National Institute of Dental Research

ANNUAL REPORT

Fiscal Year 1986

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

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CONTENTS

INTRODUCTION

Fiscal Year 1986 marked a period of growth and research progress in the dental sciences. Accomplishments, both intramural and extramural, were many and touched upon a great variety of the biomedical and behavioral disciplines. Significantly, seeds for future progress were sown this fiscal year through the development of major cooperative initiatives among NIDR and her sister Institutes, other Federal agencies, and private industry. These collaborative efforts expand the research boundaries of the Institute, maximize the use of available resources, and provide the opportunity to explore new research directions.

REPORT OF THE DIRECTOR

Research Directions

During FY 1986, the Director set the tone for research planning and program objectives through a variety of administrative accomplishments. These include:

- The completion and transmittal to Congress of the report, "Use of the Center and Related Large Grant Mechanisms of Support in Meeting the Nation's Dental Research Needs." This report, developed with broad input from the dental research community, outlined an action plan for the future.

- Completion of the first stage of a collaborative planning effort involving the NIDR, the National Institute on Aging (NIA) and the Veterans Administration (VA) to produce and distribute widely the "Research Agenda on Oral Health in the Elderly." This effort is bringing a coordinated Federal focus to this important research area.

- Initiation of and oversight for a new Institute effort to work more closely with the private sector.

- Initiation of the early phase of development of a 10-year research plan for the 1990's including strategy, timetable and budget requirements.

Research and Training Support

The Director initiated a new centers program, Research Centers in Oral Biology (RCOB), designed to further strengthen the fundamental science base in dental research. In concert with the National Advisory Dental Research Council, Dr. Löe identified three NIDR grantees to be recipients of the Institute's first MERIT (Method to Extend Research in Time) awards. With Council support, the Director also implemented the NIDR Minority Research Supplement Grant Program to increase the number of under-represented minorities actively pursuing research objectives of NIDR.

Dr. Löe initiated the first meeting held by the NIDR to explore strengthening NIDR-industry collaborative research activities. Over 50 companies, associations, and foundations were represented at the one-day meeting. An internal NIDR task force on industry and academic relations has been established to identify further action that can be taken to foster mutually beneficial collaborations involving all segments of the research community.

In the summer of 1986, the Director initiated the Second National Survey of Oral Health in U.S. School Children. This is a follow-up to the NIDR 1979-80 survey to determine if the reduction in dental caries among school children is continuing, and to provide the basis for targeting preventive disease research activities.

Assigning a high priority to AIDS-related research, Dr. Löe assembled a meeting of representatives from the NIH, other governmental agencies, and academia who were knowledgeable about AIDS research and the oral implications of this infection. New clinical research activities have been initiated

within the intramural program and, where appropriate, ongoing fundamental research has been directed to AIDS and AIDS-related problems. Additionally, the portfolio of AIDS and AIDS-related projects supported by extramural grants was significantly expanded.

In January 1986, the Director assembled a meeting of a group of internationally known experts in vaccine development and/or dental caries to assess NIDR caries vaccine research activities and provide guidance on future directions.

Employee Opportunity

The Director actively encouraged the recognition of all employees for outstanding performance and renewed NIDR's commitment to the full utilization of talent among minority and female staff. Special recognition of employee contributions was given at the annual Awards Ceremony.

Working directly with the Equal Employment Opportunity Manager and Committee on the affirmative action program, the Director has supported the sponsorship of safety and self-development courses for NIDR staff. Other activities included establishing forums for guest speakers to discuss important educational opportunities for NIDR employees; providing EEO training on Federal equal employment opportunity laws and regulations which prohibit discrimination in employment; and nominating four outstanding employees for the NIH EEO Special Achievement Award--an award of merit recognizing individuals in the area of equal employment opportunity.

The Director also has committed the Institute to moving aggressively to increase its minority biomedical research support (MBRS) and to attract minority students to the Institute. For the first time, three Minority Access to Research Careers (MARC) students and one MBRS student were provided the opportunity to work in the Institute's laboratories during the summer session. In addition, the Institute sponsored a workshop inviting representatives from other BIDs to give an overview of their strategies for attracting minorities to biomedical and behavioral research.

Organizational, Public and Professional Communication

The Director continued implementation of the Operational Goals Project begun in 1985. This organizational development project is designed to determine what could be done to increase the effectiveness of the NIDR as an organization, what obstacles diminish its effectiveness, and how the NIDR environment could be altered to enhance working conditions in the Institute. Among milestones that have been accomplished, or which are in the final stagee, include regular Director visitations with senior staff, and regular visits by Personnel Office staff to program areas. In addition, employees are now offered greater opportunity for open communication in the position review process by having both notice of review and their position descriptions in advance.

The NIDR Incentive Awards Program is also being strengthened by the development of an awards profile and a review of the internal award process.

Emphasizing awareness of supervisory responsibility, a mandatory supervisory element has been added to all FY 1986 performance plans for SES/SSS/PMRS staff. Supervisors also are required to attend NIDR supervisory training sessions.

The Director provided numerous personal interviews to major print and broadcast media on a broad range of topics covering NIDR programs and research advances. These included Newsweek, USA Today, Business Week, Chicago Tribune, Boston Globe, Newhouse News Service and other general circulation newspapers and magazines.

Dr. Löe serves as chairman, Dentistry Section of the American Association for the Advancement of Science (AAAS) and represents the NIDR at meetings of the Association of American Medical Colleges, the American Dental Association, the American Association of Dental Schools-Deans meeting, the American Association for Dental Research and the American Academy of Periodontology. He attended the Government-University Industry Research Roundtable Conference, National Academy of Sciences, and conferred with the Institute of Medicine President regarding mechanisms to improve long-term funding for the NIDR and the NIH.

International Activities

International activities of the Director included: serving as a member of the Oral Health Research Advisory Committee to the World Health Organization (WHO) and as a member of the Commission on Oral Research and Epidemiology of the Federation Dentaire Internationale; acting as a consultant to the following organizations: Council on Graduate Studies of Canada to appraise the MSc/PhD program in dentistry, the Karolinska Institute in Sweden to discuss the impact of research on the future of dentistry, and the Council on International Relations of the American Dental Association.

