combined efforts of scientists, practitioners and the public.

FY1987, however, also held challenge. Special oral health needs of an increasingly older population, the rising prevalence of AIDS and its oral complications, and the continuing search for better ways to treat--and ultimately prevent--the full range of oral health problems tested the investigative talents of NIDR's intramural and extramural researchers. Although much was accomplished, it is the nature of science to answer each achievement with a new challenge. The progress made in FY1987 will point the way to new research directions in the coming year.

REPORT OF THE DIRECTOR

Research Directions

In FY1987, the Director shaped and guided NIDR research planning and program objectives through a variety of administrative accomplishments. Prominent among these are:

Defining and developing NIDR program strategies to stimulate research initiatives in the following areas: AIDS, geriatrics (in collaboration with the National Institute on Aging and Veterans Administration), oral epidemiology of U.S. employed adults and seniors, and oral health promotion for targeted populations.

Initiation of major program evaluations to determine effectiveness of NIDR research investment in an international context, to assess the relationship of investment in biomaterials to private sector development, and to examine further the nation's dental research training and manpower development capacity.

Continuation of the development of a 10-year research plan, including a collaborative effort with the American Association of Dental Research (AADR) on a series of papers addressing anticipated research developments and their impact on the practice of dentistry.

Research and Training Support

The Director initiated a range of interagency collaborative efforts in AIDS research, including a study of the natural history of HIV infection, a program of nationwide monitoring of oral health problems of AIDS patients in Veterans Administration hospitals and treatments received, and the development of professional educational materials on infection control in the dental setting.

In other activities, the Director oversaw the development of RFAs (requests for applications) for specialized research centers on periodontal diseases, caries, and oral health in aging; and a RFP (request for proposals) to evaluate the safety and efficacy of intravenous premedication regimens in dental patients. The development of a clinical protocol for a major two-year study of periodontal diseases in older adults also was completed.

The Director supported the establishment of an ad hoc panel to determine the most effective use of National Research Service Awards in an effort to avert shortages in dental research personnel, and fostered the planning of two consensus development conferences on implants and oral health complications of head and neck cancer treatment.

The Director also initiated and participated in an NIDR Interdisciplinary Conference on Diagnostic and Therapeutic Technology in Dentistry, NIDR/AIDS Task Force, NIDR centennial symposium on "Oral Biology--Biomedicine: Mutual Research Objectives," the Institute's history of dental research project, and the annual Seymour J. Kreshover Lecture on advances in research on tooth enamel genes. As senior investigator, the Director continued his participation in an ongoing longitudinal study of the natural history of periodontal diseases in male populations in Norway and Sri Lanka.

Organizational Activities

In FY1987, the Director filled two key positions in the Institute. Dr. Preston A. Littleton, Jr. was named Deputy Director, NIDR. This appointment will enable increased delegation of line responsibilities, and will strengthen both the Institute's representation to the extramural community and internal decision making. A new Deputy Director for the Epidemiology and Oral Disease Prevention Program, Dr. Thomas F. Drury, was also selected.

During this fiscal year, the Director strengthened NIDR commitment to AIDS research by initiating plans for a new Oral Ecology and AIDS intramural program, and guided the review and development of a plan to renovate the NIDR research building. The Director also supported the 1987 NIH Combined Federal Campaign through NIDR's role as the lead Institute, and continued implementation of the Operational Goals Project to increase NIDR effectiveness and to enhance the physical and interpersonal working environment.

Employee Opportunity

The Director reaffirmed the Institute's commitment to equal employment opportunity through a variety of activities and programs throughout the fiscal year. The Director fully supported the EEO Advisory Committee's career enhancement activities, which led to increased NIDR applications to NIH training programs. The Director's encouragement of the recruiting of minorities, women and handicapped individuals for summer programs resulted in an employment roster of 53 percent minority and 46 percent women in the Undergraduate Program, and 36 and 49 percent respectively in the Graduate Program. Three MARC (Minority Access to Research Careers) students were also employed for the summer in the Institute's research laboratories.

In other activities, the Director supported NIDR participation in a major two-day symposium at Meharry Medical College in Tennessee designed to attract minority students to careers in biomedical sciences; supplied minority schools with relevant scientific materials; and facilitated the loan of dental equipment to Meharry Dental School. During this fiscal year, the Director also fostered the publication of "NIDR Guide to Personnel and Employment Information" and "Opportunities for Minorities in Research," and nominated women and minority representatives to NIDR's advisory bodies.

Professional and Public Communications

The Director presented preliminary findings of the "National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986," at major professional meetings throughout the fiscal year. In related activities, the Director initiated planning strategies for a national health promotion program to publicize improvements in the oral health of the U.S. public and to ensure that these gains will be maintained; and directed the development of monographs detailing national and regional data and analyses of the survey.

The Director provided numerous personal interviews on a variety of subjects in the dental sciences to major broadcast and print media, including the Washington Post, USA Today, Sell, Atlanta Constitution, US News and World Report, Newsday, Wall Street Journal, Associated Press and United Press International. Information presented by the Director on the status of adult

oral health was covered on national radio, the Today Show, Cable News Network, NBC affiliate in Chicago and the CBS Washington affiliate.

The Director pursued the expansion of the "NIDR Online" system to enhance the dissemination of dental research information to the scientific community, and maintained links with the international research community through presentations to professional groups in Europe and the Far East.

Honors and Awards

During FY1987, the Director received the following honors from the academic community:

Alpha Omega International Dental Fraternity Achievement Medal Award,

Honorary Doctorate, University of Medicine and Dentistry of New Jersey,

Honorary Professorship, Beijing Medical University, Beijing, China.

PRESENTATIONS

NIDR Interdisciplinary Conference on Diagnostic and Therapeutic Technology in Dentistry, Concluding Remarks, Baltimore.

American Association for Dental Research/National Affairs Committee Meeting, Remarks, Washington, D.C.

AADR/Washington Section, "Future of Dental Research," Bethesda.

Institute of Medicine Annual Meeting, Remarks, Washington, D.C.

NIH Centennial: Opening Ceremonies.

International College of Dentists Convocation, "The Responsibility of the Professional to Science," Miami.

American Dental Association, Adult Survey Data Presentation: Opening Remarks, press conference.

Karolinska Institute, Symposium: Oral Health During the Nineties, "Future Research on Oral Diseases," Stockholm, Sweden.

Kanagawa Dental College, "The Impact of Dental Research on Dental Education and Practice," Kanagawa, Japan.

Sunstar International Symposium on Periodontal Disease, keynote speaker (program title: Future Prospects for the Control of Periodontal Diseases), address: "Trends in the Prevalence and Severity of Periodontal Diseases; Research on Oral Diseases and its Impact on Dental Education and Practice;" and Concluding Remarks," Kobe, Japan.

Alpha Omega International Dental Fraternity Achievement Medal Award, "The Omega Challenge of Dental Research: A Natural Dentition for a Lifetime," Washington.

University of Southern California, School of Dentistry, 13th Annual USC Periodontal Symposium: A Critical Review of Antiplaque Agents--Topical and Mechanical, Address, Los Angeles.

University of Connecticut School of Dental Medicine, Anthony L. Neely Protocol Defense, Hartford.

American Dental Association Trustees, Adult Survey Presentation, Chicago.

American Association for the Advancement of Science 1987 Annual Meeting, Symposium: Viral Infections of the Mouth: From Herpes to AIDS (Chairperson), Chicago.

NIH Centennial: Dental Symposium: Opening Address.

University of Tennessee College of Dentistry, Dental Research Symposium, keynote address: "Dental Research - Preparing for the Nineties," Memphis.

House Hearings: NIH Overview.

Senate Appropriations Committee Hearings--Opening Remarks and Testimony.

House Appropriations Subcommittee Hearings--Opening Remarks and Testimony.

American Association of Dental Students/AADR Joint Symposium, Adult Survey Presentation, Chicago.

American Dental Association Dental Student Research Meeting, University of Michigan, keynote address: "Dental Research--Preparing for the Nineties," Ann Arbor.

State University of New York, Discussion: Change and the Future of Dentistry, Buffalo.

Norwegian Research Council, Discussion: Dental Research in Norway, "General Evaluation," Oslo/Bergen, Norway.

Connecticut State Dental Society, University of Connecticut, Address, Farmington.

University of Medicine & Dentistry of New Jersey, Honorary Degree.

University of Florida College of Dentistry, Commencement Ceremony, convocation address: "Of Alligators and Awe," Gainesville.

State University of New York at Stony Brook, Commencement Ceremony, convocation address: "Science and the Practicing Dentist."

University of Rochester, Cariology--An Update Symposium: "Adult Oral Health Survey--Its Implications."

Louisiana State University School of Dentistry, Research Day, "The Impact of Research on Dental Education and Practice," New Orleans.

American Dental Association Council on Government Affairs and Federal Dental Services, "Adult Survey Presentation," Annapolis.

University of California, Los Angeles/Robert Wood Johnson Scholars Program, NIDR/NIH.

American Society of Dentistry for Children: "Toward an End to Caries and Other Plaques of Childhood: The Role of the NIDR in Improving the Dental Health of Children."

Beijing Medical University, School of Stomatology, Lecture, Beijing, China.

University of Bergen, 25th Anniversary of the School of Dentistry, Lecture: "Challenges for the Nineties--The Role of Oral Research," Norway.

Norwegian Society of Periodontists, "New Concepts and Old Realities in Periodontology," Bergen, Norway.

University of North Carolina DRIC 20th Anniversary Celebration, "Research in Oral Biomedicine: An Agenda for the Year 2000," Chapel Hill.

University of Iowa College of Dentistry Faculty Retreat, "Research in Oral Biomedicine: An Agenda for the Year 2000," Galena, Illinois.

Swiss/Dutch Society of Periodontology, "Epidemiology of Periodontal Disease," Montreux, Switzerland.

Annual Seymour J. Kreshover Lecture, Opening Remarks, Bethesda, Maryland.

Gothenburg University Dental School (20th Anniversary), "The Influence of Dental Research on Dental Care and Dental Education in the Future."

PUBLICATIONS

Löe, H. (Ed.) 1986. Chlorhexidine in the prevention and treatment of gingivitis. In Journal of Periodontal Research Supplement No. 16, Vol. 21, Munksgaard, Copenhagen.

Holm-Pedersen, P. & Löe, H. (Eds.) 1986. Geriatric Dentistry, Munksgaard, Copenhagen.

Löe, H. & Morrison, E. 1986. Periodontal health and disease in young people: screening for priority care. International Dental Journal 36:162-167.

Löe, H. 1986. Dental Plaque Control Measures and Oral Hygiene Practices, Concluding remarks, Proceedings from a workshop at NIDR, Löe, H. & Kleinman, D. (Eds.) IRL Press, Oxford.

Löe, H. 1986. Periodontology in the past 20 years. Tandlaegebladet 90:788-794.

Löe, H. 1986. Present and future trends in dental research. Zobozdravstveni vestnik 41:9-23.

Löe, H. 1986. The Omega challenge of dental research: a natural dentition for a lifetime. Alpha Omegan 70:17-21.

Löe, H. 1986. Progression of natural, untreated periodontal disease in man. The Borderland Between Caries and Periodontal Disease III, Lehner, T. & Cimasoni, G. (Eds.), pp. 11-29, Editions Medecine et Hygiene, Geneve.

Löe, H. 1987. Oral health during the nineties: Future research on oral diseases. Journal of the American College of Dentists 54:4-7.

Löe, H. 1987. Toward an end to caries and other oral plaques of childhood: The role of the NIDR in improving the dental health of children. The Journal of Dentistry for Children (in press).

Löe, H. 1987. Preventive dentistry. Phillip Journal (in Press).

Miller, A.J., Brunelle, J.A., Carlos, J.P., Brown, L.J., & Löe, H. 1987. Oral Health of United States Adults: National Findings. NIH Publication No. 87-2868.

ASSISTANT DIRECTOR FOR INTERNATIONAL HEALTH, NIDR

The Assistant Director for International Health coordinates global health activities for the Institute, facilitating the development of new initiatives, assisting and monitoring existing cooperative biomedical and behavioral research activities, and fostering communications among scientists throughout the world. The Assistant Director for International Health also serves as the principal advisor to the NIDR Director on international aspects of dental research.

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

During Fiscal Year 1987, Assistant Director for International Health developed a request for proposal (RFP), sole-source justification, Office of Management and Budget submission, and State Department clearance forms with the contract officer for ICS-II and began work on the RFP for US replication; worked with the National Center for Health Services Research and the Indian Health Service on their project submissions, and with the Centers for Disease Control on its proposed submission; and also coordinated the latter two projects with the Center for Health Administration Studies (University of Chicago) and the World Health Organization (WHO).

In related activities, negotiations were held in New Dehli with the Indian Council for Medical Research for ICS-II participation using Rupee Funds; with the WHO representative from the USSR for potential US-USSR bilateral cooperation for ICS-II; and with Israeli co-investigators to submit to the National Institute on Aging for funding of their ICS-II participation. During the fiscal year, the Assistant Director for International Health continued ICS-II negotiations in Cairo with the Office of International Health and the principal investigator, and took part in a special meeting held at the National Academy of Sciences on Egyptian-Israeli participation in the US Agency for International Development Regional Cooperation Program.

Conference Activities

The Assistant Director for International Health represented the Director, NIDR, at the Oral Health Research Review and Advisory Group meeting of WHO held in Manila, and maintained coordination of NIDR as WHO Collaborating Center. At the World Dental Congress in Manila, the Assistant Director for International Health organized a special session on "Lowering Barriers to Dental Care"; chaired the Working Group on Oral Health Promotion and produced draft guidelines for national dental associations; served on the Scientific Programme Committee and as a consultant to the Commission on Oral Research and Epidemiology; represented the Behavioral Scientists in Dental Research at the General Assembly; and negotiated with the Federation Dentaire Internationale and the American Dental Association (ADA) for space and time on the 1988 meeting agenda for NIDR's 40th anniversary activities.

The Assistant Director for International Health served as a consultant to the new ADA Council on Scientific Programs and International Relations, drafting for them proposed ADA initiatives, providing reports on NIDR activities with particular reference to interactions with China, and facilitating dental participation in meetings of the National Council on International Health (NCIH). The Assistant Director for International Health also attended an NCIH seminar on international health needs as perceived by lesser developed

countries; and participated in the American Sociological Association Program on cross-national research and in the Public Health Conference on Records and Statistics session on "Public Policy and International Data Needs," working toward the development of a policy paper for NIDR on international research.

Fogarty International Center (FIC)

In FY 1987, the Assistant Director for International Health represented the Institute at the BID international representatives meetings coordinated by FIC; submitted the NIDR annual international report; and transmitted FIC comments on a variety of circulated documents including WHO, Pan American Health Organization and Western Pacific Regional Office plans and reports, and the International Classification of Diseases. The Assistant Director for International Health also attended FIC Advisory Board meetings; assisted the international issues program and encouraged dental topics; and served as a consultant on contracts dealing with program evaluation and international allocation of biomedical resources.

Other International Activities

Assistant Director for International Health provided staff reviews for applicants seeking support from the US-Israel Binational Foundation and for potential project collaboration with Chile; provided orientations to international visitors from Australia, Brazil, China, Federal Republic of Germany, India, Israel, Saudi Arabia, Thailand and the USSR; and facilitated planning for WHO and PAHO activities related to acquired immunodeficiency syndrome (AIDS). During FY 1987, information on specific dental research opportunities was provided in response to inquiries from Kenya (resulting in a sabbatical appointment with a US investigator), China, Israel, Colombia, United Kingdom, the Netherlands and the Federal Republic of Germany (resulting in a WHO Travel Fellowship to the US); and two requests were handled for the Exchange Visitor Waiver Review Board. The Assistant Director for International Health also monitored dental research progress under US-Italy and US-Mexico Agreements; and worked with the NIDR Director to generate a policy paper on NIDR's objectives for international dental research.

Papers and Presentations

Assistant Director for International Health presented seminars and technical assistance to Harvard School of Dental Medicine on opportunities for a new international health track for fifth year dental students; to Forsyth Dental Center for long-range planning, including social science research; to the Sparkman Center for Health Education at the University of Alabama; and to the University of Michigan School of Dentistry.

A paper on a topic of international relevance, "Societal Expectations for Oral Health: Response of Dental Care Systems," was presented at the Dunning Symposium at Columbia University in New York.

A paper on the International Collaborative Study of Oral Health Outcomes, presented at the International Sociological Association meeting in New Dehli, was accepted for publication in International Sociology.

A presentation was made in Manila at the World Dental Congress, "Converting Unmet Need for Dental Care to Effective Demand." The paper will appear in the International Dental Journal.

A poster presentation was given at the annual session of the International Association for Dental Research on "Tracking the Long-Range Research Plan of the National Institute of Dental Research."

FY 1987 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. Planning and evaluation functions are coordinated with both budget and information systems in concert with operating programs of the Institute.

Planning and Evaluation Activities

Staff continued to track and evaluate the NIDR Long-Range Research Plan, "Challenges for the Eighties," and presented preliminary findings from its evaluation at the 1987 annual session of the International Association for Dental Research/American Association for Dental Research in Chicago, Illinois. PES has proposed a structure, table of contents, and process for the development of a research plan for the 1990's. Implementation of this effort will begin in early FY 1988.

Staff developed and coordinated the annual research planning session with the Director, NIH, in December, 1986; the NIDR component of the NIH Research Plan; and the NIDR FY 1988-1989 Evaluation Plan. PES worked with the Director, NIDR and the Budget Office in preparing the FY 1988 Budget Justification narrative and opening statements by the Director for the House and Senate Appropriations Subcommittee hearings, and coordinated responses to questions submitted for the record. Staff worked with the Director in planning, writing, and editing formal presentations before a variety of audiences and written materials for professional journals. PES, working with representatives of the National Institute on Aging and Veterans Administration continued work to develop an Implementation Plan for collaborative research on oral health in the aging.

Projects completed during FY 1987 include: an evaluation of the research training of dental clinical investigators by the NIDR under the National Research Service Award (NRSA) program; a follow-up assessment of NIDR-supported research trainees and fellows who received their training between FY 1975 and 1979; a bibliometric analysis of the extent of support by the NIDR of U.S. and foreign-based dental research investigators; and an evaluation of articles published during 1985 in the highest impact English-language clinical and research dental journals, regarding countries and institutions of authors, funding sources, and subfields of research. Projects started during FY 1987 include an assessment of NIH, academia, and private industry relationships in the support of research on restorative dental materials; and an evaluation of NIH/NIDR-supported social and behavioral science research.

Other Activities

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

- Technical Review Committee, National Cancer Institute, for "An Assessment of the Factors Affecting Critical Cancer Research Findings".

- Evaluation Project Advisory Committee, National Library of Medicine for "TOXLINE Evaluation".

- Site Visit Committee, National Library of Medicine, to review "Technical Evaluation of the Clinical Literature", Harvard University, School of Public Health, Department of Health Policy and Management.

- Planning committee for an NIDR-sponsored three-day international conference on diagnostic and therapeutic technology in dentistry.

- Planning group for an international workshop on the significance of oral health research in HIV infection.

- Core staff of the NIA-NIDR-VA collaborative project for research on oral health in aging.

- Advisory/review group for NOVA program on dental research.

- Advisory/review group for the NIDR history project.

- AAAS dental research section representative at meeting to discuss ways to improve science and mathematics education for undergraduate liberal arts majors.

Staff also represented the NIDR at an exhibit at the annual meeting of the American Association of Medical Colleges.

Presentations

Jim Lipton

"A Qualitative and Quantitative History of the T32 and F32 NRSA Programs Sponsored by the NIDR", NIDR ad hoc NRSA Training Committee meeting.

"Tracking the Long-Range Research Plan of the NIDR", International Association for Dental Research/American Association for Dental Research annual meeting.

"The World of the Facial Pain Patient", Greater New York Dental Meeting, New York City.

"New Methods and Approaches to the Diagnosis of Chronic Facial Pain, and Potential Epidemiological Studies of Chronic Facial Pain," NIH/NIDR Pain Clinic Staff.

"Differential Diagnosis of Orofacial Pain", seminar for the postgraduate endodontics training program, Bethesda Naval Hospital.

"Issues in the Diagnosis of Chronic Facial Pain", lecture to NIDR Fellows.

"How Sociologists Can Help Your Agency", seminar for Federal Managers and Personnel Officers sponsored by the American Sociological Association.

"Attitudes and Behavior of Dental Directors of Migrant Health Centers Concerning Preventive Dentistry", annual meeting of the Migrant Health Centers Association.

Joan Wilentz

"Tracking the Long-Range Research Plan of the NIDR," International Association for Dental Research/American Association for Dental Research annual meeting.

"New Methods and Approaches to the Diagnosis of Chronic Facial Pain, and Potential Epidemiological Studies of Chronic Facial Pain, NIH/NIDR Pain Clinic staff.

"Oral Health in Aging," a report and update on the NIDR, National Institute on Aging and Veterans Administration Collaborative Project for the NIDR Programs Advisory Committee.

Publications

Marbach, J.J. & Lipton, J.A. "Biopsychosocial Factors of the Temporomandibular Pain Dysfunction Syndrome: relevance to restorative dentistry." Dental Clinics of North America, 31(3):473-486, 1987.

RESEARCH DATA AND MANAGEMENT INFORMATION SECTION (RDMIS)

The mission of this Section is primarily one of processing information for and about the Institute. What follows in this report partially documents the variety of activities undertaken by the Section in order to collect, process, retrieve, compile, and report all manner of data in many formats for a wide range of uses and users. It also illustrates the endless pursuit of new approaches, new equipment, new skills and new techniques for accomplishing our goals faster and better.

IBM PS/2 and Streamlining the Research Project Management System (RPMS)

The recent introduction of the International Business Machines (IBM) Personal System Two (PS/2) system was greeted with enthusiasm by members of the RDMIS. The increase in Random Access Memory (RAM) from 640 thousand bytes (K) to 16 megabytes, coupled with increased disk storage of up to 300 megabytes provides a PS/2 user with an incredibly powerful computer. Two of the many enhancements in the PS/2 are of particular interest. A new operating system (OS/2) takes full advantage of the 16 Megabytes of core and an advanced proprietary hardware/software interface provides a "seamless" marriage to the IBM DB2 Database Management System that resides on the NIH Division of Computer Research and Technology (DCRT) mainframe.

RDMIS now envisions being able to convert the present RPMS, which was conceived about 12 years ago, into a DB2 application. This conversion will extend the power of an easy to use, user friendly DBMS to members of the NIDR at all levels. RDMIS will be working with staff of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to convert their existing DB2 application (Desktop) for use by the NIDR. This approach will save months of research and development time, software generation costs, mainframe computer costs and provide compatibility between NIDDK's Desktop system and the NIDR's RPMS. Thereafter, any DB2 software enhancement by either NIDDK or NIDR will be readily available for installation in the other Institute's system.

Continuing Developments in the Budget Tracking Reports

The use of the Division of Financial Management's Allotment Ledger Master File (ALMF) continues to be the NIDR's primary method for tracking monthly expenditures for both obligated and committed amounts by allowance or Common Account Number (CAN). Tasked with the responsibility of tracking the additional influx of funds for research in acquired immunodeficiency syndrome (AIDS), the RDMIS has responded by further enhancing a special AIDS report. This enhancement will allow immediate inclusion of an intramural laboratory in the report upon receipt of an AIDS CAN. Additionally, one of the other reports that deals with travel costs was modified to reflect cumulative domestic and foreign travel as well as foreign travel as a separate column, thereby assuring that the NIDR meets foreign travel ceilings.

Conversion from ISAM to VSAM at the Division of Computer Research and Technology (DCRT)

The DCRT has announced its intention to convert from two older methods of accessing data, Indexed Sequential Access Method (ISAM) and Basic Direct

Access Method (BDAM), to be replaced by Virtual Storage Access Method (VSAM). The impact of this change was made easier by the development, by RDMIS, of a VSAM model thereby saving considerable amounts of time. Working on this conversion whenever there was time, available members of the RDMIS staff have now completed work on all major software subsystems in the Research Project Management System (RPMS). The RPMS is the software system which does all budgetary and programmatic tracking of grants, contracts, and intramural projects as well as the NIDR operating expenditures.

Continued Savings on Delegated Procurement (DELPRO)

The RDMIS continues to provide advice and technical assistance to the various administrative offices of the NIDR in the use of Personal computers as DELPRO workstations. The use of personal computers, which have the ability to provide 3270 emulation, eliminates the high cost of previously rented AT&T DELPRO dedicated equipment. The changeover to personal computers, which began in Fiscal Year 1986, is not yet complete, but the rental costs of the remaining AT&T terminals have been reduced dramatically from $25,000 in 1985 to approximately $10,000 this fiscal year, realizing a 60 percent savings. In addition to the savings, the use of personal computers allows NIDR staff expanded capabilities in the areas of word processing, spreadsheets, database management systems and access to various mainframes.

Remote Information Facility (RIF) Improved

The Remote Information Facility (RIF) has maintained its importance as a major tool for the dissemination of research data throughout the Institute. The addition of several new information modules in the system has brought the RDMIS one step closer to becoming a "paperless office." In response to feedback and suggestions made by the RIF users, we have incorporated the ability to access eight different contract monthly reports and summaries of ADP account/sponsor charges for the Institute. This has significantly reduced costly charges incurred in widespread report distribution.

New features of RIF are continuously being designed and developed as time allows. One noteworthy enhancement will be the use of RIF as an "umbrella" system. Specifically, this will enable staff to use RIF as a focal point for accessing all other systems designed by the RDMIS. Upon its final implementation, the NIDR community will only have to remember one acronym to access a multitude of systems. Among those to be included are NIDR ONLINE, the NIDR Meeting Scheduler, CRISP abstracts, NIH telephone directory, DRG Snap Shot routines and Operational Goals.

NIDR Data Information Vocabulary Evaluation

For many years the Institute has used several vocabularies to index its research projects. With the help of an information scientist, Dr. David Batty, and Professional Management Associates, our needs were reassessed and the various vocabularies evaluated. The result has been to reduce the NIDR Scientific Classification System to its five major categories and to use the National Library of Medicine's Medical Subject Headings (MeSH) for all projects. Arrangements are being made to have a professional indexer assign the subject headings to each project. This arrangement will also be used to augment the DENTALPROJ data base.

NIDR ONLINE

The Institute's own online data system has received more attention as our constituents learn of its existence and value. Demonstrations at national meetings have prompted more requests through libraries and the registration of individual end users. A number of new features, such as the addition of newly awarded grants and awards to the choice of data available, have been enthusiastically received and the comments, which are solicited at the end of each session, have been positive. Several other data bases, such as the American Dental Network, Cleveland Free-Net, and St. Josephs's Hospital and Medical Center have requested permission to copy news items for their own systems.

DENTALPROJ

One of the most challenging projects being attempted with the help of the National Library of Medicine and the Division of Research Grants (DRG) is the development of a data base of abstracts of ongoing dental research projects. Such a data base existed between 1969 and 1980 through the Smithsonian Science Information Exchange and was the source of much valuable information about research in progress. The new data base, to be called DENTALPROJ, will contain the same kinds of information gathered from the IMPAC and CRISP files of DRG, the Veterans Administration, the Department of Defense, and others as available, and will be accessible through an online data base at NLM. It is also proposed to have an annual printed version of the data base on a fiscal year basis.

PaperChase

One of the most exciting additions to our FEDLINK interagency agreement with the Library of Congress has been a new vendor called PaperChase. This online service developed by Beth Israel Hospital has made MEDLINE available to anyone with access to a communicating terminal. A menu-driven program takes the mystery out of database searching and allows the end user, the investigator, to search the literature without an intermediary.

Program to Rename Data Sets

As a result of IBM's decision to enforce specific data set naming conventions, the RDMIS designed a procedure which facilitated the renaming process, and made it available to all WYLBUR users. The program identified any data set name which needed to be changed due to the imposition of new rules, and gave the individual a chance to rename it. This program proved to be a very helpful and time saving measure which expedited the tedious task of manually searching one's datasets.

Technical Reports

One of the continuing services provided by the Section is the collection, publication and distribution of data in the form of technical reports. The current list of reports, which includes "NIDR Programs," "NIDR Indexes," "Selected List of Technical Reports," and "Trainees and Fellows Supported by the National Institute of Dental Research," are compiled on a fiscal year

basis. Automatic distribution includes dental schools and advanced education institutions and their libraries in the United States and Canada, administrators of research institutions and dental organizations, NIDR Staff and Council.

One technical report that is compiled regularly but not broadly distributed is the "NIDR Annual Report" for the Deputy Director for Intramural Research, NIH. This report, which describes individual intramural research projects and summarizes yearly activities by program, is coordinated by the Section and is the source of intramural data for the Institute. Outside individuals must request this report, for nongovernmental purposes, through the Freedom of Information Act Coordinator.

Lead Users' Activities

Other activities include the involvement of three employees as representative "Lead Users" for the Institute. Ron Ruben, Carla Flora, and Mary Ann Williamson have provided ongoing support and technical assistance to personal computer users throughout the NIDR. In addition, Carla Flora has participated in the Personal Workstation Associate Instructor Program, where she assists in the teaching of microcomputer training courses to individuals at the NIH.

Staff Activities

We are represented on the NIH Handicapped Employees Committee (HEC) by Janet Pomerantz who chairs the Telephone Committee and serves as Secretary to the Reasonable Accomodations Committee for that group. Dolores Karamian has been commended for her contribution to TEN, the electronic bulletin board sponsored by the Office Technology Coordinators. Deane Hill serves as the Secretary for the NIH ADP/EP Coordination Committee.

All members of the programming staff have received some additional training in the use of our FEDLINK vendors (BRS, DIALOG, MEDLARS, and PAPERCHASE).

Facility Improvement

The shortage of space and the particular needs of the Section for computer equipment and programmer privacy were greatly improved by the addition of modular furniture. The cubical arrangement seems to meet these needs attractively and efficiently.

Privacy Act/Freedom of Information Act

The Chief of the Section continues to coordinate all Privacy Act and Freedom of Information Act requests received by the Institute. These requests are logged on to an NIH Tracking System and then processed by the appropriate program area. There has been very little activity related to the Privacy Act, and the Freedom of Information Act accounts for about 20 requests during the year.

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.

Special Projects

PIRS planned and coordinated activities for the NIDR 40th anniversary: staff negotiated an agreement for the preparation of a film treatment on dental research advances for NOVA; acquired a producer and arranged interviews with national and international investigators whose research will form the basis of the production; continued negotiations with outside organizations assisting in the project, and with NOVA about airing the film to coincide with the Institute's fortieth anniversary.

In other anniversary-related projects, information staff directed and assisted the research, writing and editing of a historical monograph on the NIDR; established a committee to monitor the direction of the history and to review written material completed to date; and opened negotiations with several publishers concerning printing of the history.

PIRS also initiated negotiations that secured the agreement of both the Smithsonian and the National Library of Medicine to produce exhibits commemorating NIDR's fortieth anniversary, and preliminary planning was begun.

The office initiated planning and research for production of a historical slide-tape program for viewing at national meetings of dental practitioners and researchers and at NIDR anniversary functions.

PIRS coordinated the Institute's participation in NIH centennial activities which included a special research symposium and the development of a commemorative poster. Staff also directed the production of a portable centennial exhibit which was displayed at national professional meetings.

In conjunction with NIH's 100th anniversary, PIRS conceived and directed the development of a permanent historical exhibit on fluoride for the NIH Museum of Medical History. This included building a collection of original artifacts and materials, writing copy, and incorporating video and a fluoridated drinking fountain. Staff also prepared a press release, and planned and hosted a reception for the formal dedication of the exhibit in May.

PIRS staff coordinated the logistics for and staging of a major press conference at the IADR/AADR meeting in Chicago to announce the results of the National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986. Staff developed a videotape for distribution to television reporters, and prepared separate print and broadcast media kits for the approximately 50 representatives of the lay and trade press who attended.

PIRS served with NIH senior staff and EEO advisors on a steering committee to plan the "National Symposium on Career Opportunities in the Biomedical Sciences" at Meharry Medical College in Nashville, Tennessee. Information

staff developed an NIH exhibit for display at Meharry and handled all press registration and activities for the conference.

In FY 1987, PIRS added two new components to the NIDR diabetes education effort. Staff produced "Periodontal Disease and Diabetes: A Guide for Patients," and coordinated the development of a clinical slide presentation for health care providers on the oral health complications of diabetes. Staff also initiated a meeting with CDC's dental representative to enlist assistance in the distribution of these materials through that agency's dental and diabetes control programs. The distribution arrangement with the National Diabetes Information Clearinghouse remains in effect.

Staff provided technical assistance to NIAID and NIDDK in the development of multi-image slide productions for those Institutes. The office has developed an extensive collection of laboratory and clinical slides and serves as a resource for audiovisual needs of NIH staff, and the lay and trade press.

PIRS planned, coordinated and directed all arrangements for the 1987 Seymour J. Kreshover Lecture delivered by Dr. Harold Slavkin, who highlighted advances in understanding the development and mechanism of action of tooth enamel genes.

PIRS coordinated publicity for the NIH Lecture presented by the NIDR intramural research director. Activities included the preparation of an article for the NIH Record and introductory comments for the Director, NIH, as well as the design and production of a poster and tent cards.

Public and Professional Education

During FY 1987, PIRS carried out the following activities in the areas of public and professional education: prepared press summaries on NIDR-supported research advances for the American Association for Dental Research/International Association for Dental Research meeting; 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; contributed articles on recent research advances to the Journal of the American Dental Association for the continuing NIDR series; distributed four dental health articles to the nation's minority media, including print, radio and television; and prepared a series on dental health for publication in the syndicated "NIH Search for Health" column.

In related activities, PIRS staff provided background material for articles on NIDR research activities to professional journals and health-oriented publications including JADA, Dental Management, Dentistry Today, Dental Health Advisor, Dental Student Magazine, Medical Tribune, American Health, Health Confidential, and Your Health Magazine.

PIRS provided two articles on pain research for the Pharmacy Times continuing education series; developed an extensive article on NIDR-supported AIDS research for dental practitioners and the research community; and prepared a major patient education feature for Diabetes Forecast on the oral complications of diabetes.

Staff consolidated into a single videotape highlights of more than two hours of NIDR research news stories aired on local and network television. The tape

was available for viewing by the research community at the IADR/AADR annual session and at NIDR staff seminars.

In FY 1987, PIRS negotiated a joint agreement between NIDR and People's Drug Stores to prepare, print and distribute, at no cost to the Institute, a pamphlet promoting oral health. Staff provided background materials and a detailed outline for the publication, and reviewed copy for the pamphlet which is in press.

PIRS effectively orchestrated a multi-media patient recruitment effort for the NIDR clinical research program in the areas of third molar removal, headache, herpes, pain, TMJ, orthognathic surgery, and periodontal disease.

PIRS also exhibited at the annual scientific sessions of the IADR/AADR, AADS, ADA, Federation of American Societies for Experimental Biology, and the American Academy of Family Physicians; conducted tours of NIDR research facilities for visiting practitioners, students and scientists; and continued periodic publication of "NIDR Research News."

Publications

In FY87, PIRS developed and produced several new publications:
"Dentist Scientist Award,"
"Periodontal Disease and Diabetes: A Guide for Patients,"
Biennial Report for the Institute and the NIDR Advisory Council.

The following publications and fact sheets were updated, rewritten and reprinted:
"Seal Out Dental Decay,"
"Tooth Decay,"
"Fluoride to Protect Your Children's Teeth,"
"Periodontal Disease."
Institute, Intramural and Extramural brochures.

Print Media

In FY 1987, PIRS arranged interviews and/or provided background information on dental research topics to numerous general circulation magazines, including Children's Magazine, Family Circle, Self, Harper's, Vogue, Parenting Advisor, Glamour, Woman's Day, and Woman's World; and to virtually every major metropolitan newspaper and wire service.

The Director and other Institute staff were interviewed as well for articles in the trade press, news and general interest magazines, and newspapers including the Washington Post, USA Today, Self, Atlanta Constitution, US News and World Report, Newsday, Wall Street Journal, Associated Press and United Press International.

Broadcast Media

Announcement of the results of the National Survey of Oral Health of Employed Adults and Older Americans was covered extensively on national radio, and was reported on the Today Show, Cable News Network, NBC affiliate in Chicago, and CBS Washington affiliate.

NIDR pain researchers were prominently featured in a half-hour PBS (Boston) program entitled, "Mastering Pain," one of the 26-part "Bodywatch" series on health issues.

Local networks covered several NIDR-supported research activities on such topics as herpes, dry mouth and prevention of AIDS transmission in the dental setting.

The Voice of America interviewed Institute staff on canker sores.

Reports

PIRS coordinated the recording, writing and printing of the Biennial Report, the minutes of the National Advisory Dental Research Council and the Institute's Program Advisory Committee meetings during FY 1987.

Staff contributed to seven Special Reports to Congress, including research advances in the following areas: cystic fibrosis, diabetes, arthritis, digestive diseases, AIDS, maternal and child health, and international activities.

General Communications Activities and Services

PIRS responded to approximately 8,200 requests for information on a broad range of topics from the public, professionals, Congress and the media. Over 517,000 publications were sent out during FY 1987.

Staff provided medical arts, photography, graphics and printing services to the Institute for activities such as the Operational Goals Bulletin, EEO Bulletin, NIDR Calendar, and the NIDR Awards booklet. PIRS also arranged the production and printing of health promotion materials for the Science Transfer and Research Analysis Branch.

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

Other services included arranging a series of presentation skills seminars for NIDR staff to enhance public speaking abilities; 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 Science Transfer and Research Analysis Branch.

PIRS also contributed regularly to the "NIH Record" and "NIH News and Features." Staff provided editing assistance for several proceedings, including conferences on oral hygiene practices and the microbiology of caries and periodontal disease, as well as for intramural research papers.

Personnel

During the summer of FY 1987, PIRS arranged to have the services of four journalism interns from Harvard University, Temple University, University of Maryland, and Northwestern School of Journalism. The interns performed a variety of writing and public information assignments.

FINANCIAL MANAGEMENT OFFICE (FMO)

The Financial Management Office 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 FMO is also the Institute's repository for accounting and payroll records, statistical data and legislative reports, and serves as principal staff advisor to the Director on all financial matters relating to the Institute's appropriations.

NIDR Budget: Fiscal Years 1988 and 1989

During FY 1987, the FMO formulated the Institute's FY 1989 budget request by generating the initial budgetary levels. Staff continued to advise the Director on all changes made during negotiations as the budget was transmitted for incorporation into the President's budget submission to Congress. The FMO also prepared all supporting documents, justifications and statistical materials that supported the request.

Because of the overlap in the budget cycles, the FY 1989 budget is being negotiated as the FY 1988 budget request is being reviewed by the Congress for legislative enactment. If approved, the request is incorporated into an appropriation bill. During this process, the FMO staff accompanies the Director to the Congressional hearings and provides additional justification materials if needed. After action on the request by both houses of Congress, the FMO prepares effects statements to summarize Congressional action which becomes the operating budget to carry out the Institute's program plans for the next fiscal year.

While the formulation and legislative processes were in progress, the Institute conducted its current year business. The FMO provided managerial and financial support for the Institute's extramural and intramural programs and for direct operations. Staff maintained payroll records, generated program expenditure reports, tracked the funding of grants and contracts, requisitions and purchase orders, and worked with Institute administrative staff to ensure that reprogramming actions were initiated in areas where additional funds were required. The FMO apportioned monies by quarter to fund planned activities and, at the end of the year, balanced the books.

Automation

The FMO has expanded its activities through the use of microprocessors and automated data systems, automating its financial operations and initiating new uses for personal and mainframe computers. The office continues to be an innovator in enhancing productivity through the use of electronic and automated equipment.

Other Activities

The FMO provides special reports and monitors the Institute's trans-NIH activities which include research projects in diabetes, arthritis, nutrition, disease prevention, and acquired immunodeficiency syndrome (AIDS). Forecasts for strategic planning purposes are prepared by this office. The FMO is also responsible for the acquisition of materials in response to requests for program and financial data from Congress, the Office of Management and Budget, and other Federal and nonfederal agencies.

EQUAL EMPLOYMENT OPPORTUNITY PROGRAM (EEO)

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 Office. This office serves as the principal source of information for and advisor to the Institute Director and to senior management on matters of equal employment opportunity, affirmative action, Federal Equal Employment Opportunity Recruitment Plan, civil rights, and contract compliance. In addition, the EEO office is responsible for the special emphasis program for minorities, Hispanics, women and the handicapped.

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

Discrimination Complaints

The Institute had no informal or formal discrimination complaints filed in 1987. 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 EEO Council, the NIH Handicapped Employees Advisory Committee, and the NIH Hispanic American Advisory Committee.

During 1987, the Committee invited four guest speakers to various meetings to discuss important educational opportunities for NIDR employees. As a result of their presentations, three employees submitted applications to the Management Intern Program, one employee applied to the Stride Intern Program, and two employees submitted applications to the Career Curricula Program.

In addition, the Committee nominated to the Director two deserving individuals for the NIH EEO Special Achievement Award. These individuals will be recognized for their invaluable contributions to Equal Employment Opportunity at the Institute's Awards Ceremony November 1987.

April 1987, the Advisory Committee sponsored a seminar on "Safety and Theft in NIH Buildings and Parking Areas," a topic of major importance to the NIH community. It provided an opportunity to heighten awareness of all NIH

employees about protecting themselves, as well as government and personal property. Approximately 80 employees attended the seminar.

The Advisory Committee compiled and designed an NIDR Employee Guide to Personnel and Employment Information which became available for distribution in January 1987. The Guide highlights key NIDR personnel, OPM and NIH pamphlets, and various services available to NIH employees. All NIDR employees have received a copy of this handy document and Personnel is now including this information in their orientation packages for new employees.

Each year the NIDR EEO Office sponsors training for the EEO Advisory Committee on the Federal equal employment opportunity laws and regulations which prohibit discrimination. The Handicapped Program and Disabled Veterans Program are highlighted during the training session.

Minority Brochure

The EEO Manager developed a new brochure entitled "Opportunities for Minorities in Research." It identifies the various programs--the Minority Research Supplement Program, the Minority Access to Research Careers Program (MARC), and the Minority Biomedical Research Support Program (MBRS)--available to minorities in NIDR. The brochure represents an effort by the Institute to stimulate interest and increase the participation of minority students, particularly those who are considering careers in research or medicine. The publication was mailed to all program directors of the MARC and MBRS programs and has also been shared with participants at dental-related conferences and meetings.

MBRS and MARC Programs

Through cooperative agreements with the National Institute of General Medical Sciences and the Division of Research Resources, the NIDR supports components of the Minority Biomedical Research Support and the Minority Access to Research Careers Programs that relate to the overall mission of the Institute.

The new minority brochure motivated three students participating in the MARC Program to consider the National Institute of Dental Research as a part of their enrichment assignments for the 1987 summer session. Two students are currently enrolled at Howard University and one student is attending Morgan State University. Institute staff participated in and exhibited at the NIH Centennial MBRS-MARC Symposium held in fall 1987.

Recruitment and Awareness

The EEO Manager continues to identify and communicate with minority, women, and handicapped organizations and associations concerning our mission and activities. In addition, the manager continues to disseminate information through other NIH EEO Offices participating in conferences.

To increase awareness of the special needs of a handicapped person, the EEO Office sponsored a sign language course. The course was designed to help NIDR employees to understand the culture of the hearing-impaired; develop sign language communication skills; and to gain knowledge of signs of laboratory

terminology, finger-spelling, and counting. Approximately 25 employees participated in the training.

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

Community Outreach

The EEO Office continues to provide Morehouse College, Meharry Medical College School of Dentistry, and the University of Puerto Rico School of Dentistry with the Institute's surplus scientific books, journals and slides. This service has now been expanded to include a public school in the District of Columbia--Ballou Senior High School. Since the inception of the Resource Collection, approximately 128 journals have been identified for distribution and over 300 books have been contributed to the project.

Two scientists from the Intramural Program and the EEO Manager represented the NIDR as special judges in the District of Columbia Annual Science Fair. The group reviewed 16 dental-related science projects. Four deserving students were recognized for their outstanding participation in the Annual Science Fair. Each student received an award, a book, and several publications on the mission of NIDR.

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.

ANNUAL REPORT OF THE ACTING ASSOCIATE DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Epidemiology and Oral Disease Prevention Program's (EODPP) major emphasis this past year has been on national activities. The monograph of the National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986 has been completed. This survey has demonstrated the success the country is having in maintaining their oral health. However, it also showed that the older persons need particular attention for both caries and periodontal diseases. In addition, efforts are needed to maintain the low rate of edentulism seen in those younger than sixty-five as they age.

With preliminary findings of the above survey in hand, a group of senior advisors was convened in early April to discuss future directions necessary to improve the nation's health. They recommended the development of a national oral health promotion program coordinated by the NIDR. Program staff are developing a plan for such a program with the help of outside consultants. It is critical to maintain the gains achieved in the nation's oral health, while focusing on those groups who are in need of special attention. A collaborative effort across practitioner, consumer, academic and research groups will be required.

The follow-up of the 1979-80 survey of schoolchildren was conducted this past year. Data collection was completed in June for the 1986-87 National Survey of Oral Health in U.S. Schoolchildren. The comparison of the findings between these two surveys will permit a much-awaited analysis of the trends in dental caries in U.S. children over the past six years. In addition, the 1986-87 survey will provide first-time detailed information on the periodontal health, fluorosis, and oral mucosal lesions in the five to seventeen-year-olds. A questionnaire on smokeless tobacco, tobacco and alcohol was administered, as well as salivary samples were taken to assess the levels of S. mutans and lactobacillus. These latter findings will be compared to the dental caries status.

While analyzing one national survey and conducting another, Program staff have been involved in planning and developing the oral health component of the National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) III. Through the help of consultants and the involvement of all parts of the Program, the staff have provided the details of data collection procedures, forms and diagnostic criteria, and have trained the examiners for the pilot tests to be conducted in FY 1988. The NHANES III oral health component is extremely broad, encompassing not only dental caries, periodontal diseases and oral mucosal tissues assessments, but also occlusal and dentofacial characteristics, traumatic injuries to teeth, assessment of tooth conditions and prostheses, and subgingival plaque and salivary sample analyses. As a household-based survey, the NHANES III will provide needed information, especially for pre-kindergarten children and older persons.

In order to establish specific points of reference from which to determine trends in oral helath over time, Program staff have been active in analyzing existing data bases. The National Study of Dental Health Outcomes Related to Prepayment: 1981, has been undergoing review and comparisons have been made

with findings from NHANES I and the 1985-86 survey of U.S. adults.

No one survey has all the essential elements needed to answer all the questions being asked by the public health, practitioner and research communities. Through the above efforts a good basis has been established for monitoring and determining the nation's oral health. However, there are special populations such as the institutionalized, the homeless, the home-bound, those in college, etc., which have not been surveyed. Plans are under way to stimulate the collection of pertinent data from such groups. This requires an extremely broad-based effort involving multiple examiners and multiple studies.

A major step taken by the Program has been in initiating the development of a cadre of individuals trained in the application of the NIDR epidemiologic examination methods. Two four-day courses were conducted with dental directors of state and local health departments as well as dental school faculty in attendance. All these individuals were about to embark upon surveys in their own geographic areas. The perpetuation of such courses should facilitate the conduct of surveys utilizing similar data collection methods and will aid in comparisons between such surveys.

New methodologies for oral epidemiologic research have been evolving. Analyses of the effect of partial recording in the estimation of periodontal attachment loss in population studies have shown that there is minimal bias in two sites per tooth in half the mouth examination relative to estimates from full-mouth examinations. Program staff also have been developing and improving methods for the collection, storage, and culturing of salivary samples. Work has proceeded in investigations to determine ways in which to assess levels and types of periodontal pathogens. The evolution of these methodologies is critical in the delineation of microbial risk factors in large-scale population-based surveys. In the area of clinical trials, staff participated with the American Dental Association in the development of guidelines to establish uniformity in the comparative clinical testing of fluoride dentifrices at low disease levels. These guidelines should help with appropriate determinations of differences between dentifrices.

The Program has broadened its studies of periodontal diseases. In adolescents incipient periodontal disease is being investigated through a longitudinal study incorporating clinical and microbial factors. To determine the prognostic value of bone loss in adolescence for adult periodontal status a study in Sweden was initiated. Phase II of a clinical and laboratory study of risk factors in adults, 55 years of age and older, has been initiated through a joint venture between scientists at SUNY-Buffalo, University of Texas, San Antonio, and the NIDR. In addition, the membrane-controlled delivery system has been evaluated for tetracycline, and prototype controlled-release pellets for chlorhexidine have been fabricated. Both of these therapies could be applied to periodontal diseases. Analyses of treatment needs projected from periodontal health status reported in national surveys have been researched.

While studying the risk factors associated with and treatment of periodontal diseases in adolescents and adults, the Program is pursuing ways in which to prevent gingivitis and maintain periodontal health. A new clinical trial was initiated to determine the effectiveness of gingival bleeding compared to traditional plaque control for the long-term maintenance of optimal oral

hygiene in adolescents. New educational materials have been developed for use by the profession and include a free-loan exhibit on preventing periodontal diseases and a user's guide for NIDR's film entitled "Prescription for Periodontal Health."

The EODPP has continued efforts toward research and health promotion activities related to oral health manifestations of human immunodeficiency virus (HIV) infection. The controlled-release delivery system is being investigated for the application of antifungal agents for the treatment of oral candidiasis, a common lesion found in early stages of the infection. The final protocol for a natural history study of oral conditions in HIV-infected individuals is being reviewed. This study will be one part of a larger natural history study conducted by the Army at Walter Reed Army Medical Center. Data collection for this investigation is due to begin in early winter. Program staff also have established a collaboration with the Veterans Administration (VA), the Centers for Disease Control (CDC) and the WHO Collaborative Centre on Oral Manifestations Associated with HIV Infection. Within the VA a surveillance system for documenting the oral lesions, diseases and treatment needs of HIV-infected individuals frequenting the VA dental clinics has been launched. In September a visit was made to CDC's Center on Infectious Diseases and to the Dental Disease Prevention Activity to exchange current activities and plans. Also in September a planning group was convened to develop a state-of-the-science workshop on research needs to better prevent, diagnose and treat oral health conditions associated with HIV infection. This workshop is projected to be held in late spring/early summer 1988.

Appropriate infection control in health care settings has gained visibility of late due to HIV infection. Program staff have been active in analyzing data related to infection control practices, knowledge and attitudes of dentists and dental hygienists. Findings will be used to develop educational strategies for dental practitioners. For example, together with the American Dental Association (ADA), and VA and the CDC staff have worked on the development of audiovisual materials on infection control practices. In addition, collaborating with the National Institute of Allergy and Infectious Diseases, a poster on the need for infection control practices has been published.

The following Branch reports detail additional specific activities. Dental caries prevention and the appropriate application of fluorides and sealants continue to play a key role in the Program's activities. Also, the reasons for and the magnitude of the health problem of fluorosis have been investigated. New study initiatives in facial and dental injuries; the establishment of an oral component to a long-term study of cardiovascular disease in children, the prevalence of oral mucosal lesions in adults; the relationships between oral health status, treatment needs and utilization of dental services; and research in patterns of tooth loss and their treatment implications.

The EODPP Visiting Scientist Program has been successful. This year the Program has benefited from scientists from Minnesota, Norway, Finland, Denmark, China and Colorado. Working with staff throughout the EODPP, they have served to augment international collaboration, initiate studies, and expand the research base.

Dr. Thomas Drury joined the Program on September 14 as the Deputy Director, coming from the National Center for Health Statistics, Division of Epidemiology and Health Promotion. Dr. Drury, in addition to Program management, will augment the Program's research base with his experience in chronic pain epidemiology.

ANNUAL REPORT OF THE DISEASE PREVENTION BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Disease Prevention Branch was active in establishing several new studies and in providing major support for the salivary analyses for the National Survey of Oral Health in School Children. The position of Branch Chief remained vacant throughout the year, and Drs. Stiles and Heifetz shared the responsibility as Acting Branch Chief in addition to their Section-specific activities.

CLINICAL TRIALS SECTION

Because more foods and beverages are being processed in fluoridated communities and use of various fluoride vehicles for caries prevention has become widespread, there is concern whether the ingestion of fluoride has significantly increased, with a concomitant increase in dental fluorosis. To help determine if any recent changes have occurred in the level of dental fluorosis, a survey was conducted in 1985 among children in four areas of Illinois with water fluoride concentrations of 1, 2, 3, and 4 times the optimal and findings compared with those of a prior survey made in the same areas in 1980. Results showed that the distributions of fluorosis scores of young children (8-10 year olds) in 1980 and 1985 were quite similar. However, older children (13-15 year olds) in 1985 showed a greater prevalence and severity of fluorosis than did their cohorts in 1980. For example, in 1980 fluorosis scores involving staining and/or pitting affected more than 6% of the maxillary labial surfaces of teeth only in the four times optimal area, whereas, in 1985 all above-optimal areas showed at least 6% of these surfaces with severe forms of fluorosis. Despite the observed increase in fluorosis among older children, in the area with the optimal water fluoride concentration the type of dental fluorosis detected was still limited to the less advanced forms (whitish blemishes of enamel) which have only cosmetic effects.

Several sources of fluoride have been identified as contributing to systemic fluoride ingestion by infants and young children. These sources include commercial infant formulas, fluoride dentifrice and dietary fluoride supplements prescribed in excess dosage or without knowledge of the amount of fluoride already present in the drinking water. However, because of a number of recent actions that have been taken to limit fluoride intake from these sources, any increase in the extent of fluorosis being reported now may prove transitory. Further prevalence surveys, similar to those conducted in Illinois, are needed to help monitor future levels of dental fluorosis among U.S. school children.

Continuing progress toward control of dental caries is likely to come from use of certain combinations of fluoride procedures in conjunction with dental sealant therapy; fluorides are relatively most effective in inhibiting decay in smooth surfaces of teeth, whereas sealants have demonstrated their efficacy 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 general application to a greater proportion of the population.

Results of an 11-year, school-based dental health program, consisting of weekly rinsing with a 0.2% NaF solution, daily ingestion of a 1 mg fluoride tablet and home use of a fluoride dentifrice, in Nelson County, Virginia, showed a 65% reduction in caries prevalence compared with baseline findings. However, the

data showed that more than 90% of the remaining decay occurred in pit and fissure tooth surfaces. Accordingly, in 1983 a sealant program was added to the ongoing fluoride program to protect the highly susceptible pits and fissures in occlusal, lingual, and buccal surfaces. Newly erupted permanent teeth of children in selected grades (when teeth are erupted enough to treat but not erupted long enough to experience decay) 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. The combined preventive program has the potential of virtually eliminating dental caries among Nelson County school children.

Additionally, because it has not yet been established that the use of sealants is cost-effective, records have been kept on major factors that influence cost, including longevity of the sealant placement, number of teeth treated, time required to carry out the procedure, salary of operators (dental hygienists) and cost of materials.

The pronounced anticaries effects of the combined fluoride regimen in Nelson County are greater than those usually reported for any of the individual components, suggesting an additive effect. However, because all children in Nelson County received the entire regimen, no certain claims of additive effectiveness can be made. To specifically compare the combined regimen of weekly fluoride rinsing and daily fluoride supplements with each procedure when used alone, an eight year study was initiated in Springfield, Ohio. Interim two-year findings showed that the combined procedure produced a 33% lower increment in dmfs (primary teeth) than one of the positive controls (fluoride rinse group) which already may itself have had an effect in lowering dental caries. Thus, the data not only suggest additive effectiveness but increase the estimate of benefits for the combined fluoride regimen. For permanent teeth which were all newly erupted on the two-year examination, incremental caries scores were too small to permit a valid assessment of effectiveness. However, the latest interim examinations in 1986 should provide DMFS increments large enough to detect treatment effects and to determine if benefits begin to parallel those observed in the primary dentition.

Although gingivitis does not necessarily always lead to the development of progressive periodontal disease, the reduction in gingivitis is still the most important approach to the promotion of periodontal health. A reduction in gingivitis is best achieved by means of effective mechanical oral hygiene measures. Experience from a number of studies indicates that it is difficult to motivate children and maintain good oral hygiene over long periods of time when the emphasis is placed on removing dental plaque. According to Dr. Jukka Ainamo, Professor of Periodontology, University of Helsinki and a Visiting Scientist with the Clinical Trials Section, the elimination of gingival bleeding may produce better results than plaque control as a motivational tool for long-term maintenance of optimal oral hygiene. Under Dr. Ainamo's direction, a two-year clinical trial was initiated in York County, Virginia to test this hypothesis. Ninth and tenth grade children in one group received a manual for self-assessment of gingival bleeding, whereas those in another group received the traditional instructions in plaque control. Children in both groups are supplied with soft toothbrushes and special wooden toothsticks for interdental cleaning and receive instructions in their proper use. 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.

In contrast to the York County study, a chemical approach using stannous fluoride to prevent the development of plaque and reduce gingivitis is being investigated under contract with the University of Minnesota (NO1-DE-52556). Several animal and short-term human studies have demonstrated clear-cut antimicrobial effects for stannous fluoride, thus warranting renewed attention. Adults with gingivitis but without any bone loss (periodontal case Type I) brushed twice a day with a 0.4% SnF_2 gel for a period of 18 months. Results of the SnF_2 group will be compared with those of a NaF gel group and a placebo gel group. Final examinations were completed in the fall of 1987. The data are currently being analyzed and the contractor's report will shortly be submitted to NIDR.

Existing guidelines for the conduct of clinical trials of fluoride dentifrices are not well structured either to determine if a new formulation is superior or equivalent to a currently accepted product or to test for relative anticaries benefits among subjects with a low prevalence of dental decay. Today, both problems confront investigators attempting to evaluate "improved" fluoride dentifrices. In order to develop fluoride dentifrices to its maximum decay preventive potential and thus help to further reduce the level of dental decay among the U.S. population, additional guidelines to establish uniformity in the comparative clinical testing of fluoride dentifrices at low disease levels are needed. With the support of NIDR, the Council on Dental Therapeutics, American Dental Association, held a small conference on June 15 and 16, 1987 to develop guidelines for the design, conduct, and analysis of clinical trials that compare the anticaries effectiveness of new with existing fluoride dentifrices. Investigators from a cross-section of the scientific community were in attendance. A consensus report is currently being drafted.