The Director also participated in the activities of the Butterfield Committee on behalf of the British Society for Dental Research (London), in the annual meeting of the International Association for Dental Research in The Hague, and in the International Conference on Periodontal Disease in Zurich. In addition, Dr. Löe presented papers at the 3rd European Symposium on Caries and Periodontal Disease in Geneva and at the National Council for International Health in Washington, D.C. He also presented a seminar for Latin American dentists convened by the Pan American Health Organization (PAHO).

Ongoing international research activities include data collection in association with the study of the natural history of periodontal disease in tea plantation workers in Sri Lanka and general oversight for all NIDR international activities, including the development of a WHO International Collaborative Study of Oral Health Outcomes.

PUBLICATIONS

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Löe, H. 1985. Dental health of older Americans: Planning for the Future. Gerodontics 1:288-290.

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Löe, H. 1986. The fluoridation of U.S. public water supplies. Public Health Reports, 101:157-162.

Löe, H. 1986. Hearings before the Subcommittee of the Committee on Appropriations House of Representatives, FY 1987, 99th Congress, National Institute of Dental Research, NIH, March 12, 1986, pp. 731-858.

Löe, H. 1986. Hearings before the Senate Subcommittee of the Committee on Appropriations; FY 1987; 99th Congress, March 6, 1986 (in press).

Löe, H. 1986. Butterfield Report-The right foot forward. British Dental Journal, 160:224.

Löe, H. 1986. Periodontology: Memories and Hopes. In Parodontologie in Beweging 50 jaar A.R.P.A-N.V.v.P. 1936-1986, Veldkamkp, D.F. (ed.), pp. 156-159, Nederlandse Vereniging voor Parodontologie 1986.

Löe, H., Anerud, A.,, Boysen, H. & Morrison, E. 1986. Natural history of periodontal disease in man: Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. Journal of Clinical Periodontology, 13:431-440.

HONORS AND AWARDS

During FY 1986, Dr. Löe received the following honors from the academic dental community:

First Annual Penn Dental Journal Lecturer, University of Pennsylvania, February 1986.

1986 Goldstein Lectureship, Emory University, April 1986.

Honorary Doctorate, University of Maryland, May 1986.

PRESENTATIONS

NIDR Workshop/Seminar on the Development of Clinical Trials, Introductory Remarks, Bethesda, Maryland.

Interagency Meeting on Mercury Toxicity-Dental Amalgam, Opening Remarks and Summary Remarks, Bethesda, Maryland.

IADR/AADR National Affairs Committee, Remarks: NIDR Update, Washington, D.C.

Symposium on Periodontal Diseases, "Chlorhexidine Therapy in the Treatment of Gingivitis: Overview Comments", San Diego, California.

Meeting on the Oral Aspects of AIDS, Introductory Remarks, Bethesda, Maryland.

Symposium on Geriatric Dentistry and Nursing Home Care, "Toward Optimal Oral Health in Aging: A New Research Agenda", Farmington, Connecticut.

Dental School Deans 1985 Conference, American Association of Dental Schools, Comments, Bahamas.

The British Society for Dental Research, The Independent Committee on Dental Research (The Butterfield Committee), Remarks, London, England.

NCI/NIDR Smokeless Tobacco Consensus Conference, Greetings--Opening Remarks, Bethesda, Maryland.

Washington Metropolitan Academy of General Dentistry Meeting, Presentation/Lecture, Bethesda, Maryland.

3rd European Symposium: Caries & Periodontal Disease, "Progression of Natural Untreated Periodontal Disease in Man", Geneva, Switzerland.

Canadian Council on Graduate Studies, "Advanced Dental Education Needs", Toronto.

First Annual Penn Dental Journal Lecture, University of Pennsylvania, "The Once and Future Dentistry", Philadelphia, Pennsylvania.

Baltimore County Dental Society, "Progress in Dental Research and Its Impact on Dental Education and Practice", Baltimore, Maryland.

Georgetown University School of Dentistry, Faculty Retreat, "Impact of Research on Dental Education", Washington, D.C.

Tri-Service Dental Society Annual Meeting, "Putting Science Into Practice", Ft. Meade, Maryland.

Senate Appropriation Committee Hearings, NIDR, Opening Remarks and Testimony, Washington, D.C.

House Appropriations Subcommittee Hearings, NIDR, Opening Remarks and Testimony, Washington, D.C.

American Association for Dental Research Annual Meeting, Student Research Group, "Excitment of Dental Research", Washington, D.C.

American Association for Dental Research Annual Meeting, "NIDR Forum", Washington, D.C.

University of Minnesota, Annual Dental Students Conference on Dental Research, Keynote address--"From Molecules to Mouths", Minneapolis, Minnesota.

American Student Dental Association, 1986 Goldstein Lectureship, "Of Dentistry, Dynasty, and Destiny", Atlanta, Georgia.

Lancaster Dental Society Meeting, "Advances in Periodontal Disease Diagnosis and Treatment", Bethesda, Maryland.

The Excitement of Dental Research: An ADA Briefing for Congressional Staff, "Recent Advances in Dental Research", Bethesda, Maryland.

Conference on Evaluation and Management of Salivary Gland Dysfunction, Welcoming Remarks, Bethesda, Maryland.

NIDR/Industry Collaborative Research Conference, Opening Remarks, Bethesda, Maryland.

Conference on Microbiological Diagnosis in Dental Caries and Periodontal Disease, Opening and Closing Remarks, Bethesda, Maryland.

American Dental Hygienists' Association Annual Meeting, Greetings, Washington, D.C.

University of Texas, Dental Branch Annual Faculty Retreat, "Overview of the Structure and Purpose of NIDR; Funding Opportunities from the Institute", Houston, Texas.

National Council for International Health, "Prevalence and Severity of Oral Diseases in a Sri Lankan Population", Washington, D.C.

Karolinska Institute, "The Future of Dentistry", Stockholm.

Pan American Health Organization, "Advancement of Dental Research and Future of Dentistry", Washington, D.C.

NIDR Clinical Staff Fellowship Conference, "The Future of Dentistry", Bethesda, Maryland.

International Conference on Periodontal Disease, Ittigen, Switzerland.

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

Conference on Diagnostic and Therapeutic Technology in Dentistry Conference, Opening and Closing Remarks, Baltimore, Maryland.

ASSISTANT DIRECTOR FOR INTERNATIONAL HEALTH, NIDR

Accomplishments in the international health area for FY 1986 include administrative coordination of NIDR-related activities and program initiatives. A total of eight foreign extramurally-funded projects were supported, for a total of $459,336, in Canada, Israel and in the United Kingdom. Six of these were regular research grants and two were fellowships awarded to U.S. citizens. Some of the areas of research include orofacial pain, dental implants, collagen, mineralization and the role of dental mechano-receptors in mastication.

Administrative staff coordination involved review of international meeting reports and travel orders, and monitoring and tracking of staff participation in official international agreements. Orientations were provided to visitors from abroad.

Fogarty International Center (FIC)

In coordinating between NIDR and FIC, the Assistant Director for International Health and staff participated in meetings of the BID International Representatives; prepared and submitted NIDR annual international activities reports; obtained staff comments on circulated documents; maintained contact with the FIC Advisory Board; prepared a letter to U.S. dental school deans concerning the Senior International Fellowship program of FIC; participated in an agreement between NIH and the Mexican Center for Research and Advanced Studies initially to involve the Neurobiology and Anesthesiology Branch; participated in the U.S.-Italy Science and Technology Agreement involving a project in osteonectin variation and visited the Italian coordinator in Rome to discuss collaboration objectives; facilitated reviews for several dental research projects under the U.S.-Spain Science and Technology Agreements and for one project under the U.S.-Israel Binational Science Foundation; consulted on FIC evaluation projects, particularly those related to international allocation of biomedical research support.

World Health Organization (WHO)

The Assistant Director for International Health continued to work with the World Health Organization to strengthen the protocol for the WHO International Collaborative Study of Oral Health Outcomes (ICS-II); visited Egyptian and Israeli Government officials and proposed principal investigators to explore use of tri-lateral funds to support the inclusion of these countries; visited with Government of India officials and researchers to explore use of U.S.-India funds to support India's participation and presented a paper on the collaborative project at the World Sociological Congress in New Delhi; obtained concept clearance for the global project and the U.S. replication from the NIDR Programs Advisory Committee and the National Dental Research Advisory Council; explored potential collateral support with World Bank officials; and prepared contract justification forms for the two U.S.-related proposed projects.

The Assistant Director for International Health and staff also provided technical assistance to WHO (Europe) on draft Guidelines for Self, Family and Lay Care in Oral Health; provided assistance to Pan-American Health Organization (PAHO) for a presentation on social sciences and dentistry to a

group of Latin American pedodontists; and maintained activities associated with WHO Collaborative Center Status.

Federation Dentaire Internationale (FDI)

The Assistant Director for International Health served as consultant to the FDI Scientific Programme Committee, organizing and presenting a special session and workshops on "Improving Access to Dental Care" for the Congress in Manila; provided technical assistance on program evaluation projects and on abstracts submitted for review for the Congress in Buenos Aires; and prepared a tentative program agenda for the 1988 Congress in Washington, D.C. at which NIDR's 40th anniversary will be celebrated.

Other FDI activities included serving as Task Leader for the Working Group on Oral Health Promotion, Commission on Oral Research and Epidemiology, providing coordination and guidance on three sub-projects and the preparation of a draft set of guidelines for use by national dental associations; and serving as official delegate to the FDI-General Assembly, representing the Behavioral Sciences in Dental Research.

Council on International Relations, American Dental Association (ADA)

The Assistant Director for International Health served as consultant to the Council on International Relations and on their behalf participated in the development of a survey of dental schools' international activities; prepared background materials for a proposed film on international oral health opportunities; served as liaison to WHO for the Council's project on audio-visual continuing education materials; and assisted on follow-up of ADA Delegation Visit to China (including setting up a de-briefing for NIDR of a UNICEF consultant).

National Council on International Health (NCIH)

The Assistant Director for International Health participated as a member of the Program Planning Committee of the annual meeting of the NCIH and organized a special session on "Global Research Dimensions of Oral Health and Disease," stimulating interest among dental scientists in NCIH programs; and circularized to the dental community a program announcement for a forthcoming meeting highlighting health promotion.

Papers and Presentations

Presentations were made at Joint Singapore/Malaysian Dental Association meeting on oral health promotion and contributions of behavioral and social sciences to changes in oral health and disease.

The Assistant Director for International Health addressed the First International Congress on Community Dentistry and Pedodontics in Alexandria in December on contributions of socio-dental research to community oral health and the role of the WHO International Collaborative Study of Oral Health Outcomes in public health policy.

A presentation was given on international oral health programs of the NIDR at the Conference on International Oral Health Problems and Challenges, Harvard

School of Dental Medicine.

Information Inquiries

The Assistant Director for International Health provided responses to inquiries regarding international research support to the University of Groningen, The Netherlands; Health Education Council, U.K.; University of Aberdeen, Scotland; Indian Association for Dental Research; Harvard University; University of Michigan; and MD Anderson Cancer Center in Texas.

Information and technical assistance also were provided to University of Dublin on analysis of their national survey of children's health, to Columbia University on Dunning lecture nominees (international dental care delivery systems), to the University of Melbourne on sabbatical research project interests, to a Singapore dental journalist for publicity materials on dental research, to Japan regarding placement of a sociologist in a health services research center in the U.S., to Brazil about institutions providing master's degrees in dental public health, and to China on post-doctoral fellowship support.

In addition, nominees were provided for speakers and potential participant lists for the World Dental Conference held in Jerusalem in July 1986.

PLANNING AND EVALUATION SECTION (PES)

The function of the Planning and Evaluation Section, OPEC, is to coordinate all planning and evaluation activities for the NIDR, originate special projects, and respond to internal (NIDR) and external requests for information relevant to planning and evaluation.