Dr. Jukka Ainamo's appointment as a visiting scientist in the NIH Visiting Scientist Program terminated in June 1987, after almost two years with the Clinical Trials Section. He returns to the Institute of Dentistry, University of Helsinki, as Professor and Head, Department of Periodontology, the position that he held before taking sabbatical leave from the University. Also, Dr. Stanley B. Heifetz, Acting Chief, Clinical Trials Section retired from the Public Health Service October 1, 1987. Because of the departure of these two key investigators, the Clinical Trials Section finds itself insufficiently staffed with professional personnel.

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Heifetz, S.B., Horowitz, H.S., Meyers, R.J., and Li, S-H. Evaluation of the Comparative Effectiveness of Fluoride Mouthrinsing, Fluoride Tablets, and Both Procedures in Combination: Interim Findings After Two Years. Pediat Dent. 9:121-125, 1987.

Horowitz, H.S.: Indexes for Measuring Dental Fluorosis. J Public Health Dent. 46:179-183, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00070-15 EODP

PERIOD COVERED

October 1, 1986 to September 30, 1987 CT 0600045

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.	Senior Field Investigator	EODPP, NIDR
Heifetz, Stanley B.	Chief, Clinical Trials Section	EODPP, NIDR
Nowjack-Raymer, Ruth	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

Nelson County, Virginia Public School System

LAB/BRANCH

Disease Prevention Branch

SECTION

Clinical Trials Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.40	.18	.22

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 October 1972, a self-administered dental health program was started in Nelson County, Va., a fluoride-deficient community. Children in the County's schools, under teacher supervision, chew and ingest daily a 1 mg F tablet and rinse weekly with a 0.2% NaF solution. A fluoride dentifrice is provided for ad libitum use at home. Baseline DMFS examinations were made of 2138 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 the final examinations will be made in September 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00310-07 EODP

PERIOD COVERED

October 1, 1986 to September 30, 1987 CT 0600144

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Evaluation of fluoride mouthrinsing and fluoride tablets when used separately and in combination

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

Heifetz, Stanley B.	Acting Chief, Clinical Trials Section	EODPP, NIDR
Driscoll, William S.	Senior Field Investigator	EODPP, NIDR
Nowjack-Raymer, Ruth	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(If any)*

LAB/BRANCH

Disease Prevention Branch

SECTION

Clinical Trials Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.84	.30	.54

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

In September 1981, a self-administered dental program 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 dissolve and ingest daily a 1 mg F tablet; the children in Group III rinse weekly with a 0.2% NaF solution; and Group II carries out both procedures. The assigned treatments are self-administered under the supervision of the teacher.

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. The procedures will be continued through 1989 and followed by a clinical examination. Three to four years following the cessation of treatments, a post-treatment examination will be conducted in high school to determine the extent of continued protection.

Supplies have been ordered, schedules arranged and local personnel identified for the start of the seventh school year of treatments in October 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00396-03 EODP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Caries, fluorosis, gingivitis and calculus in areas with different water-fluoride levels.

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

Driscoll, William S.	Senior Field Investigator	EODPP, NIDR
Heifetz, Stanley B.	Acting Chief, Clinical Trials Section	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention Branch

SECTION

Clinical Trials Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.24	.22	.02

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 involves the same seven communities in Illinois that had been previously surveyed by the NIDR in April 1980. The communities were grouped according to the relation of their water fluoride concentration for the area (1, 2, 3, or 4-times optimal). Study participants consisted of children in two age groups, 8-to-10 and 13-to-15 years of age. The 8-to-10 year age group comprised children who received parental consent to participate and whose histories indicated that they had resided continuously since birth in their respective communities. The 13-to-15 year old group comprised only those children who were examined as 8-to-10 year olds in 1980. Examinations for the survey were conducted in April 1985. Dental caries was assessed with the DMFS index and fluorosis was measured with the Tooth Surface Index of Fluorosis, an index developed by NIDR for the 1980 survey. These indices were applied in both age groups by the same examiners who had used them in 1980. A new examiner assessed the prevalence of gingivitis and calculus in the entire dentition in the 13-to-15 year age group. A report presenting findings on dental caries and fluorosis has been submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00439-01

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

Heifetz, Stanley B.	Acting Chief, Clinical Trials Section	EODPP, NIDR
Ainamo, Jukka	Visiting Scientist	EODPP, NIDR
Nowjack-Raymer, Ruth	Clinical Trials Specialist	EODPP, NIDR
Driscoll, William S.	Senior Field Investigator	EODPP, NIDR
Suomi, John D.	Contract	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Disease Prevention Branch

SECTION
Clinical Trials Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.02	49	53

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 April 1987 a study was begun to evaluate two approaches to the prevention of gingivitis in teenage children. Baseline examinations for periodontal health (plaque, pocket depth, calculus, gingival bleeding), DMFS and gingival recession were conducted on 500 ninth and tenth graders in the York County High Schools, Virginia. Questionnaires regarding oral hygiene methods and professional care practices were completed by each student. 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. Every subject participated in small group sessions for instruction in the self-assessment procedures and supply distribution. Two weeks later, individual instruction was held to ensure that the procedures were understood.

Examinations of periodontal health will be conducted in October and April of the next two school years. DMFS and gingival recession examinations will be repeated at the final examination in April 1989. Each participant will receive an oral prophylaxis in May 1988.

LABORATORY METHODS SECTION

Whereas the Laboratory Methods Section staff continued research in oral ecology and in oral disease prevention, the focus of this research has broadened from a focus primarily on dental caries to diseases of the periodontal and oral mucosal tissues. Methods and materials for epidemiological studies of periodontal disease and dental caries; e.g., FA reagents for periodontal pathogens, biochemical tests, improved cultural methods, and ion sensors have been developed and applied. Substantial progress has been made in improving the delivery of known preventive agents and in developing new treatments.

Research has continued on the development of the intraoral controlled-release delivery systems for the prevention and treatment of oral diseases. Controlled-release delivery systems, which are designed to deliver a therapeutic agent at a controlled rate to a specific anatomical site, can be a convenient and efficient means of drug administration. Research has shown the feasibility of controlled intraoral delivery of fluoride from a system fabricated with biocompatible hydrogel copolymers. This system, the Intraoral Fluoride Releasing Device (IFRD), produced significant reductions in the incidence of experimental dental caries in rats and was used for six months in adolescents without significant adverse reactions. Currently work with the Southern Research Institute is being conducted on the development of an improved manufacturing method for the IFRD. This new method should make it possible to produce the large numbers of IFRDs at a reasonable cost. It is expected that a new system for retaining and protecting the IFRD in the mouth will be developed and clinically evaluated during the next 18 months under a separate contract. Completion of these projects will make it feasible to conduct a large-scale two year clinical trial of the IFRD.

The evaluation of a membrane-controlled delivery system for tetracycline that should be suitable for the intraoral (local) treatment of periodontal disease is continuing. This tetracycline controlled-release pellet (TCRP) was developed using the same biocompatible copolymers employed in the IFRD. In a study in primates, 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 in subgingival plaque samples associated with periodontal disease. The TCRPs recovered from the monkeys at end of the treatment period still released biologically active tetracycline. Tetracycline concentrations of 1 to 3 ppm were found in monkey saliva samples upon analysis by high performance liquid chromatography.

Prototype controlled-release pellets for chlorhexidine also have been fabricated using the same biocompatible copolymers used with the tetracycline and fluoride systems. These pellets released biologically active chlorhexidine for at least two weeks. The demonstrated ability of the hydrogel copolymers to release complex organic molecules such as chlorhexidine and tetracycline suggests that these copolymers could be used to fabricate controlled-release systems for antifungal agents that might be useful for the prevention or treatment of opportunistic mycotic infections.

A calcium phosphate solution, CPS, (0.7M Ca, 1.9M PO4), has been shown to increase the uptake of fluoride from topically applied solutions (> 0.5%F). The resultant enamel-bound fluoride was associated with a decreased incidence in dental caries in rats. Recent studies show that topically applied CPS augmented the fluoride uptake and caries restriction attendant to use of an intraoral fluoride-releasing device. The device provided protracted low levels of fluoride to the oral cavity. From these findings one could speculate that CPS treatments alone might enhance the benefits of ambient fluoride from food and drinking water.

There is interest among clinicians, epidemiologists and researchers to determine the surface extent and tissue loss in incipient caries lesions. Members of the section have investigated a method for determining the porosity of enamel white spots termed the fluorescein permeability (Fp) method. This method, which is a variant of the iodide permeability method (Tavares, 1985), has the advantage of greater reliability. Furthermore, the fluorescein, imbibed into porous, caries-active areas of the white spot, fluoresces strongly under blue light (450nm) unlike iodide which is imperceptible under any light source. This allows the documentation of the surface extent of the fluorescein-treated white spot by fluorescent photography.

Members of the Laboratory Methods Section have developed methods of assessing the pH levels of dental plaque in situ in living and dead rats. These procedures have several unique advantages. The electrodes are calibrated in vivo. They are inexpensive, rugged and thin enough for monitoring specific sites on the dentition of the rat.

Methods of saliva collection, transportation and microbiological analysis for S. mutans and lactobacilli are being evaluated and adapted to large scale clinical studies where on-site screening is not possible. Procedures must be devised to effectively collect large numbers of specimens and ship them to the laboratory under conditions that maintain bacterial viability. The use of saliva has been established as an acceptable method for monitoring whole mouth levels of S. mutans and lactobacilli; however, specimen collection can be difficult and time consuming. A mouthrinsing procedure has been examined and found to greatly facilitate the sampling procedures. The problem of specimen shipment has been approached through two basic mechanisms. Rapid freezing of samples at the site of collection has been used successfully in situations where large numbers of specimens are collected over several days and sent to the laboratory for sequential processing. In studies where on-site freezing is not possible, the use of several transport media is being evaluated. In addition, the use of multiple selective media to improve the recovery of S. mutans is being investigated.

Dr. Horace Stiles left the Program after 18 years with the NIDR. As the Chief of the Laboratory Methods Section he provided critical leadership and guidance during a period of organizational change. He has joined the Division of Research Grants as the Executive Secretary of the Epidemiology Study Section.

Kingman, A.; Little, W.A.; Gomez, I.M.; Heifetz, S.B.; Driscoll, W.S.; Sheats, R.; and Supan, P. Salivary levels of S. mutans and Lactobacilli and dental caries experiences in a U.S. adolescent population. Community Dent. Oral Epidemiol., in press.

Mirth, D.B. Controlled-release therapeutic systems. Technology applicable to the treatment of oral disease. Adv. Dent. Res., in press.

Shern, R.J. Effects of various organic compounds and fluoride on dental plaque and caries in rats. Caries Res., in press.

Shern, R.J.; Chow, L.C.; Schreiber, C.; Brunelle, J.A.; Groh, R.K. Effects of flushings with an acidic calcium phosphate solution on fluoride binding and caries in the rat. Caries Res., in press.

Shern, R.J.; Little, W.A.; Kennedy, J.B.; and Mirth, D.B. Effects of octenidine on dental plaque and gingivitis in monkeys. J. Periodontol., in press.

Shern, R.J.; Mirth, D.B.; Emilson, C.-G.; Adderly, D.D.; Bowen, W.H. Evaluation on an intraoral controlled-release delivery system for fluoride in primates. Community Dent. Oral Epidemiol. 15: 113-116 (1987).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00112-14 EODPP

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Little, Wayne	Microbiologist	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Brunelle, Janet A.	Supvy Statistician	EODPP EB NIDR
Li, Shou-Hua	Statistician (Health)	EODPP EB NIDR
Monell Torrens, Esteban	Biology Lab Technician	EODPP LMS NIDR
Mirth, Dale B.	Research Chemist	EODPP LMS NIDR
Bartkiewicz, Andrea	Chemist	EODPP LMS NIDR

COOPERATING UNITS *(if any)*
Clinical Investigation and Patient Care Branch, NIDR (Michael W. Roberts)
American Dental Association Health Found. National Bureau of Standards, Gaithersburg, MD (L.C. Chow)

LAB/BRANCH
Epidemiology and Oral Disease Prevention Branch

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.11	0.72	0.39

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 principle objective of this project is to systematically screen agents that might promote oral health using a selective battery of in vitro and animal tests. Secondary objectives include the development of testing procedures. Recent studies indicate that the anticaries benefits of the intraoral fluoride releasing device can be improved if the teeth are pretreated with a calcium phosphate solution developed by Chow and coworkers (1974). We have identified mechanisms that might contribute to therapeutic effects of the antiplaque agents, alexidine and octenidine. We have developed an in vivo method for measuring the pH of rat plaque in situ.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00282-08 EODPP

PERIOD COVERED

October 1, 1986 - September 30, 1987

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	EODPP LMS NIDR
Bartkiewicz, Andrea	Chemist	EODPP LMS NIDR
Shern, Roald J.	Principal Investigator	EODPP LMS NIDR

COOPERATING UNITS *(if any)*

Southern Research Institute, Birmingham, AL 35255

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Laboratory Methods Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.21	0.11	0.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Intraoral Fluoride Releasing Device is a new intraoral therapeutic system being developed by the National Institute of Dental Research. The IFRD is designed to provide continual topical fluoride for periods of up to six months for the prevention of dental caries.

The IFRD 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 objectives of this project are to refine the shape and method of attachment of the IFRD in order to make it more durable and easier to use in humans and to investigate various treatment regimens in animals in an attempt to optimize the cariostatic benefits from the topical fluoride provided by the IFRD.

Findings from recent animal studies indicate that the marked caries reductions produced by the IFRD were due to topical effects of fluoride and the results also suggest that the cariostatic effects may persist for a significant time period after an IFRD is removed from the mouth.

An improved manufacturing process for the IFRD is being developed and new methods for retaining and protecting the IFRD in the mouth are being reviewed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00408 03 EODPP

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Short-term clinical trials of oral therapeutics N01 DE 52484 CT 0600075

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

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Li, Shou-Hua	Statistician (Health)	EODPP EB NIDR

COOPERATING UNITS *(if any)*
ADA Health Foundation, Research Unit, NBS, Gaithersburg, MD 20899
School of Dentistry, College of Medicine, National Taiwan University, Taipei

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.78	0.20	0.58

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.)*

The objectives of this project are: (1) to identify, adapt or develop, and pretest simple methods of measuring the bacterial and chemical composition of dental plaque and saliva, (2) develop methods of assessing host resistance (3) to conduct short-term clinical studies of agents which might promote oral health. Recent in vitro studies have identified a reliable, clinically feasible method of determining the tissue loss and the surface extent of active incipient dental caries. Tissue porosity was determined by measuring the amount of fluorescein which could be extracted from the fluorescein treated lesion, and lesion extent was documented by fluorescence photography. Short-term clinical studies of CPS will begin when approved by the appropriate review boards. Testing of biochemical tests are being conducted as collaborative efforts within the section and with other groups within the NIDR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00417-02 EODPP

PERIOD COVERED: October 1, 1986 - September 30, 1987

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	EODPP LMS NIDR
Bartkiewicz, Andrea	Chemist	EODPP LMS NIDR
Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Little Wayne A.	Microbiologist	EODPP LMS NIDR
Gomez, Irma M.	Microbiologist	EODPP LMS NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology and Oral Disease Prevention Program

SECTION: Laboratory Methods Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.19	0.52	0.67

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objective of this project is to develop intraoral controlled-release therapeutic systems for antibiotics/antimicrobial 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) have been 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 Intraoral Fluoride Releasing Device also can be used for the delivery of large organic molecules in vivo. In a study in primates, 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 in subgingival plaque samples associated with periodontal disease.

Prototype controlled-release pellets for chlorhexidine have been fabricated using the same biocompatible copolymers used with the tetracycline and fluoride systems. These pellets released biologically active chlorhexidine for at least two weeks. The behavior of these pellets in the presence of saliva is being evaluated.

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 individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00442-01 EODPP

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Evaluation of Methods of Saliva Sampling, Transport and Analysis

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

Little, Wayne A.	Microbiologist	EODPP LMS NIDR
Stiles, H.M.	Chief, Lab Methods Section	EODPP LMS NIDR
Kingman, A.	Statistician	EODPP EB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Laboratory Methods Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.72	.02	.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this study was to examine methods of saliva collection, transportation and microbiological analysis for S. mutans and lactobacilli, which could be adapted to field situations where on site screening is not possible. Experiments were carried out to evaluate the following:

1) The effect of quick freezing and rapid thawing of whole saliva on bacterial counts. This method is applicable to field situations where large numbers of samples are collected over several days and shipped to the laboratory for sequential processing. The average drop in counts after 30 days was 5% for S. mutans and 8% for lactobacilli, indicating that on site freezing of whole saliva is a practical approach for this type of study.

2) The use of a mouth rinsing procedure to facilitate saliva collection yielded counts within the range of variation of whole saliva counts.

3) The effect of combining the saliva rinse with Reduced Transport Fluid for shipment to the laboratory in situations where on site freezing is not possible. S. mutans counts dropped less than 0.1 log10 units after 3 days in RTF, but lactobacillus counts were less predictable, suggesting other transport media should be evaluated.

4) Comparison of three selective media (MSB, GSTB, TSY20B) for recovery of S. mutans. Results vary from sample to sample, possibly due to strain differences, and suggest the use of more than one selective media for S. mutans recovery.

ANNUAL REPORT OF THE EPIDEMIOLOGY BRANCH,
NATIONAL INSTITUTE OF DENTAL RESEARCH

Staff of the Epidemiology Branch have been extensively occupied with the conduct of two national surveys of oral health. Data collection for a study of the oral health of U.S. adults was completed in 1985 and the analyses and reporting of results at scientific meetings and in monograph form were accomplished during the past year. Significant findings included the fact that edentulism was markedly lower among those aged 18-64 years than had been the case 15 years earlier, and that the prevalence of advanced destructive periodontal disease in these age groups was lower than anticipated. However, in persons aged 65 and older, edentulism, root-surface caries and destructive periodontal disease constitute major oral health problems.

Data collection was also completed on a probability sample of over 40,000 U.S. school children, ages 5-17 years, in a repeat of a 1980-81 study of dental caries prevalence. In addition, information was gathered on the presence of calculus, gingivitis, periodontal attachment loss, oral soft tissue conditions, smokeless tobacco use, and dental fluorosis. Analyses of these data will be carried out during FY 1988.

A major interest of the Branch is the study of the prevalence and incidence of incipient periodontal disease in adolescents, with the goal of identifying risk factors, as well as predictors of susceptibility to more advanced disease. A longitudinal study of Navajo Indian high school children continued, and focused on the relationship of clinical (calculus and gingivitis) and microbial factors (B. gingivalis, B. intermedius and H. actinomycetemcomitans) to loss of periodontal attachment and loss of alveolar bone. Analyses thus far suggest that the combination of calculus, gingivitis and B. intermedius increases the relative risk of early attachment loss significantly, though most of the inter-subject variance in attachment loss remains unexplained. Risk factors for bone loss are now being studied.

A complementary study involved the clinical and radiographic examination of 250 adults, aged 30-33 years, in the county of Västerbotten, Sweden. Because radiographs taken at age 15 are available for these subjects, the data will permit assessment of the prognostic value of bone loss in adolescence for adult periodontal status.

The oral health effects of the use of chewing tobacco and snuff were identified as high priority research questions by the U.S. Surgeon General. Staff of the Epidemiology Branch studied this question in Navajo Indian adolescents and found that the use of these products was higher among these teenagers than ever previously reported. The risk of oral leukoplakia, a possibly pre-malignant condition, was more than eight times greater among smokeless tobacco users than in non-users. However, there was no evidence of an increased risk of periodontal disease associated with the use of smokeless tobacco.

The final protocol and manual of procedures were completed for a comprehensive clinical and laboratory study of risk factors in adult periodontitis. This research involves joint collaboration with scientists at SUNY-Buffalo and the University of Texas, San Antonio.

Staff of the Biometry Section carried out an analysis of the effect of partial recording in the estimation of periodontal attachment loss in population surveys. The results confirmed that estimates of prevalence and average severity of attachment loss obtained by examination of two sites per tooth in half the mouth have minimal bias relative to estimates from full-mouth examinations. To reduce examination time, the partial recording method is recommended for large epidemiologic surveys.

Branch staff continued their involvement with the Microbial Strain Data Network, and with the Hybridoma Data Bank, two international collaborative efforts to develop and maintain computer software and data bases on a worldwide scale. Work continued on analyses of phenotypic data on oral microbiota to improve taxonomy and identification data for these organisms.

Several new research projects were begun. One involves a study of trends in product-related facial and dental injuries, using data reported to the National Electronic Injury Surveillance System. Another project begun in collaboration with staff of the School of Dentistry, Louisiana State University, was the addition of a dental examination component to a long-term study of cardiovascular risk factors in a large cohort in Bogulusa, Louisiana. Baseline clinical and radiographic examinations of over 1000 children were completed under the supervision of staff of the Field Studies Section. A study of the prevalence of oral mucosal lesions in adults was also initiated, in collaboration with the V.A. Hospital at Perry Point, Maryland. Finally, administrative arrangements and a protocol were completed for a longitudinal study of oral health in patients with AIDS, in collaboration with the Walter Reed Army Medical Center.

The Branch conducted two 4-day courses on "Clinical Diagnostic Methods for Epidemiologic Studies." A total of 23 attendees from state and local dental public health departments and academic institutions received training in the use of NIDR study methodologies for the diagnosis of dental caries, periodontal disease and oral mucosal lesions.

Most of the Branch staff participated as consultants in the development of the protocol and the training of examiners for the dental section of the forthcoming NHANES-III, to be conducted by the National Center for Health Statistics. Consultation was also provided to several state health departments and to epidemiologists in West Germany, the Republic of Ireland, Hong Kong and China in connection with the planning of population surveys of oral health, and to the Veterans Administration and the American Dental Association regarding statistical analysis of data from clinical trials.

The Branch Chief organized and directed a course in "Epidemiology: Methods and Applications" under the auspices of the ORCA Summer School of Cariology, in Bergen, Norway.

Dr. Jens Pindborg continued to provide consultation in studies of oral mucosal lesions, and in the area of oral conditions related to HIV infection.

Dr. Edith Morrison, who was with the Field Studies Section for two years, returned to the University of Michigan in September 1987.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00044-17

PERIOD COVERED
October 1, 1986-September 30, 1987

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)*

Krichevsky, Micah I.	Research Chemist	MSS, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, EODPP, NIDR

COOPERATING UNITS *(if any)*
See attachment

LAB/BRANCH
Epidemiology

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	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.)*

Microbial strain data are being entered into a data bank to provide: data on specific organisms, indentification of unknown isolates, cluster analysis definition of parameters of taxa, data management and report writing aids, aids in quality control of tests, methods, and laboratories, and communication of data via common format. Data files of primary data on microorganisms found in the oral cavity and related types are established, providing a resource for asking both ecological and epidemiological questions in dental research. Coding conventions have been developed to relate oral clinical parameters with the incidence and distribution patterns of specific microflora. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

Programs are being developed to enter, retrieve, and analyze the data for epidemiological, diagnostic, taxonomic, ecological uses. The long term goal is to establish a world-wide data network at a series of cooperating centers. The original bacterial system now includes the algae, yeasts, molds, protozoa, and hybridomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00250-10

PERIOD COVERED: October 1, 1986-September 30, 1987

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.	Computer Scientist	MSS, EB, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, EB, EODPP, NIDR
Mercer, Paula	Computer Programmer	MSS, EB, EODPP, NIDR

COOPERATING UNITS (if any)
See attachment.

LAB/BRANCH
Epidemiology Branch

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.12	2.12	

CHECK APPROPRIATE BOX(ES)

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

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

Algorithms are being developed and tested for aiding in numerical taxonomy of feature by strain matrices too large to be analyzed by existing programs. Both segmentation and heuristic approaches are being investigated.

A program has been developed and is being tested to compare and evaluate methods and/or laboratories when characterizing the same set of strains. The usual statistical packages are not useful because of the predominantly binary. (i.e., discontinuous) nature of the data. The algorithm allows comparison of tests or laboratories at the levels of the individual strain (with replicable determinations), species, genus, and overall set for determination of test method equivalences and/or inter-laboratory consistency. The program is being used to evaluate TB reference laboratories world-wide.

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 for computer database building of valuable archival paper records of phenotypic strain data.

Computer graphic algorithms are being tested to aid microbiologists in visualizing individual similarities as well as hierarchical group memeberships among strains.

COOPERATING UNITS: V. Jones, Food and Drug Administration
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, Veterans Administration
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
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-00387-04

PERIOD COVERED

October 1, 1986-September 30, 1987

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

Relationship Between Specific Microorganisms in Saliva and Dental Caries

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

Kingman, Albert	Statistician	EB, EODPP, NIDR
Little, Wayne	Microbiologist	EB, EODPP, NIDR
Gomez, Irma	Microbiologist	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology

SECTION

Biometry

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.10	.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.)*

Potential associations between the levels of micro-organisms in saliva and dental caries prevalence and incidence for a population of U.S. adolescents were investigated. Initial findings indicated that there was a significant positive correlation between the levels of S. mutans and lactobacillus in saliva and the prevalence of dental caries. The initial caries prevalence for participants having low levels of these micro-organisms in saliva were 32% and 34% lower than those having low levels for S. mutans and lactobacilli, respectively.

During the 1st 17 months of the study these groups of subjects developed 54% and 67% fewer new DMF surfaces than those with high levels, respectively; by the end of 36 months they developed 44% and 48% fewer DMF surfaces than those with low levels, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: A01-DE-00388-04

PERIOD COVERED: October 1, 1986-September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Evaluation of a New Surface Index of Fluorosis

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

Kingman, Albert	Statistician	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Epidemiology

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.02	.02	

CHECK APPROPRIATE BOX(ES)

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

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

A new index for scoring dental fluorosis, denoted by TSIF, recently developed at the NIDR, was evaluated for reproducibility by the two examiners who performed the fluorosis examinations. Each examiner was able to reproduce his fluorosis diagnosis upon repeated evaluation of a subsample of participants. The examiners experienced some difficulty in agreeing between themselves, especially in making the differentiation between the presence or absence of the very mild form of fluorosis.

In spite of the difference between examiners, this index was able to detect statistically different levels of fluorosis between groups having optimal, 2, 3 and 4 times optimal levels of fluoride in their water supplies, after adjusting for examiner differences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE00399-03

PERIOD COVERED
October 1, 1986-September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Periodontal Disease 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	FS, EB, EODPP, NIDR

COOPERATING UNITS *(if any)*
School of Dentistry, SUNY at Buffalo
Department of Oral Biology

LAB/BRANCH
Epidemiology Branch

SECTION
Field Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	.4	1.1

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.)*

A longitudinal study of risk factors for incipient periodontal disease in adolescents continued in 226 Navajo Indians, aged 14-18. The second of three rounds of clinical, radiographic and microbiologic data was completed.

Analysis of the baseline data indicated that the risk of loss of periodontal attachment is increased by exposure to both B. gingivalis and H. actinomycetencomitans; both associations are confounded by the presence of dental calculus and gingivitis. Therefore, both stratified and multiple logistic regression analyses have been undertaken to remove the effect of confounding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00402-03

PERIOD COVERED: October 1, 1986-September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Analysis of Incomplete Longitudinal Dental Data

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

Li, Shou-Hua Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Epidemiology

SECTION
Biometry

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	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.)

Much of the literature on the analysis of longitudinal data using the intermediate observations assumes that the data are complete, i.e., no missing observations in the repeated measurements of the same individual. Yet longitudinal studies typically have some missing data that make standard repeated measurement procedures inapplicable. Often dental data of this kind are analyzed by looking at only one interval, the longest available. Wei and Johnson (1985) have proposed a procedure which allows the investigator to determine whether a new treatment consistently maintains an improvement over the standard therapy for the entire study period. The test procedure allows different patterns of missing observations in the comparison groups.

Data collected in a dental clinical trial to evaluate DMFS increment of two treatment groups is used to illustrate this method. The test is based on a linear combination of individual t-test statistics on increment data collected at three different time points. The weights in the linear combination are chosen to produce locally the most powerful test when the sample size is large. The weights depend on the patient attrition rate at each time point and the covariance structure of the t-test at different time points. By combining DMFS increment from multiple time points in this clinical trial, the method is able to show that one group is consistently better than the other group over the entire period ($p=0.03$). This result could not be shown statistically by analyzing the data in the conventional way, i.e., by testing the difference in increment between the baseline and final follow-up only ($p=0.09$).

· DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00403-03

PERIOD COVERED
October 1, 1986-September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Collection and Analysis of Oral Health of U.S. Adults

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

Brunelle, Janet A	Chief, Biometry Section	EB, EODPP, NIDR
Miller, Ann J.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Biometry and Field Studies

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.15	.95	2.2

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 National Survey of Oral Health in U.S. Adults was designed to establish the prevalence of coronal caries, root surface caries and periodontal destruction in a readily accessible adult population with sufficient precision to permit future detection of changes by geographic region and within five year age intervals. The sampling frames for this survey included U.S. business establishments listed by Standard Industrial Codes (SIC) and maintained by Dun & Bradstreet and county rosters of multipurpose senior centers compiled from lists confirmed by the state and local area agencies on aging. The primary sampling units were counties, the second stage sampled business establishments or senior centers, and the third-stage sampling unit were the employees or seniors. The categories of Agriculture and Mining, the military, the permanently unemployed, and persons who are not employed outside the home were excluded from the sample.

Both samples were stratified into seven geographic regions of the contiguous 48 states. For employed persons the sample was also stratified by urban/rural, mean income and percent minority in the sampled counties and by size of business establishments. The final sample consisted of 15,132 persons aged 18-64, representing approximately 100 million employed adults in these age groups, and of 5686 retired persons, aged 65-80+, representing 4 million seniors.

The survey was conducted under contract with Westat Inc., of Rockville, Maryland. The data were processed by the Biometry Section, Epidemiology Branch, NIDR. The detailed findings are presented in two 1987 monographs. "Oral Health of U.S. Adults, National Findings: NIH #872868 and Oral Health of U.S. Adults, Regional Findings: NIH #882869.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00410-03 EODPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

The Natural History of Periodontal Disease in Man

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

Löe, Harald	Director, NIDR		NIDR
Morrison, Edith C.	Senior Staff Fellow	EODPP	NIDR
Smith, Jacqueline I.	Statistician	EODPP	NIDR

COOPERATING UNITS *(if any)*

University of Texas Dental School, San Antonio, Texas

LAB/BRANCH

Epidemiology

SECTION

Field Studies

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.77	.77	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The study of the natural history of periodontal disease in Sri Lankan tea laborers is continuing. Three sub-populations have been identified, based on rates of periodontal disease destruction and tooth loss due to periodontal disease: (1) ~10% of the group had rapid progression (RP) and extensive tooth mortality; (2) ~10% showed no progression (NP) of the disease and (3) ~80% showed moderate progression (MP) of periodontal destruction.

Bacteriological, immunological, radiographic and genetic studies of the three populations are under way.

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 the genetic determinants in the three groups.

Building of two master data sets in SAS for the longitudinal Norwegian surveys 1968-1985 (15) was completed. Data bases are: LOE.NOR.AVO (all valid observations) and LOE.NOR.IAS (in all surveys).

Continued studies have focused on the stability of the gingival lesion, the mean number of years needed for an inflamed gingival unit to convert to progressive loss of attachment and the factors which may influence the continuity and discontinuity of the destruction of the periodontium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE00418-02

PERIOD COVERED: October 1, 1986-September 30, 1987

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	EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 pocket 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 signficiant deterioration for shallow sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00420-02

PERIOD COVERED

October 1, 1986-September 30, 1987

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, J. A.	Chief, Biometry Section	EB, EODPP, NIDR
Miller, A. J.	Epidemiologist	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology

SECTION

Biometry

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	.55	1.95

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.)*

A national survey of the oral health of school children was implemented in 1986. A probability sample of approximately 52,000 school-aged children in Kindergarten through 12th grade was selected. Thirteen examiners were trained and calibrated by NIDR staff in criteria for measurement of coronal caries, periodontal disease, fluorosis and presence of lesions of the oral soft tissues. Dental exams on approximately 41,000 children were conducted during the 1986-1987 school year by the 13 dental teams. A questionnaire on use of smokeless tobacco and alcohol was also administered and a salivary sample was collected. The overall response rate for participation was 78%. Data processing of forms and questionnaires is being done. Data analysis for regional and national estimates of the presence of each disease will be made during the coming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00425-02

PERIOD COVERED
October 1, 1986-September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Leukoplakia and Use of Smokeless Tobacco in Adolescents

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
Epidemiology Branch

SECTION
Field Studies

INSTITUTE AND LOCATION
NIDR, NIH

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.8	.3

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project was to examine the relationship between smokeless tobacco use and periodontal status in Navajo Indian adolescents.

Two hundred and twenty-six Navajos, ages 14-19, were examined for evidence of gingival bleeding, calculus, pocket depth, gingival recession, attachment loss, and oral leukoplakia. They were also questioned about their use of smokeless tobacco products. Of the subjects (75.4% of the boys and 49.0% of the girls), 64.2% were users of smokeless tobacco. Of the users, 25.5% had leukoplakia. The duration (in years) and frequency of smokeless tobacco use (days per week) were highly significant risk factors associated with leukoplakia. No consistent relationship was observed between the use of smokeless tobacco and gingival bleeding, calculus, gingival recession, or attachment loss, either when comparing users to non-users or when comparing the segment where the tobacco quid was habitually held to a within-subject control segment. These results confirm that smokeless tobacco is significantly related to the etiology of leukoplakia. The results of this project have been published.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-00429-01

PERIOD COVERED

October 1, 1987 - September 30, 1987

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

Periodontal Health & Oral Soft Tissue Lesions in an Adolescent Population

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

Bhat, Mohandas Visiting Scientist EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

Louisana State University - School of Dentistry, New Orleans, LA
Musselman, R.J.
Cassingham, R.J.
Dummett, Clifford O.
Gardiner, James F.
Schneider, Paul E.

LAB/BRANCH

Epidemiology Branch

SECTION

Field Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.50	.20	.30

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this longitudinal study are: 1) to determine whether early periodontal lesions (attachment loss and horizontal bone loss) indicate a predisposition to chronic periodontitis, 2) to determine the relationship between prevalence of various types of oral mucosal lesions and patterns of consumption of alcohol, cigarettes and smokeless tobacco (snuff and chewing tobacco), and 3) to determine the prevalence and causes of fractured anterior teeth, in an adolescent population.

The study is being conducted in a biracial (35% black), semi-rural community of Bogalusa, Louisana, utilizing junior high school and high school students participating in a longitudinal study of heart risk factors. This study population is unique in that there is access to previously collected longitudinal data on physical and anthropometric examinations, blood biochemistry, 24-hour dietary recall, tobacco and alcohol consumption habits, to name a few, available on these subjects. Hence, there is a potential for testing several secondary hypotheses, besides the main objectives of this study.

Baseline data on the prevalence of dental caries (DMFS), loss of attachment, calculus, soft tissue lesions, traumatized incisor teeth, and crestal alveolar bone height determined by bitewing x-rays have been collected. Current data on alcohol and tobacco usage, as well as history of injuries to teeth has also been collected through questionnaires. The study population consisted of about 1700 children of whom approximately 1000 children participated in the baseline exams. The data are now being processed for computer analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-00430-01

PERIOD COVERED

January 1, 1987 - September 30, 1987

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)*

Bhat, Mohandas	Visting Scientist	EB, EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

Consumer Product Safety Commission, Bethesda, MD

LAB/BRANCH

Epidemiology Branch

SECTION

Field Studies Section and Biometry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.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 objective of this study is to do a trend analysis of product-related data on injuries to teeth, face and jaws, including an analysis of 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 (CPSC).

NEISS collects almost daily data on product-related injuries from a propability sample of all hospital emergency departments in the U.S. and its territories. From these data estimates can be made of product-related injuries associated with, but not necessarily caused by, consumer products and treated in hospital emergency departments. This system excludes injuries associated with automobile accidents, but includes injuries associated with sports and recreational vehicles. Although the system does not provide for collection of data on specific types of injuries to teeth, it is possible to obtain these data through an analysis of the comments on the injuries included in the data base.

Data tapes obtained from CPSC have been processed to record data on specific types of tooth injuries and a preliminary analysis of the data has been carried out. Analysis of summary statistics on data from 1979 through 1985 showed a decreasing trend in mouth and face injuries after a slight peak in 1981. There was, however, a consistent but slight upward trend seen in injuries to teeth during this period. Plans call for more detailed analyses of the data.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00432-01

PERIOD COVERED

October 1, 1986-September 30, 1987

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	FS, EB, EODPP, NIDR

COOPERATING UNITS *(If any)*

University of Umea
Umea, Sweden

LAB/BRANCH

Epidemiology Branch

SECTION

Field Studies Section

INSTITUTE AND LOCATION

NDR, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.2	.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.)*

The epidemiology of Periodontal diseases is not clearly understood, largely because of inadequate diagnostic methods and an incomplete knowledge of possible risk factors associated with the onset of an subsequent exacerbation of the condition. 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 subject. The University of Umea 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 Umea. Dental examinations were conducted in November on 240 subjects at four dental clinics in the surrounding county of Vaserbotten. Clinical components consisted of examinations 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. Also, a brief questionnaire regarding past periodontal treatment was administered. Bite-wing radiographs, taken in adolescence, were retrieved and are being compared to those taken at the 1986 examination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00435-01

PERIOD COVERED: October 1, 1986-September 30, 1987

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

Systematic Errors in Assessing Periodontal Disease using Partial Scoring Systems

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

Kingman, Albert	Statistician	EB, EODPP, NIDR
Morrison, Edith C.	Senior Staff Fellow	EB, EODPP, NIDR
Loe, Harald	Director	NIDR
Smith, Jacqueline	Programmer	EB, EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH: Epidemiology

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.6	.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.)*

An investigation was made regarding the degree of bias and relative bias in estimating the prevalence and severity of periodontal disease that results from employing partial mouth examinations. The partial mouth exams that were considered were all based on the random half-mouth technique. This technique consists of examining sites in two quadrants, one randomly selected from the maxillary arch and one from the mandibular arch.

Three large epidemiologic data sets in which full mouth periodontal measurements were collected were used in this study.

True prevalence and severity of the disease was based on the full-mouth examination. This consisted of diagnosing 112 sites, based on four sites for each of the 28 teeth (excluding third molars). The sites examined were the mesiobuccal, midbuccal, distobuccal and midlingual.

Four separate partial-mouth examinations were considered: the 1st evaluated mesial sites only (M), the 2nd the mesiobuccal and midbuccal sites (MB), the 3rd the mesiobuccal, midbuccal and distobuccal sites (MBD), and the 4th the mesiobuccal, midbuccal, distobuccal, and midlingual sites (MBDL).

All four partial-mouth examinations produced underestimates of the prevalence of periodontal disease, whereas the estimates of severity were dependent upon which sites were selected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00436-01

PERIOD COVERED: October 1, 1986-September 30, 1987

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

Repeated measurement analysis of microbiological changes in dental plaque

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

Li, Shou-Hua Statistician (Health) EB, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Epidemiology

SECTION: Biometry

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.15	.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.)

It is common in the laboratory to measure microbiological changes in dental plaque in subjects (animals) over periods of time. It is usually assumed that the repeated measurements from each subject are correlated rather than independent observations. The statistical procedures when correlation is present in the repeated measurements are different from those used under the independence model. There are three different statistical approaches that may be used in this situation. The simplest method is to use the univariate analysis of variance approach. The second approach is based on the multivariate analysis of variance technique. A third approach is the growth curve analysis. The growth curve analysis is based on a separate polynomial growth curve of time that is fit to each group of data. Data on microbiological changes in dental plaque from three groups of rats measured repeatedly over time were utilized to illustrate three types of analyses of repeated measurements. This study focuses on how the growth curve method can be successfully applied to analysis of microbial growth data in dental plaque since this approach has not been used by dental researchers.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-00443-01

PERIOD COVERED
October 1, 1986-September 30, 1987

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.	Dental Epidemiologist	EB, EODPP, NIDR
Kleinman, Dushanka V.	Special Assistant to the Associate Director for Program Coordination	EODPP, NIDR

COOPERATING UNITS *(if any)*
Perry Point VA Medical Center
Perry Point, MD

LAB/BRANCH
Epidemiology Branch

SECTION
Field Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.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 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 12-month period. It is expected that about 2,000 patients will be examined during the study year. Pathologies that cannot be definitively diagnosed by the examining dentist will be 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 and to characterize the range and severity of the pathologies noted. Information will also be collected from existing records regarding risk factors such as dental prostheses, medical condition, medications, and use of tobacco and alcohol.

ANNUAL REPORT of the SCIENCE TRANSFER and RESEARCH ANALYSIS BRANCH, NATIONAL INSTITUTE OF DENTAL RESEARCH

INTRODUCTION

The Science Transfer and Research Analysis Branch (STRAB) conducts: (1) activities designed to promote oral health by transferring the results of scientific research to other scientists, health care providers, and the general public; and (2) research concerning the relations among the changing patterns of oral diseases and economic, social, and personal characteristics of the population and the profession.

RESEARCH ANALYSES

Three internationally known scholars, Drs. Richard Oliver, Leif Heloe, and Lawrence Meskin, each spent several months as visiting scientists on the staff of STRAB. Dr. Oliver, former Dean at the University of Southern California and the University of Minnesota Schools of Dentistry, researched the measurement of treatment needs resulting from periodontal diseases for the U.S. population. Dr. Heloe, former Minister of Health and Social Affairs for Norway, investigated the relationship between oral health, treatment needs, and the characteristics of different dental delivery systems. He also lectured extensively to NIDR staff and to faculty at dental schools and schools of public health throughout the country. Dr. Meskin, Dean of both the School of Dentistry and the Graduate School, University of Colorado Health Sciences Center, investigated patterns of tooth loss and their treatment implications among U.S. adults.

STRAB staff collaborated extensively with each visiting scientist to complete several studies. Using data from a national household survey, studies of the epidemiology of periodontal diseases and the cost in time and money for treating those diseases were completed. Several analyses of patterns of tooth loss and their treatment implications among U.S. adults using data from four national surveys were completed. A study comparing oral health, treatment needs, and the dental delivery system characteristics of the United States and Norway was also completed.

A pilot study of the relation between oral health status, treatment needs and utilization of dental services was conducted by STRAB in cooperation with the Dental Outpatient Clinic of the Boston Veterans Administration (VA) and Department of Oral Ecology, Harvard School of Dental Medicine, using longitudinal data from the V.A. Normative Aging Study. The pilot study was successful; a full analytical study is anticipated for the coming year.

A study initiated and supported by STRAB and conducted by Dr. John Newman of Georgia State University to inventory all existing national oral health and dental databases and assess their analytical potential both singly and in combination will be completed in the fall, 1987. A workshop to discuss the implications of the findings of this project for additional research is being planned for the fall, 1987.

STRAB staff participated in the analyses of sociodemographic data from the National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-86 conducted by EODPP and reported the results of the analysis at a symposium during the annual session of the International Association for Dental Research.

NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY III (NHANES III)

Staff was responsible for the development of diagnostic criteria and preliminary training for the oral clinical examination of the third cycle of the National Health and Nutrition Examination Survey (NHANES III) for the restorations, tooth conditions, and prostheses assessment. Initially, purchase orders were awarded to the University of North Carolina at Chapel Hill School of Dentistry for the development of clinical criteria for recording unaggregated conditions and the preparation of a training manual. Refinements were made by staff to the criteria and the manual was completed in preparation for pilot testing of the dental examiners as a component of the NHANES III. Staff also has been active in developing the dental component of the household interview of the third cycle of the NHANES III that will be conducted by the National Center for Health Statistics in FY 1988.

HEALTH PROMOTION AND SCIENCE TRANSFER

Staff has been active in developing a national plan to promote oral health for the U.S. adult population. Using existing data sources, major oral health issues and key target groups are being identified. Objectives, which describe potential disease reduction, are being developed based on observed needs. Individual, professional, and environmental interventions are being identified. The plan is being revised based on reviews by dental and health promotion experts. Working groups are planned for the fall, 1987 to provide further expert input for the development of a complete plan early in 1988. Staff collaborated with the Clinical Trials Branch, EODPP, on a demonstration project to determine the effectiveness of a bleeding index as an educational tool for plaque reduction among high school aged students.

Staff has been active in working with other agencies, providing health promotion and research analysis expertise. One member assisted the Indian Health Service and the Head Start Program in the study design for a health promotion demonstration project for baby bottle caries. Additionally, staff has worked with the National Institute on Aging (NIA) on the evaluation of an Institute of Medicine proposal on health promotion and the elderly and served on a special review group to evaluate nutrition conference grant applications.

STRAB staff continued activities in support of public health agencies in states, cities, and other countries, that are involved in the promotion,

implementation, or extension of dental health programs using fluorides. In FY 1987, these activities have included provision of technical information for judicial and regulatory proceedings; performing liaison with other health and technical entities (U.S. and foreign) for the development, synthesis, and exchange of needed information; maintaining working contacts with professional groups in public health and related fields; continuous review of relevant publications, research reports, and findings of judicial, regulatory, and legislative deliberations. To further some of these activities, a STRAB staff person is a member of the American Dental Association (ADA) National Advisory Committee on Fluoridation and an Invited Observer at the meetings of the U.S. Preventive Services Task Force.

STRAB staff prepared a variety of new educational materials including: a free-loan exhibit, a leaflet and bookmark on preventing baby bottle tooth decay; a free-loan exhibit on preventing periodontal diseases; prepared a user's guide for NIDR's film, "Prescription for Periodontal Health"; a poster on barrier techniques and prepared new materials for the NIA's "Age Page" on oral health. Staff revised "Fluoride to Protect Your Children's Teeth" (for Information Office) and "A Healthy Start...".

Staff worked with non-Federal groups in the preparation of a video-tape on the use of sealants and a manual for using sealants in private practice or public settings. Staff is working with the VA on the development of audiovisual materials on infection control practices in dental settings.

The analysis of data obtained by surveys conducted by the ADA, American Dental Hygienists' Association (ADHA) and University of Ohio regarding the knowledge, attitudes and practices of dental care providers vis-a-vis the prevention of the transmission of infectious diseases in dental settings has been received and will be used in collaborative efforts to develop educational strategies to promote change among practitioners.

Staff efforts continue to provide consultation and assistance to educational and health agencies in the US and abroad regarding oral disease prevention and health promotion. These activities include preparing manuscripts, giving presentations and providing scientific information and educational materials.

STAFF DEVELOPMENT

From November 1986 to May 1987, the STRAB staff organized and presented 9 seminars. The purpose of the seminars was to familiarize STRAB staff and other NIDR staff with the activities of individual members and to provide opportunities for expert input and discussion. The topics that were discussed were broad and included epidemiological data and treatment needs, a plan to improve the oral health of the nation's adults, the difficulty in determining treatment needs from epidemiological data, dental radiology, and expenditures for caries and prosthetics-related treatment needs.

Two demonstrations of a computerized dental record system were organized by STRAB for 25 people throughout the PHS as well as representatives from the Department of the Navy and the VA. The software was developed by Drs. Howard Bailit and Terry Truax, and Dr. Truax demonstrated the computerized clinical record system which is an electronic substitute for paper records. As

demonstrated, the system collects information on a variety of important office activities including dental status, treatment plans, and treatment rendered.

One staff member completed and graduated from a 10-month Grant Associate Seminar Series.

OTHER ACTIVITIES

The members of STRAB staff has been active on several committees that deal with science transfer and health promotion. One member is responsible for evaluation of scientific programs and membership services for the Federation Dentaire Internationale (FDI) as well as serving on several committees of that organization: Oral Health Promotion Working Group, Marketing Working Group and Scientific Program Committee. This person is also President of Behavioral Scientists in Dental Research (BSDR) and organized and led a workshop on research methodologies for health promotion for BSDR at the IADR annual meeting in March, 1987. Also, this individual is on the Future of Dental Research Committee of the IADR. Staff participated in two contract review groups for Office of Planning, Evaluation and Communication (OPEC) and assisted two intramural scientists in questionnaire design for ongoing projects. This member serves on the NIH Women's Health Issues Committee, is NIDR's representative to the R&W Executive Council, and is on the R&W Board, serving as corresponding secretary.

Another staff member is a member of the Executive Council of the American Association of Public Health Dentistry and chairs the prevention committee through which science transfer activities take place. Staff worked with NIDR and Office of Medical Applications of Research (OMAR) personnel to facilitate two forthcoming consensus development conferences.

PUBLICATIONS:

Gift, H.C.: Current utilization patterns of oral hygiene practices. Dental Plaque Control Measures and Oral Hygiene Practices. H. Loe and D. Kleinman (eds.). Oxford, England: IRL Press Limited, 1986.

Gift, H.C.: Demand for dental care and treatment. Dentistry and Social Change. A. Schuller, E. Witt and B. Bergmann-Krauss (eds.). Koln, Germany: German Dental Association, 1986.

Gift, H.C.: Prevention of dental caries--the sum of community, professional and individual efforts. Evaluation and the Health Professions. August, 1987.

Glasrud, P.H., Frazier, P.J. and Horowitz, A.M.: Insurance reimbursement for sealants in 1986: report of a survey. Journal of Dentistry for Children. March-April, 1987

Horowitz, A.M. and Frazier, P.J.: Effective oral health programs in school Settings. Clinical Dentistry. Clark (Ed.), J.B. Lippencott Co.

Miller, A., Brunelle, J., Carlos, J., Brown, L. and Loe, H.: Oral Health of United States Adults, National Findings. NIH Pub. No. 87-2868, Bethesda, Maryland, 1987 (in press).

Staff provided logistical support to the Clinical Investigations and Patient Care Branch and the two co-chairman of the Conference on the Evaluation and Management of Salivary Gland Dysfunction held in May 1986. The proceedings of the Conference were published as a special issue in the February 1987 Journal of Dental Research through the continued working relationship with the co-chairmen and the director of publications for the Journal of Dental Research.

PRESENTATIONS:

Brown, L.J.: "Sociodemographic and Other Dental Characteristics." presented during the Symposium on Oral Health of U.S. Adults: NIDR 1985 National Survey at the 65th Annual Session of the International Association for Dental Research, March, 1987.

Brown, L.J.: "The Relationship Between Clinical Need and Demand for Dental Services," presented at the 65th General Session of the International Association for Dental Research, March, 1987.

Brown, L.J.: "A Survey of Dental Health and Dental Utilization Associated with Prepaid Dental Care," a paper presented at the 114th Annual Meeting of the American Public Health Association in Las Vegas, Nevada, October, 1986.

Frazier, P.J., Loupe, M.J., Horowitz, A.M., Kleinman, D., U. of MN, Minneapolis, and NIDR, Bethesda, MD, "Science Transfer: Impact of NIDR on Dental Hygiene Educators' Caries-Prevention Knowledge and Opinions".

Gift, H.C.: "Research Agendas in Health Promotion--Application to Dentistry," Behavioral Scientists in Dental Research Special Workshop at the 65th General Session of the International Association for Dental Research, March 1987.

Glassrud, P., Frazier, P.J., Horowitz, A.M.: School of Dentistry, University of Minnesota, Minneapolis, MN and NIDR, NIH. "Insurance Reimbursement for Sealants in 1986: A National Survey."

Horowitz, A.M., Gift, H.C., NIDR, Bethesda, MD, C.E. Mounts, ADHA, Chicago, IL, "Knowledge and Reported Use of Hepatitis B Vaccine by Dental Hygienists," presented at the 65th General Session of the International Association for Dental Research, March, 1987.

Horowitz, A.M.: "Keeping Your Patients Caries Free," presented at the Montgomery Dental Hygienists Association in Rockville, MD, October 14, 1986.

Horowitz, A.M.: "The Use of Fluorides in Community-based Programs," Department of Operative Dentistry, Georgetown University, Washington, D.C., November 19, 1986.

Horowitz, H.S. and Horowitz, A,M.: Two-Week Course in Preventive Dentistry, Intercountry Centre for Oral Health, January 5-16, 1987.

Horowitz, A.M.: "Keeping Your Patients Caries Free: The Role of Dental Hygienists," Columbia, S.Carolina, January 31, 1987.

Horowitz, A.M.: "The Appropriate Use of Fluorides," D.C. Dental Society, Washington, D.C., February 10, 1987.

Horowitz, A.M.: "Implementing Effective, Comprehensive, Community-Based Programs," Department of Health Ecology, School of Dentistry, University of MN, Mpls, MN, February 19-20, 1987.

Horowitz, A.M.: "School Based Caries Preventive Regimens," Department of Community Dentistry, U. of Penn., Philadelphia, PA, March 18,1987.

Horowitz, A.M.: "Promoting Oral Health Through Preventing Periodontal Diseases," Annual Migrant Health Conference, South Padre Island, TX, April 10, 1987.

Horowitz, A.M.: "Appropriate Use of Fluorides" and "Update on Dental Health Education Materials for the Preschool Child," Dental Head Start Workshop, Sedona, AZ, August 13, 1987.

Horowitz, A.M.: "What's New In Caries Prevention," Conference on Nutrition and Dental Health Through the Life Cycle, Sedona, AZ, August 14, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00444-01

PERIOD COVERED
January 1, 1987 - September 30, 1987

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	STRAB NIDR
Kleinman, Dushanka	Spec. Asst. to Assoc. Director for Pgm. Coordination	EODPP NIDR
Oldakowski, Richard	Computer Programmer	STRAB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.31	0.3	.01

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 survey and interview data collected from a household sample of the U.S. population and describes the associations among attitudes, behaviors and oral health. The data analysis 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: Z01DE00445-01

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

Gift, Helen	Sociologist	STRAB NIDR
Houghton, Joan	Education Specialist	STRAB NIDR
Horowitz, Alice	Education Specialist	STRAB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.60	.05

CHECK APPROPRIATE BOX(ES)

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

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

The activities involved extensive literature review, analysis of existing data bases, and consultations with experts in the dental sciences and health promotion fields. The objective of the project is to identify the key diseases, target audiences and strategies available to prevent oral diseases. The plan will be the basis of publications on the state of the art, research projects to improve knowledge of existing oral health strategies and programs to encourage oral health in identified populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00446-01

PERIOD COVERED: February 1, 1987 - July 1, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
TOOTH LOSS PATTERNS IN U.S. ADULTS

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

Brown, L. Jackson	Acting Chief	STRAB NIDR
Meskin, Larry	Mem. Natl Advs Den Res Cncl	STRAB NIDR
Warren, Galen	Dental Research Ana	STRAB NIDR
Brunelle, Janet	Supvy Statistician	EODPP NIDR
Oldakowski, Richard	Computer Programmer	STRAB NIDR

COOPERATING UNITS (if any)
School of Dentistry and Graduate School, University of Colorado Health Sciences Center

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The study used data from four national surveys of the U.S. population to describe tooth loss patterns and to indicate the treatment needs generated by the tooth loss. The results from the different surveys are being compared to detect trends in tooth loss patterns and associated treatment needs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00447-01

PERIOD COVERED
September 15, 1986 - October 1, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
EPIDEMIOLOGY OF PERIODONTAL DISEASES AND IMPLICATIONS FOR TREATMENT NEEDS

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

Brown, L. Jackson	Acting Chief	STRAB NIDR
Oliver, Richard	Special Expert	STRAB NIDR

COOPERATING UNITS *(if any)*
University of Minnesota School of Dentistry

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.95	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The study used epidemiological data on periodontal disease collected with a household survey of the U.S. population which describe the prevalence, severity and extent of periodontal diseases. These epidemiological data were then used to estimate the periodontal treatment needs of the U.S. as well as the time and dollars required to provide the treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00448-01

PERIOD COVERED
October 1, 1986 - June 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
THE RELATIONSHIP BETWEEN ORAL HEALTH AND VARIOUS SOCIAL AND DEMOGRAPHIC FACTORS

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

Heloe, Leif	Visiting Scientist	STRAB NIDR
Warren, Galen	Dental Research Ana	STRAB NIDR
Brown, L. Jackson	Acting Chief	STRAB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	1.0	.05

CHECK APPROPRIATE BOX(ES)

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

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

The work involves an investigation of the relationship between oral health, treatment needs, and the characteristics of different dental delivery systems. The dental health policy of a country is determined by a variety of factors, economic, demographic, social, and cultural. The influence of such factors in shaping dental care delivery and financing were highlighted particularly in reference to the U.S. and Norway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00449-01

PERIOD COVERED: September 1, 1986 - October 1, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
KNOWLEDGE, ATTITUDES, PRACTICES OF DENTAL DIRECTORS RE INFECTIOUS DISEASES CONTROL

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

Horowitz, Alice — Education Specialist — STRAB NIDR

COOPERATING UNITS (if any)

University of Ohio

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project was designed to analyze data regarding infection control from a national survey of local and county dental directors. Data regarding their knowledge, attitudes and practicers about infection control in dental settings will be used as baseline information to develop appropriate educational efforts for change.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00450-01

PERIOD COVERED: September 15, 1987 - October 1, 1987

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.): Knowledge, Attitudes, Practices of Dental Directors re Infectious Diseases Control

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

Horowitz, Alice — Education Specialist — STRAB NIDR

COOPERATING UNITS (if any)

American Dental Association

LAB/BRANCH: Science Transfer and Research Analysis Branch

SECTION:

INSTITUTE AND LOCATION: NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	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.)