Planning and Evaluation Activities

Staff developed and implemented procedures for tracking and evaluating the NIDR Long-Range Research Plan, "Challenges for the Eighties," which was published in FY 1984. All extramural grants, intramural projects, and research contracts funded by the NIDR since FY 1982 have been classified according to their primary and secondary focus regarding the research objectives and 14 sections delineated in the plan. Research funded before, during and after the plan's implementation will be compared according to the classification system. Progress made in attaining the objectives will be tracked through the use of such criteria as number and quality of applications received, dollars obligated, number of projects funded, papers published and impact of these publications, and peer assessment of accomplishments.

New evaluation projects include a bibliometric evaluation of behavioral and social science research funded by the NIDR and the NIH (to be conducted in conjunction with the Chief, Program and Evaluation Branch, Office of the Director, NIH); an assessment of international dental research, including sources of financial support, and the contributions made by various countries to dental science; the impact of the NIDR on knowledge advances in the biomedical sciences; and the interaction among private industry, academia, and the Federal government in research involving restorative dental materials. Other new projects include an evaluation of NIDR activities in basic and clinical pain research (including sensory-motor dysfunctions directly related to orofacial pain) and an analysis of past NIDR extramural and intramural research and training efforts, including changes and trends in the various oral research subfields. This effort will assess contributions to knowledge about the epidemiology, etiology, pathology, prevention, diagnosis, and treatment of oral diseases and dysfunctions. The latter project will be conducted in connection with the 40th anniversary celebration of the NIDR.

Staff developed and coordinated the annual program planning meeting with the Director, NIH, in December 1985; the NIDR component of the NIH Research Plan; and the NIDR FY 1987 Evaluation Plan.

Other Activities

Staff compiled the annual budget justification for the NIDR, wrote many of the sections, and provided other assistance to the NIDR Budget Office and the Director, NIDR.

In coordination with the National Institute on Aging and the Veterans Administration, staff wrote, designed and formatted the "Research Agenda on Oral Health in the Elderly" and "Catalog of Resources". Staff also served as principal contact for follow-up on production and printing, and arranged distribution to the major constituencies of the NIDR. In connection with this project, a member of PES staff was the primary author of an article for

Gerodontics, based on this collaborative effort to stimulate and foster research on oral health in the elderly.

Staff, in association with the Assistant to the Director, NIDR intramural research program, planned, developed an agenda, and coordinated follow-up for a NIDR/Industry Collaborative Research Conference held in May 1986 and served on an internal NIDR/Industry Relations Task Force. A staff member served on the Planning Committee for the Conference on Diagnostic and Therapeutic Technology in Dentistry held in September 1986.

Staff prepared or assisted in the preparation of the following material: a summary of NIDR cancer research for inclusion in a report of the National Cancer Institute; AIDS reports; the NIDR Biennial Report (section on future initiatives); NIDR research related to women's health issues; program analysis projects which will be used for the NIDR 40th anniversary; and "New Challenges, New Realities," a brochure describing NIDR funding patterns.

Staff acts as Division of Legislative Analysis representative for the NIDR and serves on the following NIH committees: Manpower Evaluation Advisory Committees, Committee to Investigate the Average Costs of Research Grants, ad hoc Working Group on Economic Impacts of Research Advances Supported by the NIH, Technical Review Committee for Evaluation Projects Funded Through 1 Percent Set-Aside Funds, and Planning and Evaluation Officers.

Staff took part in discussions with the Director and senior staff on how dental research, along with changing patterns of disease and demographics, can be expected to affect dental education and dental practice in the years ahead. Staff provided resource material and references to the Director in developing such themes for major policy statements and journal articles.

Presentations

The following were presented by the PES staff during FY 1986 at various conferences, committee and other professional meetings:

L. Jackson Brown

"The Relationship Between NIDR Supported Research Training and Subsequent Grant Activities," NIDR Dental Research Programs Advisory Committee Meeting.

"Economics of Regulation," Annual Session of the Eastern Economic Association.

"The Current Status of Forecasts of the Dental Sector," Annual Session of the American Public Health Association.

"The Economic Performance of Dental Markets: An Empirical Investigation of the Relationship Between Costs and Prices," Annual Session of the American Association of Dental Research (AADR).

James Lipton

"An Assessment of the Effectiveness of the K04 Grant Program," NIDR Dental Research Programs Advisory Committee Meeting.

"An Evaluation of the Performance of NIDR-Supported Trainees and Fellows," NIDR Dental Research Programs Advisory Committee Meeting.

"The History of the NIDR Contract Use," NIDR National Advisory Dental Research Council.

"An Assessment of the NIDR Intramural Research Program," NIDR National Advisory Dental Research Council.

"Sociocultural Considerations When Assessing Pain: A Proposed Model and Methods of Investigation," Annual Meeting of the American Association of Dental Research.

"Cultural Aspects of Pain Assessment," NIH Consensus Conference on the Integrated Approach to the Management of Pain.

"Dental Caries Prevention Activities and Attitudes Among Migrant Health Center Dental Service Directors," Annual Meeting of the U.S. Public Health Service Commissioned Officers Association.

"Recent Accomplishments in Providing Dental Prevention Services and Other Essential Dental Care at Migrant Health Centers," Annual Meeting of the National Advisory Council on Migrant Health.

"The Role of the Social Sciences and the Sociologist in Dental Research Sponsored by the NIDR," Annual Meeting of the American Sociological Association.

Joan Wilentz

"Collaborative Research Project on Oral Health in the Elderly," Annual Session of the American Association of Dental Research.

RESEARCH DATA AND MANAGEMENT INFORMATION SECTION (RDMIS)

The Section continues to support the technical and administrative information needs of the Institute. Collecting and processing the various data elements of every research and training project supported by NIDR occupies considerable time and effort. Similar attention is given, though less successfully, to projects of dental interest with support from other sources.

A variety of devices have been developed to retrieve and disseminate these data in an expeditious and meaningful manner with major emphasis on the "end user." Published technical reports and online computer programs have transported most of this information beyond the boundaries of the Institute and into the dental research community.

NIDR ONLINE

A computerized online dental research information system to improve the lines of communication between the Institute and the research community became fully operational during this fiscal year. Users include the libraries of dental schools, advanced dental education institutions, Veterans Administration, Department of Defense, Public Health Service, industry, and individual investigators. Over half of the dental schools and advanced dental education institutions are registered users. Statistics are being collected on a weekly basis to determine the level of use and the areas of greatest interest.

Remote Information Facility (RIF) Enhancement

The Remote Information Facility's (RIF) widespread savings continue to accumulate as more and more individuals are accessing and utilizing its full capabilities. As a result, personnel and budgetary resources have been more efficiently expended. Work has already begun on a new and enhanced edition of RIF (Version 3.0), whose initiatives are based on feedback and suggestions from current users and the staff of the Research Data and Management Information Section. The systems analysis design and development phases have already been accomplished, and the next step will be the actual programming and implementation.

One significant module has already been incorporated into the present system this fiscal year. This software enhancement allows management and administrators to track NIDR expenditures for Acquired Immunodeficiency Syndrome (AIDS) research, both in the extramural and intramural programs. This has resolved a budgetary tracking problem of increasing magnitude by accounting for this special allocation of funds.

The RDMIS has designed and developed a brand new system which gives the Administrative Officer of the Intramural Research Program the ability to directly change personal compensation, personal benefits, and other object dollars to reflect the volatile situation of ever fluctuating budgetary requirements. Previously, such changes took several days of staff time and effort, as well as that of the budget and administrative officers. Now, within minutes, budgets can be changed and dollars re-allocated among the different laboratories and branches, an immediate balance of funds is automatically calculated, and reports are run and printed at a location convenient for fast retrieval. Hence, up to-date information is readily

available.

Other Systems

Three other systems have been developed to better meet the needs of the Institute. The NIDR SCHEDULER records all meetings and conferences, both present and future, that management plans to attend. The system was designed in response to the expanding interests of the dental community and the scheduling problems this entailed. For example, problems arose when one scientist organized a conference only to find that chief speakers were scheduled elsewhere. Each executive secretary is responsible for entering the data and the resulting current reports can be accessed at any time.

The NIDR LOGGING SYSTEM was developed mainly for in-house use, but its capabilities are many. It enables the Chief, RDMIS to review the number and type of reports being prepared for various time frames, requestors, and/or subjects. It also alleviates duplication of effort as one can see if a similar request has been processed, where the information or program can be found, and how the report was prepared.

One other system that has been designed, developed, programmed, and now is being tested and debugged is an NIDR REPORT WRITER. This program will help in the preparation of various research "subject" requests, as it will totally automate the entire process. In addition, it will allow "non-programmers" the flexibility to "design" their own reports, with a multitude of options open to them. Final implementation is projected for early Fall.

Savings on DELPRO

The RDMIS also has provided assistance to the Administrative Offices in converting from dedicated 3270 equipment used exclusively for delegated procurement (DELPRO) to an IBM PC-based system. This new system provides DELPRO 3270 emulation as well as normal PC operations which include spreadsheets, data base management, word processing and mainframe communications. This conversion is being done in an effort to eventually eliminate $25,000 worth of yearly DELPRO equipment rental fees while simultaneously taking advantage of the superior capabilities of personal computers.

FEDLINK

The interagency agreement with FEDLINK, the Federal Library Information Network operated by the Library of Congress, has been expanded in-house to include users in all three of the Institutes' operational programs. Through participating vendors like DIALOG and BRS, NIDR staff has access to well over 300 computerized data bases on a variety of subjects from Agriculture and Nutrition to the Social Sciences and Humanities.

Electronic Mail

Electronic mail, which has been a practical means of communication within the Institute for some time, is now available to the research community at large through the "Message to NIDR" option of NIDR ONLINE and world-wide via DIALMAIL. Other electronic mail services, conferences, and bulletin boards

are likely to become available within the next year.

Technical Reports Published

The following technical reports which are published and distributed annually were made current. Due to the lag-time inherent with the collection of data on trainees and fellows and changes in the computer program to produce these data, it was necessary to go back to fiscal year 1981 and complete three years at one time. In order to produce data on three consecutive years, computer program procedures were rewritten and improved to streamline the process of accessing IMPAC (Information for Management, Planning, Analysis, and Coordination), the Division of Research Grants computer based information system, the Trainee Appointment Files, as well as the NIDR data files.

"NIDR Programs"
A comprehensive listing and analysis of dental research projects supported by the dental Institute on a fiscal year basis.

"Selected List of Technical Reports in Dentistry"
Citations to all of the final technical reports submitted to the National Technical Information Service (NTIS) related to dentistry.

"Trainees and Fellows: Supported by the National Institute of Dental Research"
A collection of trainees and fellows by name, program/sponsor, discipline/field, and fellowship project title.

"NIDR Indexes"
An index to all dental research projects supported by the Institute during the fiscal year by name of principal investigator, project number and subject.

Private Act/Freedom of Information Act

The number of PA/FOIA requests appear to have leveled off this year with no particularly large or unusual requests. The bulk of the Privacy Act requests are from dental patients in the Clinical Center seeking copies of their x-rays. More effort is expended reporting, updating system notices, and performing related administrative duties related to the Act rather than responding directly to requests. Freedom of Information Act requests are about evenly divided between third party requestors for copies of successful contract proposals and awarded grant applications and progress reports. Our average response rate is about half of that for all NIH and the other statistical measures are equally favorable.

Staff Activities

Mrs. Karamian received a commendatory letter from the NIH Office Technology Coordinator for her service to that group. Mrs. Hill continues to serve as the Secretary for the NIH ADP/EP Coordinating Committee. Mrs. Flora and Mr. Ruben represent Lead Users for the Institute. These individuals have provided considerable assistance to personal computer (PC) users within various operational components of the NIDR in terms of the purchasing, setup, and effective utilization of PC's.

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 staff planned and coordinated activities for the NIDR 40th anniversary program: directed the solicitation and procurement of services for the development and publication of a formal Institute history; and initiated the program concept, prepared a proposal and handled negotiations with professional staff of NOVA for the production of a feature on research advances in dentistry to coincide with the Institute's 40th anniversary in 1988.

Under special agreement with the NIH Office of Communications, NIDR information specialist developed, produced and directed a slide-tape presentation on the NIH for its upcoming centennial celebration. In addition, plans are under way to develop a new exhibit depicting the history of fluoride in the United States. This exhibit will be a significant part of the NIDR's contribution to the NIH centennial celebration.