This project was designed to analyze data concerning infection control from a larger, national, cross sectional survey. Data regarding dentists' knowledge, attitudes and practices about the prevention of the transmission of infectious diseases will be used as baseline information to develop and implement educational programs on the topic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00451-01

PERIOD COVERED
September 1, 1986 - October 1, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
KNOWLEDGE, ATTITUDES, PRACTICES OF DENTAL DIRECTORS RE INFECTIOUS DISEASES CONTROL

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

Horowitz, Alice	Education Specialist	STRAB NIDR
Gift, Helen	Sociologist	STRAB NIDR

COOPERATING UNITS (if any)
American Dental Hygienists Association

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

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

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

This project was designed to analyze data concerning infection control from a larger national cross sectional survey data regarding dental hygienists' knowledge, attitudes and practices about the prevention of the transmission of infectious diseases in dental settings will be used as baseline information to develop and implement educational strategies for change.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00452-01

PERIOD COVERED
September 1, 1986 - October 1, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Knowledge, Attitudes, Practices of Dental Directors re Infectious Diseases Control

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

Horowitz, Alice Education Specialist STRAB NIDR

COOPERATING UNITS *(if any)*
Dental Section, Department of Health, State of Georgia

LAB/BRANCH
Science Transfer and Research Analysis Branch

SECTION

INSTITUTE AND LOCATION
NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	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 project is designed to analyze state data regarding infection control of dentists. Their knowledge, attitudes and practices regarding the prevention of the transmission of infectious diseases will be used to develop and implement a broad based educational effort.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00453-01

PERIOD COVERED: November 1, 1986 - September 30, 1987

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	STRAB NIDR

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Science Transfer and Research Analysis Branch

SECTION:

INSTITUTE AND LOCATION: NIDR NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

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

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

The potential value of existing data sources for further analyses is being investigated utilizing a purchase order mechanism. National dental survey and oral clinical exams conducted over the past 30 years have been identified and contacts have been made with sponsors and principal investigators to determine the status of data files. Reviews of publications and other mechanisms are being used to determine the appropriateness of additional work, with emphasis on possible trend analysis and interpretation for public policy.

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 tissue. It is the only NIH Branch or Laboratory devoted to bone research. In the late fall of 1985, the Branch was reviewed by the NIDR Board of Scientific Counselors and achieved a uniform rating of excellence. Indeed, the scientific efforts of the Bone Research Branch are widely recognized by the international biomedical research community. This past year, two organizational changes were recommended for the Branch. The nuclear magnetic resonance spectroscopy program was to be separately recognized as the Protein Biophysics Unit, Dr. Dennis A. Torchia, Chief. The Skeletal Biophysics Section was to be renamed the Mineral Chemistry and Structure Section, Dr. E. David Eanes, Chief. A number of significant research advances were achieved this year. These are detailed below.

Skeletal Biology Section

The features that distinguish one connective tissue from the other arise from the cells and extracellular matrices that comprise them. The major functions of the bone forming cells are, in fact, to synthesize, secrete, organize and mineralize a particular set of primary gene products, the precise proportions of which are unique to this tissue. All of the structural, biomechanical and metabolic properties of bone either originate directly or are modulated by these gene products. Further, over twenty different hormones, vitamins and growth factors are known to regulate bone metabolism. In normal circumstances, bone formation is tightly regulated by the concerted efforts of these regulatory agents. Imbalances in either the intrinsic features of bone turnover or the regulatory systems that control it lead to heritable and acquired diseases of the skeleton. These diseases are marked by changes in bone cell synthetic patterns that can be followed experimentally. Studies of such phenomena are organized in the Skeletal Biology Section (SBS), Dr. John D. Termine, Chief, along three interdependent disciplinary lines of investigation; protein chemistry, cellular biochemistry and molecular biology.

The protein biochemistry group, headed by Dr. Larry W. Fisher, completed the purification of sufficient quantities of human bone proteoglycan I and II, bone sialoprotein I and II, osteonectin, and 24K phosphoprotein to allow amino terminal sequencing of all these proteins. Antisera were produced to both the intact proteins and, for both the small proteoglycans and the sialoproteins, to synthetic peptides constructed from microsequence data. These antisera have proven useful for: 1) production of quantitative assays; 2) biosynthesis studies in cultured osteoblastic cells (Dr. Gehron Robey); 3) immunolocalization of these and related proteins in the mouse and rabbit; and 4) purification of full length human cDNA clones for complete sequencing of the proteins' primary structures. The N-terminal sequences and the antisera showed, for the first time, that PGI and PGII and also BSPI and BSPII were all completely separate gene

products. Additionally, the V8 protease cleavage site of tendon/skin/cartilage PGII was located between residues 17 and 18 of its amino terminal sequence. It was shown also that the 24K phosphoprotein of bone and dentin is the N-propeptide of α1(I) collagen. In addition to being a potentially strong marker of matrix turnover, this bone protein is of interest because its single serine residue (in bovine) is stoichiometrically phosphorylated. Preliminary biosynthetic data (Dr. Gehron Robey) suggest that skin cells in culture, unlike bone cells, may not phosphorylate this serine, thus suggesting that this particular post-translational modification of type I collagen may be tissue-specific.

Studies in the Section's bone cell biochemistry group, headed by Dr. Pamela Gehron Robey, were aimed at the elucidation of mechanisms governing the growth and differentiation of bone cells in culture. After immunoprecipitation of radiolabeled cultures with monospecific antisera and SDS gel electrophoresis, pulse-chase analysis of the biosynthetic products of bone cells suggested that they differ in both compartmentalization and kinetics of secretion. In addition, the bone cell proteoglycans were identified and found to contain a 600,000 M_r species containing chondroitin sulfate and a core protein of 350,000 M_r, a 400,000 M_r species containing heparan sulfate, and 250,000 and 135,000 M_r species (PGI and PGII, respectively) containing dermatan sulfate and core proteins of ~45,000. Many, but not all, of these proteoglycans were incorporated into the extracellular matrix. Finally, by electron microscopic analysis, <u>in vitro</u> bone matrix mineralization was found to occur by hydroxyapatite deposition onto both collagen fibers and matrix vesicles.

The active form of vitamin D_3, 1,25-$(OH)_2D_3$, was found to inhibit human bone cell growth. Further analysis showed that at physiological doses of 1,25 $(OH)_2D_3$, osteonectin levels seemed unaffected, while at high doses, osteonectin mRNA and biosynthesis were markedly decreased. These results contrasted with the effects of this metabolite on collagen and osteocalcin synthesis which were elevated at most doses studied. Thus, a major effect of vitamin D_3 on human osteoblastic cells is to inhibit their growth and modulate their biosynthetic pathways. Fetal bovine bone cells were found to have message for, to actively secrete, and to respond to transforming growth factor beta (TGF-β). These studies, done in collaboration with Drs. Michael Sporn and Anita Roberts of the NCI, suggest that this agent may be an autocrine or paracrine factor for bone cells. TGF-β was found to be a mitogen for fetal bovine cells, but inhibited growth of two transformed, osteosarcoma cell lines. Fetal bovine cells had both high and intermediate affinity receptors for TGF-β and responded to its presence when only 1% of the receptors were occupied. These studies have important implications for bone cell metabolism <u>in vivo</u> and point toward eventual use of specific agents such as TGF-β to promote bone formation in pathological conditions.

The Section's molecular biology group, headed by Dr. Marian F. Young, continue their efforts to study the nature and regulation of the bone genome. The complete primary structures of bovine bone PgII and osteonectin have been determined and revealed distinct structural domains in these proteins. Bone PGII shares extensive homology to a small PGII made by human fibroblasts and appears to process a pre-pro sequence at its NH_2 terminus. The primary sequence of bone PGII is identical to a bovine

skin form except for two amino acid substitutions at residues 139-140. Osteonectin on the other hand, contains two potential high affinity Ca^{++} binding structures along with a dominant cysteine-rich domain. Protein sequence comparisons indicate that osteonectin is homologous to the recently described protein SPARC made by parietal endoderm cells, to a "culture shock" protein whose synthesis is induced in long-term endothelial cell cultures, and to BM40, a component of the EHS basement membrane tumor. Because the underlying characteristics of these homologous systems seems to be rapid proliferation and growth, the distribution of osteonectin was determined in a human tissue with these traits namely, the decidua of the placenta. This work was done in collaboration with Dr. U. Wewer, NCI. In this tissue, osteonectin was expressed mainly by newly differentiated, intermediate cells and showed specific temporal and spacial induction, being shut down at a terminal stage of differentiation both at mRNA and protein levels.

Parathyroid hormone was found to inhibit osteonectin mRNA expression as much as 90% in the osteosarcoma bone cell line UMR106. In view of this and the above vitamin D regulation of osteonectin expression, genomic DNA was isolated and characterized in detail. Several bovine genomic libraries were constructed and the entire coding portion of the osteonectin gene obtained. Electron microscopic and DNA sequence analyses reveal that the gene is divided into 9 exons, several of which encode discrete protein domains. Studies are now underway to characterize the potential cis and trans activating elements associated with osteonectin gene expression.

Proteoglycan Chemistry Section

Studies this year by the Proteoglycan Chemistry Section have shown that monoclonal antibody 1-C-6 recognizes two tryptic peptides in the cartilage proteoglycan core protein, one in the N-terminal globular domain with the hyaluronate-binding site and one in the second N-terminal domain. Sequence analyses of these two peptides indicate that they have an identical stretch of 9 amino acids which contains both V8- and chymotrypsin-sensitive sites and a tyrosine residue, all known to affect reactivity with 1-C-6. Thus, this region, which is highly conserved in proteoglycans which interact with hyaluronate, appears to contain the epitope and is likely to be critical for hyaluronate-binding activity. Further, the results indicate that the two N-terminal globular domains share considerable sequence homology even though the function of the second domain is presently not known.

The initiation step for chondroitin sulfate chain biosynthesis on the core protein, namely the transfer of xylose from UDP-xylose to acceptor serine residues, was shown to be a late event in the post-translational maturation of the core precursor to a proteoglycan in chondrocytes. Thus, ^{3}H-labeled serine residues in the core protein precursor were shown to have less than 15% as many substituted serines as are present in chondroitin sulfate linkages on the completed proteoglycan. This result contradicts a long standing hypothesis in the literature that xylosylation of core protein occurs at the level of the nascent polypeptide in the rough endoplasmic reticulum. Preliminary evidence, however, has suggested that xylosylation of the core, probably in an early Golgi compartment, does precede the addition of the remainder of the chondroitin sulfate chain. Xylosylation,

then, may be an important regulatory step in determining numbers and sites of glycosaminoglycan substitution on core proteins.

Chondrocytes in organ culture of articular cartilage maintain steady state metabolism of proteoglycans whereby biosynthesis is balanced by catabolism. The insulin-like growth factor-1, IGF-1, at 20 ng/ml was shown to be sufficient to maintain steady state proteoglycan metabolism in the absence of serum for at least 5 weeks. Transforming growth factor-β, TGF-β, at 5 ng/ml in the absence of serum, was shown to be able to increase proteoglycan synthesis to the maximum levels achieved with serum. Thus, normal chondrocytes residing in an established extracellular matrix have receptors which recognize these small polypeptide growth factors and which are critically involved in regulation of proteoglycan metabolism.

Studies on the synthesis and catabolism of hyaluronate showed that this glycosaminoglycan is metabolized with the same kinetic parameters as for the proteoglycans. Thus, hyaluronate in native, undissociated aggregates is labeled with ^{3}H-glucosamine in a ratio relative to labeled chondroitin sulfate in monomers equivalent to their chemical ratio in the aggregate. Further, the labeled hyaluronate is catabolized from the matrix at the same rate as for the bound proteoglycans. This evidence suggests a novel mechanism for normal proteoglycan catabolism in cartilage, one in which the chondrocytes actively remove hyaluronate from the matrix via cell surface associated processes which at the same time disrupt proteoglycan monomers from their aggregation to hyaluronate. Thus, the chondrocytes appear to have a normal mechanism for turning over entire proteoglycan aggregate structures through cell surface mediated events.

Ion exchange and hydrophobic chromatography methods developed for studying chick cornea proteoglycans have been applied to analyses for individual human corneas, obtained at keratoplasty, which have been labeled with appropriate isotopic precursor. These procedures have permitted a complete analysis of the structures of the dermatan sulfate proteoglycan and of the modified keratan sulfate proteoglycan synthesized by a portion of a single cornea from a patient with corneal macular dystrophy. The analyses confirmed that the biosynthetic defect is an inability to add sulfate esters to keratan sulfate and showed that the modified keratan sulfate proteoglycan does carry short lactosaminoglycan side chains that are sensitive to endo β-galactosidase digestion. The procedures show excellent potential for detecting and characterizing additional proteoglycan anomalies, particularly for keratan sulfate proteoglycans, which appear to lead to clinical disorders requiring corneal transplantation.

Studies with a Chinese Hamster Ovary (CHO) cell mutant, clone 13, which has impaired ability to antiport transport UDP-galactose from the cytoplasm into the Golgi complex have shown that the defect greatly reduces the cells net capacity for glycosaminoglycan synthesis. This result is consistent with the evidence that the addition of the two galactose residues in the glycosaminoglycan chain linkage region required for subsequent chain elongation occurs in the Golgi. A previous observation that the rate of proteoglycan synthesis in the mutant cell line was nearly the same as for the parental cell line is now explained by: a) the fact that the parental cell line has a capacity for net glycosaminoglycan synthesis which is 2 times the endogenous proteoglycan synthesis rate whereas the mutant has no

excess capacity, and b) the likelihood that the galactosyl transferase enzymes involved in glycosaminoglycan synthesis have preferential access or affinity, i.e., lower Km values, than the galactosyl transferase enzymes involved in oligosaccharide synthesis, which is more severely affected in the mutant.

Work continued on the structural characterization of ovarian granulosa cell proteoglycans. Monoclonal antibodies have been raised against proteoglycans isolated from whole ovarian tissue and they were screened using highly purified proteoglycans radiolabeled in granulosa cell cultures. Antibodies which recognize DS-PG have been isolated and the characterization of epitope structures is underway. Work has been also initiated toward developing cDNA probes for the ovarian granulosa cell proteoglycans.

During the preparation of monoclonal antibodies against granulosa cell proteoglycans, we, along with other investigators, found difficulties raising antibodies against membrane-associated HS-PG. This may suggest the presence of structurally similar membrane-associated proteoglycans in antibody producing cells as those used for antigen. Characterization of hybridoma proteoglycans indicated these cells synthesize two major types of proteoglycans; CS-PG (with overall MW ~120,000 and a core protein of MW ~42,000) which is secreted into culture medium, and a CS/HS hybrid proteoglycan (overall MW ~90,000 with a core protein of MW ~74,000).

Cell membrane-associated proteoglycans have been implicated to play some important roles in association with the cytoskeletal system. A parathyroid cell line has been chosen to study this aspect of proteoglycan function. These cells show a dramatic rearrangement of cytoskeleton along with the changes in parathyroid hormone production under physiological changes in the environmental calcium concentrations. Parathyroid cells synthesize two major species of proteoglycans; a) HS-PG-I with MW ~130,000, O-linked and N-linked oligosaccharides and a core protein of MW ~120,000, and b) HS-PG-II with MW ~140,000, O-linked oligosaccharides and a core protein of MW ~75,000. The former appears to be an intracellular species and the latter to be primarily a cell surface and medium species. The distribution of the latter changes on the cell surface significantly with the different environmental calcium concentrations.

Protein Biophysics Unit

Work during the past year focused upon studies of the structural dynamics of keratan intermediate filaments (IF), staphyloccocal nuclease and selected model compounds, with the purpose of attaining a better understanding of protein function.

The ^{13}C and ^{2}H nmr studies of the keratan IF have provided the first experimental demonstration that IF filaments contain both kinetically free end terminal domains and a more structured central core domain that is most likely a flexible rod. The data suggest that the flexibility of the IF filaments enhance the flexibility and rheological properties of the cells that contain them.

Solution studies on S. nuclease provided the first experimental evidence that this protein contains cis X-Pro peptide bonds in solution, and support the hypothesis that cis-trans isomerization is a rate limiting step in the protein folding pathway. Although studies of the interaction of Ca^{++} with the protein remain puzzling, they do demonstrate that it is possible to identify and assign signals in the ^{13}C spectrum to specific atoms in the protein sequence, and to investigate the interactions of the labeled atom with ligands of interest.

The solid state studies of S. nuclease provide the first experimental evidence that proteins consist of a manifold of interconverting structures even in the crystalline state. Clearly, it will be necessary to understand solid state studies of model compounds now that the details of the complex dynamics of protein sidechains are better understood. Such work is important because it provides us with models for motions within proteins and because the detailed information obtained can be used to test theoretical predictions of molecular interactions and dynamics.

Mineral Chemistry and Structure Section

The use of artificial lipid vesicles (liposomes) as in vitro models for studying membrane-directed precipitation of calcium phosphates continues to be the principal focus of the research conducted in this Section. This work is being done with the intent of acquiring a more complete understanding of the nucleation and growth processes which occur during the initial stages of mineral formation in vertebrate hard tissue. Previous work under the project showed that liposomes can be made suitable for this purpose by prefilling their interior aqueous compartments with concentrated phosphate solutions and then making their lipid membranes permeable to ionophore-driven Ca^{2+} fluxes. The ensuing precipitation events are similar to those seen in biological matrix vesicles. Present work showed that neither the course nor the rate of this precipitation was significantly affected by the number of concentric membrane bilayers making up the lipid walls of the liposomes. On the other hand, current studies with acidic phospholipid (APL)-containing liposomes showed that lipid composition can have a varied but sometimes substantial impact on precipitation. Phosphatidylserine (PS) and phosphatidic acid (PA), for example, had a moderate to severe inhibitory effect on precipitation events which occurred outside the liposome, whereas the effect of phosphatidylglycerol (Pg) and phosphatidylinositol (PI) on these extraliposomal events was minimal. TEM analysis showed, moreover, that mineral particles, both inside and outside the liposomes, localized most closely to membranes that contained PA, the most inhibitory of the APLs. The mineral in Pg- and PI-liposome suspensions established generally only point contact with the membrane surface. These data suggest that APL-membrane binding to crystal surfaces was the principal inhibitory factor, most probably through immobilizing potential and seed crystals at their site of formation, i.e., within the liposomes, or by rendering active surface sites on the crystals unavailable for nucleation and/or growth. PA-containing membranes were most effective in this regard because the terminal ligand on PA is a strong Ca-binding monester phosphate group. The phosphate moiety on the other APLs is blocked by more-weakly interactive zwitterionic or non-binding neutral terminal groups. These results suggest that certain membrane-specific

agents could have major regulatory roles in matrix vesicle calcification *in vivo*.

Detailed analyses were carried out on eleven hydroxyapatite preparations suitable for use as standards and controls for studies of tooth enamel composition and surface reactions. Further studies focused on the water component in these preparations, indicated that about one water molecule per unit cell may be structurally incorporated in these hydroxyapatites that have nearly stoichiometric complements of calcium, phosphate and hydroxide. The structural location of this water and to what extent it alters the chemical and physical properties of hydroxyapatite are yet to be determined. Water incorporation of this type may also occur in biological apatites along with additional types due to nonstoichiometry and impurities in these systems.

It was discovered previously that octacalcium phosphate forms double salts with certain dicarboxylic acids. In a collaborative study with M. Markovic and W. E. Brown of the American Dental Association, this was confirmed by preparing and characterizing one of these new compounds, the octacalcium phosphate-succinate double salt. This salt had a compositional formula of approximately $Ca_8H(PO_4)_5(C_4H_4O_4).5.5H_2O$ and an octacalcium phosphate-like structure with an expanded crystallographic a-axis. If further study shows that these compounds, and other analogs, have low solubilities, they may have importance in biological systems where calcium, phosphate, and dicarboxylic acids (Krebs cycle) are present. Finally, preliminary results from a collaborative study with J. M. Antonnucci of the NBS indicate that calcium metaphosphate, $Ca(PO_3)_2$, may have application as a filler in resin based dental materials.

Bibliography for Bone Research Branch FY87

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00012-25 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Fowler, Bruce O. Research Chemist BRB NIDR

COOPERATING UNITS *(if any)*

Univ. of Copenhagen, Denmark
ADAHF, NBS, Gaithersburg, MD
NBS, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

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

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

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-15 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Fisher, Larry W.	Senior Staff Fellow	BRB NIDR
Termine, John D.	Chief	BRB NIDR
Tuross, Noreen C.	NIH Postdoctoral Fellow	BRB NIDR
Kinne, R. W.	Guest Researcher	BRB NIDR
Maloy, W. Lee	Staff Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

SIU, School of Dentistry, Edwardsville, IL
Univ. of New Mexico, Albuquerque, NM
FDA, Bethesda, MD

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.70	3.10	1.60

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binging, RIA, ELISA, etc.) have been developed to quantitate the levels of noncollagenous proteins in bone including osteonectin, bone sialoproteins I and II, bone proteoglycans I and II, and the N-propeptide of type 1(I) collagen (formerly known as 24K phosphoprotein) 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 immunoprecipitation studies, immunolocalization, immunodetection on Western blots and in the isolation of full length cDNA of human bone osteonectin, proteoglycans I and II, and bone sialoproteins I and II.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00088-14 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Eanes, Edward D.	Chief, Mineral Chemistry and Structure Section	BRB NIDR
Heywood, Brigid R.	Visiting Fellow	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 Institute of Dental Research, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Calcium phosphate salts provide the hardness and rigidity which uniquely characterize normal, health 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. Topics currently being pursued include (1) 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, and (2) the growth dynamics and size/shape parameters of apatite crystals prepared under physiological-like aqueous solution conditions. 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. The purpose of the crystal growth experiments is to better elucidate the physicochemical and physiological factors delimiting the size and shape apatite crystals can attain in various hard tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00134-13 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Hascall, Vincent C.	Chief, Proteoglycan Chemistry Section	BRB NIDR
Morales, Teresa I.	Senior Staff Fellow	BRB NIDR
Midura, Ronald	NIH Postdoctoral Fellow	BRB NIDR
Sayed, Atef K.	Senior Staff Fellow	BRB NIDR
Campbell, Margaret	Visiting Fellow	BRB NIDR
Calabro, Anthony	Guest Worker	BRB NIDR

COOPERATING UNITS *(if any)*

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

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	5.25	.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of the project is to study the chemical and physical properties and biosynthesis of proteoglycans in a number of tissue and cell systems. Topics of present interest include: 1) Protein chemistry and immunology of the hyaluronic acid-binding region of proteoglycans from the Swarm rat chondrosarcoma; 2) Biosynthesis of core protein precursors and processing to mature proteoglycans; 3) Effects of bacterial endotoxins (lipopolysaccharides), interleukin 1 on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilages; 4) Characterization of the keratan sulfate-proteoglycan and dermatan sulfate-proteoglycan in chick and human cornea.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00157-12 BRB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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)*

Torchia, Dennis A.	Chief, Protein Biophysics Unit	BRB NIDR
Hiyama, Yukio	Visiting Associate	BRB NIDR
Sparks, Steven W.	Staff Fellow	BRB NIDR
Mack, James W.	NIH Postdoctoral Fellow	BRB NIDR
Cole, Holly	Staff Fellow	BRB NIDR

COOPERATING UNITS *(if any)*
York College, SUNY, Jamaica, NY; DB, NCI, NIH; LC, NHLBI, NIH
University of Maryland; LB, NCI, NIH

LAB/BRANCH
Bone Research Branch

SECTION
Protein Biophysics Unit

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	4.00	.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

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) Mouse epidermal keratin subunit. Carbon-13 and deuterium nmr are being used to study the structure of keratin intermediate filaments obtained from mouse epidermal cells; 2) Calcium binding proteins. We are using multinuclear nmr to study (a) the molecular dynamics and (b) the interactions of staphylococcal nuclease with calcium, and with inhibitors and model substrates, and 3) We are continuing our program of detailed studies of molecular dynamics of various small molecules which serve as dynamic models of proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00379-04 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Young, Marian F.	Senior Staff Fellow	BRB NIDR
Termine, John D.	Chief	BRB NIDR
Day, Agnes A.	Staff Fellow	BRB NIDR
Findlay, David M.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.95	3.35	1.60

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The matrix proteins of bones 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 were isolated using expressing 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00380-04 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

Gehron Robey, Pamela	Senior Staff Fellow	BRB NIDR
Termine, John D.	Chief	BRB NIDR
Heywood, Brigid R.	Visiting Fellow	BRB NIDR
Fedarko, Neal S.	IRTA	BRB NIDR
Kopp, Jeffrey B.	Staff Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

LC, NCI, NIH, Bethesda, MD

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.35	1.80	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.)*

Bone cells (human and bovine) 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 parathyroid hormone and 1,25 dihydroxy vitamin D3; 3) elucidate production and interaction of potential growth factors such as transforming growth factor-β, and 4) serve as a source of mRNA and DNA for studies of these proteins at the genomic level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00431-01 BRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Metabolism of Proteoglycans

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

Yanagishita, Masaki	Visiting Scientist	BRB NIDR
McQuillan, David J.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*

Rush-Presbyterian St. Lukes Medical Center, Chicago, IL; HGB, NICHD, NIH; Toranomon Hospital, Tokyo, Japan; NIADDK, NIH; Univ. of West Virginia; University of Maryland

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	2.00	.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The 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) Metabolism of sulfur containing amino acids as sulfate sources in chondrosarcoma and granulosa cell proteoglycan biosynthesis, (2) Analysis of proteoglycan structure in hybridoma and myeloma cell lines, (3) Analysis of proteoglycans in a parathyroid cell line.

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

The Clinical Investigations and Patient Care Branch functions as the nucleus of the Institute's clinical activities. As such it has multiple major and varied responsibilities. These include the following: (1) to conduct high quality, clinically oriented research programs; (2) to render clinical care to specified patients of the Clinical Center; (3) to offer consultation on oral and dental problems to other institutes; (4) to encourage and to provide support, consultation and facilities for clinical research activities of other branches and laboratories within the Institute; and (5) to sponsor an oral medicine training program, the Clinical Dental Staff Fellowship, aimed at developing academic and research oriented dental clinicians.

The past year has seen considerable progress by laboratory research programs with our overall effort directed at studying the regulation of glandular epithelial cell secretory processes (eg. protein routing and release; ion fluxes and fluid movement). Likewise the strong center of clinical research has been expanded around the dry mouth/xerostomia program and second, around diagnostic and management problems posed by specific compromised patient groups (oncology, congenital disorders). Collaborative interactions with other laboratories or branches, both within and outside NIDR, have continued to expand. There is an extremely high level of effort, cooperation, flexibility and understanding by Branch personnel which has allowed us to make great strides towards reaching our overall goals.

Patient Care Section

The Patient Care Section 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, dental hygienists and nurses routinely participate in medical rounds and patient care discussions thus integrating oral health care concerns to total patient management.

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

This past year has seen a solidification of section research programs. This effort has been tangibly aided by a close working relationship with the Clinical Investigations Section, the Clinical Studies Unit as well as by collaborations with other programs at NIH. Two major protocol based study areas have been developed and are central to this Section. The first addresses management problems of compromised patients under going 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. The second general area involves patients with congenital disorders; particular attention being directed towards the characterization and management of dental-craniofacial manifestations. For example, Section staff are involved in efforts to better diagnose patients with ectodermal dysplasia, osteogenesis imperfecta, neurofibromatosis, precocious puberty and nevoid basal cell carcinoma syndrome through roentgencephalometric analyses. Expanded management research is directed at standardizing fixation methods following orthognathic surgery and at rigorously evaluating endosseous titanium implants in edentulous persons including persons with congenital dental agenesis. In addition, Section staff are integral to several projects based in the Clinical Studies Unit, eg. protocols related to human aging, management of the immune aspects of primary Sjogren's syndrome, and the development of oral candidiasis in AIDS patients (see below). Furthermore, Section staff collaborate with the Clinical Investigations Sections 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 besides NIDR, four other research centers; Emory University, Harvard University, University of Michigan and University of Texas-San Antonio. Strict clinical and radiological diagnostic parameters are used to determine if flurbiprofen can restrict the progress of advanced periodontal lesions.

The Patient Care Section 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, and this past year two senior dental students (University of Pennsylvania, Georgetown University) served in an eight week program which emphasized providing dental care to medically compromised patients in a hospital environment and an introduction to clinical oral health research.

Clinical Investigations Section

The general theme of this section's investigative efforts is understanding the regulation of membrane-associated, epithelial cell secretory events. The model tissues studied are mammalian salivary glands and the focus of study includes (1) understanding the mechanisms involved in controlling trans-acinar cell transport (serosa to mucosa) of fluid and electrolytes; (2) understanding mechanistic steps in gene expression, protein processing, routing and secretion; and (3) understanding the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders. To better accomplish these activities we have established, this year, within this Section, a Membrane Transport Unit and a Clinical Studies Unit.

Considerable effort 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 valuable to our understanding of basic concepts of secretion as well as to appreciating pathogenesis in conditions such as cystic fibrosis. At present we primarily utilize rat salivary glands with both in vitro and in vivo systems.

As noted above one main thrust of study is directed towards understanding secretory events related to water and electrolyte movement. These responses are primarily under α_1-adrenergic and muscarinic-cholinergic control. Because these autonomic receptors elicit their effects in great part due to changes in membrane ion permeability, we have directed considerable effort at clarifying ion fluxes associated with early events in secretion.

Much of our effort to study ion fluxes has utilized a dispersed improved acinar cell preparation and both basolateral and endoplasmic reticulum membrane vesicle preparations from rat and rabbit parotid glands. Studies have been directed primarily at understanding transport mechanisms for Cl^-, Na^+, K^+ and Ca^{2+}. For example we have extended studies, reported last year, which demonstrated the existence of a Cl^- uptake system sensitive to K^+ and Na^+, and to loop diuretics. We suggested that this was likely the $Na^+/K^+/Cl^-$ cotransporter and that it was very important to driving fluid secretion in the acinar cell. By using vesicle preparations we have firmly established the existence of this cotransporter in acinar cells and studied its functional characteristics in detail.

During the past year we have utilized the loop diuretic binding site of the cotransporter as a tool to both isolate the cotransporter and probe its structure. The [^{3}H]-bumetanide binding site in parotid membranes, and KCl-dependent $^{22}Na^+$ transport, were monitored in parallel, and we found the $K_{0.5}$ for bumetanide inhibition for both was identical. This indicates that the high affinity loop diuretic binding site is identical with the inhibitory site of the Na/K/Cl cotransporter. Binding of radiolabeled bumetanide follows hyperbolic dependence on both [Na^+] and [K^+], consistent with binding stoichiometries of 1:1. Conversely, binding dependence on [Cl^-] was biphasic, indicating a competitive interaction of bumetanide with the Cl^- site on the cotransporter. We have partially purified the cotransporter by following [^{3}H] bumetanide binding, and in so doing established in vitro conditions necessary to stabilize its binding activity. On continuous sucrose gradients, the cotransporter sediments as a single band and exhibits a molecular weight of ~200-300 Kd.

In addition to this cotransport mechanism, during this past year we have shown that it is likely that salivary fluid secretion also occurs in part in response to parallel electroneutral Cl^-/HCO_3^- and Na^+/H^+ exchanges in parotid basolateral membranes. These act in concert to exchange NaCl for CO_2 and H_2O. We demonstrated, in rabbit parotid basolateral membrane vesicles, the presence of a potent, Na^+-independent Cl^-/anion exchanger. This is shared by HCO_3, NO_3, Br, F and formate; is inhibited by SITS ($K_{0.5}$=0.05 mM) and exists in the same vesicles which

contain the Na/K/Cl cotransporter. Also, we showed that both rat and rabbit parotid basolateral membrane vesicles possess a Na^+/H^+ exchanger. Na^+ Uptake into vesicles is markedly dependent [~10X) on an outwardly directed pH gradient (pH_i=6.0; pH_o-8.0) and is 90% blocked by amiloride (K_I=3.2μM). Na^+ Uptake can be driven against its concentration gradient by a H^+ gradient, supporting existence of secondary active H^+-coupled Na^+ transport. The amiloride sensitive component of Na^+ flux exhibits Michaelis-Menten type kinetics (K_m=8mM Na^+). Initial experiments show that Na^+/H^+ exchange activity in membranes prepared from parotid acini treated with a muscarinic agonist is 2-3X greater than that found in control membranes. This stimulation can be completely blocked if acini are pretreated with a muscarinic antagonist. In aggregate, these studies argue for the likely significant role of Cl^-/HCO_3^- and Na^+/H^+ exchangers in the salivary fluid secretory process. Thus current models of fluid secretion must accommodate these transporters, as well as the Na/K/Cl cotransporter.

All models proposed to describe the formation of primary saliva require the existence of an apical conductive pathway for Cl^-. This conductance would allow Cl^-, which had entered the cell via the cotransporter and neutral exchangers on the basolateral membrane, to exit the cell at the apical face, "pulling" Na^+ and water to generate the primary saliva. During the past year we have provided additional extensive evidence for such a Cl^- efflux channel. Chloride efflux ($^{36}Cl^-$) from parotid acinar cells is rapid ($t_{1/2}$~10 sec) and dramatic (~50%). Thereafter a slow ($t_{1/2}$~2 min) recovery of intracellular chloride occurs to ~80% original levels. Diphenylamine-2-carboxylate, a putative Cl^- channel blocker, blunted the initial Cl^- loss and enhanced the subsequent Cl^- recovery, while bumetanide, an inhibitor of the cotransporter, only blocked Cl^- recovery. Calculation of salivary secretion rate from in vitro measures of sustained muscarinic stimulated Cl^- loss yields a value of 14 ul/gland. min, in excellent agreement with in vivo salivary secretion rates. These data strongly support a central role for Cl^-fluxes in salivary secretion.

It has long been known that Ca^{2+} plays a key role in stimulus - secretion coupling. Since stimulation of parotid fluid and electrolyte secretion by muscarinic cholinergic and α_1-adrenergic agonists is associated with elevated intracellular calcium levels, a number of experiments were carried out to examine the role of calcium in these parotid fluid secretory responses. Both types of neurotransmitter stimuli cause a comparable pattern of Cl^- efflux from parotid cells. When ^{36}Cl loaded acini were exposed to carbachol or epinephrine plus propranolol, (to yield muscarinic, or α_1-adrenergic stimuli), in calcium free (ie. +EGTA) medium, the acini rapidly recovered their full chloride content, indicating that there was no sustained chloride release and hence no sustained salivary secretion in the absence of extracellular calcium. The Ca^{2+} ionophore, A23187, causes a similar loss of intracellular Cl^- as is seen with the sustained neurotransmitter response. Also, acini loaded with $^{36}Cl^-$, and with the Ca^{2+} chelator/fluorescence indicator Quin2, show blunted Cl^- efflux responses. To further study the role of Ca^{2+} in salivary fluid secretion, we have also undertaken a collaboration with Drs. Kevin Foskett and Pamala Gunter-Smith (Dept. of Physiology, Armed Forces Radiobiological Research Institute) using digital video imaging microscopy to follow stimulation induced changes in acinar intracellular calcium and acinar cellular morphology with high temporal and spatial resolution. When intracellular calcium is monitored using Fura-2 fluorescence (while a second

video camera images the acini with Nomarski optics) carbachol caused a rapid increase in intracellular calcium which peaked within 2-3 sec and was followed by a relaxation toward control levels over the subsequent 5 min despite the continued presence of the agonist. A depolarization of the intracellular electrical potential was typically observed simultaneously with the calcium response. Stimulation was also associated with biphasic cell volume changes. Cell shrinkage began 4 sec following the initial increase in intracellular calcium. Maximum shrinkage was reached 10-14 sec later followed by a partial volume recovery during the subsequent 5 min to somewhat less than control values. These studies have directly shown that stimulus-secretion coupling is associated with a well-defined sequence of changes not only in intracellular Ca^{2+} but in cell volume and cell ion content as well.

We have also continued our intensive studies aimed at understanding, in detail, Ca^{2+} handling mechanisms in rat parotid plasma and endoplasmic reticulum membranes, as well as examining steps in the neurotransmitter signaling processes which regulate intracellular Ca^{2+} levels. Considerable effort has been directed at clarifying the mechanistic nature of ATP-dependent Ca^{2+} transporters in both membrane systems. These "Ca^{2+} pumps" appear to function coordinately to set the intracellular free $[Ca^{2+}]$, thus allowing for neurotransmitter responsiveness. Previously, we showed that the plasma membrane pump was electrogenic. This year we have shown that furosemide inhibits ATP-dependent Ca^{2+} transport, only in the presence of K^+ and Cl^-. KCl can stimulate initials rates of $^{45}Ca^{2+}$ uptake, maximal stimulation being achieved at [KCl] >50mM. In the absence of KCl, in a mannitol (non-ionic) or NMDG-gluconate (ionic, impermeant) media, only 40-50% of the transport activity was maintained, and this was unchanged by furosemide. In addition, evidence for a K^+ conductance affecting Ca^{2+} uptake into parotid vesicles, was obtained. This K^+ gradient effect could be seen in the absence of Cl^-, or in the presence of a Cl^- gradient, but not when Cl^- was at equilibrium. These results suggest that parotid basolateral membranes possess a Cl^- dependent, furosemide sensitive, K^+ flux which is associated with the ATP-dependent Ca^{2+} transport activity, likely providing charge compensation and thus optimizing Ca^{2+} movement. We also observed that parotid basolateral membranes isolated in medium without the reducing agent, dithiothreitol (1mM), lacked the KCl sensitivity of ATP-dependent Ca^{2+} transport. Also dithiothreitol was shown to activate the transport activity directly. Together, these results suggest an important role for SH groups in the parotid plasma membrane Ca^{2+} pump.

In an effort to better characterize this Ca^{2+} transport mechanism a simple reconstitution techique, previously successfully used for reconstituting various eukaryotic and prokaryotic membrane transport systems, was used to reconstitute the parotid basolateral membrane ATP-dependent Ca^{2+} transporter. Basolateral membrane vesicles were solubilized with octylglucoside, in the presence of lipids and glycerol. Three kinds of lipids were tested, E. coli phospholipids, phosphatidylcholine, and asolectin. ATP-dependent Ca^{2+} transport activity was obtained in all three vesicle preparations. Highest activity was obtained in the order asolectin > E. coli lipids > phosphatidylcholine. Maximum loading of Ca^{2+} (at 2 min.) obtained in asolectin proteoliposomes was 8-10 fold higher than in native membrane vesicles, while the initial rate of Ca^{2+} uptake was approximately similar in the native and reconstituted membranes. ATP-Dependent transport of Ca^{2+} was sensitive to vanadate (50 μM) and addition of the calcium ionophore A23187, but

not the addition of extravesicular Na^+ (30mM), released the intravesicular Ca^{2+}. Addition of valinomycin to the proteoliposomes induced a 50% stimulation of Ca^{2+} transport. These results are all consistent with findings in native membranes.

We have, this year provided a detailed characterization of the ATP-dependent Ca^{2+} transport mechanism in parotid microsomal membranes. It is the role of this transporter to load the Ca^{2+} trigger pool which is the target for neurotransmitter stimulation, thus mediating physiological responsiveness. This transporter follows Michaelis-Menten kinetics and shows a high affinity (Km∿38 mM) and high Ca^{2+} transport capacity (∿30 nmol/mg protein min.). Activity is unaffected by mitochondrial inhibitors and steady state velocity is stimulated ∿10 fold by oxalate. Manipulations of vesicle K^+ diffusion potential suggested that ATP-dependent Ca^{2+} transport in these membranes is electrogenic.

The role of neurotransmitter signaling in regulating parotid Ca^{2+} handling was also examined. We have extended earlier studies demonstrating an age-related deficit in α_1-adrenergic receptor ability to mobilize intracellular Ca^{2+}. Young adult animals, in response to an α_1-adrenergic stimulus, increase cytosolic Ca^{2+} from ∿100-150 nM to ∿600-650 mM in <10 sec. Senescent rats have unchanged basal Ca^{2+} levels but on average, following $\alpha_1$adrenoreceptor activation, peak $[Ca^{2+}]_i$ is only ∿400nM. This was unrelated to the ability of older cells to generate inositol trisphosphate (IP_3), after α_1-adrenergic stimulation. Using permeabilized parotid acini, we showed that it is likely that the microsomal target Ca^{2+} pool in older cells is not capable of responding to exogenously added IP_3 such as seen with young adult preparations. This indicates the defect in this signal transduction event may be at the level of the putative IP_3 receptor. In addition to these studies, we also have examined the role of β-adrenergic receptor activation on cellular Ca^{2+} responses. This receptor is coupled to cyclic AMP. Our earlier work has shown that cyclic AMP-coupled stimuli can directly alter the kinetic behavior of the parotid basolateral membrane ATP-dependent Ca^{2+} transporter (increase Km and Vmax). This year we have shown that stimulation at the β-adrenoreceptor leads to an increased cytosolic free Ca^{2+}, which is ∿3 fold lower, and ∿10 fold slower in rate, compared to changes observed after α_1-adrenergic and muscarinic stimulation of parotid acinar cells. These beta responses were mediated by the β_2-receptor subtype and were unlikely occurring via a cyclic AMP mechanism since neither cyclic AMP analogs, nor forskolin, elicited any response. The Ca^{2+} mobilized by β-adrenoreceptor stimuli appeared to be derived from an intracellular pool which could also be released by muscarinic stimuli.

In addition to secreting water and electrolytes, parotid acinar cells secrete the majority (>80%) of parotid salivary proteins. As in the past, our primary focus in this investigative area has been on β-adrenoreceptor regulation of secretory glycoprotein synthesis, processing, routing and release. This work has however been greatly expanded in scope during this last year. We had previously reported that β-adrenoreceptor activation enhanced parotid protein synthesis (ie. $[^{14}C]$-leucine incorporation). In order to test the hypothesis that short exposure of parotid acinar cells to β-adrenergic agonists increases the expression of certain genes, we have begun studies identifying and cloning the genes of two major parotid N-linked glycoproteins, ie 220 Kd and 32 Kd proline-rich glycoproteins. Following construction of a cDNA library in λgt 11, we were able to identify seven clones for

parotid proline-rich-proteins. These cDNA are now being sequenced. Besides the increased synthesis of these proteins, it has long been known that β-adrenoreceptor stimulation can increase parotid DNA synthesis. Since oncogenes have been implicated in growth regulation, stable translational changes and differentiation, we examined the expression of specific oncogenes in parotid acini in vitro after β-adrenoreceptor stimulation. We observed that the expression of oncogenes encoding 3 nuclear proteins (c-fos, c-myc and p53) and encoding 2 "growth factors" (c-abl and c-sis) was increased after treatment with the β-agonist isoproterenol. The increased expression followed a specific time course for each oncogene and was blocked by the β-adrenergic antagonist propranolol. Only one oncogene, c-abl, showed increased expression after chronic isoproterenol treatment in vivo. In particular c-fos gene expression was enhanced ∿20 fold within 60 min.

We have also extended our past efforts on protein packaging and localization by both biochemical and immunocytochemical approaches. For example we have investigated secretory granules and their limiting membranes after in vitro isolation. The protein content of the purified granules was comparable to that of rat parotid saliva. The granules membrane protein profile was quite distinct from the contents although some "exocrine" proteins were present. Two proteins, or 68 Kd and 23 Kd, appeared to be characteristic of the granule membrane. Immunocytochemical studies have utilized the antibody: protein A-gold method to evaluate the subcellular localization of several salivary gland proteins including regulatory subunits of cyclic AMP dependent protein kinase (cAPK-R), lingual lipase, amylase and a high molecular weight salivary agglutinin. Interestingly, the cAPK-R subunits were observed to be preferentially packaged in secretory granules of parotid serous acinar cells. Similarly, lingual lipase was localized only to serous demilune cells, and not mucous cells, of Von Ebner's gland. Additionally we have examined the distribution and location of two proteins (β_1, 26 Kd; C, 89 Kd) which are secretory products of the rat submandibular gland. Protein β_1 was found exclusively in type III acinar cells while protein C was present essentially in type I cells. Results of developmental studies following these proteins suggested that some type III cells in neonate animals differentiate to form adult acinar cells, while other, variant type III cells and type I cells form the intercalted ducts. The conclusion regarding type III neonate cells as progenitors of acinar cells was reinforced when the distribution of mucin and a submandibular glutamic acid-rich secretory protein was followed by the immunogold method.

We have also continued efforts to assess the permeability of junctional complexes (joining adjacent acinar and ductal cells). Recent studies have extended our past work on the rat parotid gland to the rat submandibular gland. We have shown that following β-adrenoreceptor stimuli junctional permeability is observed and remains evident for at least 24h after a single stimulus. We have, in addition, provided further support for the notion that both intercalated and striated ductal cells of the rat parotid are capable of the endocytosis of proteins from the apical luminal face. This appears to be a specific process, being dependent on chemical properties of the proteins presented. Importantly, our results suggest that salivary secretory proteins (eg. immunoreactive to amylase and protein β_1) are found in endocytic structures of duct cells after release from acinar cells by isoproterenol stimuli.

In order to better study the variety of salivary physiological events described above (ie. water and electrolyte secretion; protein synthesis, processing and routing), we have tried to develop stable salivary gland epithelial cell lines. During this reporting period we have continued to make substantial progress in this area. Efforts have been directed towards the development of both acinar and ductal cell lines. Because salivary cells are responsive to many factors in serum, we have emphasized cell growth in low-serum or serum free medium. We have had some success in obtaining acinar-like cells (based on morphology and growth pattern) from rat and minipig parotid explants. These, however, survive for only short periods in vitro. Conversely ductal-like cells are more readily obtained, and maintained, in culture.

Last year we reported the development of a cell line RSMT, from rat submandibular gland ductal elements, following chemical transformation with 3-methylcholanthrene. We have, this year, cloned a line from RSMT, termed A-5. This clonal line has characteristic epithelial morphology and excellent growth properties. A-5 also contains both α_1 and β_2-adrenoreceptors (no muscarinic receptors) which are functionally coupled to phosphatidylinositol metabolism and cyclic AMP generation, respectively. We also have begun to characterize two previously described cell lines obtained from Japanese colleagues. One, HSG-PA, has biochemical and immunochemical markers of intercalated duct cells while the other, HSG-MY, appears to be like myoepithelial cells. Both of these cells possess β_2-adrenergic and muscarinic-cholinergic receptors, without evidence for α-adrenoreceptors. The latter 3 cell lines provide us with an excellent opportunity to study glandular epithelial cell physiology in vitro.

The focus of this Section's human clinical research is understanding the etiology and oral sequelae of conditions which result in salivary dysfunction. Central to this activity is the dry mouth, xerostomia study which now has involved over 400 patients. We have established diagnostic approaches to the evaluation of patients with complaints suggestive of salivary gland dysfunction. Most patients seen have diminished gland secretory capacity. Approximately sixty individuals per year are admitted as inpatients for intensive study under the protocol "Evaluation and treatment of salivary gland dysfunction". During this past year we have initiated two new treatment protocols for salivary dysfunction (based on results reported by us earlier). In one protocol, we utilize steroids (alternate day 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. In the other protocol, we employ the parasympathomimetic drug pilocarpine to preserve salivary function during head and neck irradiation. Approximately 50,000 Americans each year are diagnosed with a head and neck cancer. Most are treated, at least in part, with irradiation. The single greatest area of post-treatment complaint in surviving patients is related to oral damage reflective of salivary dysfunction. Thus both of these new protocols address significant clinical management problems. In addition we are continuing 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). Thus far more than 30 patients have been treated under this extensive protocol.

During this reporting period we have made considerable progress in improving clinical diagnostic capabilities in patients that subjectively complain of "dry mouth". Using a standardized questionnaire, we evaluated the responses of 97 patients with respect to objective measurements of saliva output from major glands. Certain, specific questions were found to be highly significant predictors of actual salivary gland hypofunction. Interestingly, subjective responses were most influenced by submandibular output. Thus, a specific series of questions can be selected which are useful in distinguishing between individuals with true salivary dysfunction and those with xerostomia unrelated to fluid output deficits. This provides a means of selecting patients requiring further evaluations, suggests those individuals who may be best studied for specific compositional changes which may contribute to their subjective reports, and those who likely will experience subjective relief from management approaches which result in increased salivary fluid secretion.

We have also considerably expanded our efforts in the area of aging and oral physiology. More than 80 normal volunteers from the National Institiute of Aging's Baltimore Longitudinal Study of Aging have now been evaluated with respect to major salivary gland function, oral motor and oral sensory performance. We have been able to demonstrate that parotid protein secretion, from a variety of salivary cells including acinar as well as ductal elements, remains functionally intact across the human lifespan. Several proteins, all of significant importance to oral health maintenance, were examined, including lysozyme, lactoferrin, secretory IgA and the anionic proline-rich proteins. Furthermore, initial analysis of fluid output from submandibular/sublingual glands of this healthy population, indicates no change occurs with aging. Earlier studies with this population have reported that only modest, quality-specific changes in gustatory function occur with increased age. This past year our work on olfactory function has shown this chemosensory function responds to age differently. With increased age there appears to be a marked reduction in olfactory recognition occuring about ages 60-70. We also have applied our experience in evaluating chemosensory performance in healthy populations to two specific pathological entities, Sjogren's syndrome and Alzheimer's Disease. For example, we observed that gustatory function in patients with Sjogren's syndrome did not differ from control subjects with respect to measures of intensity perception. However, more patients than controls showed impairments in at least 1 taste threshold measure and many more patients (75%) than controls (18%) showed threshold impairment for two or more taste qualities. With Alzheimer's disease (AD) patients we carefully examined olfactory and gustatory function because recently it has been hypothesized that among the earliest signs of the disease are olfactory performance changes. In 10 men with early AD, we observed no response difference in measures of gustatory thresholds and perceived intensity, or in measures of olfactory detection, when compared with 10 age-matched controls. Conversely, the AD patients were notably impaired with respect to olfactory recognition. These results suggest that the earliest changes, with respect to olfactory function, occuring in AD take place at a central, not a peripheral, site. Parallel studies, examining oral motor performance (particularly the oral phase of swallowing) have been initiated in AD

patients and patients with multi-infarct dementias. These studies have been built upon our earlier work which showed a strong positive correlation between salivary secretion and oral swallow performance and between increased age and the time required to complete an oral swallow. All of these motor studies make use of ultrasonic visualization of the oral swallowing phase, previously developed in collaborative efforts by ourselves with the Clinical Center Departments of Rehabilitation Medicine and Diagnostic Radiology.

We have also expanded our efforts examining the oral condition of patients with the acquired immunodeficiency syndrome (AIDS). Patients with AIDS have numerous oral complications as a consequence of their underlying immune dysfunction. The early appearance of Candidiasis, specific oral lesions, and xerostomia have been reported. Specific questions studied involve the presence of HIV-I, the causative agent of AIDS, in salivary secretions; the effects of AIDS on salivary gland function; the general oral health status of AIDS patients and alterations by current therapies; and oral Candida Albicans colonization in AIDS. The latter studies involve determination of salivary levels of histidine-rich proteins (HRP), which have been shown to kill C. Albicans, in vitro. We have also instituted a longitudinal component to these protocols and patients will be seen every 6 months.

To date, HIV-I has not been identified in pure gland secretions of 7 patients known to be serum HIV-I positive. We have analyzed major salivary gland secretions of a large group of 36 patients with early stage AIDS for electrolyte and protein concentrations. There were no marked differences found with respect to electrolytes, total protein and salivary flow rates when compared to non-AIDS controls. However, there were dramatic increases in the frequency with which albumin was detected in samples (48/73 AIDS; 0/64 control) and levels of lysozyme from the AIDS group. These data demonstrated specific salivary gland dysfunction in early-diagnosed AIDS. Therapy with AZT had little effect on the salivary findings.

Studies of salivary fungal forms isolated from AIDS patients showed that >99% were C. Albicans. Also, AIDS patients had higher concentrations of C. Albicans in saliva than controls. The patients also had a much higher incidence of hyphae found on mucosal surfaces, even in the absence of frank oral lesions. This is consistent with the high incidence of oral thrush seen in the AIDS group. Our studies of salivary HRP are continuing. Antisera have been produced and an ELISA has been developed to quantitate these proteins in saliva. Studies on the effects of whole and individual gland secretions on C. Albicans viability and infectivity are ongoing, as well.

Besides these obvious studies of clinical problems, within this section there is a strong effort to develop suitable experimental models to test etiological or management hypotheses obviously necessary prior to reaching the clinical trial stage. Most activity in this regard has focused on animal models of Sjogren's syndrome. We have utilized two mice strains which spontaneously develop autoimmune dysregulation and we also have begun work with an induced model of autoimmune disease in rats. Additional activity has focused on characterizing the sites, and nature, of iatrogenic perturbation of salivary glands following various drug, and radiation, regimens. Another particularly important line of investigation developed this past year is attempting to localize, using anti-idiotypic antisera, the

antigenic site(s) of human minor salivary glands involved in the autoimmune reactivity of Sjogren's syndrome.

Success in the unique mission of the Clinical Investigations and Patient Care Branch in the NIDR is made possible by the blending of the academic-, clinical problem oriented-Patient Care Section together with the strongly basic science oriented, yet clinical problem appreciating, Clinical Investigations Section. We continue to make substanial progress since the reorganization of the Branch in 1982. We have the opportunity to make many contributions to clinical dentistry, to oral science as well as to fundamental biology. Our Branch recognizes this opportunity and works creatively and with considerable enthusiasm and effort towards meeting this goal. We anticipate continued forward movement in our efforts to address questions of importance to the understanding and management of oral diseases, thus contributing to the future development and direction of the dental profession.

CLINICAL INVESTIGATIONS AND PATIENT CARE BRANCH PUBLICATIONS 1986-1987

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00028-20 CI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructure and Cytochemistry of Secretory Cells

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

Hand, Arthur R.	Dental Director	CIPC, NIDR
Moreira, Jorge E.	Guest Researcher	CIPC, NIDR
Lotti, Lavinia V.	Visiting Fellow	CIPC, NIDR
Takamo, Kunio	Guest Researcher	CIPC, NIDR
Field, Ruth Bisen	Sr. Staff Fellow	CIPC, NIDR

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:	PROFESSIONAL:	OTHER:
6.55	4.75	1.80

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 mechanisms of the secretory process are studied in rat and human salivary gland cells. Techniques utilized include light and electron microscopy, enzyme-and immunocytochemistry, radioautography, and biochemistry. Major areas of investigation are: (1) localization of secretory and cellular proteins in developing and adult salivary glands using fluorescent and colloidal gold immunolabeling procedures; (2) structure and permeability properties of junctional complexes in rat salivary glands; and (3) the role of salivary duct cells in protein reabsorption.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-11 CI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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
Koss, Elizabeth	Senior Staff Fellow	LNS	NIA

COOPERATING UNITS *(if any)*

LN, NIA

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.55	1.05	.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.)*

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 chemosensory perception. Functional variation under pathologic circumstances are now being measured. Currently, objective evaluations are being made of oral sensory disturbances occurring in association with systemic disease, salivary gland dysfunction or as an isolated complaint. Assessments of olfaction and of oral tactual sensitivity are obtained when they can contribute to an understanding of oral sensory function in relation to the complex stimuli encountered in everyday life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00332-06 CI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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/Chf Patient Care	CIPCB	NIDR
Brahim, Jaime S.	Senior Staff Fellow	CIPCB	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin	CIPCB	NIDR
Folio, John	Senior Staff Dentist	CIPCB	NIDR
Tylenda, Carolyn A.	Senior Staff Fellow	CIPCB	NIDR
Wright, William E.	Senior Staff Dentist	CIPCB	NIDR
Atkinson, Jane C.	Dental Staff Fellow	CIPCB	NIDR
Haller, Julie M.	Dental Hygienist	CIPCB	NIDR

COOPERATING UNITS *(if any)*

Laboratory of Clinical Science, NIMH;Pediatric Branch, NCI; Inter-Institute Genetics Program, CC; Arthritis and Rheumatism Branch, NIADDK; Clinical Research Branch, U.S. Army Institute of Dental Research; Letterman Army Institute of Research, San Francisco, CA

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.05	4.1	2.95

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☒ (a2) Interviews

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

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00336-06 CI

PERIOD COVERED
October 1, 1986 - September 30, 1987

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
Horn, Valerie J.	NRSA Fellow	CIPC NIDR
Melvin, James E.	NRSA Fellow	CIPC NIDR
Roth, George S.	Research Chemist	LCMB NIA
Wellner, Robert B.	Sr. Staff 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:	PROFESSIONAL:	OTHER:
4.50	3.50	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 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 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 major specific areas of study with these preparations include (1) mechanistic aspects and functional correlates of α-adrenoreceptor mobilization of cellular calcium and (2) characterization of neurotransmitter receptor-coupled signalling mechanisms in a cloned rat salivary ductal epithelial cell line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00337-06

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oral Physiologicial 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.	Dental Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC	NIDR
Hubbard, Van	Medical Officer	DDDN	NIDDK
Lane, H. Clifford	Medical Officer	LIR	NIAID
Ship, Jonathan	Dental Staff Fellow	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC

*See Additional Investigators

COOPERATING UNITS *(if any)*
RM, CC; DR, CC; DDDN, NIDDK; LIR, NIAID; Columbia University; Boston University; SUNY, Stony Brook

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.20	4.20	.70

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

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 with demonstrable functional gland mass yet inadequate basal salivary performance 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 sydrome, an autoimmune exocrinopathy, have been initiated. An initial treatment protocol for primary Sjogren's sydrome has been started. 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. A major effort has been instituted to characterize oral alterations associated with the acquired immune deficiency syndrome (AIDS). Studies focus on soft tissue changes, salivary gland function, and salivary-fungal interactions in AIDS, ARC and pre-AIDS (HIV-positive state).