At the request of National Institute of Diabetes and Digestive and Kidney Diseases, a member of PIRS staff addressed a meeting of the National Diabetes Advisory Board about NIDR's planned diabetes-related education activities. Over the course of the fiscal year, the professional guide "Detection and Prevention of Periodontal Disease in Diabetes," a patient hand-out "Dental Tips for Diabetics," and a coordinating professional education exhibit were produced. Arrangements also were made with the National Diabetes Information Clearinghouse to handle mailing and distribution of the new publications.

In FY 1986, PIRS also sponsored a science writers seminar on bone research attended by 65 journalists, and provided background materials, press information and scientific papers to participants; initiated, planned and conducted the first NIDR walking tour for attendees at the American Association for Dental Research meeting in Washington, D.C., and developed and produced a guidebook for visitors to the Institute's research facilities; and coordinated development of NIDR events for the NIH centennial, including a day-long scientific symposium for the NIH community on dental research.

PIRS also planned, coordinated and directed all arrangements for the 1986 Seymour J. Kreshover Lecture honoring Dr. Irwin Mandel and highlighting the role of saliva in monitoring oral homeostasis.

Public and Professional Education

During FY 1986, 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 and International Association for Dental Research meetings; prepared regular 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; prepared four dental health articles for distribution 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 Danish, Norwegian and Swedish dental journals, ADA News, and General Dentistry; prepared a major article on fluoride for Public Health Reports; prepared a special edition of "NIH News and Features" devoted entirely to the Institute's pain research activities; and converted the NIDR slide-tape show to video for use at professional meetings including the AADR, American Dental Association and the American Association of Dental Schools.

PIRS also exhibited at the general sessions of the AADR, AADS, ADA and the American Diabetes Association; continued to distribute, on a free-loan basis, Dr. William Wright's slide program for patients undergoing treatment for cancers of the head and neck; and continued periodic publication of "NIDR Research News."

Publications

In FY 1986, PIRS developed and produced the following new publications:

"Graduate Training Programs Supported by the National Institute of Dental Research,"
"Pain Research from Laboratory to Clinic,"
"Detection and Prevention of Periodontal Disease in Diabetes,"
"Dental Tips for Diabetics."

"Dry Mouth" and "Seal Out Dental Decay" fact sheets, and the Institute, Intramural and Extramural brochures were updated and reprinted.

Print Media

In FY 1986, PIRS arranged interviews and/or provided background information on dental research topics to a variety of general circulation newspapers and magazines, and specifically to a number of women's journals. These include Glamour, Family Circle, Woman's Day, New Woman Magazine, McCall's, Working Woman Magazine, Vogue, Self and Washington Woman Magazine.

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, Chicago Tribune, Business Week, Boston Globe, Newsday, Newsweek, Newhouse News Service, Reader's Digest,

Prevention, Associated Press and the Wall Street Journal.

As a result of PIRS publicity efforts, Reader's Digest featured NIDR's mussel glue story in a recent issue.

Broadcast Media

In FY 1986, local channels 4, 7 and 9 provided coverage of NIDR-supported research on a variety of topics including pain, recent advances in periodontal disease, orthognathic research, mechanism of fluoride action, and nitrous oxide. The highlight was a week-long feature on the Pain Clinic by channel 7 news.

The Voice of America conducted interviews with Institute staff on cleft lip and palate research, fluoride and periodontal diseases.

Reports

PIRS coordinated the recording, writing and printing of minutes of the National Advisory Dental Research Council and the Institute's Program Advisory Committee meetings during FY 1986.

Staff contributed to 10 Special Reports to Congress, including research advances in the following areas: cystic fibrosis, diabetes, arthritis, digestive diseases, AIDS, maternal and child health, endocrinology, immunology, sleep disorders, and international activities.

Information specialists prepared the FY 1985 annual report, the FY 1985-1986 biennial report, and contributed to the EEO FY 1986 Report on the Decade of the Disabled.

General Communications Activities and Services

PIRS responded to approximately 12,000 requests for information on a broad range of topics from the public, professionals, Congress and the media. Over 413,000 publications were sent out during FY 1986.

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.

This office also 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 in all program areas 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 Health Promotion and Science Transfer Section.

In addition, PIRS prepared the NIDR submission for the NIH centennial publication on recent research advances; contributed regularly to the "NIH Record" and "NIH News and Features"; provided information services for the pain management consensus conference and developed a videotape for TV broadcast; and provided editing assistance for several proceedings, including the pain consensus conference, as well as for intramural research papers.

PIRS fluoride specialist continued activities in support of public health agencies in states and cities involved in the implementation, extension or promotion of dental health programs using fluorides. In FY 1986, these activities included provision of technical information for judicial and regulatory proceedings; performing liaison with other health entities (U.S. and foreign) for the development, synthesis and exchange of needed information; maintaining working contacts with professional groups in public health and in the related technical fields; and continuous review of relevant publications, reports on ongoing research, and the findings of judicial, regulatory and legislative deliberations for additions to the existing records system.

Meetings

PIRS provided a range of planning, logistical and communications services for the following meetings:

January 1985	District of Columbia Academy of General Dentistry seminar.
March 1986	Tri-service Dental Society Annual Meeting.
April 1986	Lancaster County Dental Society seminar.
April 1986	NIH Consensus Development Conference, "Anesthesia & Sedation in the Dental Office."
May 1986	American Dental Association Briefing for Congressional Staff
September 1986	Seymour J. Kreshover Lecture.

Personnel

The secretarial position in PIRS was filled permanently in fiscal year 1986. In addition, PIRS had available the services of a summer intern from Harvard University for a variety of writing projects.

In June 1986, the position of fluoride specialist was transferred from PIRS to the Epidemiology and Oral Disease Prevention Program.

HEALTH PROMOTION AND SCIENCE TRANSFER SECTION (HPSTS)

The Health Promotion and Science Transfer Section was part of the Office of Planning, Evaluation, and Communications for the first eight months of FY 1986. This report covers that time frame. On June 1, 1986, the Science Transfer and Research Analysis Branch was established. Staff and activities of the Health Promotion and Science Transfer Section were incorporated into the new Branch in the Epidemiology and Oral Disease Prevention Program.