Additional Investigators

Tylenda, Carolyn	Senior Staff Fellow	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wolff, Andy	Visiting Fellow	CIPC	NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC	NIDR
Macynski, Alice A.	Clinical Nurse	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00372-05CI

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
N-Linked Protein Glycosylation and B-Adrenoreceptors

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

Kousvelari, Eleni	Senior Staff Fellow	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf Clin	CIPC NIDR
Huang, Lan-Hsiang	Staff Fellow	CIPC NIDR
Mirels, Lily	Guest Worker	CIPC NIDR
Yeh, Chih-Ko	Visiting Associate	CIPC NIDR
Louis, John	Visiting Fellow	ODDCBD NCI

COOPERATING UNITS *(if any)*
ODDCBD, NCI; Roche Inst. of Molecular Biology

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.05	2.65	.40

CHECK APPROPRIATE BOX(ES)

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

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

Saliva plays a pivotal role in maintaining the integrity of the hard and soft tissues. Salivary gland cellular activities such as protein exocytosis, synthesis and processing are regulated by β-adrenergic receptors. To study the mechanisms involved in synthesis, processing and secretion of proteins carrying N-linked oligosacchrides we have utilized in vitro cell and microsomal membrane preparations from rat parotid glands. We have shown that β-adrenoreceptor stimulation increased N-linked protein glycosylation through a c-AMP-mediated mechanism. This appears to be due to an increased synthesis and utilization of oligosaccharide-PP-dolichol and enhanced activity of specific glycosyltransferases. In addition β-adrenoreceptor stimulation modulates the rate of processing on a high molecular weight (220kD) secretory glycoprotein. We have also shown that changes in parotid saliva protein composition after treatment with β-adrenergic drugs influence the adherence and aggregation of oral bacteria. During this reporting period we have 1) characterized the oligosaccharides associated with four secretory glycoproteins (220,38,32 and 17kD); 2) isolated secretory granules and secretory granule membranes of a high purity and partially characterized these membranes; 3) constructed cDNA libraries from Poly(A) RNA isolated from control and isoproterenol treated rat parotid glands; 4) synthesized oligonucleotide probes corresponding to the parotid proline rich protein (PRP); 5) identified seven positive PRP clones and 6) demonstrated that β-adrenergic receptor stimulation regulates protooncogene expression (c-fos, c-myc, p 53, c-abl and c-sis) in rat parotid acinar cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00411-02 CI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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
Haller, Julie M.	Dental Hygienist	CIPC	NIDR
Harlow, Shelley A.	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 Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.90	0.15	0.75

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.)*

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, oriented 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

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00412-02 CI

PERIOD COVERED: October 1, 1986 - September 30, 1987

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	CIPCB	NIDR
Folio, John	Senior Staff Dentist	CIPCB	NIDR
Wright, William E.	Senior Staff Dentist	CIPCB	NIDR
Fox, Philip C.	Dental Officer	CIPCB	NIDR
Guckes, Albert D.	Chief, CODC	CODC	CC
Gracely, Richard H.	Research Psychologist	NA	NIDR
Li, Shou Hua	Statistician (Health)	EB	NIDR

COOPERATING UNITS (if any)
Rehabilitation Medicine Department, CC; Nutrition Department, CC;
Surgical Services Department, CC
Commissioned Officers Dental Clinic, CC

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	1.55	2.20

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.)

Evaluation of Rigid Versus Nonrigid Fixation Following Orthognathic Surgery

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 radiograghic, cephalometric and clinical assessment. Any changes in the height of the gingiva or the width of the attached gingiva will be recorded. Post-operative changes in facial contours and occlusion will be recorded. Pre- and postoperative speech and swallowing will be assessed.

Clinical Study of Oral Endosseous Titanium Implants in Edentulous Subjects

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 a coronal segment attached to each root analogue. A complete denture is constructed to restore the mandibular dentition. Cephalometric radiographs, the Cornell Medical Index, the Minnesota Multiphasic 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 will be used to obtain data. The information obtained will be utilized to determine if implant supported mandibular dentures significantly effect loss of vertical 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.

OTHER PROFESSIONAL PERSONNEL

Rudy, Susan F.	Clinical Nurse (General)	CC	NIDR
Morgan, Victor L., Jr.	Dental Laboratory Techn	CIPCB	NIDR
Stables, Gloria	Dietitian	NURT	NIDR
Sonies, Barbara C.	Speech Language Pathologist	RM	CC
Gaston, Gerald W.	Oral Surgeon (Consultant)	Univ. Of MD-Baltimore	
Tilghman, Donald M.	Oral Surgeon (Consultant)	Johns Hopkins University	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00415-02 CI

PERIOD COVERED
October 1, 1986 - September 30, 1987

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
Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Helman, Yossi	Visiting Fellow	CIPC NIDR
Manganel, Michel	Visiting Fellow	CIPC NIDR
Melvin, James E.	NRSA Fellow	CIPC NIDR
Corcelli, Angela	Guest Worker	CIPC NIDR
George, Janet N.	Chemist	CIPC NIDR

COOPERATING UNITS *(if any)*

Armed Forces Radiobiological Research Institute, Bethesda, MD.

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.60	3.60	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.)*

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 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), whose transmembrane and transepithelial movements are involved in primary salivary fluid secretion, was studied in vitro in a rat parotid acinar suspension and/or in isolated rabbit and rat parotid basolateral membrane vesicles.

(2) Intracellular events (changes in calcium concentration, electrical potential and cell volume) associated with muscarinic cholinergic stimulation of individual parotid acini were studied using electrophysiological techniques and digital video imaging microscopy.

(3) The bumetanide binding properties of a Na/K/Cl cotransporter in the rabbit parotid basolateral membrane were characterized. This transporter is plays a major role in fluid secretion in a number of exocrine glands.

(4) The rabbit parotid Na/K/Cl cotransporter was solubilized and partially purified using conventional protein separation procedures.

(5) Primary cultures of parotid acinar cells grown in defined media were studied in order to establish minimal requirements for cell viability, proliferation and differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00438-01 CI

PERIOD COVERED: October 1, 1986- September 30, 1987

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	NRSA Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*: Department of Physiology, Johns Hopkins University, School of Medicine

LAB/BRANCH: Clinical investigations and Patient Care Branch

SECTION: Clinical Investigations Sections

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	1.20	.40

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Saliva, a complex fluid secreted by salivary glands, is of critical importance to the health of the oral cavity. Neurotransmitter mediated calcium mobilization in salivary glands regulates the formation and secretion of saliva and is of primary importance to the salivary cell. To investigate the mechanisms regulating cytosolic calcium during secretory events, we have utilized in vitro dispersed cell and cellular membrane vesicle preparations from rat parotid glands. These studies have been directed towards understanding at a molecular level, (i) the basic mechanisms of calcium transport involved in the establishment of cellular calcium homeostasis; and (ii) the modulation of these processes during neurotransmitter induced calcium mobilization. It has been shown previously that α_1-adrenergic and muscarinic receptor stimulation lead to calcium efflux from parotid cells. It was also shown that basolateral membrane vesicles isolated from parotid gland possess an ATP-dependent calcium transporter, which is one of the major systems regulating cytosolic calcium.

In the present reporting period we have characterized the calcium transporting activities associated with two major membrane systems in the parotid cell. In the basolateral membrane we have (i) studied the modulation of the ATP dependent calcium transport by secondary ion movements and by membrane sulfhydryl group modification, (ii) developed a reconstituted system of this transporter in artifical lipid vesicles, (iii) established the electrogenic nature of the sodium/calcium exchanger. In the endoplasmic reticulum, we have characterized the ATP + magnesium-dependent calcium pump and its ionic correlates. We have also studied β-adrenoreceptor mediated calcium mobilization in rat parotid cells in order to identify the signalling events leading to modification of calcium transport.

REPORT OF THE DIAGNOSTIC SYSTEMS BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Diagnostic Systems Branch (DSB) is concerned with the isolation and identification of factors limiting performance obtainable from predominantly noninvasive diagnostic systems.

Particular emphasis is directed toward development of image-based systems designed primarily for dentistry, but the scope is broad enough to include research applicable to a variety of biomedical tasks.

In keeping with DSB's multidisciplinary approach to systems optimization, existing resources are deployed preferentially in collaboration with other agencies having common interests.

The task-specific systems focus of the Diagnostic Systems Branch, (DSB), is reflected in the description of continuing research activity into three general areas depending on the most significant factor limiting diagnostic performance. These areas correspond to: 1) Tasks limited by the data-sampling strategy i.e. so-called sample-limited systems, 2) Tasks limited by the amount of data acquired relative to the intrinsic variability of the observations i.e. so-called capacity-limited or noise-limited systems, and 3) Tasks limited by the ability of the system to appreciate or understand the diagnostic significance of the sampled data i.e. so-called interpretation-limited systems.

All continuing research efforts are encompassed by three broad project designations which evolved from early efforts to classify research according to the factors limiting performance as described above. These designations classify our research in terms of: 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 methods for manipulating diagnostic data in specific, task-dependent ways; whereas the second is more global in scope, involving all aspects of data acquisition and interpretation essential to the diagnostic process. The third is more explicit, involving the sampling of new or unusual kinds of diagnostic information.

These efforts also represent extensions of a precedented commitment to design and produce a clinically useful, computer-based x-ray system having primary use and application in dentistry, and to develop new and more

efficient methods for gathering diagnostic information . This commitment complements previous efforts to broaden the systems base of our research effort.

Recent additions to our staff in keeping with this more balanced approach to systems science include Dr. Robert van den Berg, a Visiting Fellow from the Netherlands, who has some experience in computer image analysis as well as being a dentist and protege of another visiter to DSB, Dr. Paul van der Stelt who served as a Visiting Fellow in previous years. Also new this year are Dr. Harvey Himel, a physician having research interests in osseous changes demonstrable through digital subtraction radiology who is working in a temporary capacity on a joint project with DSB and LDBA, and Dr. Makoto Tsuchimochi, a Visiting Scientist from Nippon Dental University in Niigata Japan. Dr. Tsuchimochi is an Oral Surgeon with research interests in nuclear medicine and magnetic resonance imaging. We also were particularly fortunate to have Dr. Aaron Tzukert spend a year with us while on sabbatical from Hebrew University, Hadassah School of Dental Medicine, Jerusalem, Israel. Dr. Tzukert's research expertise is in system optimization, and he put his skills in this area to productive use in several projects described later in this report during his year's tenure at DSB.

System-development research is an extension of that reported in previous years wherein a prototype miniaturized tomosynthetic x-ray system is being fabricated with extramural support, in collaboration with private interests, the United States Army, and the National Bureau of Standards. The device is unique in that it contains no moving parts and is capable of producing a video image in near-real time which can be fed back to a computerized x-ray generator to make possible task-specific control of exposure. Such a system has many advantages which facilitate reliable detection and spatial location of tiny changes in the teeth and supporting bone, occurring over relatively long periods.

Progress in this area has been systematic involving efforts to procure a state-of-the-art x-ray tube having multiple focal spots which can be activated selectively under computer control. After innumerable administrative delays an appropriate tube has been custom designed and is being fabricated via a subcontract from the National Bureau of Standards to the Kevex Corporation. At the same time, work continues in parallel to develop practical high-performance intraoral x-ray detectors derived from two new technologies, one based on the use of a two-dimensional, solid-state, pin-diode array, and the other making use of chips similar in design to high-density dynamic random access memory used in modern computers. The latter development is being sponsored by the General Imaging Corporation

and also was delayed somewhat this year by financial constraints which now reportedly have been alleviated through an extensive internal reorganization within the company. A third detector system, also described last year, is based on more bulky and expensive optical components, i.e. a fluorescent-screen which covers a fiberoptic taper which in turn is coupled to a microchannel-plate image intensifier. In light of the many drawbacks intrinsic to this design enumerated in last year's report, it was decided to table its further development in order to focus limited resources on the two far more promising alternatives described above.

The ultimate goal of this work remains unchanged, and it is anticipated that the prototype emerging from this effort will have an even superior potential for synthesis of any desired x-ray projection from the radiographic information acquired during a single automated scanning sequence. To this end DSB has continued its systematic efforts to develop improved tomosynthetic algorithms which can be programmed into the prototype system once the transduction hardware is finally in place. This work includes fundamental research being done primarily by Drs. Ruttimann and Qi involving the design of optimized spatial frequency filter kernels to minimize ringing artifacts intrinsic to the tomosynthetic reconstruction process. If successful, this work could reduce the need for time-consuming iterative algorithms to eliminate unwanted blur.

Work continues on the implementation of a method for eliminating the present need for using a stent to stabilize projection geometry when generating radiographs intended for subtraction. The method is based on the theoretical realization that only one component of the registration error created by changes in projection geometry is irreversible. This irreversible component is determined by the spatial relationship existing between the x-ray source and the irradiated tissues of diagnostic interest. Earlier experiments done in collaboration with investigators at Harvard University confirmed the theoretical prediction that sagittal projections can be stabilized adequately with the aid of a conventional cephalometric x-ray positioning system in lieu of an occlusal stent. More recent work has centered on the design of a new type of cephalometric head holder that does not require the use of ear rods to aid in the positioning of the patient. Indeed, the new design requires no intrusive physical contact between the patient and x-ray machine at all. The positioner's design is the product of a joint effort between DSB staff and the General Imaging Corporation, and a patent search is currently under way to facilitate exploration of potential opportunities for marketing such a device.

The two-dimensional warping algorithm described last year has been implemented successfully. This algorithm corrects images which have been distorted by movement of the image detector relative to the irradiated tissues of diagnostic interest. Such distortions are better known as projective transformations of which affine distortions are a special case. It already has found application in the correction of preliminary radiographs taken as a part of a large, multicenter clinical trial of a new drug intended to prevent periodontal bone loss.

Other research dealing primarily with diagnostic factors limited by sampling strategy is exemplified by the spectroscopic analysis of hemoglobin through differential attenuation of tooth-scattered light using a narrow band-pass optical filter. Recent progress has centered around the testing of a prototype device designed to determine tooth vitality using the noninvasive optical method described above to detect oxygenated hemoglobin in the dental pulp. Controlled investigations have now confirmed conclusively that tiny changes in tooth color measured in this way can be used to predict degenerative changes in the pulp invariably seen in recently extracted teeth after storage for a week or more in saline at 37 degrees C. Related research done in collaboration with Dr. Maret Maxwell of BEIB also has shown that the optimum-signal-to-noise ratio for demonstrating these degenerative changes in the dental pulp is manifest when the test and control band-pass filters are centered at 575 and 595 nm respectively. During his sabbatical year at DSB, Dr. Tzukert extended these findings by doing similar but more tightly controlled experiments which involved the surgical removal of intact pulps from recently extracted teeth after noninvasive optical measurement to quantify changes expressed in terms of actual hemoglobin assayed from the extracted tissues. These data show that optically-detectable amounts of blood extractable from vital pulpal tissue can be extremely small, often being measured in microliters.

Particularly promising in terms of modality-specific applications is research based on the use of radiopharmaceuticals to predict loss of periodontal bone. Earlier findings suggested that the uptake ratio of ^{99m}Tc-MDP measured with a tiny cadmium telluride probe applied to active sites relative to control areas in a beagle dog model anticipated loss of supporting bone months before it could be detected radiographically. Recent work has involved efforts to use much more sensitive, quantitative methods to track bone loss radiographically combined with a more refined nuclear medicine technique in these animals. In this way we hope to establish a small data base which can be used to model the healing dynamics of normal bone expressed as lesion volume per unit time. Such data have never been available before because previous methods accurate enough to be used for recording such changes

have required that the animals be sacrificed thus precluding controlled dynamic analysis.

Research pertaining to capacity- or noise-limited systems is usually reflected in analyses of system efficiency. For example, the quantum yield of devices used to sample the distribution of photons produced by any radiographic or nuclear-medicine based system directly influences the exposure required to produce data with consistent statistical properties. The nuclear-medicine-based work described above also qualifies when considered in this way i.e. as a means for improving what currently may be considered to be a capacity-limited system. This is to say that existing noninvasive measurement methods tend to be so noisy that the only traditional way to gain measurement precision is to increase the number of observations, usually at the cost of a massive project which is cross-sectional rather than longitudinal in design.

Other projects exemplifying DSB's approach to problems of this sort include analysis of the significance of photon energy in quantitative determinations derived radiographically. Specifically, we are taking a hard look at the effects of beam hardening caused by differential attenuation of the various tissues being irradiated by the central beam, and are exploring two different approaches for eliminating some of the intrinsic variance in quantitative measurements caused by this inescapable source of ambiguity. One approach makes use of a tissue equivalent attenuating ramp of known gradient and dimensions which is used to calibrate a series of fiducial wedges which are simultaneously exposed with the tissues of diagnostic interest. By matching tissue densities in the region of interest with a portion of the ramp which has comparably filtered the beam overlying the image of one of the fiducial wedges it is possible to "tune" the system to an appropriate range of energies for purposes of densitometric calibration.

Another approach involves the recording of two radiographic projections simultaneously on films having differing x-ray energy sensitivities. This is accomplished by separating the two films with an attenuating filter and using emulsions having compensatingly different speed characteristics. In theory this makes it possible to separate the two predominant energy-specific photon attenuation mechanisms involved; photo-electric, and Compton scattering. In this way we are planning to use the data produced with dual energy spectra and a computer to synthesize projections from which artifactual changes attributable to soft tissues can be largely eliminated.

Interpretation-limited systems research perhaps is best exemplified by DSB's continuing effort to implement and augment quantitative methods for tracing changes in the size and shape of specific tissues over extended periods of

time. Many useful algorithms have been developed and tested which facilitate the detection and measurement of lesions recorded in spatially registered images. Specific applications range from quantification of induced bone loss in the extremities, to tracing volumetric changes in bone attributable to periodontal disease or wound healing. In the latter case, recent in vitro experiments based on the use of cadaver material confirm preliminary results reported last year which indicated that volume estimates are precise to one mm^3, a value unprecedented using conventional noninvasive methods. New also this year is a method applicable to the integrated measurement of multiple diffuse lesions. The basic approach involves finding an optimum threshold separating two overlapping gray-level distributions through the use of Fisher's linear discriminant function. The methodology used is a generalization of the symmetric-axis transformation developed by the late H. Blum at DCRT.

This work also compliments research reported last year involving an automated procedure to estimate retrospectively the projection angle of a radiograph with respect to a set of spatially registered projections taken at another time. The mean error of this angle-estimation technique was determined in a controlled experiment and found to be 0.5 degrees. The method has the advantage that no multiplications are required for its determination and, hence, it is quickly computable on an image processor designed to automate image registration and analysis. These projects also demonstrate the increase in statistical power afforded by improved sampling techniques intrinsic to the methods used.

Work also continues on the development of methods for automating the process of lesion detection. One aspect of this process involves the need to limit consideration to only tissues of diagnostic interest. Recent efforts have focused on combining quad-tree image characterization with split-and-merge procedures for image segmentation. Gray-level statistics are being used to test homogeneity of subareas, and for establishing region borders. The use of second-order gray-level statistics and methods employed in texture analysis, as well as scale-invariant transformations, are being investigated likewise.

Another area of continuing investigation involving research directed toward the development of improved systems concerns the determination of the predictive values of existing diagnostic methods used to detect routine dental pathoses. This work extends that reported last year which dealt specifically with the role of disease prevalence in estimating cost-effectiveness of dental radiographic screening. This year the diagnostic performances of panoramic, bitewing, and fullmouth radiographic examinations were investigated. The results of comprehensive analysis of diagnostic efficacy

based on computed predictive values place in question the utility of radiography for the detection of dental caries and periodontal disease in the general population (although bitewing radiography may be efficacious when administered to a caries-prone population). Also, these data confirm results suggested last year which indicated, for most common diagnostic objectives, the performance of panoramic radiography is inferior to intraoral radiography. These findings contradict existing patterns of dental practice and point to the need to modify currently accepted dental diagnostic protocols and implement newer more effective diagnostic modalities.

Decision theory is being applied to two other common diagnostic tasks as well. The first clinical situation assesses the decision to administer prophylactic antibiotics (as per the recommendations of the American Heart Association and the American Dental Association) to a patient who presents for dental treatment with a history of rheumatic fever. The second situation evaluates the common clinical decision to extract asymptomatic impacted third molars in young patients rather than risk an extensive surgical procedure in the patient's later years. For each of the above clinical problems, a decision tree was structured in order to display the alternative actions available to the decision-maker, to show the consequences of these actions, and to illustrate all reasonable outcomes for the patient. The various probabilities associated with chance events was derived from existing medical literature and the possible outcomes (payoff measures) were computed using standard probability theory. The data analysis showed: if penicillin was prescribed for dental patients with histories of rheumatic fever, the resulting death rate would be 220 deaths per 100,000. The principle cause of mortality would be anaphylactic drug reaction or infective endocarditis. On the other hand, if antibiotics were not administered, the mortality rate would be <1 death per 100,000. This finding contradicts established treatment recommendations. In the instance of third-molar surgery, the data also contradicted established practice patterns. The data analysis demonstrated that if surgery were deferred until a patient became symptomatic (even if the patient were in poor health), overall mortality and morbidity would be markedly lower than if surgery were performed on asymptomatic young adults.

Other new statistically motivated research involves a collaborative effort between DSB and the NIDR Epidemiology and Oral Disease Prevention Program. Specifically, data of tissue attachment level measured from a population in Sri Lanka are being analyzed for changes attributable to periodontal disease. The aim is to obtain a dynamic description of the progression of the disease using the attachment loss measurements as proxy variables. In order to estimate parameters of interest from the available data,

a continuous-time Markov process is used as a model characterizing the evolution of the disease. Current efforts center on the build-up of the necessary software from standard mathematical libraries, and the determination of special software needs to be developed de novo.

A theoretically related but independent research effort involves continuing collaboration with physicians at Children's Hospital National Medical Center. This investigation involves the prediction of survival or death of patients admitted to an intensive care unit (ICU). The mortality risk predictor is based on the measurement of 34 physiologic variables and was validated prospectively by ROC performance analysis using data obtained from a national sample of pediatric ICUs. Despite highly significant differences in the respective patient populations in terms of age, medical/surgical or emergency/scheduled admission ratios, extent of underlying chronic disease, and mortality rates, the predictor showed no loss in performance compared to the ICU population from which it was derived. It also demonstrated that the observed sixfold differences in mortality rate among the institutions could be explained fully by the differing levels of severity of illness measured at the admission day in the respective populations. In order to make this predictive instrument more widely applicable and easier to use, efforts were undertaken to reduce the number of variables to be included in the severity of illness index without losing its predictive power. Application of logistic regression techniques to a national sample of 2500 pediatric ICU patients permitted reduction of the number of variables required from the original set of 34 to 15, all of which representing noninvasive measurements. This simpler predictor can be used for quality of care comparisons among a wider variety of institutions.

Considerable effort continues to be expended in the transfer of technology developed earlier by DSB. This year DSB technicians have started to transfer software developed on our VAX minicomputer onto a newly acquired IBM-based image-processing microcomputer. A commercially available high-level image-processing language (Media Cybernetics' Image Pro) is being used which has the advantage of supporting a large variety of microcomputer-based, image-processing systems. In this way, it is anticipated that many other investigators and institutions will be able to use software developed by DSB scientists without extensive rewriting to accommodate differences in hardware.

Efforts to improve image-processing efficiency have been also extended to the rather inglorious but essential task of debugging commercial software. A particularly large investment in time has been devoted toward the elimination of logic errors in programs for the computation of two-dimensional Fourier

transforms and related image display computations. Of particular importance also is the rectification of scaling and truncation artifacts associated with software used to perform digital filtration of images.

Because DSB is a very small laboratory, research productivity is keyed to collaborative efforts with other groups both inside and outside the intramural program at NIH. The visibility afforded by these collaborations coupled with the significance of associated research has fostered a considerable demand for DSB investigators as methodological consultants in the areas of systems optimization and image processing. For example, this year two DSB scientists were selected to represent the image-processing community at NIH in an international "teleconference" sponsored by the International Society for Photoscopy. Also, DSB assumed a leadership role in the NIDR sponsored "Conference on Diagnostic and Therapeutic Technology in Dentistry" held on September 29-October 1 of last year which focused on new technological opportunities in dentistry.

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Glass, N.L., Pollack, M.M., and Ruttimann, U.E.: "Pediatric Intensive Care: Who, why, and how much?" Crit. Care Med. 14: 222-226, 1986.

Jeffcoat, M.K., Reddy, M.S., Webber, R.L., Williams, R.C., and Ruttimann, U.E.: "Extraoral Control of Geometry for Digital Subtraction Radiography". Accepted for publication in J. Period. Res. Vol. 22, 1987.

Pollack, M.M., Ruttimann, U.E., and Getson, P.R.: "Accurate Prediction of Outcome of Pediatric Intensive Care.- A New Quantitative Method". N. Engl. J. Med. 316: 134-139, 1987.

Pollack, M.M., Ruttimann, U.E., Glass, N.L., and Yeh, T.S.: "Monitoring Patients in Pediatric Intensive Care". Pediatrics. 76: 719-724,1985.

Ruttimann, U.E.: "Computer-Based Reconstruction and Temporal Subtraction of Radiographs". Accepted for publication in Advances in Dental Research. 1987.

Ruttimann, U.E., Albert, A., Pollack, M., Glass, N.L.: "Dynamic Assessment of Severity of Illness in Pediatric Intensive Care", Crit. Care Med. 14: 215-221, 1986.

Ruttimann, U.E., van der Stelt, P., and Webber, R.L.: "Use of Image Similarity for the Selection or Synthesis of Projections for Subtraction Radiography". Proc. SPIE., MEDICINE XIV, Vol. 626, pp. 301-307,1986.

Ruttimann, U.E., and Webber, R.L.: "Fast Computing Median Filters on General Purpose Image Processing Systems". Optical Engineering, 25: 1064-1067, 1986.

Ruttimann, U.E., and Webber, R.L.: "Three-Dimensional Imaging in Dental Radiography". Accepted for publication in Proc. IEEE Annual Conf. EMBS, Boston, 1987.

Ruttimann, U.E., and Webber, R.L.: "Volumetry of Localized Bone Lesions by Subtraction Radiography." J. Periodontal Res. 22: 215-216, 1987.

Ruttimann, U.E., Webber, R.L., and Saffer, A.: "Calibrated Volume Determination of Localized Bone Lesions by Subtraction Radiography". MEDINFO '86, Elsevier Science Publishers, pp. 301-307, 1986.

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Ruttimann, U.E., Webber, R.L., and van der Stelt, P.F.: "Automated Registration and Selection of Radiographs Suitable for Subtraction". Accepted for publication in Symposium on Computer Applications in Medical Care, 1987.

van der Stelt, P.F., Ruttimann, U.E., and Webber, R.L.: "Enhancement of Tomosynthetic Images in Dental Radiology". J. of Dental Research, Vol. 65, pp. 967-973.

van der Stelt, P.F., Webber, R. L., Ruttimann, U.E. and Groenhuis, R.A.J.: "A Procedure for Reconstruction and Enhancement of Tomosynthetic Images". IADMFR 15: 11-18, 1986.

Webber, R.L. "Factors Limiting Dental Diagnosis: A Conceptual Overview", (Ms. T-21), Accepted for publication in Advances in Dental Research, 1987.

Webber, R.L., Jeffcoat, M.K., Harman, J.T., and Ruttimann, U.E., "MRI Evidence of Simplicity and Stability in the Nasal Cycle of a Beagle Dog", Accepted for publication in the Journal of Computer Assisted Tomography, March 1987.

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Welborn, L.G., Ramirez, N., Oh, T.H., Ruttimann, U.E., Fink, R., Guzzetta, P., and Epstein, B.S., "Postanesthetic Apnea and Periodic Breathing in Infants". Anesthesiology, 65: 658-661, 1986.

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Zeichner, S.J., Ruttimann, U.E., and Webber, R.L.: "Dental Radiography: Efficacy in the Assessment of Intraosseous Lesions of the Face and Jaws in Asymptomatic Patients". Radiology 162: 691-695, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00065-16 DS

PERIOD COVERED

October 1, 1986 - September 30 1987

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)*

Webber, Richard L.	Chief, Diagnostic Systems	DS	NIDR
Ruttimann Urs E.	Bio-Medical Engineer	DS	NIDR
Zeichner, Samuel J.	Senior Staff Fellow	DS	NIDR
van den Berg, Harry R.	Visiting Fellow	DS	NIDR
Tsuchimochi, Makoto	Visiting Associate	DS	NIDR

COOPERATING UNITS *(if any)*

Childrens Hospital National Medical Center
Radiation Physics Group, National Bureau of Standards
United States Army Institute for Dental Research, Washington, D. C.

LAB/BRANCH

Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.41	0.84

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 in vitro modeling of promising systems 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 designed to be used both fluoroscopically and off-line to produce images which can be subtracted to show small changes in tissue occurring over long intervals of time, and combined in ways permitting tomosynthetic display of specific slices of the teeth and jaws.

Progress continues in the development of both hard-ware and soft-ware for this system. Particularly noteworthy is an automated technique recently created to estimate the projection angle of a radiograph relative to a set of standardized projections. The algorithm produces the correct solution from data characterized by angular discrepancies as large as 20°. Other software exploits differences in the mechanism of energy dissipation produced by hard and soft tissues enabling separation of these components for purposes of improved image analysis.

More general research concerns decisions underlying the entire diagnostic process in dentistry. Recent data place in question prophylactic extraction of impacted third molars, and routine premedication with antibiotics of dental patients scheduled for surgery having a history of rheumatic fever.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00211-11 DS

PERIOD COVERED
October 1, 1986 - September 30, 1987

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)*

Webber, Richard L.	Chief, Diagnostic Systems	DS	NIDR
Ruttimann, Urs E.	Bio-Medical Engineer	DS	NIDR
van den Berg, Harry R.	Visiting Fellow	DS	NIDR
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COOPERATING UNITS *(if any)*
Radiation Physics Group, National Bureau of Standards

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.20	2.27	0.93

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 involving the creation, development and testing of image-processing techniques designed to improve diagnostic performance. Current work has centered on methods relevant to the processes of radiologic image subtraction and tomosynthesis. Work continues in the area of automatic manipulation to facilitate registration. Particular emphasis has been placed on: 1) methods for automatically segmenting radiographic images into regions of diagnostic interest, in anticipation of the automated detection of associated lesions, 2) methods for quantifying the apparent size of lesions from these images, 3) methods for increasing the efficiency of complex, spatial-frequency-dependent manipulations essential for optimization of diagnostic performance of specific tasks.

The recognition and delineation of areas showing trabecular bone was set as a primary target because of its importance in the diagnosis and monitoring of periodontal and other lytic bone diseases. Efforts continue in quad-tree image characterization using split-and-merge procedures for image segmentation. Second-order statistics and scale-invariant transformations are also being investigated in this context in order to increase the specificity of the segmentation process.

Other work involves the use "shaded aperture" sampling techniques to eliminate "ringing" artifacts associated with high-pass filtered tomosynthetic reconstructions. Recent findings show that optimum weighting of 25 projections permits suppression of the first side lobe of the transfer function to about 1%.

Future activity will continue coordinate image-processing efforts with research directed toward the development of complete diagnostic systems.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00373-05 DS

PERIOD COVERED
October 1, 1986 - September 30, 1987

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)*

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Ruttimann, Urs E.	Bio-Medical Engineer	DS	NIDR
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COOPERATING UNITS *(if any)*
Harvard School of Dental Medicine, Boston, MA
Biological Engineering and Instrumentation Branch, NIH
Hebrew University, Hadassah, Israel
Laboratory of Developmental Biology and Anomalties, NIDR

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.35	1.01	0.34

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 a variety of new and existing diagnostic techniques which have yet to be applied to biomedical diagnostic applications having particular relevance to dentistry. It is largely a continuation of work done in previous years which dealt with the development of quantitative methods for measuring factors believed to be associated with relatively long-term changes of diagnostic interest in dental tissues. Modalities studied include quantitative radiology, nuclear medicine (99mTc-MDP), and fiber-optic systems making use of visible light (hemoglobin-specific spectrum analysis).

New algorithms are being used to extend the application of digital subtraction radiography to include longitudinal assay of experimentally simulated, progressive, bone lesions in phalanges and femur Preliminary data have shown that spectrometrically confirmed, diffuse, changes of as little as 0.3% are readily discernible.

Ratiometric assessment of scattered light continues to be used to evaluate tooth vitality. In vitro data obtained using a prototype, filtered, fiber-optic system designed to detect the presence of oxygenated blood in the pulp chamber show that tiny changes in tooth color measured in this way can be used to predict degenerative changes seen invariably in recently extracted teeth after storage for a week or more in saline at 37° C.

Measurements of differential uptake of 99mTc-MDP in two beagle dogs following periodontal surgery are being replicated to confirm earlier findings which suggested that the dynamics of wound healing in periodontal bone can be predicted from baseline measurements in these animals.

LDBA has made notable achievements during the past years. This is particularly evident in the work on cartilage. The complete structures of the cartilage proteoglycan and link proteins have been established. An enhancer was identified in the collagen II gene which is required for transcription in chondrocytes. This element in the gene probably serves as a major site to which unique nuclear regulatory proteins bind while other cells lack these proteins and do not transcribe this gene. The identification of these regulatory proteins represents a challenging project.

Advances in our basement membrane projects include completing the structures of the B1 and B2 chains of laminin. Notable in the B1 sequence is a pentapeptide, tyrosine-isoleucine-glycine-serine-arginine (YIGSR), which we have identified as a major cell binding site in laminin. Cyclic forms of the peptide have been synthesized and shown to be potent suppressors of metastasis in experimental models. Additionally, clones have been obtained for the basement membrane proteoglycan and the promoter of the α1(IV) gene.

Receptors represent a major new emphasis in the Laboratory. Two new laminin receptors have been identified. One of these serves as a neural specific receptor. In addition, we have completed the structure of the previously described laminin receptors and of anchorin, a collagen receptor.

We have continued our studies on metastatic cells and have shown that a proteolytic cascade is involved in the activation of collagenase IV which degrades the basement membrane. Several newly developed inhibitors of collagenase which block the invasion of tumor cells through basement membranes have been tested and found to have antimetastatic activity in animals. The in vitro model we have developed to study the invasion of tumor cells through basement membrane is being used to screen for other compounds which might have antimetastatic activity.

Cells from Kaposi's sarcoma have been found to invade through reconstituted basement membranes to resemble smooth muscle cells, and secrete a factor(s) that is an attractant for endothelial cells. These observations suggest that the transformed cells in these lesions are smooth muscle-like and attract endothelial cells which give the lesions their highly vascularized character. Synthetic peptides and anticollagenase compounds are being tested for their ability to block the invasive activity of the Kaposi's sarcoma cells.

We noted in the 1985-1986, last year's Annual Report, that Dr. Mark Bolander formerly of LDBA has been asked by the National Institute of Arthritis Muscular Skeletal and Skin Diseases to form an Orthopaedic Research Unit. This Unit is currently located in LDBA space and is involved in active collaborations with LDBA staff. Notable progress in studying the molecular events in fracture healing has been made by this Unit. A description of their activities is included in this report.

Finally, the Scientific Counselors of NIDR plus ad hoc advisors conducted a two day site visit of LDBA in April 1987. Some 26 members of the staff presented the broad program of LDBA for the site visitors.

CARTILAGE

Due to its important role in growth and development, the structure and function of cartilage proteins are an important focus of our investigations. Cartilage contains an abundant matrix composed of a specific proteoglycan, collagen II and several other glycoproteins including the link protein. Current projects include cloning and characterizing various cartilage proteins including the link proteins, the cartilage proteoglycan and anchorin. We are also investigating the regulatory elements in the collagen II gene to obtain a molecular model for the tissue-specific expression of cartilage genes.

Structure of the Proteoglycan Cartilage

We have determined the complete sequence of the cartilage proteoglycan. This is a large molecule (Mr = 2 X 10^6) primarily carbohydrate but with a large core protein. Using recombinant DNA technology, we have cloned and sequenced the entire core protein. These studies show that the core protein comprises some 2220 amino acids including a signal peptide. Analysis of the sequence indicates that it forms a number of distinct domains arranged linearly along the molecule. Two globular structures, the hyaluronic acid binding domains, are present at the N-terminus of the protein. This domain is followed by a domain rich in proline, likely to be a keratin sulfate binding region based on past chemical studies. Adjacent to this is a region of 1114 amino acids containing 119 serine-glycine sequences, likely to be the attachment sites for the chondrocytin sulfate chains on the molecule. The sites of glycosaminoglycan attachment are present in the ser-gly sequences three distinct domains. Finally, the most carboxyl domain in the core protein is a globule with lectin activity. Thus the proteoglycan can attach to various cartilage components via the globules at each end and these interactions are likely to fix the molecule into the matrix.

In addition, we have isolated several overlapping genomic domains coding for the most proteoglycan core. Attempts will be made to determine gene structure and to define the regulatory regions of this gene. We will also screen for human and mouse clones to the proteoglycan to use for studies on genetic disorders of cartilage.

Alternative Splicing of the Link Protein Generates Distinct Proteins

Link protein stabilizes the interaction of hyaluronic acid with cartilage proteoglycan. Multiple forms of link protein differing in size are found in cartilage. Two cDNA clones were obtained for link protein which have identical 5' and 3' sequences but an additional 159 bp sequence is present in one of the clones. Subsequently, we isolated genomic clones for the rat link protein and found that the 159 bp internal segment was coded for by a single exon. Using this exon as a probe, RNA blot analysis showed that the mRNA containing this additional sequence is much less abundant than the mRNA lacking the exon. The 53 amino acids encoded by this exon are notable for a high net positive charge, and three cysteine residues, two of which are adjacent. Our data suggest that alternative splicing allows a single gene to encode at least two different forms of link protein, one having a predicted molecular weight of 38,000 and the other having a molecular weight of 43,000. Attempts will be made to establish the tissue distribution and function of these alternative forms.

Identification of a Cartilage Specific Enhancer on the Collagen II Gene

Previously we isolated the promoter of the collagen II gene. When we constructed plasmids containing the promoter of collagen II gene linked to the gene for the chloramphenicol acetyl transferase and introduced them into chondrocytes and limb bud cells, we found that the promoter showed little ability to drive transcription. These results indicated that additional positive regulatory elements necessary for efficient transcription were located elsewhere in the gene. Various fragments of the first intron gene were incorporated into the collagen II promoter-CAT construct and a 450 base sequence from the first intron was found to strongly stimulate transcription in chondrocytes and in differentiating limb bud cells. Further studies showed that this 450 base sequence had characteristics of a classic enhancer; ie, it showed stimulatory activity when placed in the construct in either orientation as well as when placed either before or after the promoter. Other studies established that it is "cartilage specific" being active in chondrocytes and differentiating limb bud cells but not in a variety of other cells. The enhancer was sequenced and the importance of several regions in the enhancer established by deletion analysis. Preliminary studies have established that chondrocytes contain distinct nuclear proteins that bind to this enhancer. Attempts will be made to identify and characterize these proteins since they may represent the super genes regulating the chondrocyte phenotype.

Establishment of a Transgenic Mouse Facility

With the availability of gene constructs for various extracellular matrix molecules, it is now possible to test the function of these molecules, the regulation of their expression, and their use in gene replacement therapy by incorporating these constructs into mouse eggs, thus creating transgenic mice. A transgenic mouse facility has been established and staff have been trained to carry out timed breeding of donor and foster mothers. Several different strains have been tested for oocyte suitability for injections. The complex techniques involved in DNA injection have been mastered and injections are carried out on a daily basis. The capacity of the facility will be doubled in 1987.

We are starting to use the collagen II constructs in which various lengths of the 5′ flanking region are coupled to the gene for chloramphenicol acetyl transferase (CAT) with or without putative enhancer sequences. The DNA construct is injected into the male pronucleus of fertilized oocytes which are transferred to pseudo-pregnant foster females. Viable young which have incorporated the injected DNA into their chromosomes are then assayed for CAT activity to examine tissue specific expression. A construct without the enhancer (2 Kb of the 5′ flanking DNA from rat collagen II gene fused to CAT) has been successfully established as a stable transgenic line but has not shown the tissue expression.

We anticipate that this system will be useful in testing recombinant constructs of cartilage and of basement membrane genes as possible in vivo models for gene regulation and gene therapy. When full length cartilage proteoglycans are available, we plan to use mutant (CMD) mice unable to produce cartilage specific proteoglycan as a test system. Other mutant mouse lines such as congenital and juvenile polycystic kidney disease,

hypochondroplasia, chondrodysplasia, progressive ankylosis, and fragilitas ossium are potential targets for this type of genetic intervention when the molecular basis of their defects is determined. We also plan to generate mutant transgenic mice by introducing in vitro mutagenized genes as models for hereditary disorder defective in genes for connective tissue proteins.

BASEMENT MEMBRANES

Basement membranes are thin sheets of matrix which underlie epithelia and endothelia, and surround muscle, nerve, and fat cells. They serve a structural role, create barriers which prevent the passage of cells and proteins, and guide the formation and regeneration of tissues. LDBA has been studying basement membranes for several years. Our work was facilitated by the discovery of a tumor that produced copious amounts of basement membrane which allowed the isolation of basement membrane components in quantity including collagen IV, laminin, and heparan sulfate proteoglycan. Current activities include determining the structure of laminin chains from cDNA clones, determining its gene structure and identifying regulatory regions, expressing cDNA for prokaryote and eukaryote systems, and determining active sites in the molecule. A cell attachment domain has been identified in the B1 chain of laminin. Additional studies are being conducted on other basement membrane components including collagen IV and the heparan sulfate proteoglycan. Application of these studies is made to development and to disease.

Structure of Laminin

Laminin is a large basement membrane-specific glycoprotein composed of three chains: A (400 Kd), B1 (230 Kd), and B2 (200 Kd). Its size plus difficulty in separating its chains have impeded the characterization of its structure by conventional chemical approaches. To study this protein, we have obtained clones for all three chains and have completed all of the B1 and B2 chains. The nucleotide sequence of the cDNA for the B1 chain shows a 5,358 bp open reading frame with the potential to code for 1,786 amino acids including 20 amino acids of a presumptive signal peptide. The sequence of the B2 chain clones shows an open reading frame of 4,821 bp coding for a protein of 1,607 amino acids including a presumptive signal peptide plus 2.5 Kb of 3' untranslated sequence. Analysis of the deduced protein sequences predicts that the B2 and B1 chain have six or seven distinct domains respectively which show similar structural features. These include domains with α-helix, cysteine-rich repeats, and globular structures, suggesting that these chains are homologous components as indicated by electron microscopy of the molecule. One of the major differences between these chains is that the B2 chain lacks a small domain composed of thirty amino acids with six cysteine and eight glycine residues. In order to localize the functional domains of laminin in more detail, we are also attempting to express cDNA segments of the B1, B2, and A chains in both eukaryotic and prokaryotic systems.

Characterization of the B1 Chain Gene

We previously isolated and characterized cDNA clones encoding the entire B1 chain of murine laminin. Using these cDNA clones as probes, we screened a mouse genomic library and isolated nine overlapping clones which spanned 80 Kb of contiguous DNA. R-loop analysis revealed that the gene for the B1 chain

was 63 Kb in size and contained at least 36 exons ranging from 70 to 400 bp in size. DNA sequence analysis revealed that several of the cysteine-rich repeats were coded for by a single exon while others were split between two. Three different transcription initiation sites were identified by S1 mapping and primer extension. One Kb of the 5' promoter region was characterized by DNA sequence analysis. The promoter activity of the gene was examined by transfection of CAT-constructs containing various length of the 5' flanking sequence into F9 cells. Preliminary results suggest that an upstream sequence, -9 Kb to -1.9 Kb from the transcription initiation site is needed for efficient transcription of the gene.

We also studied the DNase-I hypersensitive sites and the methylation state of the B1 chain gene in both expressing and non-expressing cells and tissues. We found that the 5' end of the gene became hypomethylated in expressing cells and tissues. DNase I hypersensitive sites were also localized to the 5' flanking region and to the first intron of the gene. These hypersensitive sites could be involved in binding factors that regulate the expression of this gene.

YIGSR - A Peptide From Laminin With Cell Attachment, Chemotactic, Receptor Binding and Anti Metastatic Activities

We have sought to identify an active site(s) on laminin for cell attachment. Using the amino acid sequence for the B1 chain deduced from cDNA clones, synthetic peptides were prepared which correspond to hydrophilic sequences in the domains that form the chain. Antibodies to albumin conjugates of these peptides were raised and their reaction with native laminin was established. None of the peptides synthesized was active in cell attachment. The antibody to a sequence in domain III, a cysteine-rich region of homologous repeats near the intersection of the chains, inhibited the attachment of cells to laminin. Since the peptide itself was inactive, inhibition by the antibody was probably due to steric blocking of a nearby site. Several additional peptides from domain III were synthesized. One of the peptides, CDPGYIGSR, was found to stimulate cell attachment and migration and to block laminin-mediated cell attachment and migration. This peptide displaced the laminin receptor (Mr=67,000) from immobilized laminin and blocked the formation of lung metastases in mice injected with malignant melanoma cells. Peptides of different sizes with various amino acid substitutions were tested to define the minimal sequence necessary for biological activity. The pentapeptide, YIGSR (tyrosine-isoleucine-glycine-serine-arginine), contained the minimal sequence able to mediate cell attachment and receptor binding. These peptides are also chemotactic and antimetastatic in animal models. Current studies include the examination of neighboring sequences in domain III which may also be involved in receptor binding.

Characterization of the Basement Membrane Proteoglycan

We are studying the large basement membrane-specific heparan sulfate proteoglycan isolated from the EHS tumor. Biochemical studies indicate that the EHS proteoglycan is 60% protein and consists of a large core protein M_r= 400,000 with 3-4 heparan sulfate chains. Electron microscopic examination showed that the core protein as a linear structure formed from 5-6 contiguous globules with the heparan sulfate chains attached at one end. Defined fragments of the core are produced by trypsin or V8 protease digestion and

peptides of M_r=200,000, 44,000 and 46,000 have been isolated from the region of the core which lacks heparan sulfate chains. Amino acid sequences of 28 and 29 amino acids have been obtained from the M_r=44,000 and 46,000 peptides and corresponding oligonucleotides have been used to screen libraries, and to obtain cDNA clones of the core protein.

Heparan sulfate proteoglycans isolated from other basement membranes are reported in some cases to be smaller and may represent degradation products. Antibodies to the EHS proteoglycan react with all basement membranes and immunoprecipitate a M_r=400,000 precursor protein. Metabolic studies indicate that the proteoglycan is degraded after synthesis giving rise to a variety of smaller products. Most of the proteolytic fragments from the EHS proteoglycan except for the M_r=44,000 peptide reacted with antibody prepared to the glomerular proteoglycan suggesting that this domain may be missing from the glomerular proteoglycan. Molecular probes will be used to assess the primary transcripts for the proteoglycan gene in different cells and to investigate the occurrence of alternatively spliced forms.

Cloning the Basement Membrane Proteoglycan

We have now isolated 4 proteoglycan clones by antibody screening of expression libraries. Two clones of 313 and 228 bp were obtained from a randomly primed library and two larger clones of 2.0 and 2.2 Kb were isolated from a library primed with oligonucleotides whose sequence was based on amino acid sequences obtained from proteolytic fragments of the core protein. Each of these clones hybridizes to a single mRNA band at approximately 11 Kb, the size expected for a core protein of M_r=400,000. This message shows approximately an 8-fold increase, in teratocarcinoma cells treated with retinoic acid and dibutyryl cAMP proportional to the increase in proteoglycan synthesis. We have further characterized these clones by preparing antibodies to fusion protein produced in bacteria and by studying their reaction with the core protein and with its proteolytic fragments. Antibodies to the fusion protein of the 2.0 Kb clone react strongly with the core protein and the 200, 44 and 46 Kd peptides obtained from the core protein. Antibodies to the fusion protein from the other three clones also reacted with the core protein but with other fragments in the proteolytic digests of the core protein. When sequenced, the 228 and 313 bp clones were found to contain sequences with extensive nucleotide and amino acid homology, suggesting that there may be repetitive structures in the glycosaminoglycan attachment region of the core protein. We are now sequencing the larger clones to establish if the 2.0 Kb clone contains a sequence which matches with amino acid sequences derived from the proteoglycan. We are also using these clones to screen other libraries to obtain clones for 3′ and 5′ portions of the mRNA.

Isolation and Structure of Collagen IV cDNA Clones - Use in Studying Gene Regulation in Diabetes

We have worked on the isolation and characterization of various genomic and cDNA clones for collagen IV and have utilized these clones to elucidate the structure and regulation of these genes. Collagen IV, the major structural component of basement membranes is a heteropolymer containing two α1(III) and one α2(IV) chain. Clones to the 3′ portion of the α1(IV) chain mRNA have been obtained and used to determine the structure of this portion of the protein. In addition overlapping genomic clones for the α1(IV) chain spanning some 50

Kb of DNA have been isolated, partially characterized and found to contain multiple exons of variable size.

To obtain possible 5′ regulatory sequences, an oligonucleotide predicted by an amino acid sequence obtained from the N-terminal domain of the α1(IV) chain was used to localize exons in genomic clones. Primer extension using a sequence from one of the exons showed that the 5′ end of the mRNA was some 600 bases upstream. This cDNA segment was cloned and sequenced.

Clones to various basement membranes proteins have been used to study gene expression in several mouse tissues and in diabetic kidneys. Steady state levels of the laminin B1 and B2 chains mRNA vary markedly in different tissues of mature animals when compared to collagen α1(IV) chain mRNA. No A chain mRNA was detected in RNA from kidney, heart, or lung. The levels of B1, B2 and α1(IV) chain mRNAs were elevated in diabetic animals and were restored towards normal by high doses of insulin. These data suggest a complex pattern of regulation of these genes with elevated synthesis in the diabetic kidney requiring strict metabolic control for normalization.

Characterization of the Promoter for the Collagen α1(IV) Chain Gene

We have isolated and are characterizing the promoter for the α1(IV) chain gene. A murine genomic library was screened utilizing a 138 bp cDNA fragment containing 5′untranslated region of α1(IV) mRNA and we obtained three overlapping clones spanning 21 Kb of the gene were isolated. Each clone contained a single exon whose sequence agreed with the 5′ 225 nucleotides of the cDNA and contained a typical splice-acceptor sequence. A 1.5 Kb fragment containing the first exon and approximately 1 Kb of 5′ flanking DNA was subcloned and sequenced. Major and minor transcription-initiation sites were identified in poly(A+) RNA from differentiated F9 teratocarcinoma cells by primer extension and S1 nuclease protection analysis. The nucleotide sequence flanking these sites was extremely GC rich (>70%), and two inverted repeats resembling the Sp1 binding sequence, GGCGGG and CCGCCC, were present immediately 5′ to the major initiation site. The promoter lacks a TATA box.

To identify cis-acting regulatory sequences, a series of hybrid gene constructs has been prepared. 5′ flanking DNA fragments, measuring approximately 2.2, have been ligated in pSV0CAT immediately upstream of the prokaryotic gene, chloramphenicol acetyl transferase (CAT). These constructs showed little activity when transfected into undifferentiated F9 cells and NIH 3T3 cells. However, inclusions of portions of the first intron strongly stimulated transcription and could represent positive regulatory elements. The nature of these segments will be established and trans-acting factors in nuclear extracts will be studied by exo III protection, DNase I footprinting, and gel retardation assays.

Regulation of Basement Membrane Protein Synthesis in Development and in Mice with Polycystic Kidney Disease

The differentiation of certain teratocarcinoma cells and the transcription of various basement membrane genes is induced by treatment with retinoic acid and cyclic AMP. During normal kidney development, the mRNAs for the chains of laminin and for collagen IV increase synchronously in fetal tissues and then decrease rapidly after birth. Only a very low level of A chain mRNA is

present during the growth and development of the kidney, suggesting that it may not be a major component of kidney laminin. We have also measured the levels of these gene products in mice with a genetic form of polycystic kidney disease. The kidneys of these animals develop large cysts and the animals die with uremia by 4 weeks of age. Immunofluorescence studies show that the basement membrane around the cysts is fragmented or even missing. Kidneys from the affected animals were found to maintain much higher levels of laminin B1 and B2 chains and collagen IV chain mRNAs. In situ hybridization shows a strong expression of basement membrane genes in the cells around the cysts. These data suggest that there is a dysregulation of basement membrane genes in polycystic kidney disease possibly as a compensatory response to kidney enlargement.

STUDIES ON METASTATIC TUMOR CELLS

It is well documented that metastatic cells represent a minor population in the original tumor that have acquired the ability to escape from the tumor proper, folate in distant sites and create secondary lesions. Metastasis represents a critical event in cancer and is associated with a poor prognosis for the patient. Basement membranes represent a significant barrier to the passage of many normal cells but one that metastatic cells are able to cross. We and others have shown that metastatic cells have a high affinity for basement membrane and particularly for laminin. Recently we have developed a reconstituted basement membrane which can be used in the Boyden chamber assay as a barrier which distinguishes between invasive and non-invasive cells. In contrast to normal cells, metastatic cells are able to attach to, degrade, and migrate through the reconstituted basement membrane layer. We are currently using this assay to investigate the activation of malignancy by oncogenes and to screen for drugs which block invasiveness.

Oncogenes and the Invasive Activity of Tumor Cells

Transfection of normal cells with certain oncogenes results in cell transformation and in the expression of a malignant phenotype. Previous studies using an in vitro assay to assess cell invasion through a basement membrane barrier have demonstrated that cells transfected with the ras oncogene are invasive in vitro and highly metastatic in vivo. The aim of the present research is to investigate the role of the ras oncogene and its protein products in inducing invasiveness. Preliminary studies have shown that clone 433.3 of NIH/3T3 cells, a stable carrier of the MMTV- LTR v-ras^H chimeric DNA, is able to invade basement membrane after exposure to low concentrations of dexamethasone. We also are currently investigating two lines of adenocarcinoma cells, DM and SR, that constitutively express different levels of ras oncogene. SR cells contain one copy per cell of ras and produce low levels of p-21. DM cells contain 30 copies and produce two-fold higher quantities of p-21. These latter cells expressing high levels of p-21 are five-fold more invasive and more migratory than SR cells. A plasmid containing anti-sense ras RNA linked to the β interferon promoter was constructed by Dr. Sue Penno (John Hopkins) and was used to transfect SR and DM cells in order to modulate ras expression. Previous studies have shown that transfection with the plasmid and exposure to an activator of the promoter results in a significant decrease in p-21. The invasive ability of the anti-sense ras RNA transfected cells will be measured in the presence and absence of inducer. Levels of p-21 will be correlated with other cell

activities such as cell attachment, migration, proliferation, and synthesis of basement membrane proteins including laminin, collagen IV, and fibronectin and their specific cell receptors.

Use of a Chemoinvasion Assay to Study Malignant Cells

Previous studies showed that human breast cancer cells are invasive, particularly when transformed with the ras oncogene. Here we have studied the effect of antiestrogens on the invasive activity of these breast cancer cells. These studies show that anti-estrogens such as tamoxifen and hydroxytamoxifen stimulate invasiveness while anti-estrogens with little agonist activity do not. The possibility that specific mediators are produced by the cells in the presence of the anti-estrogens which activates their invasive behavior is under investigation.

We are also studying the invasive and chemotactic behavior of cells isolated from Kaposi's sarcoma lesions from AIDS patients. These studies show that the cells cultured from biopsies of these lesions are invasive and show a response to growth factors as do smooth muscle cells. They also secrete potent attractants for endothelial cells. It is possible that Kaposi's sarcoma arises from partially transformed smooth muscle cells. The abundance of blood vessels in these lesions could be due to the chemotactic factor(s) produced by the Kaposi's tumor cells.

Pharmacological Modification of Tumor Cell Invasion and Metastasis

Using a sensitive solid phase assay for collagenase IV, we have found that malignant but not nonmalignant cells produce this enzyme as they invade a reconstituted basement membrane. Inhibitors of plasminogen activator prevent malignant cells from producing the active form of the enzyme. Additionally, inhibitors of plasminogen activator and of collagenase IV were found to inhibit the invasion of tumor cells through a basement membrane barrier in vitro as well as the formation of metastases in vivo. These results suggest that a cascade of proteases leads to the formation of the enzyme required for degrading basement membrane and that inhibition of any step in the cascade inhibits invasiveness.

Arachidonic acid metabolism was also found to be required for tumor cell invasion and metabolism. Inhibition of both the cyclooxygenase and the lipoxygenase pathways inhibited the invasion of the cells through basement membranes. Similarly, cells treated with such inhibitors produced fewer metastatic lesions when they were injected intravenously into animals. These agents appear to reduce the chemotactic responsiveness of the metastatic cells as well as collagenase IV production.

MATRIX RECEPTORS

The interaction of cells with extracellular matrix components confluences their growth, differentiation, and migration. Present studies suggest that these interactions are mediated through specific cell surface receptors. We have been studying the matrix receptors for collagens II and IV, and for laminin. Anchorin, (Mr=34,000) a cell surface glycoprotein which binds to collagen II, has been cloned and sequenced. Similarly a collagen IV receptor (M_r=68,000) has been isolated from EHS tumor cells and attempts are being made

to clone it. The 67 Kd laminin adhesion receptor has been cloned and is currently being sequenced. Since laminin is a multifunctional molecule, it is not surprising that several cell surface ligands exist. Neural cells respond to laminin both by attachment and by neurite process formation and two additional laminin receptors, M_r=110,000 and 180,000, have been identified on neural cells. The site on laminin which binds to the receptor(s) and promotes neurite outgrowth is being investigated using proteolytic fragments, fusion proteins obtained from cDNA clones, and synthetic peptides and their corresponding antibodies. It is expected that studies on these cell surface receptors for extracellular matrix components will help to establish their regulatory roles in development and in disease.

Cloning Anchorin, A Collagen Receptor

Anchorin (Mr=34,000) is a protein present on the surface of chondrocytes and fibroblasts which binds these cells to collagen. We constructed a cDNA library from chick sternal poly-(A^+)RNA in the expression vector λgt11 in order to screen with polyclonal antibody to anchorin and obtained several positive clones. The amino acid sequence encoded by this clone contained partial amino acid sequences obtained from proteolytic fragments of anchorin. Subsequent rescreening of the library with cDNA probes allowed us to obtain a series of overlapping clones encoding nearly the whole molecule. The deduced amino acid sequence reveals a potential transmembrane segment at the carboxy terminal region of the protein with a possible tyrosine phosphorylation signal. Northern analysis showed that a 1.7 Kb anchorin mRNA is present in sterna, calvaria, and crop as well as in fibroblasts. In addition, the level of anchorin mRNA was found to increase more than three-fold in chick fibroblasts after transformation with RSV.

Collagen IV Receptor

Plasma membranes were purified from EHS tumor cells and solubilized in the non-ionic detergent, octylglucoside. These membrane extracts were first cross-absorbed on laminin-Sepharose, then passed over collagen IV-Sepharose. Bound proteins (Mr = 68,000 and 80,000) were eluted with 1 M NaCl and 0.2 M glycine-HCl, pH 2.8. Due to its abundance, the 68 Kd protein was selected for further characterization. Polyclonal antibodies to the 68 Kd protein stained the surface of cells and also blocked their attachment and spreading on collagen IV. Immunohistochemical techniques revealed that the 68 Kd protein was predominantly localized on the basal surface of intestinal epithelial cells and at neuromuscular junction. Western blot analysis demonstrated the presence of immunoreactive proteins in lysates of teratocarcinoma, neuroblastoma, and epithelial cells but not in chondrocytes, fibrosarcoma or fibroblastic cell lines, suggesting that this protein is expressed on cells of ectodermal and endodermal, but not mesodermal origin. This protein binds to native collagens I and IV but not to collagen II or to denatured collagens I and IV. These results indicate that conformation and sequence are both important determinants of binding. Preliminary experiments suggest that RGD recognition may be involved in the binding of collagen by the 68 Kd receptor. Rotary shadowing of complexes of type IV collagen and the purified receptor demonstrate that two sites on the type IV molecule were recognized by this protein, one in the major helical domain, the other in the NCI domain. cDNA clones for this putative collagen IV receptor have been isolated from λgt11 expression libraries and are presently being sequenced and characterized.

Laminin Receptor

A 67 Kd laminin binding protein was isolated by laminin-Sepharose affinity chromatography as described for the collagen receptor above. This protein appears to be the murine form of the laminin receptor described by others. Antibodies against this protein were raised which recognized the 67Kd protein also studies with synthetic peptide show that the peptide YIGSR displaces the receptor (Mr=67,000) from laminin Sepharose indicating that this is the primary laminin receptor recognition sequence. These antibodies also specifically recognized λgtll β-galactosidase fusion proteins encoding the C-terminal 10 Kd portion of the human and mouse laminin receptor. Antibodies to the laminin receptor fusion protein have also been produced and shown to bind a 67 Kd protein in membrane extracts, presumably the laminin receptor. The gene encoding this protein appears to be considerably smaller than expected, therefore we are examining this protein for post-translational modification.

Neurite Specific Receptors for Laminin

Neuronal cells respond rapidly to laminin by extension processes in generally less than one hour. A site on laminin near the end of the long arm has been shown to be responsible for this activity. This site is distinct from that responsible for epithelial cell attachment which is near the intersection of the three laminin chains. A receptor of 67 Kd has been found to bind to this latter site on laminin. Since neural cells respond to a different region on laminin, we have sought to identify those cell surface laminin-binding molecules from neural cells. Detergent extracts of membrane preparations of NG105-15 neuroblastoma x glioma cells were chromatographed on a laminin affinity column. Three major proteins bound to this column of M_r=67 Kd, 110 Kd and 180 Kd. The 67 Kd molecule eluted in the salt gradient at 0.5 M NaCl and corresponds to the previously identified laminin adhesion receptor. The 110 Kd and 180 Kd are novel. Antibodies to the 110 Kd protein react with a variety of epithelial cells but not mesenchymal cells or tissues whereas the 180 Kd molecule is neural-specific. The antibodies to the 110 Kd and to 180 Kd protein block laminin-mediated neurite outgrowth. These data suggest that these laminin-binding protein mediate neural cell responses to laminin.

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A Model for Studying Bone Repair

Fractures are a common occurrence with an estimated incidence of over 2 million fractures in the United States per year. Despite advances in surgical treatment, considerable morbidity results from malunion and non-union of fractures in up to 5% of all cases. Fracture repair demonstrates an ordered sequence of cellular events which presumably is under the control of both local and systemic growth factors. Using sensitive and specific recombinant DNA techniques, an investigation of the effects of growth factors upon fracture healing could provide beneficial insights for treatment of fractures in both normal and healing-impaired patients.

We have produced mid shaft femoral fractures in rats using a standard force followed by fixation with an intramedullary pin. The advantage of this model is that the location of the fracture is easily controlled, the force and displacement creating the fracture are identical for each limb, and fracture motion during the healing process is controlled by the intramedullary pin. As a result, the healing in each animal is very similar, and the histological pattern of repair is reproducible.

Healing in this model has been evaluated by radiographs and histology. These studies demonstrated that these fractures heal by endochondral ossification of the cartilaginous soft callus as has been previously described. Healing was also evaluated by probing for the expression of tissue-specific genes and regulatory proteins, including heat shock proteins and proto-oncogenes, on Northern blots. These investigations suggest that the expression of tissue specific genes can be correlated with the expression of specific proto-oncogenes, such as c-fos and c-myc. In the healing fracture callus, heal shock proteins are activated but in a complex non-coordinating fashion suggesting that these proteins have a role in fracture repair.

Healing has also been evaluated by digital subtraction radiography. We have developed specific radiographic techniques and allied computer programs that permit standard radiographs to be digitized and corrected for the non-linear characteristics of the radiographic film. Serial images of the healing bone, corrected in this manner, can be compared by digital subtraction to quantitate the precise changes that have occurred during the course of healing.

In preliminary studies, various growth factors have been administered to fractures, and the effect on healing investigated by the above methods. We find that the administration of growth factors can significantly alter the magnitude and pattern of the repair process. ECGF, for example, stimulates an increase in callus size on x-ray and an increase in cartilaginous tissue on histology. These experiments should help to obtain a better understanding of the molecular and biochemical events involved in the healing process. Studies with models of impaired bone repair, such as diabetes, will be carried out to look for specific defects in gene expression and to investigate the possibility of utilizing growth factors to stimulate the repair of fractures particularly in diseases.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00009- 26 DB

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Biosynthesis of Connective Tissue

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

Martin, G. R.	Ch, Lab of Div Bio & Anom.	DB	NIDR
Albinia, Adriana	Visiting Associate	DB	NIDR
Thompson, Erik	Guest Researcher	DB	NIDR
Reich, Reuven	Guest Researcher	DB	NIDR
Shima, Thomas	Guest Researcher	DB	NIDR
Ebihara, Isao	Visiting Fellow	DB	NIDR
Killen, Paul	Staff Fellow	DB	NIDR
Yamada, Yukihide	Visiting Associate	DB	NIDR

COOPERATING UNITS *(if any)* Max Planck Institute for Biochemistry, West Germany; McGill University, Canada; State University of New York at Buffalo; M.D. Anderson Hospital, San Francisco; Upjohn Co, Kalamazoo, FDA, NCI

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.19	9.35	.84

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 study the formation, function and the destruction of connective tissue components in normal and diseased states. Particular attention is directed toward various matrix molecules including collagens, glycoproteins, and proteoglycans. Current aspects of this project include (1) the invasion of malignant cells through extracellular matrix, (2) alterations in the expression of matrix genes in development and disease.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00024-21 DB

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental processes in genetically controlled malfunction

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

Brown, Kenneth S.	Medical Director	DB NIDR
Hou-Xiang, Xie	Visiting Fellow	DB NIDR
Miyashita, T.	Visiting Associate	DB NIDR
Abramczuk, J.		OM NIDR

COOPERATING UNITS *(if any)*
Howard University; University of Maryland; University of Washington, Seattle; NCI, NIH; NEI, NIH; NIAMDD, NIH; and USDA Poison Plant Laboratory

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.78	2.10	5.68

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 availability of gene constructs for various extracellular matrix molecules, makes it now possible to test the function of these molecules, the regulation of their expression, and their use in gene replacement therapy by incorporating these constructs into mouse eggs, thus creating transgenic mice. A transgenic mouse facility has been established and staff have been trained to carry out timed breeding of donor and foster mothers. Several different strains have been tested for oocyte suitability for injections. The complex techniques involved in DNA injection have been mastered and injections are carried out on a daily routine. The capacity of the facility will be doubled in 1987.

We are starting to use the collagen II constructs in which various lengths of the 5′ flanking region are coupled to the gene for chloramphenicol acetyl transferase (CAT) with or without putative enhancer sequences. The DNA construct is injected into the male pronucleus of fertilized oocytes which are transferred to pseudo-pregnant foster females. Viable young which have incorporated the injected DNA into their chromosomes are then assayed for CAT activity to examine tissue specific expression. The BS-CAT construct (2 Kb of the 5′ flanking DNA from rat collagen II gene fused to CAT) has been successfully established as a stable transgenic line but has not shown the expected tissue expression.

We anticipate that this system will be useful in testing recombinant constructs of cartilage and basement membrane genes as possible models for gene therapy. When full length cartilage proteoglycans are available, we plan to use mutant (CMD) mice unable to produce cartilage specific proteoglycan as a test system. Other mutant mouse lines such as congenital and juvenile polycystic kidney disease, hypochondroplasia, chondrodysplasia, progressive ankylosis, and fragilitas ossium are potential targets for this type of genetic intervention when the molecular basis of their defects is determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00025-21 DB

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

Yamada, Yoshihiki	Chief, MBU	DB NIDR
Doege, Kurt	Staff Fellow	DB NIDR
Sasaki, Makoto	Visiting Associate	DB NIDR
Miyashita, Tomoyuki	Visiting Fellow	DB NIDR
Rhodes, Craig	Biologist	DB NIDR
Iwamoto, Yukihide	Visiting Associate	DB NIDR
Horton, Walter	Staff Fellow	DB NIDR
Martin, George	Chief	DB NIDR

COOPERATING UNITS *(if any)* Max-Planck Institut fur Biochemie; Johns Hopkins University; NCI; Roswell Park Memorial Institute; Sagami Chemical Institute; Columbia Univ.; Stanford University; FDA; NICHD

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION
Molecular Biology Unit

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
19.25	15.95	3.30

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this project is to understand the molecular mechanisms by which genes for connective tissue proteins are regulated and expressed during normal development and in disease states. The formation of extracellular matrix is intimately associated with cell differentiation and tissue development in mammalian embryogenesis. Furthermore, alterations of extracellular matrix structure are associated with human conditions such as neoplastic disease and diabetes. We are studying genetic mechanisms controlling the expression and function of cartilage and basement membrane components.

Recombinant DNA techniques have been used to prepare molecular clones of genes and mRNA for various cartilage and basement membrane constituents. Using these molecular tools, the genetic mechanism(s) controlling the expression of the genes has been studied. The structure of some of these constituents has been determined by cDNA sequencing. Relationships between the structure and function of the proteins have been studied using synthetic peptides deduced from cDNA sequence. Sequences in these genes regulating their expression are being studied.

Ebihara, Isao	Visiting Fellow	DB NIDR
Killen, Paul	Staff Fellow	DB NIDR
Graf, Jeannette	Biologist	DB NIDR
Kleinman, Hynda	Chief, CBS, LDBA	DB NIDR
Ogawa, Kohei	Guest Researcher	DB NIDR
Brown, Kenneth	Medical Director	DB NIDR
Hassell, John	Research Biologist	DB NIDR
Tashiro, Kenichiro	Visiting Fellow	DB NIDR
Burbelo, Peter	Staff Fellow	DB NIDR
Xie, Hou-Xiang	Visiting Fellow	DB NIDR
Savagner, Pierre	Visiting Fellow	DB NIDR
Selmin, Ornella	Visiting Fellow	DB NIDR
Ogle, Roy	Staff Fellow	DB NIDR
Abramczuk, Jan	Visiting Scientist	LOM NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00149-13 DB

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Proteoglycans During Abnormal Development and Disease

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

Hassell, John R.	Research Biologist	DB NIDR
Yamada, Yoshihiko	Chief, MBU, LDBA	DB NIDR
Horton, Walter	Postdoctoral Fellow	DB NIDR
Noonan, Douglas	Postdoctoral Fellow	DB NIDR
Doege, Kurt	Postdoctoral Fellow	DB NIDR
Horigan, Elizabeth	Biologist	DB NIDR
Mosley, General	Biological Lab Technician	DB NIDR
Marks, Michelle	Postdoctoral Fellow	DB NIDR

COOPERATING UNITS *(if any)*
Upjohn, Kalamazoo, Michigan (Steve Ledbetter)
Saint Mary's Hospital, Manchester, ENGLAND (Paul Brenchley)

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.73	3.33	4.5

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.)*

The purpose of this project is to determine the structure of proteoglycans as well as to understand the role that they play in the function of tissues and during developmental events. We have been using immunology, peptide mapping and molecular biology to characterize the core protein of proteoglycans from basement membrane. Basement membrane proteoglycans from EHS tissue consists of a MW = 400,000 core protein with 3 heparan sulfate side chains attached to one end. The core protein is divided into 2 major domains: a MW = 200,000 trypsin sensitive region containing the side chains and a MW = 200,000 trypsin resistant region containing MW = 46,000 and 44,000 V-8 protease resistant sub domains. The glomerular basement membrane proteoglycan is synthesized from the same MW = 400,000 precursor protein as the EHS proteoglycan but is proteolytically processed to a MW = 250,000 core protein containing the MW = 46,000 subdomain but not the MW = 44,000 subdomain. Genetic clones to both the trypsin sensitive and trypsin resistant domains have been obtained using antibodies. The deduced sequence from these clones show the core protein to consist of a unique repeat structure with no homology to any other connective tissue component.

We are also using chondrocytes as a model system to study gene expression and determine the mechanisms by which teratogens disrupt the synthesis of proteoglycans and other matrix components during development. Retinoic acid, a teratogen which produces limb and facial malformations in vivo is also known to alter chondrogenesis in vitro. We have found that retinoic acid inhibits the synthesis of type II procollagen, cartilage proteoglycan core protein, and link protein while stimulating the synthesis of type III collagen and fibronectin. Furthermore, retinoic acid acts to change the phenotype of the chondrocytes by affecting transcriptional activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00230-11

PERIOD COVERED
October 11, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Extracellular Matrix 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 Chemist, Chief, Cell Biol Sect.	DB NIDR
Martin, G. R.	Chief, LDBA	DB NIDR
Graf, J. O.	Postdoctoral Fellow	DB NIDR
Kitten, G. T.	Postdoctoral Fellow	DB NIDR
Cannon, Francis	Postdoctoral Fellow	DB NIDR
Horn, Valerie	Postdoctoral Fellow	DB NIDR
Ogle, R. C.	Guest Researcher	DB NIDR

COOPERATING UNITS (if any) NCI, NHLBI, NIH, Max Planck Inst. Biochem., Munich; TX Tech Lubbock, Texas; University of Montreal and McGill University, Canada; Georgetown University; Howard University, MA; CNRS, Paris; Upjohn Co, Kalamazoo, Mi; Univ of British Columbia, Canada; Helitrex, Princeton, NY; U of Minn; U of Wisconsin

LAB/BRANCH Laboratory of Developmental Biology and Anomalies

SECTION Cell Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.75	5.60	.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.)

Most cells exist in tissues in contact or even surrounded by an extracellular matrix composed of collagens, glycoproteins, and proteoglycans. The interactions of the cells with the tissue-specific extracellular matrix components are important in the regulation of cell behavior. Our understanding of how the cells adhere to, grow on, migrate to, and differentiate on the extracellular matrix has been enhanced by the ability to purify the components and to test their biological effects both in vivo and in vitro. Specific molecules have been found which mediate the cellular responses both in the matrix and on the cell surface. For example, laminin promotes the adhesion and growth of various epithelial cells while it appears to have a negative action of these activities for fibroblastic cells. We are currently investigating the biological activity of certain domains of the laminin molecule using synthetic peptides and domain and chain-specific antibodies. We are also determining the laminin and collagen receptors on neuronal and fibroblast cell surfaces using molecular biological and biochemical approaches. Our studies indicate that laminin has separate active sites for cell attachment and for neurite outgrowth and that an additional laminin receptor exists on neuronal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00275-09 DB

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

Martin, George R.	Ch. Lab of Dev. Bio. & Anomalies	DB	NIDR
Brown, Kenneth S.	Medical Director	DB	NIDR

COOPERATING UNITS *(if any)*
University of Minnesota; NCI, NIH

LAB/BRANCH
Laboratory of Development Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to study the action of fluoride in various biological tests used to detect clastogenic or mutagenic substances. To date, fluoride has been examined in tests used to detect mutagens and found to be non-mutagenic. No effects on chromosome structure were noted in animals given widely different levels of fluoride. DNA repair after X-ray treatment was unchanged by fluoride. No genetic effects of fluoride were noted in a recessive lethal test of fluoride on Drosophila. The data indicate that fluoride has no mutagenic activity. Reports of fluoride's action on the metabolism and growth of cells are being monitored and where appropriate, related studies are undertaken.

The changes in major dibasic cation constituents of culture media produced by precipitation of insoluble fluoride salts does not increase mutagenesis in mouse lymphoma cells in culture.

ANNUAL REPORT OF THE LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY
NATIONAL INSTITUTE OF DENTAL RESEARCH
October 1, 1986 to September 30, 1987

This year members of the Laboratory of Microbiology and Immunology were deeply saddened by the loss of a friend and colleague, Dr. Charles L. Wittenberger. Dr. Wittenberger was killed in an accident on June 27, 1987. At the time of his death, Chuck was the Chief of our Microbiology Section and contributed in a major way to its administration, growth and development for almost two decades. Needless to say, he will be greatly missed by all of us.

The following is a summary of our research activities and accomplishments during FY 1987.

MICROBIOLOGY SECTION

Our ecology program investigates intergeneric coaggregations that occur among diverse members of the oral microbial flora and which are now widely accepted to be of major importance in the development of dental plaque. These highly specific cell-cell recognition reactions are mediated by lectin and complex carbohydrate molecules located on the cell surfaces of the interacting bacteria. The microbial ecology group has expanded its studies of two research areas related to microbial adherence mechanisms in the human oral ecosystem: (1) relationship of coaggregation to colonization of oral bacteria, and (2) isolation and chemical characterization of cell surface adhesins that mediate specific coaggregation reactions.

Members of the genus Veillonella are found in high numbers in two oral econiches, the dorsum of the tongue and subgingival plaque. Streptococcus salivarius is predominantly found on the dorsum of the tongue, whereas, Steptococcus sanguis is found primarily on the tooth surface and especially in subgingival plaque. Of 28 veillonella isolated from subgingival plaque, 24 strains coaggregated with S. sanguis but not S. salivarius. However, 10 strains of veillonella isolated from the tongue all coaggregated with S. salivarius but not S. sanguis. These results are the first direct evidence to support the notion that coaggregation is tightly coupled to the ability of a strain to colonize a specific econiche. The veillonella are particularly suited to this study because they can be easily selected on special culture media, they represent a large portion of the bacterial population in the two chosen econiches and they use the metabolic end product, lactic acid, of other bacteria in those econiches and thus are metabolically linked in the ecosystem. Metabolic interdependence among oral bacteria is considered to be likely and the veillonella are an integral part of the metabolic consortium.

A separate study of the genus Capnocytophaga revealed a phylogenetic resemblance of the surface adhesins among the three gram negative species, C. ochracea, C. sputigena and C. gingivalis. This relationship was found by investigating the coaggregation properties of each species with several gram positive human oral partner strains including S. sanguis, A. naeslundii, A. viscosus, A. israelii and R. dentocariosa. The comparison among the capnocytophagas was based on partner specificity, sugar inhibitions and blocking of coaggregations by antiserum raised against the capnocytophagas. Although the three species share little DNA homology, they appear to share

functionally and antigenically related surface adhesin properties supporting the idea that unrelated bacteria in the oral econiche may bear evolutionarily related adhesins.

A concerted effort was made this past year to identify, characterize and enumerate the fimbriae-associated adhesins of Bacteroides loescheii responsible for its coaggregation with S. sanguis 34 and A. israelii PK14. Using adhesin specific monoclonal antibodies (MAbs) prepared in collaboration with the Clinical Immunology Section, the S. sanguis specific adhesin (SSSA) and the A. israelii specific adhesin (AISA) were shown to be composed of 75 and 45kD polypeptides, respectively. The MAbs and Fabs prepared from them completely inhibited both coaggregation reactions at very low concentrations, 0.05 to 5μg of IgG protein, suggesting that they were specific for regions at or near the binding sites of the two adhesins. Using radiolabeled MAbs, the maximum number of each type of adhesin was estimated by Scatchard analyses. A maximum of 300 ± 150 adhesins per cell was obtained for SSSA. Each bacteroides cell was estimated to bear between 300 and 600 AISA. In collaboration with the Clinical Investigations and Patient Care Branch, immunoelectron microscopy was used to localize the adhesins on the surface of the cell. These studies established unequivocally that the adhesins were not integral parts of the fimbrial subunit. Both adhesins are found at the distal portion of the fimbriae and they appear to be arranged randomly in small to large clusters. The SSSA appears to possess a dual function, MAb studies have established that it not only participates in a specific coaggregation reaction, but it is also responsible for hemagglutination with human and other animal erythrocytes.

Three specific topics have been investigated in the microbial physiology and biochemistry program: (i) sugar transport and metabolism in Gram-negative oral bacteria, (ii) the isolation and physico-chemical characterization of two previously unknown amino acids from lactic streptococci, and (iii) mechanism(s) of protein inactivation and turnover in the oral streptococci.

We have continued to study the regulatory and energetic aspects of sugar transport and metabolism in Fusobacterium nucleatum. In this innovative program it has been shown that ATP, derived from the fermentation of specific amino acids (glutamic, lysine, histidine), provides the necessary energy for transport of glucose and galactose by the organism. Both sugars are transported via a common carrier, and the kinetic and stereospecific properties of the carrier have been determined. Following membrane translocation, both sugars are phosphorylated by independent and sugar-specific kinases. Glucokinase is inhibited by oxygen, whereas galactokinase is oxygen insensitive. Glucose and galactose are rapidly converted into high molecular weight polymers, and the presence of these intracellular granules has been confirmed by electron microscopy. Data obtained by chemical analysis, indicate that both glucose and galactose yield a polyglucose (glycogen) polymer, and cells of F. nucleatum must therefore contain a constitutive Leloir pathway for galactose transformation. Previous studies demonstrated the amino acid-dependent transport of sugars, but unexpectedly, it has been found that amino acid fermentation also regulates the rate of catabolism of pre-formed intracellular polymer. The molecular basis for the dual role of amino acid fermentation in synthesis and catabolism of sugar polymer is presently under study.

In the past year two previously unknown amino acids have been isolated and characterized from cells of Streptococcus lactis. The two compounds are unusual N-alkylated derivatives of ornithine and lysine, and are respectively N^5-(1-carboxyethyl)ornithine and the homologous N^6-(1-carboxyethyl) lysine. The biochemical and physiological functions of these new amino acids have not yet been established, but N^5-(1-Ce)ornithine may participate in the regulation of synthesis (or catabolism) of arginine. In vivo experiments performed with [^{14}C] and [^{15}N]-labeled lysine reveal that exogenous lysine serves as precursor for N^6-(1-Ce)lysine, and that the ε-N atom is conserved during biosynthesis of the alkylated derivative. The absolute stereochemical configurations of both carboxyethyl amino acids have been determined by chiral synthesis. Comparative ^{13}C-NMR spectroscopy has confirmed the exclusive (2S,7S) and (2S,8S) stereochemical configurations of natural N^5-(1-Ce)ornithine and N^6-(1-Ce)lysine, respectively. The biochemical and regulatory functions of these novel amino acids are presently under investigation.

Studies on the 'marking', and subsequent proteolytic degradation of enzymes have continued using fructosyltransferase (FT) as the model for this oxidative inactivation process. An in vitro system has been developed for directly assessing the role of NADH oxidase in the Cu^{2+}-dependent inactivation of cell-associated enzyme. From the accumulated data a molecular mechanism has been advanced for FT inactivation. In this model, copper first binds to FT at, or near, the catalytic centre. Subsequently, the copper ion is reduced by O_2^- generated by NADH oxidase. The reduced copper is then reoxidized by H_2O_2, but at the same time there is localized formation of the highly reactive OH· radical. The OH· radical is apparently responsible for the oxidative modification of one or more amino acid residues at the active center. The oxidative and catalytic inactivation of the enzyme serves to 'mark' fructosyltransferase as a target for degradation by proteolytic enzymes.

The molecular biology program in this section is using the techniques of genetic modification by gene cloning and recombinant DNA technology to: 1) elucidate the organization and sequence of the lac operon of gram-positive bacteria, 2) develop transformation systems and vectors for lactobacilli, 3) clone and characterize fimbrial adhesins of Actinomyces viscosus, and 4) characterize Actinomyces phages.

The DNA sequences of the gene encoding the Factor III lactose of the lactose PEP:PTS and the enzyme β-D-phosphogalactoside galactohydrolase (P-β-gal) were completed. The P-β-gal was demonstrated to share homology with P-β-gal genes of Staphylococcus aureus and Streptococcus lactis. It was postulated that the P-β-gal gene evolved after the divergence of these organisms, and was disseminated to them by a rapid 'horizontal evolution' mediated by conjugal plasmids and transposition of the gram-positive lac operon. The P-β-gal gene also shared homology with β-phosphoglucoside glucohydrolase gene of Escherichia coli, but not with β-galactosidase. These observations established the existence of a glycoside phosphate hydrolase gene family. The coding sequences for Enzyme II lactose was also located and its nucleotide sequence is being determined. These results will give us first insight at the molecular level into the lactose-PTS of lactic acid bacteria. The genes were found to be located in a three gene cluster comprising an operon. The regulation of this unique lac operon is under

study. The development this year of a rapid and reproducible high frequency transformation system for lactobacilli will allow the advanced techniques of molecular biology to be applied to research with oral lactobacilli such as Lactobacillus casei, L. acidophilus and L. salivarius. Electroporation, the introduction of DNA into cells by passage through transient electric field-induced membrane pores, was shown for the first time in these studies to be applicable to procaryotes. It should now be possible to use recombinant DNA techniques to re-introduce genetically modified DNA into these oral bacteria to answer fundamental questions about their pathogenicity and metabolism. It should also be possible to introduce novel phenotypes into lactobacilli by these same techniques in order to derive strains for specific medical applications.

Research on the molecular biology of Actinomyces fimbriae has continued. The sequence of the Type 2 fimbriae is nearing completion. The cloned fimbrial protein subunit has been purified to homogeneity and specific and monoclonal antisera directed against it are being used to further characterize the lectin nature of this adhesin subunit. In the course of these studies two forms of the cloned protein were recognized. Differing in size by about 2 kDa, it appears the larger form carries a hydrophobic leader sequence that is used in secretion of the fimbrial subunit. The smaller form represents the leaderless processed subunit. The completion of the sequencing and immunochemical experiments should provide a clear model of the mechanism(s) by which the Type 2 fimbrial structure acts as both a lectin and an adhesin. This work is being done in collaboration with the Humoral Immunity Section and will be discussed subsequently under this section heading.

The previous apparent association of phage sensitivity with a receptor site that might be identical to one of the coaggregation sites of A. viscosus with streptococci led to a closer examination of A. viscosus phages. The phage receptor and the coaggregation site appear indistinguishable. Three types of phage were characterized and their genomes examined by restriction analysis. The phage genomes were also used to attempt transfection of A. viscosus protoplasts. The successful development of a system for transfection will aid in establishing a transformation system for the actinomyces. A gene exchange system, not presently available, will be useful in studies of the biosynthesis and assembly of fimbrial gene products in the native host.

HUMORAL IMMUNITY SECTION

In a series of ongoing and newly initiated studies, this section is examining the molecular events involved in the attachment of oral micro-organisms to different oral surfaces and the biological consequences of the interactions of oral organisms with host phagocytic cells and mediator systems. The research activities have focused on the identification, characterization and purification of microbial surface structures that mediate attachment as well as the complementary receptors for these adhesins on other microorganisms, teeth, epithelial cells and phagocytic cells. The results of these interactions are diverse and include not only colonization of oral surfaces but also destruction of the microorganisms. A considerable amount of information has now been obtained concerning the identification and function of the fimbriae on strains of oral Actinomyces. Actinomyces

viscosus, a colonizer of tooth surfaces, possesses two antigenically distinct types of fimbriae designated type 1 and type 2. The attachment of this bacterium to the tooth surface is a property of the type 1 fimbriae. The type 2 fimbriae are associated with a lectin activity that mediates attachment to certain other oral microorganisms and epithelial cells and are found exclusively on A. naeslundii. As reported previously, they are implicated in the colonization of epithelial surfaces, and the establishment of microbial communities. In addition, these fimbriae mediate the interaction of these Actinomyces with receptors exposed on polymorphonuclear leukocytes (PMNs) by sialidase, an enzyme produced by these bacteria. This recognition process initiates phagocytosis and subsequent killing of the bacteria. These effects are inhibited by β-linked galactosides. Mutants of these bacteria that lack the type 2 fimbriae are resistant to the bactericidal activity of PMNs. While the elimination of these bacteria is undoubtedly beneficial to the host, recent findings demonstrate that this process is accompanied by the release of PMN mediators that are detrimental to the surrounding host tissues. A. viscosus stimulates the respiratory burst in PMNs resulting in the release of reactive oxygen intermediates. The type 2 fimbriae initiate this response as shown by the finding that superoxide anions are not detected in the supernatants of PMNs cultured with a non-fimbriated bacterial mutant. Moreover, β-linked galactosides inhibit superoxide production thereby implicating the lectin associated with the type 2 fimbriae in the stimulation of superoxide anion production. The type 2 fimbriae also selectively cause the release of the contents of PMN secondary granules. Lactoferrin, one of the constitutents of these secondary granules, is markedly elevated in the supernatants of PMNs cultured with A. viscosus. These secondary granules also contain collagenase and the exocytosis of this enzyme could result in the degradation of adjacent connective tissue. The non-fimbriated mutant of A. viscosus did not initiate this degranulation process. The contents of the primary granules were not released by either the parent or mutant strain.

Potential receptors for the fimbrial lectin have been identified on PMNs. Several criteria indicate that a 110 Kd surface glycoprotein may serve as a receptor. It was detected by radiolabeled lectins from Ricinus communis and Bauhinia purpurea on sialidase treated Western blots of PMN membrane extracts separated by SDS-PAGE. These plant lectins are utilized since isolated Actinomyces fimbriae do not bind directly to their complementary receptors. These plant lectins that react with β-linked galactose or N-acetylgalactosamine are of similar specificity to the bacterial lectin and are effective inhibitors of the phagocytosis of the Actinomyces. The 110 Kd band is a PMN surface component since it is labeled by lactoperoxidase catalyzed iodination of intact PMNs. The radiolabeled plant lectins only react with this band after sialidase treatment of the nitrocellulose transfers of the gels. This finding is probably related to the sialidase requirement for optimal phagocytosis of the bacteria. The plant lectins also detect bands of approximately 85 and 140 Kd on non-sialidase treated transfers. Although the 85 Kd band is surface labeled and may be a receptor, the 140 Kd band is not a surface glycoprotein and is, therefore, not being considered. Recent evidence indicates that glycolipids may also be receptors for the fimbrial lectin. Radiolabeled A. naeslundii binds directly to the gangliosides GM1,GD1b and asialo GM1 separated by thin layer chromatography (TLC). Following treatment of these TLC plates with sialidase, the bacteria also attach to GD1a and GT1b. These gangliosides all contain Galβ3GalNAc.

Attachment of the parent bacterial strain to these gangliosides is inhibited by lactose. A mutant lacking type 2 fimbriae fails to adhere. Gangliosides obtained by DEAE chromatography of glycolipid extracts of PMNs were also recognized by the Actinomyces. Radiolabeled bacteria detected two bands on thin layer chromatograms of these extracts and bound to one additional ganglioside band following sialidase treatment. One of the radiolabeled lectins from Ricinus communis, a lectin that inhibits phagocytosis, reacts with these same bands. In previous reports we have described studies concerning the identification and characterization of receptors for the Actinomyces fimbrial lectin on other cells. The receptor on Streptococcus sanguis 34 has been purified and is composed of the repeating phosphodiester linked hexasaccharide: GalNAcRhaGlcGalGalNAcGal. Evidence indicates that the epithelial cell receptor is Galβ3GalNAc. The PMN receptor clearly includes Gal and/or GalNAc. The receptor on the epithelial cells is recognized by the peanut agglutinin which is specific for Galβ3GalNac. This lectin, however, fails to recognize the PMN receptor or, as predicted by the structure, the streptococcal receptor. Thus, the Actinomyces fimbrial lectin reacts with a variety of similar but not identical receptors resulting in such divergent effects as colonization, attachment to other bacteria or bacterial destruction.

While the functional properties of the Actinocyces fimbriae have been defined and some of their receptors identified and characterized little has been known about their structure. Such studies have been hindered by the inability to completely dissociate the fimbriae by conventional methods. Therefore, cloning techniques have been utilized to determine the amino acid sequence of the subunits and to obtain these subunits for physicochemical characterization. The genes for the subunit of the A. naeslundii type 2 fimbriae as well as the gene for the type 1 fimbrial subunit of A. viscosus have been cloned into Escherichia coli utilizing pUC plasmids. The gene encoding the type 1 fimbrial subunit has been localized on a 1.9 kb fragment and a subclone containing a 1.4 kb fragment expresses the terminal 47 kd region of the protein. The gene products were detected by monoclonal antibodies against type 1 fimbriae. The cloned fimbrial protein was purified from the E. coli cytoplasmic fraction by ion-exchange, immunoaffinity and gel permeation chromatography. Polyclonal antibodies prepared against the cloned subunit and monoclonal and polyclonal antibodies against the isolated fimbriae react similarly with partially dissociated fimbriae on nitrocellulose transfers of type 1 fimbriae separated by SDS-PAGE. The entire nucleotide sequence of the 1.9 kb DNA fragment has now been determined. The type 1 fimbrial subunit gene is an open reading frame of 1602 base pairs. Based on the nucleotide sequence the encoded protein contains 460 amino acid residues and a leader sequence. Of major current interest is the finding that although high concentrations of rabbit antibodies raised against the cloned protein block type 1 fimbrial mediated attachment of the Actinomyces to saliva coated hydroxyapatite, an in vitro model of the tooth surface, the Fab fragments of these antibodies are inactive. One possible interpretation of these data is that the subunit is not the adhesin and the attachment properties of the type 1 fimbriae are due to an association of a distinct adhesin molecule with these fimbriae.

A similar strategy was applied to cloning of the type 2 fimbrial subunit of A. naeslundii. Recombinant clones were screened with monospecific antibodies against these fimbriae. A clone that carried a 16.5 kb A.

naeslundii DNA insert expressed a 60 kd subunit protein. This subunit is similar to a protein detected on Western blots of partially dissociated type 2 fimbriae. Sequencing of the gene encoding this subunit is nearly complete. Cloning of the gene for the subunit of the type 2 fimbriae of A. viscosus has been previously reported. The gene encoding the 59 Kd protein was identified on a 2.5 kb fragment. The availability of these three clones for different Actinomyces fimbriae has made it possible to perform DNA-DNA hybridization experiments. Strong hybridization occurs between the DNA fragments that encode the two type 2 subunits. Hybridization was also detected between the DNAs encoding the type 2 fimbrial subunit of A. naeslundii and the type 1 fimbrial subunit of A. viscosus. These data strongly suggest that the genes for the type 1 and type 2 fimbriae evolved from a common ancestor.

Mammalian cells possess receptors for fragments of the third component of complement (C3). Recently receptors for the C3bi and C3d fragments of C3 have also been detected on Candida albicans by rosetting with erythrocytes to which these C3 fragments are attached. The expression of the C3d receptor correlates with the conversion of yeast to the pseudohyphal form. Although treatment of pseudohyphae with Concanavalin A does not inhibit rosetting, the C3d receptors are specifically eluted from a Concanavalin A affinity column. Thus, they are apparently mannosylated but this saccharide is not directly involved in the recognition of C3d by these receptors. The C3d receptors are heat and protease sensitive. Monoclonal antibodies against C. albicans were produced and three of these inhibit rosetting. Purification of this receptor has been achieved by sequential DEAE-Trisacryl and C3d-Thiol-Sepharose affinity chromatography. The low pH eluate from the C3d-Thiol-Sepharose column contains the receptors which migrate as a doublet of approximately 70 Kd as determined by SDS-PAGE. A possible biological function of this receptor may be to increase the population of Candida at a particular site. Complement activation by Candida results in the deposition of membrane bound C3b which is then enzymatically converted to C3bi and C3d. These fragments could then be recognized by the complementary receptors on other organisms resulting in their localized accumulation.

CELLULAR IMMUNOLOGY SECTION

This section continues its investigations into the physiological, biochemical and molecular events regulating normal mononuclear cell function and aberrations of these events in immunologic disorders. Characterization of the various functions of these cells and how these functions may be altered in an inflammatory response may enable modulation of cellular immune sequelae. Monocyte migration into an inflammatory site is critical to the outcome of the lesion, yet the mechanisms of recruitment are not clearly defined. A newly identified chemoattractant is transforming growth factor beta (TGF-β), a 25,000 Mr peptide originally defined as a transformed cell product which could induce transformation of nonneoplastic cells in culture. More recently, however, TGF-β has also been shown to be a product of hemopoietic cells including platelets, lymphocytes and monocytes and to play a role in wound healing. In our collaborative studies with Dr. M. Sporn and colleagues of NCI, members of the Cellular Immunology Section have shown TGF-β to be a very potent chemoattractant for monocytes and neutrophils with peak activity between 4-40 femtomolar. Furthermore, TGF-β at higher concentrations induces gene expression of monocyte growth factors including Interleukin 1 and Tumor Necrosis Factor which are effective regulators of

fibroblast proliferation. These findings would appear to have functional significance in many situations in which macrophages participate in physiological or pathological processes of fibrosis and angiogenesis.

In contrast to its ability to promote tissue repair, TGF-β appears to be an extremely active immunosuppressive agent. The ability of TGF-β to inhibit lymphocyte proliferation at femtomolar concentrations makes it significantly more inhibitory than the T cell-specific immunosuppressant, Cyclosporin A. Furthermore, the immunosuppressive activity of TGF-β is shared by TGF-β2. Although TGF-β2 has substantial sequence homology to TGF-β1, it is a unique molecular entity whose functional significance is not yet defined. TGF-β does not interfere with the interaction of IL1 with its T cell surface receptor and does not appear to block transduction of the IL1 signal leading to T cell synthesis of IL2 and IL2 receptors necessary for DNA synthesis. IL2-mediated cell progression likely proceeds to some later stage in the cell cycle before being arrested by TGF-β. The specificity of the immunosuppressive potential of TGF-β is of significant interest. TGF-β stimulates monocytes to transcribe and translate IL1, yet suppresses the ability of IL1 to promote lymphocyte proliferation and therefore, immune responsiveness.

Monocytes are also influenced by Colony Stimulating Factors (CSF), a group of glycoproteins which regulate the proliferation and differentiation of committed myeloid stem cells into mature granulocytes and macrophages. Our recent studies have shown that expression of the proto-oncogene c-fos is induced during this monomyelocytic differentiation to macrophages and particularly enhanced at the later stages of differentiation. Using the murine myeloid leukemia cell line, M1, which can be induced by granulocyte colony-stimulating factor (G-CSF) to differentiate into mature macrophages, the mechanism of c-fos expression was investigated. Since the c-fos mRNA levels were high only in fully differentiated cells and very low in proliferating or G_1-arrested (density-inhibited and aphidicolin-blocked) cells, the expression of the c-fos proto-oncogene following G-CSF stimulation appears to be restricted to functionally differentiated cells and is not related to the position of the cells in the cell cycle. These studies provide important insight into the regulatory control of monocyte-macrophage differentiation and proliferation by CSF.

Differentiation of monocytes-macrophages also results in their enhanced functional and synthetic capabilities and another major area of interest in this laboratory concerns the role of monocytes in connective tissue metabolism. Activated human monocytes produce collagenase, a key enzyme in the remodelling and/or destruction of the extracellular matrix, through a prostaglandin E2 (PGE2) dependent mechanism. In continuing studies, we have demonstrated that recombinant gamma-interferon (γIFN) blocks collagenase production induced by the lectin concanavalin A (Con A). HPLC analysis of the arachidonic acid (AA) metabolites released by stimulated monocytes in the presence of γIFN revealed that γIFN inhibited the release of all AA metabolites by inhibiting phospholipase A2. These important new findings suggest a potential mechanism whereby γIFN might be ameliorating connective tissue destruction as described below in an experimental animal model of arthritis. An extension of these studies defining the regulation of collagenase production by human monocytes using a cDNA probe for collagenase

will provide useful information concerning the effect of immunomodulators and anti-inflammatory drugs on this important enzyme.

Chronic inflammatory lesions such as are observed in periodontal disease and arthritis are associated with immunological alterations which lead to loss of bone and cartilage. In two experimental animal models, the Cellular Immunology Section has been exploring the network of cell-cell interactions that link immune cells and connective tissue abnormalities. One of these models involves the osteopetrotic (op) rat which fails to resorb bone normally due to an apparent defects(s) in the immune system of this animal. Phenotypic analysis of the immune cells of these animals by flow microfluorimetry revealed a paucity of Ia positive cells suggesting a less mature population of immunologically relevant cells. These observations suggested that a restoration of immune function and bone resorbing capacity might be possible by inducing maturation of the cells. In this regard, exposure of spleen cells from op rats to γIFN (10-100 units/ml) for 24hr resulted in an increase in Ia expression achieving levels comparable to those seen on spleen cells from normal rats. Moreover, when the γIFN exposed spleen cells from op rats were stimulated with mitogens, immune responsiveness was significantly restored as evaluated by IL2 production and lymphocyte proliferation. These exciting observations suggest that γIFN can restore an immunological deficit in these osteopetrotic rats, and provide the basis for commencing studies to administer γIFN therapeutically in an attempt to promote bone resorption and tooth eruption in these animals.

In a second experimental model, γIFN has been found to inhibit pathologic bone resorption which is the consequence of chronic inflammatory lesions. Administration of recombinant γIFN to rats injected with streptococcal cell walls (SCW) which have erosive polyarthritis in the peripheral joints ameliorates the disease process. Whether this anti-arthritic effect of γIFN is due to its antiproliferative effects, its down-regulation of prostaglandins and collagenase, or due to some other immunomodulatory role is currently under study. In this model of arthritis which is characterized by an acute, exudative, neutrophil-mediated response followed by a chronic inflammatory destructive phase, a number of antiinflammatory agents and other immunomodulators have been used to provide information concerning the cellular and molecular mechanisms responsible for the distinct phases of the SCW-induced inflammmatory response. Cyclosporin A (CsA), a specific inhibitor of T cell function, blocks the chronic mononuclear cell dependent phase of arthritis, but has no effect on the acute neutrophilic response. In contrast to CsA, the antiinflammatory corticosteroid, methylprednisolone (MP) ablates the acute response and consequently, the infiltration of mononuclear cells. MP inhibition of arachidonic acid metabolism likely impairs SCW-induced enhancement of vasopermeability and leukocyte chemotaxis which is manifested as lack of joint swelling and leukocyte infiltration. By comparison, flurbiprofen, a nonsteroidal antiinflammatory drug, only partially suppressed both the acute and chronic arthritis. Thus, each of these agents and other site specific-inhibitors can be shown to act at different loci to variably modulate SCW-induced arthritis. By choosing drug combinations with different target specificities, it may be possible to interrupt several sites in this interdependent inflammatory process. Such combinations may allow the use of lower drug doses with improved efficacy and reduced toxicity.

These in vitro and animal model studies have provided the foundation necessary for initiating clinical trials with CsA in the treatment of rheumatoid arthritis. CsA was evaluated in rheumatoid arthritis by comparing high dose (10 mg/kg/day) and low dose (1 mg/kg/day) CsA in a 12 month randomized double blind trial of thirty-one patients with severe refractory seropositive rheumatoid arthritis. Of the patients who were anergic or responded to only one antigen, 100% responded clinically to high dose CsA. Peripheral blood anergy has previously been correlated with highly inflammatory synovial biopsies. In contrast, clinically indistinguishable patients with intact immune function and probable hypocellular synovial tissue were less likely to benefit therapeutically from CsA. Furthermore, during the course of therapy, the anergic status was reversed, indicating an immunomodulatory rather than an immunosuppressive role for CsA in rheumatoid arthritis. CsA appears to be an effective drug for refractory rheumatoid arthritis dependent upon the dose and the immunologic status of the patient. A patient who is anergic or has minimal immune response has a high probability of response to CsA concurrent with restoration of immune function.

The basic investigations into the cellular and molecular events regulating normal mononuclear cell function carried out by the Cellular Immunology Section have been extended to other disease entities besides those associated with chronic inflammation and connective tissue abnormalities. One such area currently under study focuses on the contribution of monocytes to the acquired immunodeficiency syndrome. Recent evidence indicates that the human immunodeficiency virus (HIV) binds to monocytes via the T4 antigen and infects these cells. Although it has been demonstrated that monocytes obtained from AIDS patients do not respond normally to inflammatory chemotactic stimuli, are defective in their ability to kill parasites, and are less able to support mitogen-induced T cell responses, it is unclear whether monocyte dysfunction is a direct or indirect consequence of HIV infection. Therefore, we have compared peripheral blood monocytes from AIDS patients and monocytes infected in vitro with HIV to normal monocytes for phenotypic and functional aberrations. Peripheral blood cells from AIDS patients were found to frequently contain substantial numbers of IL2 receptor positive monocytes as compared to the control subjects, suggesting prior in vivo activation of the cells. RNAs isolated from monocytes from AIDS patients were screened by Northern blot hybridization for IL2 receptor sequences using cDNA probes and in some AIDS patients, a high spontaneous level of IL2 receptor RNA was observed. Furthermore, expression of cell surface HLA-DR antigens, a marker for monocyte maturation, was also elevated on AIDS monocytes. After HIV exposure in vitro, a large percentage of the LeuM3 positive cells expressed IL2 receptors and an increase in the relative median fluorescence intensity of HLA-DR. Furthermore, infection as quantitated by reverse transcriptase was enhanced in infected monocytes cultured in the presence of recombinant IL2. These data suggest that HIV may activate monocytes and the interaction of IL2 and I12 receptors on these monocytes may influence the infective process.

In related studies, monocytes infected with cytomegalovirus (CMV), a frequent infectious agent in AIDS, also exhibit depressed effector cell functions including cytotoxicity, chemotaxis, IL-1 production and PGE_2 release. CMV causes increased release of oxygen reactive intermediates which, in contrast to uninfected monocytes, cannot be stimulated further,

suggesting that CMV activation of monocytes causes them to be refractory to additional stimuli. In addition, preliminary results indicate that CMV infection may impair antigen presentation by monocytes. These observations suggest that CMV-infected monocytes may play a role in the host's acquisition of additional opportunistic infections during CMV infection. In this regard, important observations have been made in clinical studies carried out in collaboration with NCI and NIAID. The majority of symptomatic AIDS patients have been shown to have identifiable enteric pathogens, CMV being the most common. Definition of the responsible pathogens indicates that the idiopathic AIDS enteropathy occurs far less frequently than previously suspected. Contributing to the unusual frequency of enteric infections in AIDS patients may be their impaired ability to develop de novo antibody responses to enteric microorganisms such as G. lamblia which has recently been documented. Preliminary results suggest that HIV itself may be capable of inducing malabsorption in the AIDS patients.

Another pathogen of the oral cavity and esophagus, the fungus C. albicans, is frequently encountered in AIDS patients. Twelve mutants of C. albicans have been isolated from AIDS patients and these mutants are being tested for variations in their mannosylated cell wall proteins which are thought to play a critical role in candida colonization and virulence. In a recently established assay to evaluate cytotoxicity for Candida albicans, monocyte killing of candida has been augmented by a combination of MTP and γIFN. The stimulatory effect of MTP on monocyte cytotoxicity provides a basis for clinical trials of MTP in AIDS patients.

Because of the central role of monocytes in HIV infection, these cells are a likely target for anti-viral therapy. In this regard, a study is being initiated with NCI investigators to deliver liposomes containing AZT derivatives and/or other anti-viral agents to monocytes. Devising successful immunomodulation/antiviral therapies is dependent upon understanding the role of the monocyte in the pathogenesis of AIDS.

CLINICAL IMMUNOLOGY SECTION

This section studies the mechanisms involved in secretion from cells. Some of the cells used in these studies include mast cells/basophils and pancreatic acinar cells. This laboratory has developed cultured rat basophilic leukemia cell lines for studies of the biochemical events which occur during histamine release from mast cells/basophils. The cells divide rapidly, contain histamine and serotonin, grow attached to plastic and have surface IgG and IgE receptors similar to normal basophils. The cells are activated by crosslinking of the immunoglobulin receptors to release mediators like histamine, serotonin, prostaglandins and other arachidonic acid products. The ready availability of large quantities of cultured rat basophilic leukemia cells allows direct biochemical study of the events which occur in cells following activation. Variants or mutants of the rat basophilic leukemia cells have also been selected that are incapable of histamine release. Biochemical characterization of the defect in these cells is useful for understanding the events induced in cell activation and secretion. The experiments during this past year have continued the study of the biochemical changes which occur during the release of mediators, and the characterization of the cell surface molecules important for this process.

The rat basophilic leukemia cell line (RBL-2H3) has high affinity IgE receptors ($Fc_\epsilon R$) on its surface. Monoclonal antibodies have been produced that inhibit IgE binding to this high affinity IgE receptor. One of these mAb (mAb BC4) was purified and coupled to Sepharose 4B beads. Supernatants from extracts of solubilized rat basophilic leukemia cells were bound to these affinity beads; after extensive washing in buffer containing detergent the receptors were eluted. Silver staining of the elute separated by SDS-PAGE demonstrated the 3 receptor components: the broad band at 46-65 kDa; narrow doublet at 30-33 kDa and a broad band at 20 kDa. There were also two bands at 96 kDa and 43-45 kDa. On immunoblotting with this purified material, ^{125}I-IgE reacted with the broad 46-65 kDa bands, and 2 narrow bands at 43-45. The purified receptor was also used to raise rabbit polyclonal antibodies; these antibodies immunoprecipitate the 46-65 kDa component from ^{125}I-surface labeled cells and in immunoblots they react with all the bands seen on silver stained gels. The receptor proteins have also been further purified into individual components. Following trypsin digestion of the alpha component, several peptides were isolated that were used for amino acid sequence analysis. With this information oligonucleotide probes have been synthesized and used for cloning the gene for this receptor component.

The anti-IgE receptor monoclonal antibody inhibits allergic reactions. In the *in vitro* experiments, the anti-receptor mAb BA3 and its Fab fragment inhibited ^{125}I-IgE binding to the RBL-2H3 cells. The intact mAb released histamine from the RBL-2H3 cells, whereas the Fab was inactive. The addition of the Fab to RBL-2H3 inhibited the IgE-mediated histamine release reaction; this inhibition was greater when the Fab was added prior to the IgE. The anti-receptor antibody also inhibited *in vivo* passive cutaneous reactions in rats. The Fab was injected intradermally, 2 hrs later anti-DNP IgE was injected into the same sites, and after 16 hrs the rats were injected IV with the antigen. With 0.1 µg/site of IgE there was 50% inhibition with the injection of 0.08 ± 0.02 µg Fab/site. Inhibition was less marked when the IgE was injected before the mAb Fab. Intravenous injection of the Fab prior to the injection of the IgE into the skin sites also inhibited skin reactions. The results demonstrate that anti-receptor antibodies can be used as a model for inhibiting immediate hypersensitivity reactions.

IgE-receptor complexes on rat basophilic leukemia (RBL) cells trigger cellular degranulation when cross-linked by multivalent antigen or specific antibodies. We have shown that these clusters rapidly associate with the cytoskeletal matrix, suggesting that this may be an important part of the signaling mechanism. A monoclonal antibody, mAb AA4, has been previously isolated that inhibits the binding of IgE to its receptor on RBL cells, but the binding of mAb AA4 is not inhibited by IgE. We have now found that the AA4 antigen is largely detergent-insoluble in a cytoskeleton-stabilizing buffer, remaining associated with the cytoskeletal matrix under conditions that solubilize monomeric IgE-receptor complexes. RBL cells with bound fluorescein-labeled IgE, and mAb AA4 labeled with rhodamine secondary antibody show a uniform distribution of IgE on the cell surface and patches of mAb AA4 that have been clustered by the secondary antibody. When multivalent antigen is also incubated with the RBL cells to cross-link the IgE, the resulting IgE patches and the mAb AA4 patches coincide on the cell surface. Fluorescence resonance energy transfer experiments with coumarin-labeled IgE as donor and fluorescein-labeled mAb AA4 as acceptor show that energy transfer occurs between the two probes when the IgE is

cross-linked with multivalent antigen. These results indicate that AA4 antigens become associated with cross-linked IgE receptor complexes, and these antigens may be involved in the transmembrane signal by mediating the receptor-cytoskeletal interaction.

Monoclonal antibodies to human IgE were used to define different epitopes on the IgE molecule. All 12 monoclonals were of the IgG class and by immunoblotting reacted with the ε chain. Several different types of monoclonal antibodies were isolated. The first type bound to the $C_\varepsilon 1$ region of the IgE myeloma protein PS and to serum IgE but not to the IgE myeloma ND. These monoclonal antibodies probably recognize a common IgE allotype. A second type of monoclonal reacted with heated, but not the native IgE molecule suggesting that it bound to hidden determinants. The third type bound to epitopes on the $C_\varepsilon 1$, $C_\varepsilon 2$ and $C_\varepsilon 3$-4 regions of both IgE myelomas tested (the ND and PS proteins). Four of the MAb bound to the $F(ab')_2$ fragment of IgE while 5 others bound to the Fc. Two mAb bound to both fragments indicating that they react with sites in the $C_\varepsilon 2$ domain. Competitive binding assays demonstrated that the MAb recognized 10 distinct epitopes on IgE; these sites are in 3 related groupings. The sites exposed by heating the IgE molecule are different from these groupings. There were 2 monoclonals that failed to release histamine from human basophils. These blocked the binding of ^{125}I-labeled IgE to basophils and when the mAb were radiolabeled, failed to bind to IgE on the surface of basophils. These mAb recognize domains in $C_\varepsilon 2$ and $C_\varepsilon 3$-4 that are not accessible when IgE is in its receptor. There were 2 others that reacted with the $C_\varepsilon 2$-4 domain and blocked ^{125}I-IgE binding to basophils and these mAb did bind to IgE on cells. The inhibition of binding is therefore due to steric factors. There were 2 other monoclonals that failed to release histamine and both failed to inhibit the binding of ^{125}I-IgE to basophils. These recognize sites in the $C_\varepsilon 1$ and $C_\varepsilon 3$-4 regions of IgE that are either conformationally modified when IgE binds to the receptor or alternatively their binding affinity is such that they cannot inhibit IgE binding. Several of the epitopes recognized by the other mAb in $C_\varepsilon 3$-4 are accessible when IgE is in its receptor.

Antigen or IgE-mediated secretion of histamine from RBL-2H3 cells is associated with substantial hydrolysis of membrane inositol phospholipids and a rise in the concentration of cytosol Ca^{2+} (calcium signal). Such responses differed among cloned variant lines of the RBL-2H3 cell line from undetectable to about 80% of those in the parent RBL-2H3 cells. In all but one clone (1B3 Tg^R), the intensities of the phosphoinositide response and of the calcium signal were correlated with the secretory response. The 1B3 Tg^R clone had no detectable calcium signal (as measured by quin 2 fluorescence or uptake of $^{45}Ca^{2+}$) but paradoxically showed modest rates of hydrolysis of inositol phospholipids and of secretion. The responses of the 1B3 Tg^R clone were, however, dependent on the presence of external Ca^{2+} ions. The induction of secretion with antigen, therefore, was invariably associated with the hydrolysis of inositol phospholipids but it was not necessarily associated with a change in concentration of cytosol Ca^{2+}. All antigen unresponsive clones could secrete when synergistic signals were induced by exposure to the Ca^{2+}-ionophore, A23187 and the phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA). These lines, otherwise, had a normal number of IgE receptors and had no obvious defect in their capacity to synthesize the inositol phospholipids or in their phenotypic expression of phospholipase C as measured in cell extracts. One finding of possible

relevance to the role of GTP-regulatory proteins in the activation of phospholipase C was the inability of one antigen-nonresponsive line to respond to NaF (in intact cells) or to GTP S (in electrically permeabilized cells).

Studies also investigated events involved in secretory and endocytic processes in cultured cell lines. Major emphasis has been on characterizing the pancreatic acinar cell line AR42J as a model for studying exocrine secretory cells in vitro. Cells grown on collagen gels or basement membrane are more differentiated than those grown on tissue culture dishes. The AR42J cells synthesize and secrete amylase. The morphology of the secretory apparatus and the localization of acid phosphatase and thiamine pyrophosphatase in these cells was found to be similar to that in pancreatic acinar cells in vivo. Therefore, AR42J cells can be used for in vitro studies of exocrine secretory processes. The secretory polarity of the AR42J cells is also being investigated. A monoclonal antibody, 1A2, reacts with the apical surface of rat pancreatic acinar cells and with the cell surface of the AR42J cells. By light microscopy, 1A2 gave a punctate distribution on the cell surface. By electron microscopy, the antigen was associated with regions of microvilli on the cell surface. In order to obtain other antibodies to cell surface components, monoclonal antibodies are being raised against whole AR42J cells. The endocytic pathways in the AR42J cells are also being examined using the uptake of horseradish peroxidase (HRP) and transferrin-HRP into these cells. The soluble HRP is localized in lysosomes after approximately 30 minutes uptake whereas the transferrin remains in endocytic compartments. The intracellular fate of other membrane bound ligands and their relationship to the lysosomal system is currently under investigation. The role of acid phosphatase in the exocrine acinar cells is also being examined. The enzyme has been partially purified and the lysosome associated acid phosphatase, has been separated from the Golgi associated acid phosphatase. The partially purified preparations are being used to produce monoclonal antibodies against the two forms of the enzyme. Since the exocrine acinar cells contain two populations of lysosomes, one acid phosphatase positive, and the other acid phosphatase negative, studies on the distribution of acid phosphatase in these cells may provide information on the in vivo function of this enzyme.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00034-19 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 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 Section	NIDR, LMI
Hook, William A.	Research Microbiologist	NIDR, LMI
Berenstein, Elsa H.	Microbiologist	NIDR, LMI
Kitani, Seiichi	Visiting Fellow	NIDR, LMI
Scholl, Paul	Microbiologist	NIDR, LMI

COOPERATING UNITS *(if any)*
MHLBI, Laboratory of Chemical Pharmacology, NIH (M. Beaven), NICHD, ODCPR, NIH (M. Karten).