A four-year plan for the Section was developed in FY 1985. The plan serves as a matrix for the current report of activities and accomplishments for FY 1986.

Conferences, Courses, Workshops

Staff provided support to the Clinical Investigations and Patient Care Branch for a conference on Evaluation and Management of Salivary Gland Dysfunction held May 15-16, 1986. The state-of-the-art conference, targeted to dental practitioners, focused on the problems associated with secretory functions, including the assessment and treatment of these disorders. Conference participants identified several areas in which further salivary gland research is needed. Proceedings of the conference will be published as a special issue of the Journal of Dental Research.

Staff were instrumental in organizing and supporting presentations and follow-up discussions on the subject of preventing the spread of infectious diseases in dental settings at the annual meetings of the American Dental Association, the American Public Health Association and the American Association of Public Health Dentistry.

All-day seminars featuring presentations by staff of the NIDR were organized for three different groups: District of Columbia Academy of General Dentistry, dental educators, and other local dental practitioners; Lancaster County Dental Society; and dental hygiene educators as a program component of the annual meeting of the American Dental Hygienists' Association. The purpose of these seminars was to acquaint dental health practitioners with the activities and current research programs of the NIDR.

Staff organized a workshop on the use of microcomputers in health education for individuals working in health education and health promotion in the metropolitan D.C. area. The purpose of the workshop was to emphasize the potential uses of microcomputers in health education and to stimulate interest in their use in this field. Instructors for the all-day workshop were Drs. Robert Gold and Glen Gilbert, nationally known experts in the area.

Through both lectures and presentations, staff have provided overviews and updates of current research (see attached list).

Educational Materials

During FY 1986, more than 500,000 publications, posters, and scientific papers were distributed to the public. Requests came from health care providers and from individuals in agencies and institutions such as state, county, and local

health departments, Department of Defense, Indian Health Service, Head Start, and Migrant Health. International requests also were received and processed.

A paper, "Infectious and Sexually Transmitted Diseases: Implications for Dental Public Health" by Dr. Sol Silverman, was reprinted and is being distributed upon request to health professionals.

Printed Materials

During FY 1986, staff developed a variety of printed materials to promote oral health to the public. A new poster on the prevention of bottle mouth caries was produced and made available in both English and Spanish. A leaflet that explains NIDR's new film, "Prescription for Periodontal Health" was prepared and printed. The leaflet also includes an order form for free-loan use.

"Fluoride Tablets...A Healthier Smile for School Children" was revised to incorporate current knowledge and printed. HPSTS updated two of the Public Inquiries and Reports Section publications: "A Healthy Mouth for You and Your Baby" (formerly "Healthy Teeth for You and Your Baby") and "Seal Out Dental Decay." Both have been reprinted.

Two staff members co-authored an article entitled "School-Based, Self-Applied Fluoride Programs: The Critical Role of Teachers" which was submitted to Instructor, a national teachers' magazine.

Electronic Media

In collaboration with industry, a film, "Prescription for Periodontal Health," was produced and was shown for the first time at the annual session of the American Dental Association in San Francisco. The film, "Fluoride: The Magnificent Mineral" has been translated to open caption for use by the hearing-impaired as a result of collaboration with Gallaudet College and two University of Minnesota dentists.

Two scripts, "Xerostomia (Dry Mouth)," prepared in FY 85, and "Prevent Baby Bottle Tooth Decay," prepared during the current fiscal year, were submitted to and accepted by Maryland Tel-Med. NIDR's free-loan films continue to be in great demand. The Institute's seven films were seen by nearly 167,000 viewers during this fiscal year.

Exhibits

NIDR's scientific exhibits were shown at five annual meetings: the American School Health Association, American Public Health Association, American Dental Hygienists' Association, National Association of School Nurses, and the National Education Association. These meetings provided opportunities to provide consultation, promote oral health and NIDR's objectives, answer public inquiries, and distribute our publications.

Staff developed two new exhibits. The first, "Healthy Smiles Through Research," is designed for use by NIDR staff at national meetings, and it has

been shown at the annual meetings of the American Dental Hygienists Association, the National Education Association and the American Public Health Association. The second, "Protect Your Smile," is a free-loan, table-top exhibit which emphasizes the importance of sealants and systemic and topical fluorides for optimum oral health. Six of these exhibits were developed and made available for loan late in the fiscal year. The free-loan exhibits provide the opportunity for others to promote our educational materials at public health, educational, and professional meetings as well as at health fairs. HPSTS's free-loan, table-top exhibits, including the 11 which emphasize school-based, fluoride regimens, were sent throughout the U.S. to a variety of requestors for a total of 471 days.

Plans for six free-loan, table-top exhibits aimed at increasing the public's awareness of periodontal disease have been completed. These exhibits will be available for use at health fairs, health educational meetings, and by persons at dental and dental hygiene schools.

Surveys

The final report from Health Inquiries to evaluate selected health promotion and education activities of the former National Caries Program was received. The findings will be used to design additional science transfer activities.

Staff prepared justifications for non-competitive procurement to the American Dental Association and the American Dental Hygienists' Association for copies of the analysis of specific data about the knowledge, attitudes, and practices of dentists and dental hygienists regarding the control of infectious diseases in dental settings in the U.S. The information from these data bases will be used to develop educational activities of the HPST unit.

Other Activities

A staff member stimulated the formation, and participated as a member, of a task force on sealants whose purpose is to produce a guide on the use of sealants in public health and private practice settings. Two persons in HPSTS are also members of Working Group 3 - Oral Health Promotion, Commission on Oral Health, Research and Epidemiology, Federation Dentaire Internationale, which has the responsibility for the development of guidelines for dental associations on promoting health.

A chapter on preventing oral diseases among school-aged children was prepared for a textbook for practitioners. Staff also provided consultation, technical assistance, and advice on health promotion and disease prevention to local, state, national and international groups and institutions. In addition, staff presented a variety of lectures, seminars, inservice training, and Continuing Education courses for students and staff of dental and dental hygiene schools, as well as at local, state, national, and international dental meetings (see attached).