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	1.9	2.0

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.)*

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00042-17 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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)*

Bruce M. Chassy	Research Chemist	LMI, NIDR
Emily V. Porter	Chemist	LMI, NIDR
Jeannette Flickinger	Microbiologist	LMI, NIDR
Eunice Hull	Bio. Laboratory Technician	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.99	3.0	.99

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 use genetic, biochemical and physiological approaches to investigate the pathogenicity of oral bacteria. The two specific areas of investigation are: 1) characterization of plasmid-coded and chromosomal metabolic genes from oral bacteria and 2) development of systems for genetic exchange in oral bacteria. The nucleotide sequence of the β-D-phosphogalactoside galactohydrolase gene of Lactobacillus casei was completed. It displayed high homology to the same gene isolated from Staphylococcus aureus and Streptococcus lactis. Homology was also found to the β-phosphoglucoside glucohydrolase gene of Escherichia coli, but not to β-galactosidases. Factor III of the lactose PEP:PTS was cloned from the L. casei plasmid, pLZ64, into E. coli and its nucleotide sequence determined by the Sanger dideoxy chain-termination method. The third gene of the gram-positive lac operon, encoding the Enzyme II lactose of the lactose PEP:PTS was cloned and its nucleotide sequence is being determined. The three genes were found to comprise an operon-like structure. High frequency transformation of whole, untreated, cells of Lactobacillus casei was obtained for the first time using high voltage electroporation. Broad host range vectors, capable of replication in gram-positive and gram-negative hosts, and suitable for gene cloning, were introduced into lactobacilli. Transfection and transformation of Lactobacillus bulgaricus was observed using these same vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00043-17 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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 othar professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Donkersloot, Jacob	Research Microbiologist	LMI, NIDR
Robert Harr	Bio Lab Tech (Micro)	LMI, NIDR
Eunice Hull	Bio Lab Tech	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. R.L. Cihlar, Georgetown University Schools of Medicine and Dentistry,
Dr. A.L. Delisle, University of Maryland School of Dentistry

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.9	1.1

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.)*

A previously isolated recombinant clone expressing a 59 kDa antigen (p59) that reacts with monospecific and monoclonal antibodies directed against type 2 fimbriae from Actinomyces viscosus T14V has been further characterized. The fimA gene which encodes p59 hybridized strongly with a gene encoding a subunit of Actinomyces naeslundii type 2 fimbriae and more weakly with an A. viscosus T14V gene encoding a subunit of type 1 fimbriae, which suggests that these three genes may be derived from a common ancestral gene. To define fimA in detail, a number of subclones have been isolated and sequenced; so far about 70% of the sequence of one strand has been obtained. To investigate whether the cloned subunit expresses the lectin activity that is associated with intact fimbriae and which mediates coaggregation with certain oral streptococci, p59 was purified to homogeneity in three steps and injected into a rabbit to raise antibodies. During this purification of two antigen-containing peaks were observed both after DEAE-chromatography and gel filtration in the presence of Triton X-100 which differed about 2 kDa in size. It appears likely that the smaller form is due to the removal of a hydrophobic leader by the Escherichia coli recombinant cells. In order to (re)introduce cloned genes into oral actinomyces, a number of recently isolated bacteriophages have been examined to evaluate whether they can be utilized to develop a transformation system for A. viscosus. One group of small, short-tailed, phages contained linear, non-permuted, double-stranded DNA, 18-19 kb in size. Two other phages had double-stranded DNA of about 40 and 80 kb, respectively.

In another study, the receptor for the small phages on the surface of A. viscosus has been examined to see if it is related to one of the coaggregation mediators. The results obtained with various phage-resistant mutants indicate that the receptor for these bacteriophages is identical, or related, to the carbohydrate that interacts with streptococci belonging to coaggregation groups 1 and 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00046-16 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Chronic inflammation and immunomodulation of connective tissue metabolism

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

Wahl, Sharon	Chief, Cellular Immunology Section	LMI, NIDR
Allen, Janice	Chemist	LMI, NIDR
Hunt, Denise	Microbiologist	LMI, NIDR
Wahl, Larry	Research Microbiologist	LMI, NIDR
McCartney-Francis, Nancy	Microbiologist	LMI, NIDR
Dougherty, Suanne	Microbiologist	LMI, NIDR
Wong, Henry	Staff Fellow	LMI, NIDR
Agelli, Maria	Visiting Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

D. Yocum, ARB, NIAMS; I. Katona, USUHS; M. Sporn, A. Roberts, LC, NCI; L. Ellingsworth, Collagen Corporation.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	1.85	2.40

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Mononuclear cell recruitment and activation are central to the initiation, perpetuation and resolution of chronic inflammatory lesions. Investigations in this laboratory continue to characterize mechanisms of leukocyte chemotaxis to inflammatory stimuli. Transforming growth factor beta (TGF-β) produced by platelets, lymphocytes and monocytes is an extremely potent chemoattractant for monocytes and neutrophils at femtomolar concentrations. This level of activity may make TGF-β the most potent known chemotactic substance for leukocytes. At higher concentrations, TGFβ activates monocytes to elaborate polypeptide growth factors. TGF-β induces increased transcription and translation of interleukin 1β and tumor necrosis factor consistent with the ability of TGF-β to induce fibrosis and angiogenesis in vivo. While TGF-β may promote wound healing by such a mechanism, TGF-β also functions as an extremely active immunosuppressive agent. At femtomolar concentrations, TGF-β inhibits IL1-dependent T lymphocyte proliferation. The inhibitory effect of TGF-β occurs distal to the growth factor-receptor interaction and does not inhibit signal transduction leading to IL2 or IL2 receptor transcription or expression. Thus, TGF-β selectively down-regulates lymphocyte mitogenesis, but does not appear to inhibit lymphokine production. This immunosuppressive mechanism of action distinguishes TGF-β from cyclosporin A (CsA), a fungal metabolite which specificially inhibits T lymphocyte proliferation and lymphokine synthesis. In a recently completed randomized double-blind clinical trial, the immunosuppressive activity of CsA has been found to be of therapeutic benefit in the treatment of rheumatoid arthritis. The efficacy of TGF-β in reversing erosive polyarthritis is under study in experimental animal models.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE-00061-16 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Complement Activation and Inflammation

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

Ann Sandberg	Chief, Humoral Immunity Section	LMI, NIDR
Michael Brennan	Staff Fellow	LMI, NIDR
Richard Joralmon	Nat. Res. Ser. Award	LMI, NIDR
Linda Mudrick	Microbiologist	LMI, NIDR
John Cisar	Research Microbiologist	LMI, NIDR
Chieri Kurashima	Visiting Associate	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. Richard Calderone, Georgetown University, Dr. Harry Malech, NIAID, NIH, and Julia Metcalf, NIAID, NIH.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.22	4.03	1.99

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.)*

Studies are continuing to define the molecular basis for the interaction of the oral Actinomyces with human polymorphonuclear leukocytes (PMNs) and to examine the consequences of this recognition process. Actinomyces that possess type 2 fimbriae are phagocytosed and killed by PMNs in the absence of antibody and complement. This process is inhibited by β-linked galactosides and is accompanied by the production of reactive oxygen intermediates and the selective exocytosis of the contents of the PMN secondary granules assayed by superoxide anions and lactoferrin, respectively, in the culture supernatants. The generation of superoxide anions is inhibited by β-linked galactosides and a non-fimbriated mutant fails to initiate either of these responses. These findings indicate that although the Actinomyces can be killed by PMNs, the concomitant release of inflammatory agents from the PMNs may be detrimental to surrounding host tissues. Putative glycoprotein and glycolipid receptors on the PMNs for the Actinomyces lectin have been identified. Plant lectins with specificities similar to that of the bacterial lectin detect a glycoprotein of 110 Kd on sialidase treated Western blots of PMN membrane extracts. The conditions for the appearance of this band and optimal phagocytosis are similar in that both require sialidase treatment. In addition, two PMN gangliosides separated by thin layer chromatography (TLC) bind radiolabeled bacteria and a third ganglioside also expresses this activity after treatment of the TLC plates with sialidase. Complement mediated reactions with oral organisms have also been examined. The C3d receptors on the pseudohyphal form of Candida albicans have been identified. Monoclonal antibodies specific for these receptors detect a doublet of approximately 70 Kd in extracts of Candida eluted from a C3d affinity column. These receptors may be involved in the accumulation of Candida at a particular site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00199-11 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

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)*

PI:	Oliver, C.	Research Biologist	NIDR LMI
OTHERS:	Hart, T.	Visiting Fellow	NIDR LMI
	Kleinman, H.	Research Chemist	NIDR LDBA
	Zhang, W.	Visiting Fellow	NEI LOP
	Sahara, N.	Visiting Fellow	NIDR LMI

COOPERATING UNITS *(if any)*

Dr. A. Robbins, GB, NIDDK

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.95	1.85	3.10

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.)*

Secretory and endocytic processes in several cell types are currently under investigation. Short term cultures of isolated exocrine acinar cells, a pancreatic acinar cell line (AR42J) and other cultured cells are being used to study various aspects of the secretory process. Emphasis is placed on morphological, cytochemical and biochemical characterization of the cultured cells. Uptake and fate of both soluble phase and membrane bound markers by cultured cells are being examined in vivo and in vitro. The lysosomal system and its role in endocytic processes is also under study.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00216-11 LMI

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunological Control of Connective Tissue Metabolism

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

Larry M. Wahl	Research Biologist	LMI, NIDR
Sharon M. Wahl	Chief, Cellular Immunology Sect.	LMI, NIDR
Nira Hochman	Guest Researcher	LMI, NIDR
Marta Saldarriaga	Chemist	LMI, NIDR

COOPERATING UNITS (if any)
I. Katona, M.D., USUHS, K. Eckels, M.D., Walter Reed, J. Weinstein, NCI
B. Farrar, NCI, and D. Finbloom, Walter Reed.

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.85	.85	2.0

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.)

We have previously demonstrated that Con A or LPS activated human peripheral blood monocytes produce collagenase through a prostaglandin dependent mechanism. Our recent studies have examined the manner in which biologically defined molecules influence the production of arachidonic acid metabolites and collagenase by monocytes. We have shown that recombinant gamma interferon (rIFN-gamma) inhibits both PGE2 and collagenase production. The ability of rIFN-gamma to inhibit PGE2 generation accounts for the blocking effect of rIFN-gamma on collagenase production since exogenous PGE2 will restore collagenase synthesis. Analysis of the arachidonic acid products released by monocytes which are activated in the presence of rIFN-gamma has shown that both cyclooxygenase and lipoxygenase products are inhibited. This indicates that the rIFN-gamma acts by inhibiting phospholipase A2 activity since arachidonic acid is not available for either pathway. This conclusion is substantiated by the ability of exogenous phospholipase A2 to restore both PGE2 and collagenase production in IFN-gamma treated monocyte cultures. Studies on the role of the immune system in bone resorption have utilized the osteopetrotic (op) rat. Previously we have shown that proliferation responses of spleen cells from op rats are defective and based on our present work, this is associated with low interleukin 2 (IL2) levels. However, exogenous IL2 does not correct the proliferative defect. Sorter analysis of antigenic determinates on spleen cells of op and normal littermate rats revealed that the op cells had lower levels of Ia. Since IFN-gamma can increase cell surface HLA-DR or Ia, the op spleen cells were treated with IFN-gamma and examined for IA expression. IFN-gamma caused a significant increase in Ia on op spleen cells. Moreover, IFN-gamma restored IL2 production and proliferation of Con A stimulated op spleen cells to levels equivalent to those of normal littermate spleen cells. Thus, IFN-gamma corrected the defects in Ia expression, IL2 production and proliferation associated with op spleen cells.

PHS 6040 (Rev. 1/84)

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00254-10 LMI

PERIOD COVERED
October 1, 1986 - September 30, 1987

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	LMI, NIDR
Maria K. Yeung	Post-Doctoral Fellow	LMI, NIDR
Hsu, S. Dana	Microbiologist	LMI, NIDR
Sandberg, Ann	Chief, Humoral Immunity Section	LMI, NIDR
Mergenhagen, Stephan E.	Chief, Laboratory of Microbiology and Immunology	LMI, NIDR

COOPERATING UNITS *(if any)*
W.B. Clark, University of Florida, F. C. McIntire, and A.E. Vatter, University of Colorado

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Humoral Immunity Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.10	2.10	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Studies are continuing to define the fimbriae of Actinomyces viscosus and A. naeslundii that mediate the adherence of these oral bacteria to various cells and oral tissue surfaces and thereby contribute to microbial colonization, dental plaque formation and the initiation of gingivitis and periodontal disease. The type 1 fimbriae of A. viscosus are involved in bacterial attachment to the acquired salivary pellicle of teeth. The amino acid sequence of the type 1 fimbrial subunit has now been deduced form the nucleotide sequence of the cloned gene. Certain anti-type 1 fimbrial antibodies were previously shown to block bacterial adsorption to saliva-treated hydroxyapatite; however, antibodies directed against the 59 Kd fimbrial subunit appear to be poor inhibitors of adsorption even though they reacted with the type 1 fimbriae on actinomyces. Thus, the fimbrial subunit may not be directly involved in the adhesive interaction. Type 2 fimbriae of A. viscosus and A. naeslundii have been associated with a Gal/GalNAc specific lectin activity. The type 2 fimbrial subunit of A. naeslundii was identified as a 60 kD protein by cloning and expression of the corresponding gene in Escherichia coli. DNA-DNA hybridization was observed between the genes for A. naeslundii and A. viscosus type 2 fimbrial subunits as well as those for A. naeslundii type 2 and A. viscosus type 1 subunits. Thus, the different fimbriae of Actinomyces sp. may be composed of subunits encoded by genes derived from a common ancestor. The receptor for type 2 Actinomyces fimbriae on S. sanguis 34 has been identified as a linear polysaccharide formed by identical hexasaccharide units linked through phosphodiester bridges. Different structural features of the hexasaccharide appear to be involved in lectin and antibody recognition since the specificity of bacterial coaggregation is strikingly different from that of antibody against the polysaccharide. These studies have begun to provide a structural basis for understanding the specificity of oral microbial adherence and mechanisms by which oral bacteria resist the immune system.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00273-09 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Cell-Cell Interactions Between Oral Actinomyces and Other Oral Bacteria

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

Kolenbrander, Paul	Research Microbiologist	LMI, NIDR
London, Jack	Research Microbiologist	LMI, NIDR
Donkersloot, Jacob	Research Microbiologist	LMI, NIDR
Anderson, Roxanna	Microbiologist	LMI, NIDR
Weiss, Ervin	Fogarty Visiting Fellow	LMI, NIDR
Hughes, Christopher	Dental Staff Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. A.D. Deiste, University of Maryland, School of Dentistry, Baltimore, MD.
Dr. L.V. Holderman, VPI and SU, Blacksburg, VA

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.60	2.60	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.)*

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 part of this complex community studied in this laboratory pertains especially to microbial ecology. Each of the three types of bacteria, the gram-positive actinomyces and streptococci and the gram-negative veillonella, that are thought to be early colonizers of the tooth surface are being studied. In addition, the gram-negative bacteroides and capnocytophaga, which increase in numbers during stages of gingivitis in humans, have been used to isolate adhesin molecules and to demonstrate intrageneric phylogenetic relatedness among adhesin molecules, respectively. The concept advanced in this laboratory of a bridge bacterium between two other noncoaggregating cell types has been confirmed by the identification of a 75kD and 45kD adhesin on the bridge bacterium, Bacteroides loescheii, that mediate coaggregation with S. sanguis and A. israelii, respectively.

Fresh isolates of veillonella from the dorsum of the tongue coaggregate with Streptococcus salivarius, also found on the tongue, but do not coaggregate with S. sanguis which predominates in subgingival plaque. However, fresh isolates of veillonella from subgingival plaque coaggregate with S. sanguis but not S. salivarius. These results strongly support the notion that coaggregation plays an important role in colonization of different econiches in the oral cavity.

Bacteriophage from sewage and from human dental plaque appear to recognize the same surface receptor on oral actinomyces. This receptor seems to have a second function, mediating coaggregation with oral streptococci.

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00290-8
LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

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	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Fischler, Cynthia	Medical Technician Micro.	NIDR LMI
Roberts, Barbara	Biologist	NIDR LMI
Zinsser, Frank U.	Guest Worker	NIDR LMI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIDR, NIH Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.00	3.00	2.00

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.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fc episilon 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-341- 06 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Regulation of sugar transport and metabolism in lactic and bacteria

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

John Thompson	Visiting Scientist	LMI, NIDR
Stanley A. Robrish	Microbiologist	LMI, NIDR
Charles L. Wittenberger	Chief, Microbiology Section	LMI, NIDR

COOPERATING UNITS *(if any)*

Stephan P.F. Miller	LCH, NHLBI
Henry Fales	LCH, NHLBI

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.80	.40	1.40

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A unique amino acid-dependent sugar transport system has been characterized in the Gram negative oral anaerobe, Fusobacterium nucleatum ATCC 10953. Energy necessary for the active transport of glucose and galactose is derived from anaerobic fermentation of specific amino acids (Glutamic, lysine, histidine). Kinetic analyses, and data from competition studies suggest that glucose and galactose are translocated via a common high-affinity carrier. Intracellular sugars are phosphorylated by ATP-dependent kinases prior to conversion to high molecular-weight polymers. Glutamate, lysine and histidine prevent catabolism of the sugar polymers, but in the absence of amino acids the polymers are rapidly fermented to yield butyric, lactic and acetic acids.

Two new amino acids have been identified and purified from Streptococcus lactis. These unusual amino acids, namely N(5)-(1-carboxyethyl)ornithine and N(6)-(1-carboxyethyl)lysine, have been chemically prepared and the stereochemical configurations of the natural products have been confirmed by chiral syntheses. The in vitro synthesis of N(5)-(1-carboxyethyl)-ornithine has been demonstrated by incubation of a cell-free extract with ornithine, pyruvic acid and NAD(P)H.

A basic amino acid carrier has been identified and characterized in cells of Streptococcus lactis. The transporter mediates the energy-independent entry and exchange of lysine, arginine and ornithine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00374-05 LMI

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Action and regulation of colony stimulating factors

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

Dov H. Pluznik	Expert	LMI, NIDR
Matthias Bickel	Visiting Associate	LMI, NIDR
Lynda L. Weedon	Biologist	LMI, NIDR
Stephan E. Mergenhagen	Chief	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.40	2.40	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This report describes studies on: a) Production of granulocyte-macrophage colony stimulating factor (GM-CSF) by B cell lymphoma and hybridoma cell lines stimulated by LPS or by antibodies against IgM. GM-CSF activity was assayed on the basophil/mast cell line PT-18 (GM-CSF and IL3 dependent). Antibodies against murine recombinant GM-CSF were used to identify CSF activity present in supernatants of the stimulated B cell lines. No spontaneous release of GM-CSF was found in unstimulated cells. However, when stimulated with LPS, 2 of 5 lymphoma and 5 of 6 hybridoma lines produced GM-CSF. Two lines, the lymphoma M12.4.1 and the hybridoma TH2.2 were analyzed more extensively. In these two lines production of GM-CSF was dependent on the dose of LPS used and time of exposure. Antibodies against IgM stimulated TH2.2 (IgM+), but not the M12.4.1 (IgM-) cells to produce GM-CSF. Northern blot analysis of the M12.4.1 cells showed that mRNA of GM-CSF detected in LPS-stimulated but not in unstimulated cells. Our data show that B cells can be stimulated to produce GM-CSF and thus suggest that they may take part in granulopoiesis. b) Association of the proliferation-related oncogene c-fos with the differentiation of myeloid cells by CSF. Enhanced expression of c-fos is involved in the differentiation of monomyelocytic cells into mature macrophages, which accumulate in the G1 phase of the cell cycle. Ml murine myeloid leukemia cells, which can be synchronized in early and late G1 and can also differentiate into mature macrophages when stimulated with G-CSF, were used to investigate whether enhanced expression of c-fos in differentiated monomyelocytic cells is associated with the position of the cells in the cell cycle or with their state of differentiation. Our data show that the enhanced expression of the c-fos gene occurs only in fully differentiated cells and not in proliferating or G1-arrested cells. These data imply that the enhanced expression of c-fos in Ml cells is restricted to functionally differentiated cells and not related to the position of the differentiated cells in the cell cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00381-04 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mechanisms of Colonization of Oral Bacteria

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

Ciardi, Joseph E.	Research Biochemist	NIDR LMI
Kousvelari, Eleni	Senior Staff Fellow	NIDR CIPCB

COOPERATING UNITS *(if any)*

Department of Microbiology, Georgetown University

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.13	1.03	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.)*

Adherence of bacteria to saliva-coated surfaces, saliva-induced aggregation of bacteria and highly specific interactions between different species of oral bacteria (coaggregation) are important determinants in the microbial ecology of the oral cavity. Our studies which assessed the interaction of human parotid salivary proteins with hydroxyapatite surfaces and bacterial cell surfaces support roles for different proteins, tentatively identified as proline rich proteins, in adherence (Mr 22k) and in aggregation (Mr 35k) of Streptococcus sanguis. Isolation of these proteins from hydroxyapatite (HA) and from bacteria after treatment with parotid saliva and their further purification are being carried out for physico-chemical characterization and to quantitatively evaluate their importance in bacteria-surface and bacteria-bacteria interactions. Further studies with rat parotid saliva showed that the Mr 40k protein which selectively adsorbed to HA and was associated with the adherence of streptococcal and actinomyces cells to this mineral was made radioactive after in vivo incorporation of C-14-proline. C-14-labeled Mr 40k protein neither aggregated or bound to streptococcal cells indicating that the bacteria-protein interaction occurred only when the protein was attached to the HA. A purified preparation of a high molecular weight proline-rich glycoprotein (PRG) of rat parotid saliva, which did not bind to HA, aggregated Actinomyces viscosus cells. Monoclonal antibody specific for PRG is being prepared to further study this aggregation and to obtain pure preparations of this glycoprotein. Coaggregation between S. sanguis and Propionibacterium acnes on a saliva-coated HA surface occurred, but at a reduced extent in human whole saliva when compared to that determined in buffer. Results of experiments which examined pH effects, hydrophobic effects and salt effects suggest that the higher ionic strength of saliva was, in part, responsible for this reduced coaggregation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00382-04 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Growth and Interaction of Oral Microorganisms: Amino acid and sugar use by Fusobacterium nucleatum

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

Robrish, Stanley A.	Research Microbiologist	LMI, NIDR
Thompson, Jack	Visiting Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Continuing studies with the amino acid stimulated sugar transport system of Fusobacterium nucleatum have included a determination of transport specificity, as well as new information about the mechanism of transport and patterns of use of the glucose storage product. Competition studies with a wide variety of sugars and sugar analogs demonstrated that glucose and galactose are mutual and exclusive competitors. The transport system requires the alpha-D-glucopyranose molecule but allows a single hydroxyl group at the number 4 position to be in either possible orientation (glucose or galactose). Results of competition studies using analogs of galactose were identical to the results obtained with glucose analogs: glucose being the exclusive competitor for galactose transport. The glutamate stimulated transport system proved highly sensitive to proton-conducting uncouplers suggesting proton motive force transport. Evidence was obtained for cell extracts independent glucose and galactose kinases which depended on ATP or acetyl phosphate.

The use of the preformed sugar storage product of F. nucleatum is spared when energy is available from the fermentation of amino acids. Glutamate, lysine, and histidine blocked the loss of C-14 from a washed cell suspension which had preformed glucose storage product. Aerobic conditions suppressed the use of amino acids in washed cell suspensions and resulted in a release of the glucose and galactose storage products in the presence of glutamate. Analysis of the fermentation product from the use of glucose storage product revealed major differences in the ratios of acetic and lactic acids formed depending on the presence of glutamate. Low molecular weight intermediates resulting from the use of the glucose storage product have been fractionated by gel filtration and are being characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-00392-04 LMI

PERIOD COVERED

October 1, 1986 to Sepember 30, 1987

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)*

Phillip D. Smith, M.D.	Senior Staff Fellow	LMI, NIDR
Nancy McCartney-Francis, Ph.D.	Senior Staff Fellow	LMI, NIDR
Sharon M. Wahl, Ph.D.	Chief, Cellular Immunology Section	LMI, NIDR
Larry M. Wahl, Ph.D.	Microbiologist	LMI, NIDR
Janice B. Allen	Chemist	LMI, NIDR
William L. Whelan, Ph.D.	Visiting Associate	LMI, NIDR

COOPERATING UNITS *(if any)*

Gary L. Simon, M.D., Ph.D., George Washington University; Jean Michele Delga, M.D., NIAID; Suzanne Gartner, Ph.D., NCI; Mikulas Popovic, Ph.D., NCI.

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institutes of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.81	2.10	.71

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.)*

Human immunodeficiency virus (HIV) and cytomegalovirus (CMV) can induce severe lymphocyte suppression. Whether these viruses also cause monocyte dysfunction, which could contribute the lymphocyte immunosuppression, is unclear. Therefore, the goal of this project has been to determine whether HIV and CMV infect human monocytes and whether infected monocytes exhibit impaired cell functions. Early results indicate that HIV and CMV can infect monocytes . Monocytes infected with HIV in vivo exhibit impaired chemotaxis, interleukin-1 production, cytotoxicity, accessory cell activity and surface antigen/receptor expression. These findings are currently being confirmed in monocytes infected in vitro by macrophage-adapted strains of HIV. Results also indicate that monocytes infected with CMV exhibit reduced accessory cell functions but increased surface HLA-DR and IL-2 receptors and spontaneously secrete increased amounts of oxygen reactive intermediates. An investiation of the role of C. albicans, a pathogen of the oral cavity and esophagus in AIDS patients, has been initiated. Particular attention is being directed to monocyte cytotoxicity of Candida, to identification of the subgroup of Candida infecting AIDS patients, and to the role of Candida cell wall mannon in the pathogenicity of the C. albicans isolated from AIDS patients.

In clinical investigations, the etiology and therapy of the enteric infections in AIDS is being evaluated. In the majority of symptomatic patients, identifiable enteric pathogens, particularly CMV, have been identified. Contributing to the high frequency of enteric infections in AIDS patients may be their impaired ability to develop de novo antibody responses to enteric microorganisms such as G. lamblia which we have recently documented. The mechanism of the impaired B cell response in AIDS patients will be addressed in vitro by investigating the role of HIV infection of monocytes in altering the regulation of the B cell production of IgM and IgG. Also, investigation of the malabsorption associated with AIDS indicates that HIV itself may be capable of inducing malabsorption.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00424- 02 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

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 L.	Senior Staff Fellow	LMI, NIDR
Smith, Phillip D.	Senior Staff Fellow	LMI, NIDR
Wahl, Larry M.	Microbiologist	LMI, NIDR
Wahl, Sharon M.	Chief, Cellular Immunology	LMI, NIDR
Mizel, Diane	Chemist	LMI, NIDR
Dougherty, Suanne	Biologist	LMI, NIDR
Wong, Henry	Staff Fellow	LMI, NIDR
Allen, Janice	Chemist	LMI, NIDR

COOPERATING UNITS *(if any)* Ildy M. Katona, M.D. Rhem. Sect. Dept. of Ped. Med., USUHS Thomas Folks, Ph.D., NIAID, NIH, Suzanne Gartner, Ph.D., Mikulas Popovic, Ph.D., NCI, NIH, Gary L. Simon, MD., Ph.D., George Washington University

LAB/BRANCH

Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.10	1.25	1.85

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 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 mechanisms that regulate cytokine expression. We have investigated the role of IL2 receptor expression in activated monocytes in vitro and then examined the effect of in vivo and in vitro infection with human immunodeficiency virus (HIV) on monocyte IL2 receptor expression. In previous studies we demonstrated IL2 gene expression and synthesis of the receptor in activated monocytes. The addition of recombinant IL2 to suboptimally activated IL2 receptor-positive monocytes enhanced both the production of reactive oxygen intermediates and cytotoxic activity. In addition, IL2 modulated IL1 gene expression. At low doses of lipopolysaccharide (LPS), the level of IL1 mRNA was increased in the presence of IL2. However, at higher doses of LPS (>1 μg/ml), the level of IL1 message was decreased in the presence of IL2. Normal monocytes cocultured with HIV in vitro also expressed increased surface receptors for IL2, and the addition of recombinant IL2 to these cells caused an increase in reverse transcriptase activity, suggesting that IL2:IL2 receptors may play a regulatory role in the infective process. Peripheral blood monocytes from patients with AIDS expressed increased levels of IL2 receptors as well as HLA-DR antigens. In addition, Northern blot and in situ hybridizations demonstrated an increase in the level of IL1 and IL2 receptor mRNAs in the circulating monocytes from these patients, indicating the prior in vivo activation of these cells. These studies suggest that HIV infection may contribute to the in vivo activation of monocytes in AIDS patients and that these activated cells may play an important role in the pathogenicity of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00441-01 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 cherecters 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 Investigetor.) (Neme, title, leboretory, end institute effiliation)*

Allen, Janice	Chemist	LMI, NIDR
Wahl, Sharon	Chief, Cellular Immunology Section	LMI, NIDR
Feldman, Gerald	NRC Research Associateship	LMI, NIDR
Ink, Lauri	Junior Fellow	LMI, NIDR
McCartney-Francis, Nancy	Microbiologist	LMI, NIDR
Bansal, Geetha	Guest Worker	LMI, NIDR

COOPERATING UNITS *(if any)*

E. Amento, Genentech; J. Farrar, Hoffman LaRoche; A. Hand, LOP, NIDR; L. Ellingsworth, Collagen Corportation; M. Bolander, LDBA, NIDR

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	.10

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Group A streptococcal cell walls (SCW) induce a biphasic pattern of hepatic granuloma formation and chronic, erosive polyarthritis in the LEW/N, but not the genetically similar F344/N rat. The first phase is an acute exudative response characterized by neutrophil accumulation and swelling which peaks at 3 days, recedes and is followed by a chronic destructive mononuclear cell-mediated phase. In studies to identify the cellular and molecular mechanism of differential genetic susceptibility, macrophages from the two strains were compared for their responsiveness to SCW. Peritoneal macrophages exposed to SCW in vivo or in vitro were evaluated for inflammatory mediator and monokine production. The LEW/N macrophages were found to produce elevated levels of differentiation and proliferative factors compared to the F344/N which may promote chronic inflammation and provide a basis for the differential development of erosive polyarthritis in the SCW-treated LEW/N and F344/N animals. In order to further define the cellular and molecular mechanisms responsible for the acute and chronic phases of the SCW-induced inflammatory response, we have evaluated the effect of site-specific inhibitors on these processes. The anti-inflammatory corticosteroid, methylprednisolone (MP) ablates the acute response and consequently the chronic infiltration of mononuclear cells (articular index (AI) 9.1 vs 0.2). By comparison, flurbiprofen, a nonsteroidal anti-inflammatory drug, partially suppressed both the acute and chronic arthritis (AI 9.1 vs 2.1). Thus, each of these agents appears to act at different loci to modulate SCW-induced arthritis. By choosing drug combinations with different target specificities, it may be possible to interrupt several sites in this interdependent inflammatory process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00454-01 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

London, Jack	Microbiologist	LMI, NIDR
Weiss, E. I.	Visiting Fellow	LMI, NIDR
Tempro, Paulette	NRSA	LMI, NIDR
Cassels, Fred	Warner-Lambert Fellow	LMI, NIDR
Siraganian, R.	Immunologist	LMI, NIDR
Kolenbrander, P. E.	Microbiologist	LMI, NIDR
Allen, Janet	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*
Hand, Arthur, Microscopist, PIPCB, NIDR

LAB/BRANCH
Laboratory of Microbiology and Immunology

SECTION
Microbiology Section

INSTITUTE AND LOCATION
National Institute of Dental Research

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.90	2.40	.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.)*

Monoclonal antibodies (MAbs) were prepared against the B. loescheii adhesins reponsible for this organism's coaggregation with Streptococcus sanguis 34 and Actinomyces israelii PK14 by screening hybridoma supernatants specifically for coaggregation-blocking activity. Ten hybridomas were selected for preparation of ascites fluid in mice. Of these, 4 specifically blocked the B. loescheii - S. sanguis coaggregation and 2 inhibited the B. loescheii - A. israelii interaction. MAb or Fab' fragments inhibited these coaggregations at low levels suggesting that the antibodies were specific for regions near or at the binding sites of the respective adhesins. These MAbs were used to confirm that the adhesins consisted of a 75kD (S. sanguis specific adhesin) and 45kD (A. israelii specific adhesin) subunits, respectively, using radiolabeled adhesin-containing preparations. In the native form, the S. sanguis adhesin is a pentamer with a M.W. of 380kD. Using radiolabeled MAbs in direct binding assays, it was determined that each B. loescheii cell carries a maximum of approximately 300 S. sanguis specific adhesins and 600 A. israelii specific adhesins on its surface. The MAbs, were used also to localize the respective adhesins on the surface of the cells. Both types of adhesins were shown to be associated with the distal portion of the organisms fimbriae. The adhesins were arranged randomly in small to large clusters which extended away from the fimbriae. Preliminary studies with the radiolabeled adhesins suggest that the two types of adhesins are closely associated with one another on the surface of the cell.

Employing the same strategy described above, MAbs specific for the afimbriate 250kD adhesin of Capnocytophaga gingivalis, which mediates its coaggregation with A. israelii PK16, are currently being prepared. Concurrently, the carbohydrate receptor of S. sanguis 34 which mediates coaggregation with Capnocytophaga ochracea is being purified and characterized.

ANNUAL REPORT OF THE LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

NATIONAL INSTITUTE OF DENTAL RESEARCH

The reorganization of the Laboratory of Oral Biology and Physiology planned and commenced during 1985-1986 has been further implemented. Dr. John Folk, Chief of the Enzyme Chemistry Section, continues to act as chief of the laboratory which is presently composed of two sections and a peptide service facility. The two sections, the Enzyme Chemistry Section and the Bone Cell Biology Section, which was transferred from the Bone Research Branch as part of a 1985-1986 NIDR Intramural Program reorganization, are of similar size and composition. The Peptide Facility, established this year under the direction of Dr. Frank Robey, provides a service in peptide preparation to all intramural programs. Dr. Robey, in addition, maintains an independent research program presently in a stage of development. Laboratory personnel numbers about 25 with a ratio of research to support staff of approximately 2 to 1. The ratio within the research staff of tenure to temporary staff (principally postdoctoral fellows and visitors) is about 1 to 1. More than half of the temporary scientists are supported by means that do not count against our personnel ceiling. This value has been increasing during the year and should continue to do so, if we are to maintain vitality.

A change in organization made this year, but which remains to be implemented in 1987-1988, is the transfer of Dr. Keith Robbins from the Laboratory of Cellular and Molecular Biology, NCI, to this laboratory. Dr. Robbins will commence operations here with a staff of about 7 persons after room renovations are completed. The focus of his work will be the molecular mechanisms involved in retrovirus-induced transformation and their application to naturally occurring human malignant disease.

Laboratory programs have traditionally been limited in size principally by position ceiling. Thus, our ability to recruit by other mechanisms has been of prime importance. However, space limitations now become equally important and limitations are increasing with our rapid program expansion. Collaborative enterprise must also be included in practice in measurement of program size and all of our scientific staff collaborate regularly with scientists, both in and outside NIDR and NIH, and some with several different groups. In this way mutual increase in productivity is realized. Our Peptide Facility is just now reaching its full potential with advent of the completed refurnishing of a large room in the basement of building 30. All program directors have been made aware of the availability of this service.

Original research findings related to the biochemistry and biology of normal function and of disease are the principal products generated by this laboratory. Unity is achieved through the search for molecular structure-function relationships. Productivity is conventionally measured in terms of the number and the quality of publications, standards for which we would undoubtedly meet or exceed whatever standards might apply. Senior investigators participate in a number of other professional activities in addition to laboratory research. Among those of broad importance to biological science are organization of and participation in national and international meetings, review of manuscripts for a variety of journals,

evaluation of applications for grants and fellowships, lecturing before groups within and outside NIH, and serving on committees.

To produce original scientific research is, however, the major function of the Laboratory. A number of significant research products resulting from this year's efforts are summarized below.

Dr. Robey joined the Laboratory in mid-year (March 15) at a time when little of his equipment was available and when room renovations had only begun. Although much time was devoted to setting up the Peptide Facility, he nevertheless utilized his expertise in peptide chemistry to develop approaches to several important problems. By incorporation of the proper reactive groups into synthetic peptides, it was possible to polymerize these peptides post-synthetically, thus attaining material in the range of M_r = 20,000. With the use of groupings that can be differentially reacted it is hoped to obtain polymers in excess of M_r = 50,000 that will not be readily cleared by the kidney. Use of these high M_r materials for immunization should provide good response and will circumvent the use of carrier proteins which present insurmountable difficulties as vaccine components in humans. The primary objective of this approach is to provide immunization against viral infection, and to this end it will, hopefully, be of vital importance to the AIDS problem.

HIV-1 is the causative agent of AIDS. Dr. Robey and coworkers have found regions of sequence homology on the gp41 protein of the gp160 envelope of HIV-1 and the β-chain of a human host protein, the major histocompatibility antigen class II (MHC Class II). MHC Class II is believed to bind to the CD-4 receptor on human T-4 cells. Peptides corresponding to the regions of homology on the two proteins were synthesized and used to prepare antibodies that react against MHC Class II and against HIV-1 in the commercial AIDS tests. Serum from about one-third of the AIDS patients tested displayed antibodies that crossreact with the synthetic peptides. This finding suggests that patients suffering from AIDS produce antibodies against MHC Class II that may neutralize MHC Class II activity. This indeed would be an indicator of autoimmune disease in AIDS patients.

C-reactive protein (CRP) is a prototypical acute phase protein, the physiological function of which is not known. During the onset of inflammation in humans, the serum level of CRP may increase as much as 2000-fold. Dr. Robey, with others at NIDR and elsewhere, observed that CRP contains three sequences of amino acids, each of which closely resembles the amino acid sequence of Tuftsin, a tetrapeptide Thr-Lys-Pro-Arg, with strong immunomodulating activity. They synthesized three peptides modeled after the sequences in CRP and found that they possess activities equal to that of Tuftsin in causing chemotaxis in monocytes, in producing superoxide, and in inducing mononuclear cells to produce interleukin 1. When CRP was incubated with polymorphonuclear neutrophils and the resulting digest tested for immunomodulating activity, it was found to display the same activities as those given by the synthetic peptides. These findings are strong support for hypotheses that *in vivo* digestion of CRP at the onset of inflammation results in the release of small molecules with potent immunoregulatory activity and that this is indeed a unique biological function involving CRP.

The foregoing studies illustrate the power of synthetic peptides as tools in modern bioscience and point up the diversity of their applications. Certainly, these few examples provide an understanding of the wisdom in establishment of our Peptide Facility, and, hopefully, will lead the way to its optimal use by researchers in other laboratories of NIDR.

Bone Cell Biology Section

Of central interest in bone cell biology is the control of bone cell growth and differentiation by growth factors. Bone cells are in close contact with extracellular matrix and there is increasing awareness of the vital role of matrix in dynamic modulation of bone cell function. The bone inductive factor osteogenin, which was purified earlier by the group from both bovine and human bone and shown to be a protein of apparent M_r = 22,000, is under intensive study, the aim of which is to delineate the interplay in bone and cartilage between osteogenin and various other growth and differentiation factors.

Poor fracture healing in diabetic patients is a major clinical problem. In experimental animal diabetes bone induction is suboptimal. It was found that growth hormone and insulin-like growth factor I (IGF-I), either separately or together, augment the action of osteogenin and provide optimal bone induction. These polypeptide hormones are metabolically unstable, however, and systems are under study to optimize their local delivery for eventual clinical trials. Indications that IGF-I is an autocrine growth and maintenance factor in articular cartilage was provided by the finding of rather high levels of endogenous intrinsic production of this factor and its essential role for optimal proteoglycan synthesis by cartilage slices.

For the purification of osteogenin, an *in vivo* bioassay system was employed. Like most *in vivo* assays, that for the bone induction factor is subject to modulation by uncontrollable factors. Now that purified osteogenin is available and much is known about its role in osteogenesis *in vivo*, the stage is set for *in vitro* mechanism studies. At this point a reliable *in vitro* assay that can be conducted in chemically defined medium is essential. Through a systematic approach colonization of dedifferentiated chondrocytes was found to require osteogenin; no colonies were produced in the presence of IGF-I or growth hormone alone. An immunological method for quantitation of this assay is in the final stages of testing and shows great promise.

Although transforming growth factor β is present in high concentration in bone, it was demonstrated by the group to play no role in bone induction. Its distribution in bone was examined and the results revealed its association mainly with demineralized matrix, apparently extracellular matrix. When the expression of transforming growth factor β was measured during bone formation and during chondrogenesis, the most significant increases were found early in development.

Enzyme Chemistry Section

The study of transglutaminases was instigated by the group a number of years ago as a means of gaining information on enzyme mechanism and on molecular structure-catalytic function relationships. These enzymes were chosen also because, as a group, they offered a prototype of a catalyst for posttranslational modification, the importance of which at that time was only beginning to be appreciated. For years it was believed that the primary biological function of the transglutaminases was that of catalyzing production of ε(γ-glutamyl)lysine crosslinks between protein molecules for the purpose of maintaining gross forms of structure and limiting degrees of extensibility. The classic example is fibrin stabilization. It was recently made evident, largely as a consequence of findings from this Laboratory, that these enzymes catalyze other important reactions, among which are the incorporation of polyamines and biogenic amines into specific cellular proteins and the covalent connecting of protein molecules through polyfunctional amines. Evidence that the transglutaminases may be vitally important in regulatory processes is provided by studies conducted this year.

Extravasated fibrin clots formed during wounding and in inflammatory injury, and known to be involved in hemostasis and hemorrhagic diathesis, serve as reservoirs for mitogens and chemoattractants released from platelets and other leukocytes. Fibrin stability to proteolysis and thus the local availability of these control factors is important to the normal healing process. Although the regulation of, and the enzymes involved in, vascular circulatory fibrinolysis have been extensively studied, very little study has been made of extravasated fibrin clot degradation. An animal model system was developed for the latter purpose. Using this system in conjunction with ^{125}I-labeled fibrin samples, it was found that fibrin that is fully stabilized, i.e., cross-linked in both α and γ chains through the action of the transglutaminase, blood coagulation factor XIIIa, is most resistent to fibrinolysis, whereas fibrin with α-2 anti-plasmin is covalently attached to its α chains through catalysis by this enzyme is much less stable to proteolysis. This is in contrast to the situation in plasma in which the α-2 plasmin inhibitor-fibrin complex is the most resistent to fibrinolysis. Examination of the fibrin and fibrin-inhibitor degradation products revealed pronounced differences in vascular and extravascular fibrinolysis. These findings provide evidence for one mechanism by which a transglutaminase, through its controlled action on fibrin, may enhance the healing process.

Another means by which transglutaminase action may influence wound healing is suggested by the finding that the aminopropeptide of Type III collagen is an excellent substrate for crosslinking by tissue transglutaminase. In earlier wound healing studies incorporation of radiolabeled primary amine was observed in material of larger size than the aminopropeptide. However, upon treatment with bacterial collagenase this material was degraded to a molecule the size of the aminopropeptide. Together these findings suggest that enzyme-catalyzed crosslinking between subunits of aminopropeptide and/or between aminopropeptide and other matrix components occurs during connective tissue repair. In addition, crosslinking may contribute to the high metabolic stability of this peptide, which is believed to control fibril growth of Type III collagen by

adhering to the surfaces of its fibrils. These findings of transglutaminase involvement in interactions and modifications of extracellular proteins provide a bridge to other connective tissue studies in NIDR and in other institutes of NIH.

The focus this year of studies on eukaryotic protein translation initiation factor 4D (eIF-4D) has been toward an understanding of the regulation of the posttranslational steps in the formation of its single hypusine residue. The unique amino acid hypusine is produced by transfer of the butylamine portion of the polyamine spermidine to a single lysine residue of eIF-4D precursor and subsequent hydroxylation. In normal test cells eIF-4D is one of a class of constitutively modified initiation factors, that is, modification (hypusine production) occurs either during or shortly after the translation process itself, and the modification state is not regulated thereafter. When these cells were depleted of spermidine, however, there was an accumulation of eIF-4D precursor that contains no hypusine. In these cells hypusine synthesis was regulated by the cellular level of spermidine. The possibility arises that in other cells types and/or situations there are additional factors involved in regulation. A protein from spermidine deficient cells that does not occur in normal cells has been isolated, and conversion to eIF-4D by an enzyme fraction from normal cells is evidence of its identity as the precursor. Peptides corresponding to the sequence of amino acids around hypusine in eIF-4D and around the lysine in eIF-4D precursor that is converted to hypusine were synthesized, coupled to carrier protein, and employed for antibody production. The antibodies show promise in purification of eIF-4D and its precursor, respectively. In addition the peptides were used for further purification of deoxyhypusine hydroxylase, the enzyme that catalyzes the final step in hypusine biosynthesis. Cellular control of this enzyme by polyamines was strongly indicated by in vitro inhibition studies with the purified enzyme.

Studies on the biosynthesis of hypusine arose as an outgrowth of an ongoing investigation of transglutaminase-catalyzed polyamine incorporation in cells. Hypusine formation is the most unusual example of posttranslational modification in that it occurs in all mammalian cells in only a single protein and at only a single position in this protein. The rather elaborate posttranslational mechanism for hypusine production utilizes a novel pathway of polyamine metabolism and a unique enzymic hydroxylation step. Paramount to interest in this modification reaction is the specific cellular function of the ubiquitous protein that contains hypusine and the relationship of this rare amino acid to the function of eIF-4D.

Thus, the Laboratory has had a productive and stable year despite changes that occurred and others that are anticipated. In some cases new results have led to new projects. The emphasis continues to be on growth and biological control with an eye toward an incoming program on malignant disease. It is fitting that a change in the name of the Laboratory is under consideration.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00001-35 LOBP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	LOBP NIDR
OTHERS:	Martinet, N.	Guest Researcher	LOBP NIDR
	Beninati, S.	Visiting Associate	LOBP NIDR

COOPERATING UNITS *(if any)* Dr. M. Piacentini, 2nd Univ., Rome, Italy; Dr. M. Bowness, Dept. Biochem., Univ. Manitoba, Winnipeg Man., Canada, Dr. J. Gorman, CSIRO, Aust. Natl. Health Lab., Geelong, Australia; Dr. M. Fink, Baylor Univ., Waco, Texas; Dr. R. Timpl, Max-Planck Institut-fur Biochemie, Munich, Germany.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.70	2.25	.45

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.)*

Studies on the basis for differences in specificities of the transglutaminases are underway. Specific inhibitors for these enzymes have been prepared. Several intracellular products of transglutaminase action were identified. The catabolic fate of the transglutaminase products is now understood.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00049-16 LOBP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	LOBP NIDR
OTHERS:	Cardinali, M.	Visiting Fellow	LOBP NIDR
	Kim, H.C.	Guest Researcher	LOBP NIDR
	Uchino, R.	Visiting Fellow	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. M. Lewis, DRS, NIH, Dr. F. Carmassi, Pisa, Italy.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institutes of Health, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.35	2.90	.45

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of "provisional stroma" (fibrin or fibrin-connective tissue), during tissue or bone fracture repair, and in the modulation of specific cellular processes. The coagulant seal 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 (alpha chain-polymer and gamma-gamma chain dimer) and of alpha 2 antiplasmin to alpha chains of fibrin. A number of substances, such as oxygen metabolites, sulfhydryls, triglycerides (lipophilic agents), and albumin in the plasma and tissue fluids can affect the catalytic activity of factor XIIIa and other transglutaminases. 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 cells). Proteases, known to be generated during hemostasis, induce activation of membrane-bound forms of transglutaminase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00204-11 LOBP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	LOBP NIDR
OTHERS:	Neelakandan, M.	Visiting Associate	LOBP NIDR
	Harrison, E.	Research Biologist	LOBP NIDR
	Carrington, J.	Guest Researcher	LOBP NIDR
	Luyten, F.	Guest Researcher	LOBP NIDR
	Ma, S.	Visiting Fellow	LOBP NIDR
	Katz, R.	Dental Staff Fellow	CIPCP NIDR
	Hascall, V.	Research Chemist	BRB NIDR

COOPERATING UNITS *(if any)*

Dr. J. Hollinger, USAIDR, U.S. Army; Dr. G. Bowers, University of Maryland; Dr. A. Roberts, NCI.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Bone Cell Biology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.0	2.0

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 objective of this project is to investigate the growth and differentiation factors in cartilage and bone. The research projects currently under study are: 1) purification of osteogenins from bovine and human bone; 2) actions of growth factors on bone differentiation in vivo; 3) compartmentation of transforming growth factor beta in bone; 4) expression of TGF beta during cartilage and bone development; 5) influence of human and rat tooth matrix on bone induction; 6) role of insulin-like growth factors and other differentiation factors in cartilage repair, and 7) role of osteogenin and other growth factors on chondrocytes and dedifferentiated chondrocytes in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00311-07 LOBP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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.	Senior Staff Fellow	LOBP NIDR
OTHERS:	Folk, J.E.	Acting Chief	LOBP NIDR
	Wolff, E.C.	Chemist	LOBP NIDR
	Beninati, S.	Visiting Associate	LOBP NIDR

COOPERATING UNITS *(if any)*

Dr. H.L. Cooper, NIC; Dr. Abbruzzese, 1st Medical School, Univ. of Naples, Italy.
Dr. B. Ganem, Dept. Chemistry, Baker Lab., Cornell University, Ithaca, NY
Dr. J. Gorman, CSIRO, Australian National Lab., Geelong, Australia

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.65	3.41	1.24

CHECK APPROPRIATE BOX(ES)

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

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

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. Studies are underway to relate the structure of hypusine to the function of eIF-4D and to understand the posttranslational control of hypusine biosynthesis.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00433-01 LOBP

PERIOD COVERED

March 15, 1987 to September 30, 1987

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 professionel personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Research Chemist	LOBP NIDR
OTHERS:	Wahl, S.	Microbiologist	LMI NIDR
	Ohura, K.	Visiting Fellow	LMI NIDR

COOPERATING UNITS *(if any)* N. Goldman, K.D. Jones, Division of Biochem. and Biophys., FDA: S. Futaki, N. Fujii, H. Yajima, Faculty of Pharmaceutical Science, Kyoto University, Kyoto, Japan.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

C-reactive protein (CRP) is the prototypical acute phase protein; serum levels of CRP may increase as much as 2000-fold during the early stages of the inflammatory response. Although CRP was discovered in 1930, the function of this protein is not known. This project is directed toward an understanding of the function of CRP in order to learn how the body responds to inflammatory stimuli.

A. Peptide synthesis - The primary structure of human CRP reveals three peptide sequences that may cause immunomodulation. Three peptides corresponding to three sequences were synthesized and studied for immunomodulating activity.

B. Immunomodulating assays - Assays to measure monocyte chemotaxis, superoxide production and IL-1 production were employed to test the activity of the CRP peptides.

The significance of the project lies in the identification of new mediators of inflammation.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00434-01 LOBP

PERIOD COVERED

March 15, 1987 to September 30, 1987

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 LOBP NIDR

COOPERATING UNITS *(if any)*

H. Golding, NIC, NIH; B. Golding, F.T. Gates, W. Lindner, DBB, FDA

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.65	.35	.30

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

HIV-1 is the causative agent of AIDS. We have searched the available data bases for homology which may exist between the gp160 envelope protein of HIV-1, and proteins from man. The approach was taken to learn whether HIV-1 is acting as if it is a component of a host protein.

A. Peptide synthesis - Peptides having homology between HIV-1 and a human protein, MHC Class II, were synthesized and conjugated to carrier proteins for immunization purposes. Fluorescene-conjugated peptides was prepared for visualization purposes and peptide-Sepharose conjugates were made for receptor isolation purposes.

B. Receptor studies - Attempts are being made to isolate a receptor from CEM cells to which both MHC Class II and HIV-1 are believed to bind.

The significance of this project lies in gaining an understanding of how HIV-1 binds to host cells. This may allow the targeting of anti HIV-1 drugs to specific cells which are vulnerable to HIV-1 and may identify potential targets for vaccine development

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00437-01 LOBP

PERIOD COVERED

March 15, 1987 to September 30, 1987

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 LOBP NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.52	.10	.42

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 of interest 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 on 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. These types of polymers may constitute a new generation of vaccine candidates because there would no longer be a need for the carrier proteins. Such vaccines of highly defined chemical composition may be specific for a certain pathogen, without the side effects that often occur using the peptide-carrier protein approach.

A. Peptide synthesis - Various test peptides are synthesized and polymerized for immunization purposes. Parallel to this the same peptides are coupled to carrier proteins, such as bovine serum albumin.

B. Immunization studies - Rabbits are immunized with peptide polymers or peptide carrier protein conjugates and the antibody responses are evaluated using enzyme linked immunosorbant assays.

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

ANNUAL REPORT OF THE LABORATORY OF ORAL MEDICINE,
NATIONAL INSTITUTE OF DENTAL RESEARCH

Over the last year the emphasis in the laboratory has been on herpes simplex virus, autoimmunity, and endocrine disorders. As in the past the program is disease oriented and highly interdisciplinary. The laboratory is made up of investigators who have training in a variety of disciplines including virology, immunology, molecular biology, pathology and clinical medicine and dentistry.

The trend to use molecular biological tools to study various diseases continues and molecular biological techniques are being applied to almost every phase of our work. Over the last couple of years new methods have been developed or set up in our laboratory for making human monoclonal antibodies; identifying specific cell types by use of the fluorescence-activated cell sorter; preparing and screening expression libraries for autoantigens; isolating, cloning and sequencing hormone receptors; engineering cells to express specific antigens on their surface by transfection; and making transgenic mice. Our long-term interest in insulin-dependent diabetes mellitus continues with emphasis on autoimmunity and how viruses might trigger an autoimmune response. This work which was initiated to study autoantibodies in diabetes has led to new insights into autoimmunity in general and the nature of the human B lymphocyte repertoire.

Again this year a number of new techniques were introduced into the laboratory (see below) which are making it possible to do experiments that were undreamed of just a few years ago. These rapid changes in technology mean that we must constantly retrain the people in our laboratory and recruit new people who have had exposure to these latest techniques. We now have a number of highly trained people in our laboratory. Moreover, the capability within NIDR to make both synthetic oligonucleotides and peptides, together with the know-how within the Institute to use the fluorescence-activated cell sorter and to make transgenic mice should enable us to approach some very fundamental and exciting problems in biology.

Since last year, a number of new techniques were introduced into the laboratory and existing ones modified. These include: (1) In situ hybridization of tissue sections and cells with ^{35}S-labeled RNA and DNA probes to detect viruses; (2) Techniques for studying aberrant expression of HLA antigens; (3) Detection of EBV receptors by use of biotinylated virus; (4) Labeling of small DNA probes using random oligonucleotide primers and DNA polymerase; (5) Direct sequencing of double stranded plasmids using a two-enzyme dideoxy-sequencing protocol; (6) Cloning with the pGEM-blue vector which allows direct color selection of plasmids containing inserts; (7) Purification of lambda phage DNA using lambdasorb immunoadsorbent technique; (8) DNA transfection by electrophoration; (9) Modification of methods to detect and clone oncogenes from human endocrine tumors; (10) Cloning and characterization of autoantigens by immunoscreening of cDNA expression libraries; (11) Detection of binding of chemical carcinogens to DNAase I hypersensitive regions by probing the blots of genomic DNA with oncogenes; (12) Hybridization with DNA from human-rodent somatic cell hybrids containing individual human chromosome

for chromosomal mapping of newly cloned genes; (13) In situ agarose gel RNA hybridization; (14) Biotinylated enzymes as probes for enzyme receptors; (15) Production of stable cell lines for making human monoclonal antibodies by fusing EBV-transformed cells with heteromyeloma cells.

The laboratory is involved in a number of collaborative projects including: (1) Long-term prospective study on newly-diagnosed insulin-dependent diabetes mellitus (Mt. Sinai Hospital, New York City); (2) Involvement of viruses in Sjogren's syndrome (CIPCB, NIDR); (3) Role of viruses in rheumatoid arthritis and polymyositis (NIAMS); (4) Receptors for clinical isolates of EBV (DV, CDB); (5) Collagen gene expression in transgenic mice (LDBA, NIDR); (6) Detection and cloning of oncogenes from human endocrine tumors (BRP, Littonbionetics, NCI); (7) Cloning and characterization of autoantigens (LBM, NIDDK and CGS, LB, NCI); (8) Oncogenes in chemical transformation (LEP, NCI); (9) Characterization and chemical localization of cDNA encoding brain amyloid of Alzheimer's disease (LCNSS, NINCDS, CGS, LB and LEP, NCI); (10) Detection of islet cell autoantibodies using two dimensional Western blots (LCS, NIMH); (11) POMC gene expression in lymphoyctes (LIR, NIAID); (12) Effect of corticotropin releasing hormone on POMC gene expression in human lymphocytes (LCI, NIAID); (13) Measurement of ACTH in human lymphocytes (Georgetown University, Washington, DC); (14) Preferential usage of Ig gene families by autoantibodies (LG, NCI); (15) Involvement of lymphotropic viruses in autoimmunity (LCTB, NCI); (16) Studies on double recombinant vaccine in vaccinia vector for HSV and influenza (LVD, NIAID); (17) Factors affecting reactivation of HSV (LCI, NIAID); (18) Clinical studies on epithelial irritants which reactivate HSV and agents that block reactivation (UCLA, Los Angeles, and University of Utah, Salt Lake City); (19) Characterization of islet cell antigens (University of Pennsylvania, Philadelphia); (20) Slow viruses and diabetes (CNSS, NINCDS); (21) Molecular mimicry as a mechanism for triggering autoimmunity (Scripps Clinic and Research Foundation, La Jolla, CA); (22) Long-term complications of EMC virus-induced diabetes (LDBA, NIDR); (23) Effect of interferon on the expression of IA antigens (DB, NCI); (24) Production of human monoclonal antibodies (Cetus Corporation, Palo Alto, CA); (25) Human monoclonal antibodies to rabies virus (The Wistar Institute of Anatomy and Biology, Philadelphia); (26) Human monoclonal antibodies to HIV (LTCB, NCI); (27) Studies on human Ig genes (University of Texas, Dallas); (28) Characterization of rheumatoid factor producing clones (Scripps Clinic and Research Foundation, La Jolla); (29) Characterization of human monoclonal antibodies to thyroglobulin (Mt. Sinai School of Medicine, New York); (30) Characterization of subsets of human lymphocytes that make autoantibodies (The Wistar Institute of Anatomy and Biology, Philadelphia); (31) Characterization of EBV receptors (DCT and MB, NCI and DBB, FDA).

Some of the more important findings since last year's annual report are summarized below:

I.) HERPES SIMPLEX VIRUS (HSV) AND OTHER PERSISTENT VIRAL INFECTIONS

1. Studies have continued on the molecular basis of HSV latency. Specifically we have attempted to identify latency-associated genes, that is, viral genes continuously expressed during latency. Radioactively labeled cDNAs were prepared by using as the template poly(A)$^+$ mRNA from

trigeminal ganglia of mice latently infected with herpes simplex virus (type 1). These cDNAs were used as hybridization probes for Southern blots of cloned HSV type 1 DNA fragments. Specific hybridization to fragments from the terminal repetition of the L segment was detected with probes derived from mRNAs obtained as early as 3 weeks and as late as 17 months postinoculation. Fine mapping of the region of hybridization showed that the viral transcripts originated from DNA sequences coding for the immediate-early gene IE-1 (alpha-0). At present, we cannot determine whether this transcript is indeed IE-1 mRNA, or the product of a colinear gene or an anti-sense IE-1 mRNA. IE-1 is an immediate-early polypeptide that acts synergistically with another immediate-early viral gene, IE-4, in the transactivation of the early and late viral functions. It is attractive to hypothesize that latency results from a neuron-specific uncoupling of this synergistic interaction.

2. Previously we described the development of a recombinant vaccinia virus vaccine expressing the type 1 glycoprotein gD gene of HSV. We showed that mice immunized with this vaccine were protected against both lethal and latent challenge with HSV-1 for at least 6 weeks after immunization. Over the last year we have completed our studies on the duration of immunity and the effect of booster immunization. We conclude that vaccination with vaccinia/gD produces immunity against HSV-1 lasting over one year, that this immunity can be increased by a booster with vaccinia/gD, but that prior immunization with a vaccinia recombinant virus expressing a non-HSV gene reduces the levels of neutralizing antibody and protective immunity that develops after boosting with vaccinia/gD. The demonstration that the magnitude of the immune response to gD (in animals immunized with a vaccinia/gD recombinant) depends on whether the animal has been previously exposed to vaccinia and/or primed with gD is of importance not only in developing a vaccination strategy for HSV, but has implications for other vaccines in which vaccinia is the vector.

3. Over the last year we have prepared and tested a new recombinant HSV vaccine. This one expresses the glycoprotein gB gene of HSV. This gene was isolated and modified at the 5' end by *in vitro* oligonucleotide directed mutagenesis. The modified gB gene was inserted into vaccinia and expressed under the control of a vaccinia virus promoter. The mature gB glycoprotein produced by the vaccinia virus recombinant was glycosylated, expressed at the cell surface and was indistinguishable from authentic HSV 1 gB in terms of electrophoretic mobility. Mice immunized intradermally with this recombinant vaccinia virus produced gB specific neutralizing antibodies and were resistant to a lethal challenge with HSV 1.

4. Previously, we developed a human model for studying the reactivation of HSV. Patients who have recurrent HSV are treated with ultraviolet light at site of earlier reactivations. Recurrences develop at the site of UV-exposure in about 60% of the cases. Using this model, a double blind study was initiated to examine the ability of agents such as acyclovir to block the UV-induced reactivation. Over 25 patients are now in the study and it is anticipated that the code will be broken within the next six months. In addition, a new study examining the ability of daily oral acylovir to block recurrences of herpes labialis is underway.

5. The laboratory's long-term interest in lactic dehydrogenase virus (LDV) was re-kindled over the last couple of years. This virus has certain biological counterparts to the AIDS virus (HIV) in that it produces a life-long infection, replicates in macrophages, and produces a number of immunological abnormalities. But, perhaps, even more intriguing is its capacity to raise enzyme levels in the blood. Since its discovery, LDV has remained unique as a model of long-term enzyme elevation due to impairment of enzyme clearance. The work over the last year showed that mice inoculated with silica developed an increase in plasma lactate dehydrogenase (LDH) lasting for at least six months and that the enzyme elevation was due, at least in part, to impairment of enzyme clearance. The extent of the enzyme elevation was dependent on both the dose and route of silica administration and mice that had received both silica and LDV showed a more profound impairment of LDH clearance than mice that had received either silica or LDV alone. Moreover, by use of inbred strains of mice it was found that enzyme clearance was genetically controlled and rapid and slow enzyme clearers have been identified. Based on these and other studies we conclude that impairment of enzyme clearance may be a more important factor than previously suspected in regulating enzyme levels in various disease states. This work has now been submitted for publication.

II) AUTOANTIBODIES AND THEIR PROPERTIES

1. Human monclonal antibodies of the IgM, but not the IgG or IgA class, can be readily prepared by transformation of peripheral blood B lymphocytes with Epstein-Barr virus. This is primarily due to the predominance in the peripheral blood of B lymphocytes bearing surface μ- heavy chains and committed to the production of IgM. We have now succeeded in making human monoclonal antibodies of the IgG and IgA class by isotype specific selection. B lymphocytes bearing surface antigen receptors of the γ-, α- or μ- heavy chain isotype were isolated by fluorescence activated cell sorting from the peripheral blood of healthy subjects using labeled isotype-specific antibodies as probes. The sorted cells were then transformed with Epstein-Barr virus and found to produce antibodies of the predetermined isotype. Upon expansion, cell lines producing antibodies of the IgG, IgA and IgM class to thyroglobulin, insulin and tetanus toxoid were established. To increase their stability, these Epstein-Barr virus transformed B cells making antibodies were fused with a human-mouse heteromyeloma and cloned. By this procedure, it should now be possible to generate stable cell lines producing human monoclonal antibodies of the IgG, IgA and IgM class to both self and exogenous antigens.

2. B lymphocytes bearing the Leu-1 cell-surface antigen ($Leu\text{-}1^+$), the human equivalent of mouse $Ly\text{-}1^+$ B lymphocytes, have been detected in human peripheral blood, but there was little information on their frequency and properties. This last year by analysis with the fluorescence-activated cell sorter and double immunofluorescence, we showed that $Leu\text{-}1^+$ B cells are consistently present in the peripheral blood and spleens of healthy subjects and constitute 17.0 $\pm$ 5.0% (mean value $\pm$ standard deviation) and 17.3 $\pm$ 3.9%, respectively, of total B cells. When purified $Leu\text{-}1^+$ and $Leu\text{-}1^-$ B lymphocytes were transformed into immunoglobulin-secreting cells by infection with Epstein-Barr virus and the culture fluids were tested for reactivity with self-antigens, at least two important autoantibodies, antibody to the Fc fragment of human immunoglobulin G (rheumatoid factor)

and antibody to single-stranded DNA, were found to be made exclusively by Leu-1$^+$ B cells. It is concluded that the Leu-1$^+$ lymphocytes represent a major subset of the normal human B cell repertoire and include the B cells capable of making autoantibodies similar to those found in systemic lupus erthyematosus and rheumatoid arthritis.

III) EXPRESSION OF AUTOANTIGENS

1. Sera from patients with Graves disease was used to screen a cDNA thyroid expression library prepared in λgt11. By this technique clones expressing autoantigens were isolated. Detailed experiments revealed that some of those clones were expressing the gene for the thyroid-stimulating hormone (TSH) receptor. Full length clones are now being obtained, sequenced and chromosomal location determined. This is the first isolation of the TSH receptor and should provide new insight into Grave's disease and the biology and physiology of the TSH receptor.

2. The isolation of the TSH receptor gene is making it possible to analyze this gene for polymorphism. Normal human DNA was studied for restriction fragment length polymorphism by digestion with 14 different restriction enzymes. Polymorphism has been found with three of these enzymes. DNA from patients with various diseases will now be analyzed by the same technique to look for alterations in their TSH-receptor gene.

IV) TRIGGERS OF AUTOIMMUNITY

1. The possible role of viruses as triggers of autoimmunity and the mechanisms by which this may occur has been studied in our laboratory over the last several years. One such mechanism is molecular mimicry. The idea is that antibodies raised against certain viruses cross-react with normal tissue. Previously we showed that of 600 monoclonal antiviral antibodies made against 11 different viruses, approximately 4% reacted with normal tissue. One of these monoclonal antibodies against HSV also reacted with antigens in perfectly normal pancreatic islets of Langerhans. Specifically, the monoclonal antibody reacted with beta cells, not alpha or delta cells. Further studies showed that the antigen was not insulin. The nature of this beta cell-specific, non-insulin antigen, is thus of great interest. We, therefore, made a beta cell expression library in λgt-11 and screened this library with our monoclonal antibody. By this procedure we succeeded in isolating several colonies expressing this antigen. These colonies are now being cloned and the gene and antigen studied.

2. Another way by which a virus might trigger an autoimmune response is by stimulating the expression of antigens which are normally not expressed on cells. We have found high levels of HLA DR antigens in salivary glands from patients with Sjogren's. The association between aberrant HLA expression and viral infections, in particular EBV, is now being studied. In order to identify viruses that might be involved, we have set up in our laboratory the *in situ* hybridization technique in which molecular probes for a wide variety of viruses including EBV, CMV, HTLV-I, HTLV-2, HIV, HBLV, Rubella, caprine arthritis virus and parvovirus B19 have been prepared. These probes will be used to screen tissues not only from patients with Sjogren's, but also from patients with other autoimmune diseases.

3. A model for studying the immune response to specific viral antigens inserted into the membrane of insulin-producing cells was developed. The gene coding for HSVgD was placed under control of the insulin promoter and this construct was introduced by transfection into rat insulinoma cells (RINm5F). Stable cell lines expressing gD on their surface were selected and cloned. Despite the presence of gD on their surface, the cells continued to produce insulin. When antibody to HSV was added to the cultures, it bound to the surface of the cells and in the presence of complement destroyed them. It should now be possible to study at the molecular level the immune to beta cells expressing viral antigens.

4. In humans, there have been sporadic reports, primarily circumstantial, that viruses might be involved in some cases of thyroiditis. Our earlier studies showed that in mice, reovirus type 1 infects beta cells in the pancreas and growth hormone containing cells in the anterior pituitary. The infection results in a mild and transient form of diabetes, retardation of growth, and autoantibodies to insulin and growth hormone. Treatment of the infected mice with anti-lymphocyte serum or cyclophosphamide prevented the development of diabetes and the retardation of growth, arguing that an autoimmune component plays a major role in the pathogenesis of this polyendocrine disease. Our current studies show that mice infected with reovirus type 1 also develop a mild thyroiditis characterized by focal destruction of acinar tissue, infiltration of inflammatory cells and autoantibodies to thyroglobulin and microsomal antigens. Thyroid involvement appears to be part of a generalized virus-induced polyendocrine disease. These and other studies argue that viral infections should be added to the possible list of triggers of thyroiditis.

5. The transgenic mouse system is a powerful tool for studying the expression and regulation of foreign genes. During the past year we established transgenic mouse lines carrying the HSVgD gene. Two constructs were prepared: one with gD gene under control of the rat insulin promoter (pIPEgD) and the other with gD under control of the SV 40 promoter (pSVgD). These have been microinjected into mouse embryos. Four pIPEgD transgenic mouse lines and five pSVgD lines have been obtained and are either at the stage of homozygosity, or close to it. Over the next year we will determine whether pIPEgD is specifically expressed in the islets and whether the animals develop immunologically-induced diabetes. Also the effect of introducing a foreign gene (HSV/gD) in the embryo on the development of immunologic tolerance will be evaluated. Transgenic mice with a variety of other constructs are now being made.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-00421-02

PERIOD COVERED
October 1, 1986 - September 30, 1987

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)*

J.F. Rooney	Sr. Staff Fellow	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
A. Puga	Expert	LOM, NIDR
K. Salata	Staff Fellow	LOM, NIDR
A.C. Matthews	Guest Worker	LOM, NIDR
H. Nakayama	Visiting Fellow	LOM, NIDR

COOPERATING UNITS *(if any)*
Laboratory of Viral Diseases, NIAID
Laboratory of Clinical Investigation, NIAID
Laboratory of Infectious Diseases, NIAID

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.33	3.04	3.29

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.)*

Continuing studies on the molecular biology of latent herpes simplex virus (HSV) infection in nerve ganglia have demonstrated a polyadenylated viral transcript which appears to be expressed throughout the period of latent infection. This viral transcript was accurately mapped to a region of the HSV genome where only one other gene, the immediate early 1 gene, is known to map. This important finding extends our knowledge of the state of the virus during latency and allows formulation of a hypothesis for the molecular basis of latent HSV infection.

Ongoing studies in mice with a recombinant vaccinia virus vaccine for herpes simplex have demonstrated that immunity against HSV is increased by a booster dose of the vaccine but decreased by previous exposure to vaccinia. A newly constructed double recombinant vaccinia vaccine expressing antigens from both HSV and influenza A was tested in mice and induced antibodies and protection against challenge with either influenza A or HSV.

Clinical studies examining the ability of acyclovir to prevent ultraviolet light-induced reactivation of HSV have continued and a new study examining the ability of daily oral acyclovir to block recurrences of herpes labialis was initiated.

Previous studies with lactate dehydrogenase virus have indicated that impairment of enzyme clearance is a more important factor than previously suspected in the regulation of serum enzyme levels. Studies over the past year have examined an important step in enzyme clearance, and the binding of enzymes and other proteins by macrophages. A technique for measuring enzyme binding was established and studies examining the binding of lactate dehydrogenase and other proteins were initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-00422-02

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

A. Puga	Expert	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
M.I. Lerman	Expert	LOM, NIDR
J. Srinivasappa	Visiting Scientist	LOM, NIDR
Y. Gong	Visiting Associate	LOM, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.89	2.04	2.85

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.)*

To determine if novel specific oncogenes are associated with human neuroendocrine malignancies (apudomas), we are studying three of these tumor types with varying metastatic potential: insulinoma, medullary thyroid carcinoma, and choriocarcinoma. To detect the presence of more than one oncogene in each tumor, a sib selection step was added to the recently developed bioassay based on co-transfection of tumor DNA with a selectable marker gene (neo) followed by tumorigenesis testing in nude mice. Primary nude mouse tumors were obtained from each type of human apudoma. All primary tumor DNAs were tested for the presence of human alu repeat sequences and selected DNAs are currently undergoing a second round of transfection and tumorigenesis testing. Establishing the identity of oncogenes in these three tumor types will reveal if any common oncogenes operate in apudomas and if additional oncogenes are associated with metastatic potential. Further studies are underway to establish human pancreatic beta cell lines by transfection and microinjection of oncogenes. Growth factor supplemented serum free medium is also being used to stimulate long-term growth of primary islet cell cultures. Human pancreatic beta cell lines will be invaluable for assessing the immunological status of IDDM patients.

Further studies on the mouse model of Reovirus type 1 induced polyendocrine disease have focused on the associated thyroiditis. In addition to the previously reported transient diabetes and growth retardation, infected mice develop mild thyroiditis characterized by focal destruction of acinar tissue, infiltration of inflammatory cells and the presence of autoantibodies to thyroglobulin and microsomal antigens. Thyroid envolvement appears to be another manifestation of the generalized virus-induced polyendocrine disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-423-02

PERIOD COVERED
October 1, 1986 - September 30, 1987

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

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

B.S. Prabhakar	Microbiologist	LOM, NIDR	A.B. Hartman	Guest Worker	LOM
P. Casali	Visiting Scientist	LOM, NIDR	W.L. Jackson	Staff Fellow	LOM
J.W. Abramczuk	Visiting Scientist	LOM, NIDR	M.I. Lerman	Expert	LOM
A.L. Notkins	Medical Director	LOM, NIDR	M. Nakamura	Visiting Fellow	LOM
G.P. Allaway	Visiting Fellow	LOM, NIDR	H. Nakayama	Visiting Fellow	LOM
J.P. Coutelier	Int. Res. Fellow	LOM, NIDR	E.L. Oates	Staff Fellow	LOM
S. Burastero	Visiting Fellow	LOM, NIDR	K. Salata	Staff Fellow	LOM
I. Goldfarb	Visiting Associate	LOM, NIDR	M. Shibata	Visiting Fellow	LOM

COOPERATING UNITS *(if any)*
Mt. Sinai School of Medicine, NY, NY
Cetus Co., Palo Alto, California
NIADDK and NIAMS, NIH, Bethesda, MD

J. Srinivasappa	Visiting Assoc	LOM
Y. Uchigata	Visiting Assoc	LOM
A.A. Vivino	Staff Fellow	LOM

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
21.34	14.13	7.21

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.)*

Sera from patients with Grave's disease have been used to isolate autoantigens from a thyroid cDNA expression library. Thus far three clones for thyroid antigens have been isolated. One of these clones appears to be the receptor for thyroid stimulating hormone. Work on this clone in the areas of cDNA sequencing, gene structure, chromosomal location, tissue expression and in the development of assays for autoantibodies is in progress. Several potential autoantigen clones have recently been isolated from human pancreatic islet cell expression library using diabetic patient sera.

Restriction fragment length polymorphism in the gene that encodes for thyroid stimulating hormone (TSH) receptor has been demonstrated in DNA samples obtained from normal individuals. This polymorphism is thought to be due to different alleles. Studies are underway with DNA from patients with thyroid diseases.

Using fluorescence activated cell sorter B cells that are reactive with biotinylated self and non-self antigens (e.g., thyroglobulin, insulin, tetanus toxoid, etc.) were selected and immortalized by transformation with Epstein-Barr virus. Stable cell lines have been established by fusing these cells with a mouse-human heteromyeloma. Using limiting dilution analysis and EBV transformation of B lymphocytes cell lines capable of making human monoclonal antibodies to HIV and rabies virus have been established.

Anti-idiotypic antibodies made against a panel of human monoclonal autoantibodies were used to quantitate autoantibody associated idiotopes in patients' sera with diabetes mellitus, Hashimoto's thyroiditis, systemic lupus erythematosus and normals. Our studies showed that these idiotopes are not only expressed on autoantibodies but also on immunoglobulins with unrelated specificities.

ANNUAL REPORT OF THE NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH
NATIONAL INSTITUTE OF DENTAL RESEARCH

The Neurobiology and Anesthesiology Branch is concerned with the study of oral-facial sensation, with particular emphasis on mechanisms of pain and the development of new methods for controlling pain in humans. The Branch utilizes a multidisciplinary approach that includes anatomical, pharmacological, biochemical, physiological, behavioral and psychophysical techniques to study neural function as it relates to the processing of sensory signals about tissue damage. Correlative approaches are often used to answer questions about the functional organization of nociceptive systems in normal and pathological states. The clinical component of the Branch develops new methods for measuring and assessing experimental and clinical pain and applies these methods to the study of mechanisms of acute and chronic pain in humans as well as to the evaluation of new techniques for the treatment of pain.

This year we have continued to emphasize the role of neurochemical messengers in the organization and function of pathways in the brain involved in transmitting information about pain. Our previous studies of the organization of these systems in normal states has provided a foundation for recent studies on endocrine and neural function following the development of tissue inflammation and after nerve injury. We have developed and utilized new models of peripheral tissue inflammation and nerve injury and have focused on changes occurring at the site of inflammation as well as in the central nervous system. Our animal studies provide a conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions. In our correlative studies of behavior and neural function in awake monkey, we have examined the role of the cerebral cortex in pain transmission in the normal state.

The Branch continues to coordinate the activities of the multi-institute collaborative program on clinical pain research at the NIH. This includes collaborative studies with staff of a number of other institutes including NCI, NIMH, NHLBI, the Clinical Center, and the Naval Medical Center.

Investigators in the Branch continue to receive considerable recognition for their research accomplishments. Ten scientists in the Branch are invited speakers or are organizing workshops and symposia at the Fifth World Congress on Pain held in Hamburg in August. These invitations attest to the leadership role the Branch plays in the field since this is the major international triennial meeting in the field of pain research. Dr. Dubner was selected as the chairman of the Scientific Program Committee for this meeting and Dr. Ruda, as a member of this Committee, has organized luncheon workshops. Dr. Ruda also is a member of the Scientific Program Committee of the Society for Neuroscience, the major domestic society devoted to the study of brain function. Dr. Bennett was invited to be a member of the organizing committee for a NATO Advanced Research Workshop on the spinal cord to be held in Spain in the Spring of 1988. Dr. Max continues to demonstrate his expertise in the field of cancer pain as a consultant to the WHO Cancer Pain Relief Program. Dr. Dubner was elected President of the American Pain Society for 1988 and was appointed to the Board of Directors of the International Pain Foundation.

Research acomplishments of the Branch are presented in detail below.

Neurochemistry of Nociceptive Pathways in Normal and Pathological States

With the use of immunocytochemical methods, retrograde markers, and techniques from molecular biology, we continue to determine the neurochemical mediators associated with the major components of the medullary and spinal dorsal horns: the primary afferents, the intrinsic dorsal horn neurons, and extrinsic afferent inputs originating from distant sites. This year we have continued to study the role of calcitonin gene-related peptide (CGRP) in dorsal horn function. CGRP is a 32 amino acid peptide that is found in highest density in those spinal dorsal horn laminae associated with nociception. A spared-root model consisting of one intact spinal dorsal root, flanked by 2-3 severed dorsal roots, is used to examine CGRP and SP immunoreactive axons in the denervated segments. Since CGRP appears to arise exclusively from primary afferents, the co-localization of CGRP and SP to the same axons can be used to identify SP primary afferent axons.

Neonatal capsaicin, a selective neurotoxin, has been used to destroy small primary afferents and to examine the effects of such lesions on nocifensive behavior and neural plasticity following neonatal nerve lesions. Neonatal capsaicin administration decreases levels of CGRP and SP and also alters the responsivity to some nociceptive stimuli. Using the hot-plate as a test of nocifensive behavior, it was found that hot-plate latencies increased dramatically in capsaicin-treated rats as compared to controls at 8 and 12 weeks of age. At 16 weeks, however, the latencies had returned almost to control levels. A reduction in CGRP and SP immunoreactivity in laminae I,II and V of the lumbar spinal cord was visible as early as 10 days after capsaicin administration. At 12 and 16 weeks the reduction in CGRP was less evident in the superficial laminae and there was an increase in CGRP axons migrating dorsoventrally through laminae III and IV. In contrast, there was no apparent increase in SP immunoreactive axons in laminae III and IV. These data indicate that there is a time-dependent change in thermal sensitivity after neonatal capsaicin administration and that the change may be related to alterations in CGRP content in primary afferent axons.

Immunocytochemical methods in combination with retrograde tracer methods were used to examine the presence of neuropeptides in long ascending somatosensory pathways. In continuing studies of the lamina I spinomesencephalic tract, we have found that there is a large population of lamina I neurons in the rat with projections through the dorsolateral funiculus to the midbrain. A subset of this population also sends collaterals to the thalamus. Separate populations of retrogradely labelled cells contributing both to the spinothalamic and the spinomesencephalic tract were identified as containing enkephalin (ENK), dynorphin (DYN), cholecystokinin (CCK) or vasoactive intestinal polypeptide (VIP). DYN- and ENK-containing cells projected to the medial thalamus, whereas CCK- and VIP- containing cells projected to the lateral thalamus. In all cases, the number of peptide-containing cells with thalamic projections made up only a small percentage of the total number of immunocytochemically-labelled cells. The role of peptide-containing projection neurons needs to be elucidated in future studies.

This year we have continued to study the effects of peripheral tissue injury and inflammation. Inflammation is a common component of many acute and chronic pain conditions including postsurgical acute pain, cancer pain and arthritis. We have developed two models of inflammation. An acute carrageenan model of inflammation mimics acute pain produced by tissue damage from surgery. This model allows us to directly relate animal findings to our clinical model of postsurgical pain following the extraction of impacted third molars (see below). The carrageenan model is highly predictive for detecting drugs that possess analgesic and anti-inflammatory activity in humans and this is an appropriate model to parallel the clinical studies. With this model we have examined endocrine and neural changes at the site of peripheral inflammation and have correlated these changes with behavioral hyperalgesia and tissue components of the inflammatory process. In a second model of inflammation, we have looked at changes in spinal dorsal horn neuropeptides associated with a more persistent hyperalgesia lasting at least 5 days. This model may be useful in examining neural changes associated with more persistent models of pain in humans such as cancer pain and arthritis.

A major finding has been the demonstration of a peripheral pharmacologically-specific analgesic action of opiates in the carrageenan model. Carrageenan, a polysaccharide, was injected into the plantar surface of both rat hindpaws and hyperalgesia was measured by exposing the paws to a beam of radiant heat. Paw withdrawal latencies were an index of the nociceptive threshold. Hyperalgesia as measured by the paw withdrawal latencies occurred within two hours. At one and one-half hours after carrageenan, one paw was injected with an opiate drug and the other paw received saline. At three hours, the paw withdrawal latencies of those animals receiving peripheral injections of opiates were significantly greater as compared to the contralateral control. Animals given systemic opiates showed no difference from saline controls. In another study, levorphanol in different dosages was given in the same model, and compared to saline or dextrorphan, its dextrorotary isomer, which is devoid of opiate activity. Levorphanol produced a dose-related blockage of carrageenan-induced hyperalgesia whereas dextrorphan was inactive. These studies indicate that opiates have a peripheral site of actions in inflamed tissue. These findings have direct therapeutic implications. When injected peripherally, opiates can be used in lower dosages, avoiding their undesirable side effects. Furthermore, opiates that do not cross the blood-brain barrier and therefore activate only peripheral opiate receptors, may prove to be effective analgesic agents lacking centrally-mediated side effects.

In related studies, we found that opiates such as morphine decrease edema and hyperthermia in the carrageenan model at the site of inflammation. These effects appear to be related to morphine's inhibition of plasma extravasation. The effects were stereospecific, and thus related to opioid receptor specificity, since levorphanol, and not dextrorphan, was effective. It is likely that this effect is at the peripheral site of inflammation, providing another opportunity to develop potentially clinically useful opiates that alter anti-inflammatory effects separate from pain.

The existence of a peripheral site of action for opiate-induced analgesia provides a potential target for circulating opioid peptides. Our previous studies indicate that endogenous opioid peptides increase significantly under conditions of stress and inflammatory pain. However, their physiological role is unclear. Our findings suggest that one potential target for these circulating opioids may be at peripheral sites of inflammation.

We have also investigated the role of neural mechanisms at the site of inflammation using the carrageenan model. Lesions of the sciatic and saphenous nerves in the rat partially block the development of carrageenan-induced hyperthermia and edema. This neurogenic component of inflammation appears to involve the release of Substance P, since administration of a Substance P antagonist produced a significant decrease in carrageenan-induced hyperalgesia. These results indicate that the peripheral nervous system serves not only to signal the presence of tissue damage, but via peripheral secretory mechanisms from peripheral nerve endings, promotes the development of the inflammatory response.

Inflammation also results in central nervous system changes that involve the activation of specific opioid peptides. We have shown that inflammation induced by Freund's adjuvant, yeast, phorbol ester, or carrageenan, results in significant increases in the dorsal horn content of the opioid peptide, dynorphin. This effect occurs within two days and is maximum at four days, and is localized to the segment of the dorsal horn receiving innervation from the inflamed paw. The effect lags behind the hyperalgesia which occurs within two hours and persists beyond the four day period. In order to determine whether the biosynthesis of dynorphin occurs at a time more closely associated with the hyperalgesia, we assessed the tissue content of messenger RNA coding for the prodynorphin precursor molecule, using RNA blot analysis. Prodynorphin mRNA content, shown with the Freund's inflammation model, was elevated 8 hours after the injection; by 24 hours it had increased by 200% and by day 5 by 650-800%. In contrast, proenkephalin mRNA showed only a small elevation of 50-80% between days 1 and 6. All changes were restricted to the side and segments of the spinal dorsal horn that innervated the inflamed limb. The actual location of the cells in the spinal dorsal horn exhibiting the increased biosynthesis of dynorphin was then determined using immunocytochemistry and in situ hybridization histochemistry. An increase in the transcription of prodynorphin mRNA and an increase in dynorphin peptide were identified in the medial half of laminae I and II and laminae V and VI lumbar segments receiving innervation from the inflamed limb. These findings demonstrate that an increase in dynorphin biosynthesis is associated with persistent inflammation and pain and occurs in those parts of the dorsal horn known to contain neurons involved in the transmission of nociceptive information. The future development of agents that act selectively at these sites may have unique efficacy in the control of pain associated with persistent inflammation such as in cancer pain and arthritis.

We have studied the physiology of the neurons in the superficial spinal dorsal horn in the same Freund's model of inflammation in order to examine changes in the physiological properties of the neurons associated with the behavioral hyperalgesia. The distribution of receptive field size for cells in

the superficial laminae of the dorsal horn in Freund's-treated rats included many larger receptive fields, often including the entire surface of the ipsilateral paw. Many cells exhibited complex responses including discontinuous fields, regions with different thresholds, and responses to deep structures or joint movement. Most cells demonstrated spontaneous activity. All of the above findings are rare in normal animals with uninflamed paws. These data provide evidence that a population of lamina I nociceptive projection neurons demonstrates increased activity in a model of inflammation and hyperalgesia. This increased responsiveness may be associated with a range of pain sensations, including pain in the absence of stimulation of the affected part, and referred pain.

In order to examine the effects of denervation on neuronal activity and behavior, we have developed a model of hyperalgesia and allodynia in rat. This is the first model in animals that appears to adequately mimic neuropathic pain in humans. The neuropathy is produced in rats by placing loose ligatures around the common sciatic nerve. The ligation produces a profound hyperalgesia to noxious heat that appears within 3 days and lasts for about 3 months. Responses to chemical irritants also indicate the presence of hyperalgesia. The majority of animals also showed allodynia, or a painful response to innocuous stimuli. Lightly touching the affected zone evoked nocifensive behavior. Contact with a chilled metal floor also produced behavior suggestive of pain. The animals also tended to guard the affected limb from contact. In addition, many of the animals had warm or cool skin on the nerve-damaged side. In summary, these symptoms of hyperalgesia, allodynia and changes in skin temperature, are characteristic of patients with causalgia with possible sympathetic nervous system changes. Our preliminary studies of the activity of single nerve fibers from the damaged nerve show a nearly complete loss of conduction in myelinated fibers distal to the nerve injury at postoperative intervals of 1-3 days. In contrast, a large majority of the unmyelinated fibers conducted impulses normally. Light and electron microscope observations confirmed these physiological findings. Neurochemical analyses have revealed depletion of Substance P and CGRP at day 10 which was maximal at day 20. Very small increases in the levels of enkephalin and dynorphin were seen around day 20, and studies with colchicine suggest that these increases occur in intrinsic neurons in the dorsal horn.

Rats that received injury to their sciatic nerve produced by tightly ligating a portion of it or by severing individual fascicles, showed similar behavioral changes of the experimental foot as in the model described above. Hyperalgesia was significant in rats with greater than 50% destruction or demyelination of the sciatic nerve.

Disease or trauma in human peripheral nerves sometimes produces a dysfunction of pain perception that includes hyperalgesia, allodynia and spontaneous pain. With few exceptions, painful neuropathies in humans are resistant to medical treatment. Our models are the first to produce neuropathology with behavioral changes in rats that mimics the human conditions. We, therefore, have an opportunity to investigate the mechanisms of such dysfunction and to develop potentially useful therapies.

Neural Mechanisms of Pain at Dorsal Horn and Cerebral Cortex Levels

In previous years we developed a thermal discrimination task to examine whether monkeys can make fine thermal discriminations in the noxious heat range. With this behavioral model, we studied the role of different neurons in the medullary dorsal horn in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. In addition, we employed this model to examine the effect of opioid drugs on the perceived intensity of such stimuli. This year we have continued these studies in two ways. First, we have studied the effect of noradrenergic systems on the perceived intensity of noxious heat stimuli in the task. Second, we have examined the role of the cerebral cortex in pain by studying the response properties of neurons in somatosensory cortex while monkeys performed this task.

Activation of descending noradrenergic systems exerts powerful inhibitory effects on the transmission of nociceptive information. We examined the effects of a selective alpha-2 agonist, ST-91, on the monkey's ability to perceive the intensity of noxious heat stimuli. ST-91 was directly microinjected into the medullary dorsal horn where the terminals of descending noradrenergic fibers are located. We found that ST-91 strongly attenuated the monkey's ability to perceive the intensity of noxious heat stimuli. These effects were dose and stimulus-intensity dependent. The detection of either visual or cooling cues was not modified by any of the doses of ST-91. These results show that ST-91 microinjected into the medullary dorsal horn impairs the detection of noxious heat stimuli without altering motor function, motivational or attentional variables. Future studies need to evaluate the pharmacological specificity of the effect and to evaluate the possibility of combining opiates and noradrenergic agonists in the control of pain.

The role of the primary somatosensory cortex in pain remains unresolved. We have attempted to determine its role in pain by two approaches; first, by recording from individual neurons while the monkeys were detecting small temperature changes in the noxious heat range, and second, by training monkeys to detect and discriminate noxious stimuli before and after the surgical ablation of the primary somatosensory cortex. Approximately one-half of the neurons studied increased their discharge in a graded fashion to increasing intensities of noxious heat. The remainder responded to noxious heat, but did not grade their responses in a systematic fashion. In a subpopulation of the neurons that graded stimulus intensity, the peak frequency of discharge was correlated with the monkey's speed of detection of the stimulus. We previously have shown that speed of detection is a measure of the perceived intensity of the stimulus. These findings support the conclusion that neurons in the primary somatosensory cortex are involved in the processing of sensory-discriminative information related to pain.

After bilateral removal of the primary somatosensory cortex, monkeys exhibited deficits in both detection and discrimination of noxious levels of thermal stimulation. These deficits persisted for more than one half a year in one monkey. In the other, there was a gradual recovery in its ability to detect noxious thermal stimuli. These deficits were independent of any

attentional, motoric or motivational effects since the monkeys' performance on similar visual and cooling tasks actually improved after the surgical ablation. We conclude that these studies support our electrophysiological findings that the primary somatosensory cortex plays a role in pain discrimination.

The Assessment of Experimental and Acute Clinical Pain

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. A new scaling method of pain assessment was developed last year that provides temporal information about the effects of analgesic agents on the intensity of painful stimuli. This year the sensitivity of the method was further assessed by presenting six random thermal staircases titrated at different levels of sensation including warmth detection, hot, pain threshold and three suprathreshold pain sensation levels. The six staircases stabilized at distinct non-overlapping levels with final temperatures varying from 39.5 (warmth) to 51.2 deg C. A second experiment examined the effects of placebo and three doses of fentanyl with this same method. Fentanyl attenuated thermal sensation and the effect was dose-dependent. The time course of analgesia onset also was dose-dependent. These studies provide further evidence of the sensitivity of this staircase method. The time course of fentanyl analgesia paralleled that found clinically, and the assessment of onset rate suggests that this procedure may be useful for the assessment of analgesia kinetics.

In another study, forty pain-free subjects participated in the assessment of first and second pain sensations before and after the double-blind administration of fentanyl or saline placebo. In comparison to placebo, fentanyl significantly increased the reaction time latencies to both first and second pain sensations. Previous results with chronic pain patients showed that morphine, but not deep brain stimulation, attenuated both first and second pain sensations evoked by brief, intense thermal stimuli. Thus, the morphine effect found with chronic pain patients represents a true opioid effect and not a spurious result or an effect of altered sensitivity resulting from the long-term presence of a clinical pain syndrome.

Assessment and Treatment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of pain patients, some of whom received chronic brain electrode implants for pain relief. This year only two patients particpated in preoperative evaluations. Morphine significantly decreased the magnitude of low back pain and of pain produced experimentally by heat applied to the skin. We plan to increase our observations of these patients receiving brain electrode implants by developing portable equipment that will allow us to observe the patients in their local environment rather than having them come to the NIH. Since most of the patients come from the West Coast, and many were not willing to come to the NIH, we should be more successful in recruiting patients for this study.

Some of our clinical trial studies evaluate mechanisms of pain and new treatment plans for neuropathy pain. Shingles pain is a neuropathy associated with sensory loss, hyperalgesia, allodynia and spontaneous pain. We are evaluating the effects of agents that increase the availability of serotonin or norepinephrine levels in the brain and thereby suppress the pain. This year we completed a study of amitriptyline and lorazepam in the treatment of shingles pain, or postherpetic neuralgia. Amitriptyline, but not lorazepam, was superior to placebo in relieving the pain. Pain relief increased with increasing drug dose and increasing serum amitriptyline levels. Careful assessment of patient's mood showed that only a minority of the patients were depressed. Depression was of mild severity even in those patients, indicating that pain relief was not mediated through the relief of depression. This and our previously reported study in diabetic neuropathy show that the analgesic effect is independent of effects on depression and that response increases with dose and serum drug levels through at least 150 mg. Conventional therapeutic doses for analgesia usually are lower than this and it has been suggested by others that increasing doses do not increase efficacy. Our studies suggest that analgesia can be influenced by increasing dose. There is a need to study other agents that may retain analgesic qualities at high doses without unwanted side effects.

We have surveyed pain in AIDS patients at the NIH. About one-third of patients reported pain during the previous week that was considered to be related to their disease, including lesions of Kaposi sarcoma, visceral opportunistic infections, and postherpetic neuralgia. Another one-third of patients had pain unrelated to AIDS. While reports of painful AIDS neuropathy are present in the literature, this condition will cause severe pain in only about 5% of AIDS patients, and usually occurs late in the course of the disease. Most current NIH protocols select patients early in the course of the illness.

The pain and dysfunction associated with the Myofascial Pain Dysfunction (MPD) Syndrome has been attributed to masticatory muscle spasm. We have evaluated the efficacy of an analgesic, anti-inflammatory agent (ibuprofen) and an anti-spasmodic and anxiolytic agent (diazepam) in the relief of MPD. Thirty-nine patients completed the study and significantly greater pain relief resulted from diazepam than ibuprofen or placebo. No significant changes were seen in depression or anxiety in comparison to baseline or between groups. These data indicate that diazepam results in relief of MPD in controlled trials and that anxiolytic drug therapy is effective for the relief of musculoskeletal pain.

Additional new studies recently approved will investigate the effectiveness of specific serotonin and noradrenergic agonists in neuropathic pain.

Mechanisms and Treatment of Acute Pain

We use an oral surgery model to evaluate novel agents, methods of administration, and drug combinations in comparison to standard analgesic therapy. This year we have evaluated the analgesic interaction of proglumide, an antagonist of the putative neurotransmitter cholecystokinin, and morphine.

The addition of 0.05 mg of proglumide significantly potentiated the effects of 4 mg or morphine resulting in a peak analgesic effect similar to 8 mg of morphine and a duration of action which surpassed all other groups. No difference was seen in the frequency of side effects or respiratory depression in the proglumide groups as compared to morphine. These data indicate that a low dose of proglumide potentiates and prolongs morphine analgesia without any increase in side effects. Future studies will investigate the interaction of proglumide with endogenous opioid peptides released following the stress and inflammation of oral surgical procedures. Additional new studies recently approved will investigate new analgesic agents active at the kappa opioid receptor and the effectiveness of drugs that alter bradykinin synthesis in reducing pain and inflammation.

PUBLICATIONS

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

October 1, 1986 - September 30, 1987

Dionne, R.A.: Peripheral pain mechanisms: Suppression of post-surgical dental pain with antiinflammatory analgesics. Anesth. Prog. (In press).

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Hargreaves, K.M., Schmidt, E.A., Mueller, G.P. and Dionne, R.A. Dexamethasone alters plasma levels of beta-endorphin and post-operative pain. Clin. Pharmacol. Therap. (In press) 1987.

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Mellstrom, B., Iadarola, M.J., Yang, H.-Y.T. and Costa, E.: Inhibition of met^{5}-enkephalin-arg^{6}-phe^{7} and met^{5}-enkephalin-arg^{6}-gly^{7}-leu^{8} induced antinociception. Eur. J. Pharmacol. 133:185-190, 1987.

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Nicoletti, F., Meek, J.L., Iadarola, M., Chuang, D.M., Roth, B.L. and Costa, E.: Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. J. Neurochem. 46:40-46, 1986.

Nicoletti, F., Barbaccia, M.L., Iadarola, M., Pozzi, O. and Laird, H.: Abnormality of alpha-adrenergic receptors in the frontal cortex of epileptic rats. J. Neurochem. 46: 270-273, 1986.

Nicoletti, R., Iadarola, M.J., Wroblewski, J.T. and Costa, E.: Excitatory amino acid receptors are linked to inositol phospholipid metabolism in rat central nervous system: Developmental changes and interaction with norepinephrine receptors. Proc. Natl. Acad. Sci. (USA) 83:1931-1935, 1986.

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Ruda, M.A., Bennett, G.J. and Dubner, R.: Neurochemistry and neural circuitry in the dorsal horn. Progress in Brain Res., 66:219-268, 1986.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-19 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Design and Computer Interfacing of Neurophysiologic Instrumentation

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

Brown, Frederick J. Electronic Engineer (Instru) NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology & Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

These projects involve the design and construction of electronic and electro-mechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory and central computer installations. Electronic circuit design, microcomputers, and assembly or machine language programming may be used in these instruments or interfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-13 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Pharmacological Modification of Neuroendocrine Responses to Surgical Stress

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

Hargreaves, Kenneth M.	Staff Fellow	NA NIDR
Joris, Jean	Visiting Fellow	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Flores, Christopher	Biologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

Jackson, William	Medical Staff Fellow	LOM NIDR
Schmidt, Elizabeth	Clinical Nurse	CC Nursing
Schafer, Susan	Clinical Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.65	2.30	.35

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 consists of two related lines of research evaluating 1) the neuroendocrine responses to surgical stress and inflammatory pain, as evidenced by measuring changes in circulating beta-endorphin (B-END) and bradykinin (BK), 2) the analgesic effects of prototypic and novel drugs as indicated by both behavioral effects and alterations in the activity of these substances. Stimulation of the pituitary-adrenal axis by corticotropin releasing factor (CRF) suppresses clinical post-operative pain, while inhibition of the axis by dexamethasone enhances post-operative pain. When further evaluated in animals, CRF analgesia is demonstrated to be due to secretion of pituitary B-END. Separate animal studies evaluated neuroendocrine responses to inflammation. Circulating levels of both BK and B-END significantly increase during inflammation. The hypothesis that blood-borne BK stimulates pituitary secretion of B-END is indirectly supported by observations that, 1) injections of BK into anesthetized rats stimulate secretion of B-END, and 2) that BK administered to pituitary cultures in vitro stimulates B-END secretion. Studies currently underway directly test this hypothesis. In addition, inhibition of BK synthesis blocks the development of inflammatory hyperalgesia in rats. Increased knowledge into the neuroendocrine responses to inflammatory pain should provide a logical avenue for the development of new types of analgesic drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-13 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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:	PROFESSIONAL:	OTHER:
1.20	.60	.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 objectives of this project are (1) to assess psychophysical methods of experimental pain measurement, i.e., magnitude estimation, category scaling, and cross-modality matching. Pain will be experimentally induced by electrocutaneous, electric tooth pulp, and mechanical heat stimulation; (2) to assess clinical pain measures, such as pain questionnaires and sensory matching methods, in a dental setting; (3) to determine the validity of experimental pain models by comparison of experimental and clinical pain responses; and (4) to evaluate known pharmacological and non-pharmacological pain-control agents.

The interactive computer-based staircase scaling method was used in three experiments. This method requires subjects to use a category scale to rate thermal stimuli applied to their volar forearm. Stimuli are associated with specific randomly presented staircases. Successive stimuli within each staircase are continuously adjusted to produce a preassigned level of verbal (e.g. mild, moderate, intense) responding.

The first experiment expanded the number of staircases from three to six to cover assessment of non-painful warmth sensations and a broad range of pain intensities. It successfully tracks the time course of thermocutaneous sensitivity from just-detectable warmth sensations (39 deg C) to very painful sensations (51 deg C).

The second experiment showed that both the magnitude of fentanyl analgesia and rate of analgesia onset were dose dependent. The most sensitive assessment was provided by intermediate stimulus temperatures.

The third experiment identified patterns of temporal suppression at near-threshold (45 deg C) stimulus temperatures. Subjects either showed a mild suppression of 0-1.5 deg C or a greater depression of 4-5 deg C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00246-10 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Evaluation of Pharmacological Management of Chronic Orofacial Pain

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

Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Tzukert, Arik	Visiting Scientist	NA NIDR

COOPERATING UNITS *(if any)*

Schmidt, Elizabeth A.	Clinical Nurse	CC Nursing
Lipton, James	Chief, PEC	OPEC NIDR

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.60	.45	.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 is attempting to evaluate pharmacological approaches to the clinical management of chronic facial pain and to develop better methods for its differential diagnosis. Three clinical trials have evaluated standard pharmacological agents for the management of three different variants of chronic facial pain: myofascial pain dysfunction (MPD), atypical facial pain (AFP) and headache. These studies seek to evaluate the efficacy of prototypic pharmacologic regimens under conditions of a controlled clinical trial.

Thirty-nine subjects completed the MPD study. Pain relief, as measured by the pain relief VAS, revealed significantly greater relief from diazepam than ibuprofen or placebo. No significant changes were seen in depression or anxiety in comparison to baseline or between groups. These data indicate that diazepam results in symptomatic relief of MPD when evaluated under conditions of a controlled trial, suggesting that anxiolytic drug therapy is effective for the relief of myofascial pain while NSAIDS have minimal therapeutic benefit when used alone.

Headache study: Interim analyses have not been completed.

APF study: Amitriptyline was more effective than placebo in reducing pain after four weeks of treatment. When patients were divided into depressed and non-depressed groups, based on their Hamilton depression scores, amitriptyline reduced pain in the depressed and in the non-depressed groups as compared to placebo. Amitriptyline reduced the depression scores in the depressed group but had no effect on the depression scores in the non-depressed group. These data suggest that amitriptyline is effective in the treatment of chronic oral-facial pain and that its efficacy is independent of its effects on depression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-09 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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:	PROFESSIONAL:	OTHER:
1.55	.85	.70

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The 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 experimentally induced pain will be assessed in pain-free volunteers.

The results from two patients this year continue to support previous findings from this project. Morphine significantly decreased the magnitude of both low back pain and experimentally administered heat pain and this effect was reversed by naloxone. Comparison of these results to those found after deep brain stimulation suggest that deep-brain stimulation analgesia is not opioid mediated and is either less potent than morphine analgesia or follows a different course.

A second study compared the influence of the narcotic fentanyl on reaction time measures to heat pain in normal subjects to results found previously with chronic pain patients. The results show that fentanyl decreases the magnitude of both first and second pain sensations in a manner similar to decreases found in chronic patients after administration of morphine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00286-08 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Experimental Therapeutics for Acute Pain

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

Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Lavigne, Gilles	Guest Researcher	NA NIDR
Costello, Ann	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

Schmidt, Elizabeth A.	Clinical Nurse	CC Nursing
Troullos, Emmanuel	Dental Staff Fellow	CIPC NIDR

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.55	1.30	.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 project is a series of clinical trials which are evaluating the efficacy and safety of experimental therapeutic agents used for the control of pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. Current studies are evaluating the interaction of proglumide and morphine for postoperative pain, the analgesic efficacy of spiradoline in comparison to morphine, the clinical efficacy of flumazenil for reversing the central effects of intravenous diazepam and the physiological effects of high doses of epinephrine-containing local anesthetics. 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 0.05 mg of proglumide was demonstrated to significantly potentiate and prolong the analgesic efficacy of 4 mg of morphine for post-operative pain. Higher doses of proglumide (0.5 mg and 5.0 mg) were without effect. These data thus provide indirect evidence for the hypothesis that cholecystokinin participates in the modulation of pain. Administration of relatively high doses of epinephrine in local anesthesia resulted in a greater than 25-fold increase in plasma epinephrine levels with elevated levels persisting throughout the 20 minute observation period. Results of this study confirm our previous observations and refute the traditional viewpoint that the concentrations of epinephrine in local anesthetics administered for dental procedures does not result in changes in blood levels or physiologic effects.

Taken together, the results of these studies are providing data to establish a rational basis for the pharmacologic management of acute pain and perioperative apprehension associated with minor surgical procedures in ambulatory patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-08 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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
Iadarola, Michael J.	Senior Staff Fellow	NA NIDR
Traub, Richard	Postdoctoral Fellow	NA NIDR
Cox, Marcella	Biologist	NA NIDR

COOPERATING UNITS *(if any)*

Hammond, Donna
G.D. Searle and Company
Chicago, Illinois

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.85	2.00	1.85

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 neuralgia. 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.

In situ hybridization histochemistry was used to identify dynorphin neurons in the spinal cord and chart their increased response in a rat model of peripheral inflammation and hyperalgesia. Autoradiographic localization of an oligodeoxyribonucleotide probe complementary to a portion of preprodynorphin mRNA, marked neurons in superficial dorsal horn laminae I and II and deeper laminae V and VI as responding to the peripheral inflammation by increased transcription. These data provide evidence for opioid modulation of nociceptive neural circuits in these two distinct spinal locations.

Developmental changes in primary afferent axons following neonatal administration of capsaicin, a selective neurotoxin, were examined. Initially, the thermal nociceptive threshold was significantly different in the capsaicin-treated animals as compared to controls. However, the threshold decreased between 8 and 16 weeks of age. This change was paralleled by an increase in CGRP immunoreactive axons in the dorsal horn. In contrast, a similar increase in SP immunoreactive axons was not recognized. These data indicate that there is a time dependent return of thermal sensitivity in capsaicin-treated rats between 8 and 16 weeks of age, and that the reduction in thermal nociceptive threshold may be related to alterations in CGRP immunoreactive primary afferent axons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-08 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Opiate Administration in the Medullary Dorsal Horn of the Behaving Monkey

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

Dubner, Ronald	Chief, NAB	NA NIDR
Anton, Fernand	Visiting Fellow	NA NIDR
Kenshalo, Jr., Daniel R.	Senior Staff Fellow	NA NIDR
Thomas, David Alan	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:	PROFESSIONAL:	OTHER:
1.50	.85	.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.)*

We examined the effects of ST91 microinjected into the medullary dorsal horn (MDH) on the ability of monkeys to detect temperature increases in the noxious heat range. Behavioral detection latency and the percentage of correct detections were used as measures of the perceived intensity of noxious heat stimuli. Three monkeys were trained to detect a change (T2) of 0.4, 0.6 or 1.0°C from a previous noxious heat level of 46°C (T1). Effects on attentional, motivational and motoric aspects of the monkeys' behavior were assessed by having them detect innocuous cooling and visual stimuli in tasks of similar difficulty. ST-91 (1, 3 and 10 micrograms) microinjected into the MDH produced a dose-dependent and stimulus-intensity dependent increase in the latency to detection of the T2 stimuli. There were no effects of ST-91 on the behavioral detection latencies to the innocuous cooling and visual stimuli, indicating that the effects of morphine were modality-specific and independent of changes in motivation, attention or motoric ability. These data demonstrate a pharmacologically-specific effect of alpha-2 receptor agonists on the perceived intensity of noxious heat stimuli at the earliest central relay pathway transmitting noxious information.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-06 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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
Thomas, David Alan	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanism Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.45	.15	.30

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 stimulis 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 no 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.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00366-05 NA

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analgesic Mechanisms in Patients with Chronic Pain

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

Max, Mitchell B.	Neurologist	NA NIDR
Gracley, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Kishorekumar, Ranganna	Medical Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*
Schafer, Susan, CC
Chang, Alfred, NCI

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.10	1.90	.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 project is to elucidate the principles of treatment of chronic pain syndromes that are considered resistant to conventional treatment, such as pain caused by peripheral neuropathy and nerve injury, and by advanced cancer.

Fifty-eight patients with post-herpetic neuralgia were treated with 6 weeks of amitriptyline, lorazepam, and/or placebo in a double-blind crossover study. Amitriptyline provided substantial relief in 1/3 of patients, and slight relief in another 1/3, while lorazepam and placebo did not relieve pain. The observation that only 1/4 of the patients were considered depressed by psychiatric interview, and little change in mood occurred during treatment suggests a specific pain-relieving effect for amitriptyline--i.e., analgesia was not mediated through the drug's antidepressant effect. In contrast to prevailing clinical anecdote, low doses of amitriptyline were less effective than higher doses through 150 mg/day; blood levels were also related to analgesia. Sedation and anti-cholinergic effects limited dosage, however, pointing the need for more specific and less toxic pain-relieving agents. Desipramine, a related tricyclic with fewer side-effects and a relatively specific action to promote central norepinephrine action, has been given to 20 patients thus far in a similar crossover placebo-controlled 6-week treatment. We have not yet broken the code, but dosages are 2-3 times as high as in the amitriptyline study, and more than half of patients are reporting substantial relief with one of the treatments.

A survey of 50 NIH patients with AIDS or related disorders showed that about 1/3 of the patients have pain related to AIDS, and another 1/3 have unrelated pain. Pain from HIV-related neuropathy is common in patients with advanced disease, who would benefit from more specific, nontoxic treatments, as discussed above; few of these patients are being seen yet at the Clinical Center.

Four other studies were approved by the IRB and will shortly begin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00377-04 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Role of the Primate Primary Somatosensory Cortex in the Detection of Noxious Temperature Increments.

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
Anton, Fernand	Visiting Fellow	NA NIDR
Chudler, Eric H.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David Alan	Psychologist	NA NIDR
Laylon, Lynette L.	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.10	2.45	.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.)*

The purpose of these studies is to determine the role of the primary somatosensory cortex (SI) in nociception. In the first study, we examined the response of nociceptive neurons in the somatosensory cortex to small temperature changes superimposed on noxious levels of thermal stimulation. Two populations of nociceptive neurons were found: one population encoded the intensity of noxious thermal stimulation; the other did not encode the intensity of noxious thermal stimulation even though these neurons clearly responded to the noxious thermal stimulus. We speculate that the former population might subserve the ability of monkeys to detect noxious thermal stimulation and, therefore, is involved in the sensory discriminative aspects of nociception. The latter might play a role in the motivational or affective components of nociception.

In the second study, the monkey's ability to detect and to discriminate noxious levels of thermal stimulation after the bilateral removal of the primary somatosensory cortex was examined. After bilateral excision of the primary somatosensory cortex, one monkey exhibited clear deficits in both detection and in discrimination of noxious thermal stimuli. These deficits were more pronounced at more intense levels of noxious thermal stimulation. The deficits persisted for more than a half a year. The other monkey also showed pronounced deficits in its ability to detect and to discriminate noxious thermal stimulation for the first three weeks. The second monkey exhibited a gradual recovery in its ability to detect noxious thermal stimulation. However, the animal's ability to discriminate the level of noxious thermal stimulation never returned to preoperative levels of performance.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00413-02 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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
Xie, Yikuan	Visiting Fellow	NA NIDR
Sahara, Yoshinori	Visiting Fellow	NA NIDR
Kajander, Keith	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	1.45	.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.)*

An experimental neuropathy was produced in the sciatic nerve of adult rats. The postoperative behavior of these rats indicated that hyperalgesia, allodynia and, perhaps, spontaneous pain and sympathetic dysfunction were produced. Hyperalgesic responses were evident on the second postoperative day and lasted for nearly three months. The hyperalgesia was revealed by a decrease in the threshold and in a marked exaggeration of the reflex's amplitude and duration. The presence of allodynia (pain evoked by normally inncouous stimuli) was inferred from the nocifensive responses evoked by standing on a chilled metal floor. The presence of spontaneous pain was suggested by a suppression of appetite and from the appearance of apparently spontaneous withdrawal reflexes. The presence of sympathetic dysfunction was suggested by the finding of unusually warm or cool skin temperature on the affected hindpaw. Postmortem microscopic examination of the injured nerve revealed a near total loss of myelin at the site of the injury. By 1-2 days after nerve injury, nearly all myelinated fibers can conduct impulses in the nerve proximal to the injury, but they cannot conduct through the injury. Unmyelinated fibers, however, conduct impulses through the injured region in a normal manner. Immunocytochemistry and enzyme histochemistry show that the dorsal horn levels of fluoride-resistant acid phosphatase, substance P, and calcitonin gene-related peptide all decrease. An increase in dorsal horn levels of methionine enkephalin and dynorphin has been detected by day 20 and may occur earlier. Colchicine experiments show that this increase occurs within intrinsic spinal neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-02 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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.	Senior Staff Fellow	NA NIDR
Ruda, Maryann T.	Research Biologist	NA NIDR
Flores, Christopher	Biologist	NA NIDR
Kim, Suzanne	Biologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.95	.90	1.05

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.)*

This project concerns the role of CNS peptide-containing neurons in sensory processes especially as they relate to pain and its control. A model of peripheral inflammation has been developed to investigate the relationship between spinal cord opioid-containing neurons (enkephalin and dynorphin) and abnormal primary afferent input. Alterations in opioid peptide biosynthesis are assessed by peptide and mRNA measurements and localization of the relevant neurons with immunocytochemical and in situ hybridization techniques.

In our standard adjuvant-induced inflammation model examination of the time course of mRNA change disclosed that dynorphin mRNA undergoes a rapid increase (within 24 hours) and reaches a peak by 2 days whereas the peptide elevation is significant by 2-3 days and reaches a peak at 5 days. In contrast, enkephalin mRNA is only increased by about 60% during this period and the enkephalin peptide level is unchanged. These neurochemical changes are common to several other types of inflammation which we have tested (phorbol ester, yeast and carrageenan). Two groups of dynorphin-containing neurons have been identified by immunocytochemical and in situ hybridization methods. With both methodologies the up-regulation delineates a new neural circuit which we believe may be responsible for modulating pain at the spinal level. These data indicate that, in spinal cord, dynorphin is the major peptide system affected at all time points during the inflammation and that those neurons may play a unique role in modulating inflammatory pain. The significance of these studies is that they reveal how (and which) opioid neurons are coordinated in response to inflammatory pain and possibly pain associated with arthritis and cancer. Further eludication of the pivotal role of the spinal dynorphin system may provide a new avenue for the pharmacotherapy of pain and provide insights into chronic opioid abuse and tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00426-02 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Inhibitory Processes in Antidromically Identified Spinal Dorsal Horn Neurons

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

Bennett, Gary J.	Research Biologist	NA NIDR
Sahara, Yoshinori	Visiting Fellow	NA NIDR
Xie, Yikuan	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	1.45	.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.)*

Intracellular recordings were made from antidromically-identified somatosensory projection neurons in the spinal dorsal horn of anesthetized cats. These neurons belonged to the dorsal column postsynaptic tract (DCPS), spinocervical tract (SCT), or spinothalamic tract (STT). Each neuron's responses to electrical and natural stimulation of its cutaneous receptive field were examined; the cells were thus classified as either low-threshold mechanoreceptive (responsive only to innocuous mechanical stimuli) or wide-dynamic-range (responsive to both innocuous mechanical and painful stimuli). Electric stimulation of an appropriate peripheral nerve at an intensity that was just strong enough to excite touch-responsive primary afferents produced an excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP) in nearly every neuron. The IPSPs were distinguished from other types of hyperpolarizing intracellular potentials by intracellular current injection, intracellular injection of chloride ions, and by direct measurements of membrane resistance. The EPSP and IPSP had practically identical thresholds, indicating that they were both evoked by the same afferents. The amplitude of the IPSP was constant when stimuli were delivered at 1 Hz or less, but decreased progressively in a step-like manner with frequencies of 2-30 Hz. The IPSP amplitude was virtually zero at frequencies of 30 Hz or more. These results show that the IPSP evoked by low-threshold mechanoreceptive afferents is usually sensitive to stimulus frequencies and suggest that the output of the interneuron that causes the IPSP is similarly frequency dependent. A search of the dorsal horn revealed a population of small interneurons in lamina III that exhibited such a frequency dependency. Intracellular injection of horseradish peroxidase has established the morphology of these cells and shown that their axonal terminations are found in laminae III-V. These laminae also include the dendrites of DCPS, SCT and STT neurons, thus a direct synaptic linkage of interneuron to projection neuron is plausible.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00440-01 NA

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Dorsal horn circuitry related to pain and hyperalgesia

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

Hylden, Janice L.K.	Staff Fellow	NA NIDR
Nahin, Richard L.	Postdoctoral Fellow	NA NIDR
Bennett, Gary J.	Research Biologist	NA NIDR
Anton, Fernand	Visiting Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.20	.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.)*

In the present research project, we have employed a combination of physiological, immunocytochemical and behavioral approaches to the study of somatosensory systems related to pain and hyperalgesia.

The activity of lumbar spinal dorsal horn lamina I projection neurons was studied in normal rats and in rats with an inflammed hindpaw. Inflammation was produced by injecting Freund's adjuvant into the plantar surface 4 hours to 5 days prior to electrophysiological recording. Neurons recorded ipsilateral to the inflammation had enlarged and more complex receptive fields as compared to those observed in control rats. Many cells had high background firing rates including some bursting activity and some cells responded to joint movement in addition to cutaneous stimulation. The increased activity of this population of neurons may result in a range of pain sensations. Lamina I projection neurons were retrogradely labeled in the rat following injection of tracer into the midbrain. These neurons were shown to send their axons through the dorsolateral funiculi. A subpopulation of spinomesencephalic lamina I neurons also were retrogradely labeled from the thalamus. The neuropeptide content of long ascending projection neurons was examined by combining immunocytochemistry with retrograde tracing techniques. A small proportion of spinothalamic tract neurons, that terminate in the medial thalamus, were found to contain dynorphin or enkephalin immunoreactivity. Some cells with projections to the lateral thalamus contained cholesystokinin or vasoactive intestinal polypeptide immunoreactivity. Experiments have also been initiated to examine rat models of hyperalgesia (inflammation or nerve lesion models) using behavioral, immunocytochemical and pharmacological techniques.

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

EXTRAMURAL PROGRAMS

NATIONAL INSTITUTE OF DENTAL RESEARCH

EXTRAMURAL PROGRAM

REPORT OF THE ASSOCIATE DIRECTOR

October 1, 1986 - September 30, 1987

The Extramural Program of the National Institute of Dental Research (NIDR) is responsible for the development, review, funding, and management of research, research training, and manpower development funded by the NIDR. Three program branches support a wide spectrum of research that extends from basic research to new methods of treatment and prevention. A special assistant is in charge of training and manpower development programs, while review and grants management are handled by a Special Review Branch (SRB) and a Grants Management Section respectively.

A broad array of award mechanisms is available to potential grantees ranging from small feasibility grants to regular grants to large center grants. During FY 1987 the NIDR Extramural Program (EP) made 656 research and training awards for an estimated total of $81 million. Included were $53.9 million for 391 research project grants and $14.3 million for 15 centers. The 391 research projects included 316 regular grants for $42.1 million, 11 program projects for $5.8 million, 27 young investigator awards for $1.3 million, 18 first independent research support and transition (FIRST) awards for $1.8 million, 10 Merit awards for $1.8 million, and 9 small business innovative research awards for $1 million. The center awards supported 5 noncategorical institutes, 5 periodontal diseases research centers, 3 dental caries research centers, and 2 pain research centers. Also, included were $1.1 million for 55 small grants, $125,797 for 6 conference grants, $154,864 for the Minority Biomedical Research Support Program, and $353,748 for 19 small instrumentation grants, a program new to the NIH this past year. In addition, 4 academic research enhancement awards (AREA) for $154,864 were referred to the NIDR.

In accord with funding patterns of past years, approximately 1/3 of FY 1987 funds were expended for new grants and competing renewals and the other 2/3 for non-competing continuations and supplemental grants. The new awards included 55 regular grants, 2 program projects, 1 new investigator award, 7 FIRST awards, 5 small business awards, 2 center awards, 55 small grants, and 19 small instrumentation grants. Competing renewal research awards included 47 regular grants, 3 program project grants, 6 MERIT awards, and 4 small business awards. Ten non-competing new investigator awards were converted to FIRST awards.

EP activities were varied and extensive. The most important and time-consuming effort of the year involved review of the 15 applications for Research Centers in Oral Biology (RCOB) support which the NIDR received by the December 1, 1986 deadline for funding consideration in FY 1987. The SRB was responsible for the development and execution of a rather unique review process which made use of a reverse site-visit approach and involved assignment of priority scores not only to the overall center application, but also to subprojects. A total of 4 center applications and 10 subprojects received priority scores in a fundable range. Two centers were funded this

year; the other two will be funded at the earliest date possible in FY 1988.

The change-over from the existing Dental Research Institutes and Centers (DRIC) program to the new RCOB program required special attention. While all five DRICs applied for RCOB support, only three were successful. In addition, the reduced size of the RCOB award compared to the DRIC award coupled with, in most instances, a redirection of research emphasis, exposed several more DRIC supported scientist to a sudden loss of support. The establishment of a expedited application and review process successfully resolved this special problem

Other major activities were the development of a request for applications (RFA) for periodontal diseases research centers. Eight applications, including applications from the existing five centers were received and reviewed by a special review panel established by the SRB. Staff also developed one request for proposal (RFP) calling for a collaborative clinical trial to evaluate safety and efficacy of alternative intravenous premedication regimens in dental patients. Sixteen proposals were reviewed and five contracts were awarded this year. Furthermore, EP staff issued an RFA on evaluation of orthodontic treatment strategies and a program announcement (PA) on wound healing in craniofacial injuries.

EP staff visited a number of grantee institutions as part of a continuing effort to keep the scientific community informed of institute activities. Special activities included visits to the University of Puerto Rico Dental School and to Morehouse College. Staff also participated in numerous site visits and attended scientific meetings using these occasions as opportunities for further contact with the scientific community.

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 meet the added demands for their service on peer review committees during FY 1987 was essential to the success of our many new initiatives. Their participation on the Dental Research Programs Advisory Committee, which met once this past year to advice the NIDR on directions for research on dental implants, set the stage for new initiatives. Last, but by no means least, their own research is the force that moves science forward. This report reflects some, but certainly not all, of the progress achieved during the past year.

$10.831 million were expended as follows: $5.210 million for 101 training awards through the National Research Service Awards (NRSA) Program and $5.621 million for awards related to manpower research training and development. The NRSA funds ($5.2 million) were for 58 individual awards, consisting of 52 regular postdoctoral and 4 senior fellowships; 50 institutional awards were for 28 regular training grants and 22 short-term (summer) training grants. The 28 training grants provided 18 predoctoral and 110 postdoctoral training positions, and the 22 short-term training grants supported 152 trainees; $5.6 million supported 12 career development awards, 19 physician scientist awards for dentists, 18 dentist scientist awards (individual), and 9 dentist scientist awards (institutional). We provided partial support for two MARC Ancilliary Training Activities through an agreement with the National Institute of General Medical Sciences.

Programmatic Accomplishments

The NIDR convened a special "Consultant Panel" to assist us in an evaluation of our NRSA program. The Panel was asked to make recommendations to the National Advisory Dental Research Council in regard to the content and operation of this program.

The first meeting of this panel was held on February 20, 1987. The purpose of this meeting was to obtain an overview of our NRSA program. The Panel was given information on the past and current NIDR program, including a detailed report on the follow-up of trainees and fellows from FY 75 - 79. In addition, the Panel obtained information from other BIDs in regard to their experience in evaluating their own NRSA programs.

The last meeting of the panel was held August 25-26, 1987. At this meeting the Panel was presented information on the relationship between NIDR support of research training and careers in dental research/education. The Panel then formulated its recommendations which were presented to the September, 1987 meeting of the NADRC for concurrence. The NIDR expects to present an implementation plan to the NADRC at its January 1988 meeting.

The second meeting of the program directors of the institutional Dentist Scientist Award (K16) took place in March, 1987, in conjunction with the IADR meeting in Chicago. Issues important to the efficient operation of the K16 program were discussed, such as, timing of the grant year, recruiting and the applicant pool, candidate application format, starting salaries, and the

tax issue on salaries. It was the unanimous opinion of the program directors that this meeting was very useful and should continue to be held, at least once a year.

The Physician Scientist Award for Dentists (K11) and the Dentist Scientist Awards (K15-individual and K16-institutional) continue to receive considerable interest in congress and the academic community. As a result, we were able to fund seven new K11, and nine new K15 awards this year. In addition, the nine institutional awards were each allowed to add two more additional "clinical research scholars" to their programs. Therefore, the NIDR is currently supporting approximately 79 "clinical research scholars" at some stage of their research/clinical development.

The Long-Range Research Plan indicates that a "special area of concern" is epidemiology. In this regard the NIDR has initiated the funding of another training grant in dental epidemiology at the University of California in San Francisco. This program is unique in that it is an addition to an existing, highly regarded, program in medical epidemiology at the medical school of the University of California. With this new award, NIDR is now funding three training grants in dental epidemiology with a total of ten postdoctoral and two predoctoral trainees.

New guidelines were announced for the research career development award. The most important changes were in the area of eligibility. The applicants must now have at least five years of postdoctoral research experience, including two years of experience as an independent investigator with independent peer-reviewed grant support. At the time of the award, the applicant must have independent research support sufficient for the research proposed in the RCDA application. In addition, applicants who hold a tenured faculty position or are well established in their field by virtue of their publications, are ineligible.

At the conclusion of this fiscal year, the NIDR will be supporting approximately 240 trainees, fellows, and clinical research scholars, at some stage of their research training. Of these, approximately 45% are in the Periodontal and Soft Tissue Diseases Research Branch, 33% in the Craniofacial Anomalies, Pain Control, and Behavioral Research Branch, 17% in the Caries and Restorative Materials Research Branch, and 5% in Epidemiology.

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CARIES AND RESTORATIVE MATERIALS RESEARCH BRANCH

Extramural Programs, NIDR

Recent progress in caries research includes studies on the etiology of dental caries employing recent advances in molecular biology/molecular genetics and monoclonal antibody technology; studies on specific salivary or microbial factors important in dental plaque formation; immunologic studies on the role of specific antigens and immune mechanisms in protection; replacement therapy utilizing non-virulent strains of S. mutans; and a variety of approaches to define high risk populations and to study root surface caries. Progress in restorative materials research included studies to elucidate the effects of filler variables on composite properties, human clinical trials of a new adhesive bonding technology, development of new and improved cements, studies to improve porcelain fused to metal prosthetic materials and studies to assess the biocompatibility of metals.

Caries

A01 Determine the incidence and prevalence of dental caries among all target populations.

Scientists are studying the epidemiology of caries to answer questions about the early transmission of cariogenic streptococci to infants, the effects of environmental factors on development of oral structures, the incidence and prevalence of root caries and primary and secondary coronal caries in adults, and correlates of caries in children that might be used to identify high-risk individuals and populations. During the current year, early information from a longitudinal study in Lima, Peru, indicates that in the chronically malnourished child, deciduous teeth erupted and exfoliated later and appeared to have significantly higher caries than deciduous teeth of the well-nourished child. A study in Boston, Massachusetts, continues to indicate that root surface abrasion may account for one-half of the pathology attributed to root caries and that individuals can have exposed root surfaces that do not develop caries for long periods of time if the individual controls the frequency of sugar consumption. Progress also is being made in establishing ways to identify the high caries risk child. Discriminant analysis suggests that in low fluoride areas use of fluoride supplements and salivary S. mutans counts are strong predictors of caries.

A03 Further elucidate the causes of all types of dental caries.

Several studies now are under way on the protease of S. sanguis that splits salivary immunoglobulin IgA1 and thus protects S. mutans and other microorganisms by reducing active antibody levels. The action of the

protease, which also dechains polymeric IgA, may be of considerable importance in negating host resistance to viruses. In the current year information on the amine and molecular configuration at the site of protease attack has allowed the synthesis of polypeptide analogs that are strong inhibitors of the enzyme. The gene for the protease has been cloned in E. coli to facilitate the production of large amounts of the enzyme for further studies of IgAl protease significance in human health.

A04 Further characterize the composition and chemistry of plaque, tooth enamel and other physiological systems.

There is major interest among dental scientists on the etiology of caries, including the properties and function of plaque microorganisms, plaque, tooth enamel and cementum. Many recent studies of oral streptococci are particularly exciting because they are based on use of the most advanced recombinant DNA techniques and are beginning to explain the mechanisms that in these microorganisms provide for organization and control of metabolism, development of necessary structures, and short and long term adaptation to changes in environment. Thus, for example, the DNA sequence for the entire S. mutans GS-5 gene for insoluble glucan synthesis was determined and accessory promoter sequences and ribosome binding sites identified. Immediately downstream from this gene sequence is one for soluble glucan and evidence suggests that both genes are translated from a single mRNA. Defined mutants of GS-5 are being created by introducing fragments of these genes into wild-type GS-5 which results in their incorporation into the plasmid and inactivation of the homologous gene. Similar studies are establishing the numbers of specific genes, associations between genes, and gene repressor and de-repressor mechanisms in all areas of function of oral streptococci, including the possibilities for normal inter-species transfer of genetic information by conjugative transposons and other mechanisms.

Scientists supported by the Program now are making substantial progress in understanding the interactions within complex physiological systems such as those controlling sugar transport into oral streptococci or attachment of these bacteria to dental surfaces by adhesive glucans, fimbriae or other mechanisms. To understand such systems, scientists need to study the properties of each pure component and study simple reconstituted systems containing these components. Use of recombinant DNA techniques and monoclonal antibodies are for the first time making such studies possible.

Similar techniques also are being utilized to understand the complex cellular interactions that take place in the host to establish immunity to foreign antigens. Scientists currently are cloning B cells and regulatory T cells from spleens and Peyers patch tissue and reacting these with specific types of antigen such as S. mutans serotype carbohydrate plus growth factors such as gamma interferon. Products of such cell cultures currently are being screened for suppressor and amplifier factors that establish levels and duration of antibody production.

Several scientists are actively studying the factors in saliva that protect enamel surfaces from dissolution and that promote adhesion of oral bacteria to teeth. One of the outstanding current findings in this area is the identification of the functional group of salivary statherin and proline-rich proteins that keeps salivary calcium from precipitating even though saliva is

supersaturated with respect to this mineral. The functional group is now known to be phosphoryl serine. The corresponding dipeptide, phosphoseryl phosphoserine, has been synthesized and found to have approximately 30 times more activity than statherin in inhibiting calcium phosphate precipitation.

A11 Improve antimicrobial and antiplaque agents.

A useful effector microorganism for replacement therapy of caries must be able to colonize the host for long periods of time, replacing cariogenic species and being non-virulent itself. Extensive research has revealed several potential effector strains, none of which have exhibited the requisite displacing activity. During the last year, however, an S. mutans strain (JH 1005) has been identified with powerful ability to displace indigenous S. mutans. The activity of this strain seems to depend on its production of a potent bacteriocin active against other strains of S mutans.

A05 Determine the pathogenesis of root caries and secondary caries and further elucidate the pathogenesis of smooth surface and pit and fissure caries.

A10 Investigate methods for preventing root caries.

An upsurge in interest in caries of root surfaces, specifically, and in remineralization of coronal and root caries has led to the creation of a foundation of research in this area. Several animal models, for instance, have been developed for studies of root caries. One of these is the sialoadenectomized rat. Removal of these glands is found to cause extensive smooth surface and sulcal caries. Caries of root surfaces also occur if sucrose is provided in the rats' drinking water. Another model being investigated is the young hamster that, if superinfected with S. mutans or A. viscosus, shortly develops gingival recession and alveolar bone resorption. Provision of experimental diets then lead to the development of caries on the exposed root surfaces. Various types of topical fluoride treatment (but not systemic fluoride) were found to prevent most of the root caries. Various simple carbohydrates (but not starch) were found to stimulate production of root caries. In other experiments using sterilized human tooth roots pure proteolytic enzymes such as subtilisin or collagenase were found to cause erosive-type lesions, whereas filtrates of S. mutans cultures had little effect on the tooth roots.

A12 Purify and characterize all potentially important S mutans antigens for vaccine development.

Scientists are preparing pure S. mutans antigens for projected short-term clinical trials in human adults. These trials will primarily examine the extent and duration of production of specific plasma and secretory antibodies. Antigens incorporated in lipid micells will be provided in capsules for release in the small intestine where antigen absorption into the gut-associated lymphoid system occurs. Two antigens are being purified at this time for the trials. One is the glucosyltransferase enzyme from S. mutans that makes insoluble glucan, and the other is the serotype C carbohydrate of S. mutans. A second research team is exploring the possibility of immunizing infants against S. mutans by local stimulation of labial salivary glands as the route of antigen delivery. It is interesting

that the IgG level of secretions from these glands is about 20 percent of the IgA level.

A17 Identify ways to reduce the caries-producing potential of dietary items.

Using intraoral microelectrodes to monitor pH changes, a scientist in the last year has shown that accumulation of plaque acids sufficient to dissolve tooth enamel can be prevented by chewing sialogenic materials such as paraffin wax or chewing gum sweetened with xylitol or sorbitol. Another scientist has been studying the structural characteristics of Aspartame to identify what makes this and a few other simple peptides sweet. During the past year a set of isomers of an analog of Aspartame were synthesized that agreed exactly in sweet and bitter taste as predicted by molecular structure.

Materials

P04 Determine the biocompatibility of metals used for restorative materials.

Researchers are systematically investigating the quantity, rate and mechanisms of mercury release from dental amalgams and their constituent phases by evaporation, sublimation and dissolution into the oral environment. An atomic absorption spectrophotometer was used to measure the amount of mercury dissolved from three different amalgams, as well as from certain intermetallic compounds such as Y_1, Y_2 and B_1 into saline solution. The release of mercury from the Y_1 compound far exceeded that from any of the other specimen tested in the study. Although Y_1 comprises more than 40% of the volume of most dental amalgams, the amount of mercury released from amalgams was less than 7% of that released from Y_1 alone. There are several possible explanations for this phenomenon. One may be that mercury released from Y_1 reacts with unconsumed alloy particles to repeat the amalgamation reaction, thereby reducing overall mercury dissolution. It may also be that the surface of the amalgam becomes covered by a corrosion product layer which drastically retards the liberation of mercury into the NaCl solution. Another possible explanation for the reduced mercury dissolution from amalgam, as compared with "pure" Y_1, may be that the stability of the Y_1 formed by the setting of amalgam may be higher than that of "pure" Y_1. This increased stability may be due to the presence of a small amount of Sn dissolved in the Y_1 grains of set amalgam. Although the quantity of mercury dissolved in saline solution from amalgam appears to be negligible, several factors - including the effect of time, the presence of proteins in the dissolution media, the presence of acids in food and drinks, and the effects of the continual abrasion of the amalgam surface during chewing - must be considered before conclusions can be drawn.

P07 Study adhesive bonding between tooth structures and composites.

Scientists are conducting a five year double-blind human clinical study to determine the effectiveness of a recently developed adhesive restorative technology for retention of composite restorations to both dentin and enamel surfaces. The first phase of the study, which examined the pulpal biocompatibility of the new bonding technology, utilized patients with one or more homologous pairs of sound permanent teeth which were scheduled for orthodontic extractions. Class V cavity preparations were prepared with no

retention form; that is, there were no undercuts or shapes that could provide mechanical retention. Contralateral teeth were assigned at random for treatment with the experimental and control materials. The teeth were treated for adhesive bonding by application of either the control material, light-cured Scotchbond, or the experimental materials, which included: acidified solutions of ferric oxalate, NTG-GMA (adduct of N [P-tolyl] glycine and glycidyl methacrylate) or NPG-GMA (the addition reaction product of N-phenylglycine and glycidyl methacrylate) or NPG (N-phenylglycine), and PMDM (the addition reaction product pyromellitic dianhydride and 2-hydroxy ethyl methacrylate). The teeth were filled with light-cured Silux composite resin. The test and control materials were coded so that the patients and evaluators did not know which materials were present in the study teeth. Prior to extraction, pulp vitality, marginal integrity and retention were assessed. The teeth were extracted at intervals of 3-5, 25-30, and 60-90 days and histologically studied. Results indicate that none of the tested materials produced pulpal irritation and, therefore, appear to be biologically compatible with pulpal tissue. The second phase of the project will evaluate the retention, esthetics and marginal integrity of the bonding system over a four year period of time.

P08 Elucidate the role of filler particles in the mechanical and chemical behavior of composites.

Investigations are under way to develop hydrophobic polymers derived from fluorinated and silicon-containing resins that are noted for their resistance to attack by moisture. Improved dental composite formulations of these materials have been developed based on the hardening of bulky, liquid resins with mineral fillers of various types, shapes, and sizes for esthetic anterior tooth fillings. These materials, which overcome many of the shortcomings of silicate cements and unfilled resin restorative materials, can be cured either chemically or photochemically using visible light irradiations. In addition, efforts are underway to further enhance the durability of the composite materials and to extend their use for cavities in posterior teeth or in other stress-bearing areas. These developments are expected to advance progress toward the long term goal of improved dental composite fillings.

P09 Attain basic data for the design of new polymer systems.

Dental restoratives are moving with increasing frequency to polymeric materials. Metallic and ceramic materials cannot currently compete with polymeric compositions. Low cost, ease of fabrication and speed of application make polymeric compositions much more desirable than the long used silver amalgam, gold and ceramic materials. Scientists are attempting to develop a new dental restorative material which will be superior to non-metallic materials now in use. These new materials are based upon polymeric resins that have the unique property of expansion or zero shrinkage when they are transformed from the liquid (uncured) to the solid (cured) state. It is believed that the amount of shrinkage is indirectly proportional with the amount of bio-methylene-spiro-orthocarbonate (BMSOC) in the monomer of the material and that BMSOC can therefore counteract the polymerization shrinkage that occurs during curing. Sufficient amounts of BMSOC have been prepared and dental composites containing 10% BMSOC have been formulated and tested for relevant laboratory properties. Early results indicate that there is no statistically significant difference in mechanical properties between the

control standard dental composite and the 10% BMSOC composite, but the latter did show an increased adhesion to porcelain. Further development of the monomer synthesis process to enhance yield and quality as well as development of formulations of dental resins with greater than 10% BMSOC is planned. Successful development of these new materials should significantly advance the state-of-the art of dental composites.

P13 Develop new and improved cement systems.

Although cements are used in over fifty percent of all dental restorations, these cements are far from ideal. Their relatively low mechanical properties, high solubility and lack of resistance to wear and disintegration deter their more extensive use, especially for cementation of permanent prostheses or for their use as intermediate restoratives. Recently, scientists have developed high strength eugenol-free adhesive cements and restoratives based on vanillate esters, o-ethoxy-benzoeic acid (EBA) and zinc oxide (ZOE). These cements have the following advantages compared to the presently used ZOE or EBA cements: excellent strength, much lower solubility than ZOE cements, do not inhibit free radical polymerization, can be used in conjunction with composite filling materials to which they adhere, are compatible with acrylic monomers and can be formulated in conjunction with them, and adhere strongly, even on prolonged water exposure, to non-precious metals, porcelain and composites. These cements exceed requirements of ANSI/ADA specification No. 30 for Types II, III and IV restorations. Biological assessments confirm that these cements are neither toxic nor mutagenic, and limited clinical evaluations are underway.

P15 Develop new and/or improved intraoral prosthetic materials.

Researchers are investigating porcelain-fused-to-metal (PFM) interactions which affect microcrack formation preceding clinical failure. It has been widely believed that distortion of PFM crowns is caused primarily by thermal incompatibility stresses which develop during cooling of these crowns from porcelain firing temperatures. In this study, the marginal gap between PFM crowns and their prepared dies was determined under conditions which were designed to exaggerate distortion effects. This preliminary study demonstrated that incompatibility stress induced by a positive contraction mismatch is not the primary cause of marginal or generalized distortion of PFM crowns but suggested instead that external grinding and internal abrasive blasting of crowns are more likely causes of this effect. This finding indicates that the marginal discrepancies which may cause excessive cement dissolution and recurrent caries can be minimized or eliminated by modifying the sand blasting procedure.

P99 Project does not fit any section of the Plan.

Investigations are under way to determine the feasibility of using a sealed composite restoration to arrest caries without cavity preparation and without the removal of the carious lesion. The minimal tooth preparation, a bevel in enamel to allow for greater acid etching, usually does not require any anesthetic injection and conserves tooth structure. These ultra-conservative sealed composite restorations are being compared with ultra-conservative sealed amalgam restorations and with traditional outline (unsealed) amalgam restorations. A total of 123 patients have had 156 paired study restorations

placed (several patients had more than one pair of occlusal lesions). Evaluations of the study restorations, using Ryge's clinical evaluation criteria modified by Mertz-Fairhurst, have been carried out at six month and at one, two, three and four years. Out of 129 pairs of restorations evaluated at three years, five restorations showed clinically unacceptable defects (pin point voids in the margins of the composite and enamel fractures); out of 123 pairs of restorations being evaluated at four years, a total of seven study restorations showed clinically unacceptable defects, such as the loss of the entire composite filling. The defective restorations have been properly restored and the patients involved will continue in the study for continued observation of the other tooth in each study pair. At four years, this study has estalished that the ultra-conservative sealed restorations represent a viable measure. Continued favorable results could convince the dental profession to consider these restorations as definitive treatment.

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CRANIOFACIAL ANOMALIES, PAIN CONTROL AND BEHAVIORAL RESEARCH BRANCH

Extramural Programs, NIDR

Accomplishments

C05 Explore the nature of genetic susceptibility or resistance

Nonsyndromic cleft lip with or without cleft palate [CL(P)] and cleft palate [CP] run in families but CL(P) is more common in males and CP is more common in females. The incidence of CL(P) is greater than that of CP (1.1 versus 0.7, respectively, per 1,000 births) in the United States white population and racial variation in incidence of CL(P) is greater than that of CP.

These epidemiological data prompted several studies to determine whether the inheritance of these birth defects was consistent with multifactoral inheritance or could be attributed to a major gene effect. The results were equivocal but a grantee in Hawaii has recently completed the largest comparative analysis to date, using data sets from Danish and Japanese families. CL(P) can best be explained by the combined action of a major gene together with multifactoral inheritance for Danish families, whereas multifactoral inheritance alone can account for family resemblance among the Japanese. The finding of a major gene component among Danish families explains why Caucasians, with a lower population incidence (1 per 1,000 births) of CL(P), have a higher family recurrence risk than the Japanese, who have a higher population incidence (2 per 1,000 births). Application of recently developed techniques for gene mapping of DNA isolated from affected families will be required to fully explain inheritance patterns and permit accurate and effective genetic counseling to prevent occurrence of this birth defect.

C13 Explore sensory and respiratory dysfunctions in relation to craniofacial malformations.

The relationship between mouth breathing and dentofacial deformities has been controversial for decades. Clefts of the lip and palate produce nasal deformities, reducing the size of the nasal airway. Surgical correction of clefts often further compromises the nasal airway space. Cosmetic surgery to restore symmetry to the nose reduces the size of the unaffected nostril and secondary procedures to restore function of the palate and improve speech further reduce upper airway flow. On the other hand, mouth breathing resulting from an inadequate airway is considered by many to be orthodontically harmful and is associated with a recessive lower jaw, protruding upper jaw, and other facial deformities.

Investigators at the University of North Carolina are obtaining quantitative information to resolve this controversy and provide a basis for treatment decision making, other than clinical impressions. Using a recently developed technique to measure nasal airway cross-sectional area, they have defined the minimum area necessary for adequate nasal breathing in adults. Individuals who appear to mouth breathe usually nose breathe to a significant extent. Mouth breathing is a compensating response to maintain adequate breathing pressures and upper airway pressures are maintained at a fairly constant level by opening and closing the mouth by just the right amount. Nasal braking during expiration is important in speech, especially in clefted subjects, where the velum is incompetent. Surgical correction of clefts may result in collapse of the nasal valve during inspiration and the cross sectional area is reduced by an average of 25 percent. Two thirds of cleft subjects were found to be wholly or in part mouth breathers. The investigators observed that the prevalence of mouth breathing was similar in cleft palate adults and children, despite the finding that nasal airway area increases with growth. Apparently, the increase with growth was insufficient to correct the breathing mode or the habit of mouth breathing persists, even when the airway improves.

Investigators at the University of Iowa have found that intensive daily therapy for six weeks followed by weekly therapy for the remainder of a year consistently reduced speech distortion in clefted children. Speech production difficulties appeared to be more influenced by dental malocclusion than by any other factors associated with clefting. Physical management procedures, which yielded normal or near normal oral structure and function, including satisfactory occlusion, resulted in the most improvement in speech skills.

D04 Further elaborate the molecular mechanisms involved in wound healing.

When a bone breaks it repairs itself; this massive repair is unique among all other tissues because it is scar-less; new bone is generated and integrated into the remnants of the old bone. Recent experiments indicate that adult bone is a reservoir for a large number of peptide or protein growth factors, which affect several stages of the repair process. Many of these stages recapitulate events involved in embryonic bone formation. The purification and characterization of the structure and biological activities of these factors forms an extremely active and fruitful area of intra- and extramural NIDR funded research. The sequence of repair events is roughly as follows: after fracture there is an acute inflammatory response; chemoattractants mobilize mesenchymal stem cells, which undergo mitotic expansion and aggregation into a repair blastoma; stem cells differentiate into cartilage producing cells, which stabilize and span the gap at the break site; maturation of cartilage is followed by its replacement by vascular elements and bone is formed, which then undergoes slow remodeling and integration into existing bone to provide mechanically homogeneous bone.

Investigators at Case Western Reserve University are studying the embryology of bone formation, isolating chemotactic and cartilage differentiation factors and exploring how they may be delivered back into repair sites. Their efforts are derived from those of Dr. Marshall Urist at UCLA, who has been studying the molecular and cellular aspects of bone repair with NIDR support for many years. Urist demonstrated that bone matrix contains a factor, subsequently shown to be a low molecular weight protein known as bone morphogenetic protein, which can induce new bone formation in non-bone tissues such as muscle. The CWRU group has purified a single protein, which controls conversion of mesenchymal stem cells into cartilage producing chondrocytes, and there is evidence for the existance of several other embryonic proteins which influence stem cells to become chondrocytes. Investigators at Childrens' Hospital in Boston have isolated at least six proteins from adult bone, which affect cell division of mesenchymal cells, supporting the concept of a multiplicity of factors affecting different steps in the repair pathway. These bioactive factors, which affect cell division, differentiation and eventual sculpting of bone have recently been isolated from animal bones.

Importantly, several of these repair factors have also been found in teeth. For example, epidermal growth factor activity has been demonstrated in dentin and is thought to be important in tooth development. Grantees at Northwestern University recently reported that a rat dentin matrix component is chondrogenic in vitro and osteogenic in vivo and is analogous to the morphogenetic protein of bone described by Urist. Another dentin component, phosphoryn, has been isolated and is thought to direct the deposition of mineral cations onto the structural network of collagen. In certain types of dentinogenesis imperfecta, where mineralization is drastically reduced, phosphoryn is virtually absent, confirming its importance in mineralization mechanisms. As these bone and tooth factors are purified and characterized, molecular biologists will determine their genetic origin and how they are regulated. With this information, repair protocols may be generated not only for fractures and genetic diseases of bones, but also for teeth.

E06 Study factors affecting the post-treatment stability of bones and teeth.

Several problems are sometimes encountered when mandibular advancement surgery is performed to correct developmental retrusion of the lower jaw. A group of investigators at the University of Michigan have used monkeys to understand how these problems arise and alter the surgical techniques to achieve more satisfactory outcomes. Relapse is perhaps the most common problem. The tendency of the surgically advanced monkey jaw to return to its former position was decreased when the muscles of the floor of the mouth were detached from the inner aspect of the jaw. Despite wiring together of the upper and lower teeth to hold the advanced mandible in position, backward movement of the chin often occurs. Apparently, the teeth move orthodontically, allowing the jaw to relapse. Placement of heavy wires around the monkey's lower jaw and

through the upper jaw or the use of standard orthopedic techniques to secure the surgically created bone segments of the lower jaw prevented relapse, entirely. Animals in which jaw fixation was attempted by wiring of the teeth underwent relapse, as expected.

Many patients have difficulty opening their mouths widely following lower jaw advancement and some complain that they have difficulty biting into hard foods. Immobilization of the jaws of monkeys following surgery by wiring them together for six weeks resulted in muscle atrophy leading to decreased bite force and they were never able to open their mouths as widely as before surgery. Use of the orthopedic fixation techniques, which allowed animals to open and close their mouths immediately following surgery enabled animals to regain their mouth opening ability and they had increased bite force, compared with animals whose jaws were fixed with wires.

In order to avoid the risk of disturbing normal growth, it has been customary to delay surgical jaw advancement until skeletal maturity was reached, but this has never been shown to be necessary. Periodic X-rays of the head for two years indicated that growth of young monkeys with lower jaw advancement was not impaired, relative to that of control animals, confirming that early surgery may be performed to achieve satisfactory occlusion prior to maturity.

G01 Conduct further studies on the trigeminal system with particular emphasis on pain transmission.

NIDR-supported research at the University of New Jersey Medical School and Rutgers has established several fundamental properties of trigeminal primary afferents and second-order neurons as they respond to damage within one trigeminal branch, the infraorbital nerve. These neurophysiological properties may provide important insights into clinical sensory and pain phenomena associated with neural damage in human patients.

With respect to primary afferents, experiments in neonatal rats following infraorbital nerve transection have shown dramatic differences in the capacities for reorganization within central and peripheral axons of undamaged trigeminal ganglion cells. Peripheral axons of these neurons sprout additional branches, both at the level of the ganglion and in the periphery. A large number of these fibers invade the portion of the face that is normally innervated by the infraorbital nerve and remain in place after it has regenerated. On the other hand, there is little or no central sprouting of undamaged trigeminal axons after neonatal infraorbital nerve transection. Both "bulk-tracing" experiments (which demonstrate the terminal fields of the entire nerve) and intra-axonal injection experiments provide no evidence that these fibers invade denervated infraorbital territory in the brain stem after neonatal neural damage.

The fact that primary afferent neurons with abnormal peripheral arborizations retain normal central projections may provide the physiologic basis for the sensory mislocalization and abnormal, often intractable, pain states associated with nerve damage in clinical patients.

These same investigators have demonstrated that differences between the central and peripheral plasticity of trigeminal ganglion cells are, to a considerable extent, a developmental phenomenon. If the infraorbital nerve is damaged in fetal rats, both central and peripheral reorganization takes place. Conversely, if nerve damage is induced in an adult animal, there is little evidence of either peripheral or central sprouting.

Sensory responses of second-order neurons in the trigeminal brain stem complex are also markedly altered by neonatal nerve damage. Most notable among these changes are an increase in the incidence of cells that respond to nociceptive (pain-producing) stimuli, as well as increases in the incidence of neurons with abnormally large and misplaced receptive fields. Experiments involving single-cell recordings and intracellular staining of these neurons indicate that their abnormal responsivity is probably not the result of any change in dendridic architecture. That is, cells with normal structural properties have been shown to have highly abnormal receptive fields. This finding has led this group to explore other potential substrates for the functional reorganization observed. Possible explanations include cortical input to the trigeminal brain stem complex and neural circuits intrinsic to this region. Though a complete explanation for the observed alterations in the receptive fields of second order neurons following neural injury is still lacking, these exquisitely detailed neuroanatomical and neurophysiological observations are contributing toward our understanding of physiological processes underlying sensory abnormalities and pain states in human clinical patients.

G10 Study the role of anxiety, stress, and other psychosocial factors in relationship to the etiology and symptomatology of a variety of motor dysfunctions and pain disorders.

M01 Determine how behavioral, social, and cultural factors relate to the incidence, prevalence, and distribution of oral disease and conditions.

Epidemiological studies supported by NIDR are for the first time providing information on how prevalent temporomandibular disorders are among North American populations, as well as yielding some interesting leads on clinical and psychosocial characteristics differentiating individuals who seek care from those who do not. This research is important because disorders of the temporomandibular joint (TMJ) and

associated muscles of mastication, have been identified as the major source of non-dental pain in the orofacial region. Temporomandibular disorders (TMD) are a cluster of related disorders with some common features. The most important and prevalent presenting symptom is pain localized at the temporomandibular joint, in the pre-auricular area, or in the muscles of mastication. In addition to complaints of pain, temporomandibular disorders are often associated with limited or asymmetric patterns of jaw opening and with joint sounds, usually described as clicking, popping, grating, or crepitus.

In an ongoing study, an age-stratified sample of enrollees within a large health maintenance organization responded on a mailed questionnaire (and/or a telephone interview for initial non-respondents) to carefully pre-tested items describing pain experiences and aspects of oral function, such as mouth opening and "clicking" in their jaw. In addition, standardized clinical examinations were conducted on all individuals reporting temporomandibular (TM) pain in the prior 6 months. The same clinical exam was performed on a representative sample of respondents indicating no TM pain (community control) and on 256 HMO patients who had been referred for TMD treatment.

Responses to the questionnaire indicated that approximately 12 percent of the HMO enrollee sample reported having had temporomandibular pain at some time during the prior six months. TM pain was reported about as frequently as was chest pain and slightly less than abdominal pain. TM pain was reported less frequently than headache (26 percent) or back pain (41 percent). TM pain, headache, and stomach pain (but not chest or back pain) were more prevalent among females and persons who were separated or divorced; they were less prevalent among persons over the age of 65. None of the pain conditions were significantly associated with levels of income or education.

Individuals reporting pain on the average rated TM pain at a slightly lower intensity level than did those reporting other pain conditions. Similarly, other pain conditions tended to be associated with slightly more work loss/disability days than reported for TM pain, which averaged 0.5 such days per case during the prior 6 months.

For individuals reporting TM pain, 53 percent also reported a problem with back pain, 41 percent also reported headache, 25 percent abdominal pain, and 18 percent chest pain. TM pain cases with multiple pains differed from those reporting TM pain in having lower self-rated health status, higher psychologic distress as measured by the Symptom Check List, and more family and work-related stress. They did not, however, differ in the severity of TM pain they reported.

These findings indicate that pain co-morbidity is common for individuals with TM pain, an observation which needs to be considered both in the clinical evaluation of patients, and in research on psychosocial correlates of pain.

Clinical examination results indicated that the treatment-seeking TM pain patients and non-treatment seeking individuals reporting TM pain were not consistently distinguishable on measures such as mean vertical jaw opening, extent of uncorrected deviation in jaw opening, and jaw sounds. Both groups, however, differed on the clinical measures from the control group reporting no TM pain.

Both the questionnaire and clinical exams will be repeated one year after the initial assessments to determine how signs and symptoms of TM disorders vary over time in both TM patients and in the non-treated control groups with, and without, TM pain. Such TMJ epidemiologic studies are expected to yield much-needed information on factors motivating individuals to seek treatment for TM pain, as well as the extent to which TM disorders tend, on a population basis, to be essentially self-limiting vs progressive in their course.

PERIODONTAL & SOFT TISSUE DISEASES RESEARCH BRANCH

Extramural Programs, NIDR

Recent progress in periodontal research included etiologic studies on early plaque formation, immunologic studies on the critical role of specific immune mechanisms in protection, and a variety of approaches to improve methods of diagnosis and measurement of disease. Progress in soft tissue stomatology research was evident in studies on the oral manifestations of AIDS, on oral cancer, herpes simplex virus and salivary gland function.

B01 Identify the microbial species that cause various forms of periodontal diseases.

Plaque Formation Of the many types of bacteria which inhabit the human mouth, some colonize on tooth surfaces at the gum line where they form thick masses of organisms, or plaques, which may lead to periodontal diseases. In recent years, studies from several laboratories have begun to shed light on the molecular mechanisms involved in early plaque formation. The first step is the deposition of salivary proteins, including the unique, proline-rich proteins (PRP), which have a strong affinity for adhering to the teeth. These and other salivary components form the initial "acquired pellicle", which then attracts and binds oral bacteria by specific chemical mechanisms. The studies indicate that oral bacteria recognize and specifically attach to PRP molecules adsorbed on the tooth surface. Microorganisms which possess this ability include strains of Streptococcus mutans, an organism which causes dental caries, Actinomyces viscosus, which is associated with root surface decay and gingivitis, and Bacteroides gingivalis, one of the principal organisms implicated in rapid, severe periodontal disease. Surprisingly, these organisms do not react with, or bind to the PRP when these proteins are part of the fluid saliva, because at that time, the PRPs are folded and coiled so that the potentially reactive sites are covered. However, in pellicle formation, the PRP apparently becomes uncoiled and the acidic fragment attaches to the tooth, leaving an unattached portion of the protein chain with specific PRP reactive sites exposed for potential binding to specific sites on the bacterial surfaces. These studies provide a molecular explanation for the predilection which some bacteria have for colonizing and forming plaque on the surfaces of human teeth, and suggest that it might be possible to prevent bacterial attachment and plaque formation by modifying the initial salivary pellicle which coats the teeth.

B05 Clarify the role of the host's immunological system as either a protector of the periodontal tissue or as a possible source of destruction.

Immunology Early studies on patients with AIDS and periodontal disease have drawn attention to the regulatory role of T lymphocytes in protection against infections, and subsequent studies have linked low T4/T8 (helper/suppressor) cell ratios in the peripheral blood to severe periodontal disease unrelated to AIDS. Newly developed methodologies have enabled investigators to examine these T cell subset ratios in local periodontal tissues as well as in peripheral blood. A study of 35 human subjects showed that the healthy periodontal tissues exhibited normal T4/T8 ratios which were identical to those in peripheral blood. In contrast, diseased tissues from patients with adult periodontitis and juvenile periodontitis showed T4/T8 ratios which were significantly reduced from their peripheral T4/T8 ratios, which were normal. The reduced T4/T8 ratios in local sites provided statistically significant correlations with disease severity and pocket depth. Relative recoveries of B cells from normal and disease tissues did not differ. These findings suggest that T-cell regulatory expression in the local gingival tissues is distinct from peripheral blood regulation and that in periodontal disease, there is a local immunoregulatory imbalance.

Although the reasons for the immunosuppression cited above are not clear, there is evidence that some organisms already implicated in periodontal disease etiology may play a role in suppressing different immune functions. For example, one investigator reports that Haemophilus actinomycetemcomitans (Ha) appears to reduce the T4/T8 ratio by specifically activating the T4 suppressor cells, an effect which would reduce the ability of lymphocytes to respond to invaders in general. Other periodontal organisms shown so far to exert an immunosuppressive effect include Treponema, Fusobacteria, Veillonella, and Centipeda periodontii, which can actually kill both B and T lymphocytes. The immunosuppressive effects exerted by these organisms may enhance the virulence of the organism itself or that of other opportunistic pathogens.

The influence of immunization on the colonization and potential infectivity of known periodontal pathogens has been studied in the squirrel monkey animal model in which ligatures are placed around tooth roots to induce experimental periodontal disease. Ligature placement apparently induces disease by creating an environment favorable for pathogenic flora. Squirrel monkeys were subjected to a program of immunization with B. gingivalis, and subsequently were exposed orally to the viable B. gingivalis by applying suspensions of the organisms to the ligatures placed around the tooth roots. After 10 weeks, 4 of 5 control, sham-immunized monkeys were infected with B. gingivalis, whereas none of the 5 immunized monkeys were infected with B. gingivalis. One of these, however, was infected with a black pigmented Bacteroides, which turned out to be B. intermedius. Even though the

number of animals was small, the results clearly indicate that bacterial colonization can be selectively modified by systemic immunization.

B06 Develop safe, sensitive, objective tests of disease activity and rates of progression.

Diagnosis The development of totally objective methods of diagnosing and measuring periodontal disease represent long sought goals of periodontal investigators. Such methodologies would not only be useful to clinicians in daily patient care, but would also provide highly reliable data in clinical trials to determine the efficacy of treatment and prevention programs. Although these goals have not yet been reached, considerable progress has been made in developing more accurate means of measuring both attachment loss and alveolar bone loss and in the detection of current disease activity.

Recent R&D efforts have produced two new periodontal pocket probes which are at slightly different stages of development. One of these is the Florida probe, a computer-interfaced constant-force device, which is already available for evaluation by investigators at a number of research clinics; the second is the Foster-Miller probe, also with controlled force, but in addition, with the capability of automatically locating the cementoenamel junction. Both probes are said to be decidely more comfortable for patients because of the controlled force.

The Florida probe combines the advantages of a controlled probing force with an automatic electronic readout and computer storage of the data obtained. These features enable the device to eliminate two major sources of human error: variations in the probe force and in reading the measurement. The investigators claim that an accuracy of ±0.1 mm can be achieved and that when an occlusal stent is used, loss of attachment can be detected to a certainty of 99%. Thus the device not only promises instantaneous acquisition of improved clinical data, but should also reveal actual loss of periodontal attachment in shorter periods than is now possible.

The Foster-Miller probe is similar to the Florida device in that it offers controlled force in probing with an automatic digital readout and claims the same degree of accuracy. However, it offers considerably more promise in its ability to objectively locate the cementoenamel (CE) junction, and thus to take advantage of a built-in reference point for determining attachment loss. Location of the CE junction is done in the following manner. As the probe is withdrawn from the depth of the pocket along the tooth surface, the probe tip is subjected to pulsations at a cetain frequency. When the probe reaches the CE junction, the frequency suddenly changes, marking the position of the CE junction at that point. This information is recorded and calculation of the actual attachment loss follows automatically. The Foster-Miller probe is said to be ten times more accurate than can be achieved with the conventional

manual probe. Foster-Miller probes have not yet become available for testing in other clinical settings.

Improvements in the measurement of alveolar bone loss are based on electronic scanning of standardized x-ray images and the assignment of numbers to reflect the degree of radiographic density, or film darkness. The numerical data obtained is fed into a computer and subsequently used for direct analysis, or to generate a second enhanced radiographic image from which observations can be made that cannot be discerned by human eye in undigitized images. Systems incorporating these features, together with provisions for standardizing the amount of exposure and compensating for unintentional variations in angulation have reached such a point of refinement, that investigators claim an accuracy of 0.05mm in obtaining measurements of alveolar bone height. With this ability to determine small differences between images, the new methodologies can provide an objective means of determining whether bone loss or gain has occurred in a given period of time, and thus can provide a high degree of confidence in the demonstration of clinical results by means of "before and after" radiographic images.

Attempts to develop a noninvasive, objective method of detecting current periodontal disease activity has stimulated investigators to apply an extensive array of biochemical and immunologic assays to the fluid which leaks from the gingival tissues. By this approach they hope to identify specific, easily measurable substances which reflect disease in the immediately adjacent tissues. Although studies of several substances, including myeloperoxidases and purines, have seemed encouraging, the lysosomal enzyme B-glucuronidase continues to be the most promising candidate. Measurements of this enzyme, which reflects primary granule release by polymorphonuclear leucocytes (PMNs), appear to identify patients who exhibit either generalized or localized periodontal disease activity. In recent studies, patients with generalized disease could be identified by a whole mouth measure of B-glucuronidase activity, whereas patients with only localized disease could not be identified by a whole mouth score, but could be identified by sampling specific pockets showing high enzyme activity. These results are consistent with the traditional evidence that periodontal disease activity is associated with an infiltration of PMNs. Biochemical markers for the degree of inflammation, such as the enzyme arylsulfatase, or for cell death, such lactic dehydrogenase, did not provide a sensitive measure of disease, nor did measurement of IgG, IgA, IgM, or α2 macroglobulin.

F99 Oral Manifestations of AIDS

Research on the oral manifestations of AIDS syndrome is currently focussed on epidemiology, the pathogenesis of the oral lesions which occur, and their clinical management. It is now recognized that oral lesions are serious and important complications of the immune suppression characteristic of this syndrome.

Epidemiology Early detection of HIV infection is of great value in slowing the epidemic spread of AIDS as well as providing earlier treatment of opportunistic infections associated with the disease. Presently, oral lesions such as hairy leukoplakia and candidiasis are among the first clinical signs of HIV infection. Hairy leukoplakia is a newly described white lesion observed on the side of the tongue in HIV positive individuals who ultimately develop AIDS. Candida albicans infections may occur in a variety of oral mucosal sites. Recently obtained epidemiologic data indicate that the presence of such lesions in seropositive individuals is strongly predictive of the eventual development of ARC and AIDS. Atypical gingivitis (ATYP) and AIDS-virus associated periodontitis (AVAP) may even serve as earlier indicators than hairy leukoplakia and candidiasis. Data obtained from a study of 112 patients suggest that both of these unusual periodontal diseases are related to HIV infection and have early predictive value. ATYP frequently appears in the absence of other HIV-associated signs and symptoms. AVAP is associated with low T4/T8 ratios similar to those also seen in acute necrotizing ulcerative gingivitis not related to AIDS.

Pathogenesis One of the infecting agents observed in hairy leukoplakia is Epstein-Barr Virus (EBV), which could only be demonstrated in earlier studies by biopsy techniques. Since biopsies are not desirable in HIV patients and are absolutely contraindicated in hemophiliacs, investigators have developed non-invasive diagnostic techniques. Two reliable approaches have been devised: filter in-situ hybridization (FISH), which involves the use of the pBg20 probe and cytospin in-situ hybridization (CISH), which makes use of the same probe but in a different manner. Both techniques have confirmed the presence of EBV in hairy leukoplakia lesions.

AIDS-virus associated periodontitis (AVAP) is a severe form of periodontal disease frequently seen in patients with HIV infection. It is not yet clear from preliminary studies whether this infection is caused by opportunistic organisms not usually present in the oral cavity or by the indigenous periodontal flora. One study showed that the commonly present rods and spirochetes constituted greater than 50% of the organisms observed in 20 patients studied, but unusual protozoans were also detected in 12 sites. Preliminary evidence suggests that AVAP is primarily caused by typical periodontopathogens overwhelming the severely compromised host, but other types of microorganisms could also be involved. It is hoped that future studies will correlate the types of organisms in these severe periodontal lesions with the current immune status of the patients.

Treatment In a preliminary clinical trial on the efficacy of acyclovir, a potent drug used in treating hairy leukoplakia, 7 seropositive patients with this oral lesion were treated with the agent BWA515U admininistered orally, whereas 7 similar patients received a placebo. None of the 14 patients had frank AIDS. Within 28 days, all 7 of the

patients on the active drug showed significant or complete resolution of the lesion while the seven on placebo showed no change. The study shows that BWA515U is effective on a short term basis in eliminating the clinical, histological and virological features of this frequently seen AIDS-related oral lesion.

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.

Oral Cancer Oral cancers comprise a significant fraction of total malignancies observed among world populations. Although some are associated with viruses, many are believed to be caused by environmental chemicals which affect the molecular biology of the cells which become malignant. One study has focussed on the activation of cellular oncogenes in Syrian hamster cheek pouch epithelial cells by the carcinogenic agent dimethylbenzanthracene (DMBA). When this agent is repeatedly applied topically to the hamster cheek pouch, the DMBA induces epidermoid carcinomas with characteristic histopathological changes similar to those observed in spontaneous human oral cancers. Preliminary cell culture experiments with an epidermoid carcinoma cell line derived from a DMBA-induced cheek pouch tumor have shown that DMBA activates and amplifies the cellular oncogene C-erb. This activation of the C-erb B gene occurs at a stage in tumor development beyond that of epithelial hyperplasia, and actually coincides with the onset of early invasion of the connective tissue by the dysplastic epithelium. These results implicate the activation of the C-erb B gene as a critical event in the development of DMBA-induced malignancies, and suggest that other environmental cancers might develop by similar patterns.

F05 Accelerate efforts to develop an effective herpes virus vaccine.

Herpes simplex viruses (HSV) cause common infections which can pose serious health problems, particularly in infants, elderly individuals and debilitated patients. Research to develop a vaccine against these infections has involved basic studies of the biochemical structure and immunologic properties of the proteins which make up the virus wall. So far, the strongest HSV wall component to emerge as a candidate for a vaccine is a component designated as glycoprotein D (gD), which is present in both HSV-1 (oral) and HSV-2 (genital). The scientific approach has been to study the effects of mutations in the gene for gD on the biological properties of the glycoprotein and the virus, and to investigate the synthesis and processing of gD in an in vitro system. Site-directed mutagenesis of the gD gene and the creation of several deletion and point mutants have been accomplished, and the antigenic properties of the mutant proteins have been tested following synthesis in a eucaryotic system. Subsequent experiments showed that gD and synthetic peptides that mimic gD sequences can protect mice against challenges of lethal doses of either HSV-1 or HSV-2. These studies offer great promise of providing a safe subunit vaccine effective

against human herpes simplex virus infections.

H05 Develop improved procedures for characterizing the molecular structure and function of the salivary proteins and other macromolecules important in oral health maintenance.

Recent research has established that a group of at least 7 histidine-rich salivary proteins (HRPs) function as part of the non-immune oral defense system. This work has shown that these genetically polymorphic proteins possess both antifungal and antibacterial properties. Studies are now underway to isolate and characterize HRPs from the parotid and submandibular secretions, determine their primary structure and characterize their antimicrobial properties. Characterization studies conducted thus far indicate that the basic HRPs and the neutral HRPs are coded by separate but similar genes. To test the antifungal effects of HRPs, the investigators used preparations of parotid saliva containing several different combinations of HRPs, PRPs, lysozyme and albumin against C. albicans at different stages of its life cycle. The data not only showed that parotid HRPs were effective, but in addition, indicated that parotid saliva also contains other proteins which exert deleterious effects on C. albicans. Because C. albicans has been shown to be a common opportunistic organism seen in AIDS patients, these salivary proteins could become important as an early diagnostic marker for AIDS.

H09 Identify and characterize those diseases, environmental factors and treatment regimens that affect the salivary glands.

Salivary Glands Investigations are underway to determine the long range effects of psychotropic drugs on salivary flow rate in an animal model. Preliminary results clearly show a reduced salivary flow rate upon the adminstration of amitriptyline, a commonly prescribed antidepressant. In rats, 100 μg/kg of this drug administered intravenously in a single dose decreases both submandibular and parotid salivary flow by 25%; however, a single dose of 1 g/kg intravenously decreases flow rate by 60-70%. The xerostomia, or dry mouth, produced in this manner resembles that seen in Sjogren's Syndrome. Xerostomia is often an untoward side effect resulting from the killing of salivary gland cells during irradiation for head and neck cancer. Severe xerostomia can interfere with the normal functions of speech, mastication, digestion and deglutition, and may produce extreme physical discomfort.

H04 Elucidate the cellular mechanisms involved in the synthesis, transport, storage and release of inorganic and organic salivary gland products including the transcriptional, translational and post-translational events.

The classical two-stage model for saliva production includes the production by the acinar cells of an isotonic primary secretion which is

rendered hypotonic as it flows through the ducts. This model is based on NaCl assays of samples obtained by micropuncture. However, because of the small size of the samples and the likelihood of contamination with interstitial fluid, investigators have developed a microprobe for these analyses. When the probe was used, in vivo stimulation of the rat parotid gland gave higher concentrations of Na, K, S and Cl than were obtained in similar experiments using micropuncture. In addition, in vitro experiments involving parasympathetic stimulation of the isolated gland were consistent both with the findings of the in vivo experiments, as well as with the current model for saliva formation, which is based on osmotic coupling between NaCl and water fluxes. Use of the microprobe, with its unique capability of measuring elemental concentrations in intracellular organelles, is expected to provide new insights into the ion transport processes involved in salivary fluid formation and secretory granule maturation and exocytosis.

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