HPSTS participated in the NIDR Coordinating Committee on Assessment and Transfer of Technology. Activities involved attending monthly meetings, reviewing reports from the Office of Medical Applications of Research

including reviews for potential patents, and soliciting from a variety of sources a list of topics for future NIH Consensus Development Conferences.

Staff reviewed and edited a variety of scientific manuscripts and educational materials for journals, institutions, and organizations.

Publications

Horowitz, A.M. Reaction paper, on a symposium on Building a Coalition to Improve to Public's Oral Health. J. Public Health Dentistry, 45:215-217(No.4), Fall 1985.

Horowitz, A.M. and Frazier, P.J. Promoting the use of Fluorides in a Community. Fluorides and Dental Caries, 3rd Edition, Chapter 10, E. Newbrun (Ed.) CC. Thomas.

Horowitz, A.M. and Frazier, P.J. Effective Oral Health Programs in School Settings. Clinical Dentistry, Clark (Ed.) J.B. Lippencott Co., (in press).

Warren, G.B. The Future of Dental Health as a Specialty. J. Pub. Health Dent., 45(4):238-239, Fall 1985.

Moshman, J., Warren, G.B., Blandford, D.H., Aumack, L. Geriatric Dentistry in the Predoctoral Curriculum. J Dent. Educ., 49(10):689-695, October 1985.

Presentations

Alice Horowitz

"The Use of Science Transfer to Promote Health and Prevent Disease". Canadian Society of Community Health Dental Hygienists, Ottawa, Canada.

"Keeping Your Patients Caries Free". Canadian Dental Hygienists Association, Ottawa, Canada.

"How Can We Meet the Needs of the Geriatric and Disabled Patient in Canada" panelist, Ottawa, Canada.

"Developing and Implementing Public Health Dental Educational Programs". Division of Dental Hygiene, Columbia University, NY, NY.

"Promoting Oral Health Through Disease Prevention: the Role of the Dental Hygienist". First Annual Alumni Day, Division of Dental Hygiene, Fairleigh Dickenson University, Hackensack, NJ.

"The Use of Pit and Fissure Sealants in Today´s Practice", D.C. Delta Sigma Delta, Georgetown.

"Rationale for School-Based Fluoride Regimens". U. of Pennsylvania, Philadelphia, PA.

"Dental Preventive Measures: An Update". Dental Hygiene Program, Tunxis Community College, Farmington, CT.

"Caries Prevention: An Update". Fones School of Dental Hygiene, Bridgeport, CT.

"Mechanical Removal of Plaque". Congresso Internacional De Odontologia De Mg, Belo Horizonte, Brazil.

G.B. Warren

"Fluorides and Sealants". Pennsylvania State Education Association - Department of Pupil Services Conference, Harrisburg, Pennsylvania.

Health Promotion and Science Transfer Activities at NIDR. Association of City and County Dental Directors, Washington, D.C.

"Fluorides and Sealants". Northern Virginia Community College, Annandale, Virginia.

Richard Better

"The Mission and Activities of the National Institute of Dental Research". Presented at a NIDR/EEO Advisory group-sponsored meeting for visiting students from two Washington D.C. high schools, Clinical Center, NIH, Bethesda.

"Tel-Med: A Potential Health Information System for the Military and Their Dependents". Tri-Service Dental Society Annual Meeting, Fort Meade Officer's Club, Laurel, Maryland.

"The Importance of Periodically Updating Tel-Med Tape Libraries". Tel-Med Idea Exchange, Maryland State Tel-Med Office, Anne Arundel Hospital, Annapolis, Maryland.

FINANCIAL MANAGEMENT SECTION (FMS)

The FMS coordinates the Institute's financial activities for the Office of the Director, including the development and execution of the NIDR budget. The FMS is also the repository for accounting and payroll records, statistical data and legislative reports. The Budget Officer serves as principal staff advisor to the Director on all financial matters relating to NIDR appropriations.

NIDR Budget: Fiscal Years 1987 and 1988

During FY 1986, FMS formulated the Institute's FY 1988 budget request. Staff generated the initial budgetary levels, and advised the Director on all changes as the request was transmitted through higher organizational levels, for incorporation into the President's submission to Congress. FMS also generated all supporting documents, justifications and statistical materials required for this purpose.

During negotiations for the FY 1988 budget, the FY 1987 budget request was proceeding through Congress. During this process, FMS staff provided justification materials and accompanied the Director to Congressional hearings. After action on the request by both houses of Congress, FMS prepared effects statements summarizing Congressional action that culminated in approval of an operating budget for FY 1987.

Automation

During the past year, the FMS lost the expertise of one budget analyst. Because of the Institute's FTE restrictions, the position was vacant for months, placing a greater burden on the FMS to perform tasks that are normally assigned to three people.

With the hiring of a new budget analyst, the FMS plans to expand its activities with the use of microprocessors and automated data systems. The FMS plans to continue to automate its financial operations and to initiate new uses for personal and mainframe computers. During 1986, planning was begun to have financial records and documents put on computer data bases.

Other Activities

The FMS provided managerial and financial support for the Institute's extramural and intramural programs and for direct operations. The FMS maintained payroll records, generated monthly personnel status and program expenditure reports, tracked the funding of grants, requisitions and purchase orders, and worked with administrative staff to ensure that reprogramming actions were initiated in areas where additional funds were required. The FMS apportioned monies by quarter to fund planned activities and, at the end of the fiscal year, balanced the books.

In addition, FMS provided special reports and monitored the Institute's trans-NIH activities, including research studies in diabetes, arthritis, nutrition, disease prevention and acquired immune deficiency syndrome. Staff prepared forecasts for strategic planning purposes and responded to requests for program and financial data from Congress, the Office of Management and Budget, and other Federal and nonfederal agencies.

PERSONNEL AND MANAGEMENT ANALYSIS SECTION (PMAS)

The PMAS is the focal point for these Institute functions. Personnel management activities encompass staffing and placement (including merit promotion), classification and pay management, employee relations, and employee development and training. Management analysis activities include providing advice and assistance on organizational and procedural problems, serving as the clearance and management point for consultant services, conference management, contracting out of commercial/industrial activities, and records management.

Reorganizations

Several reorganizations took place during the year. In one, a section from the Office of the Director was transferred to the Epidemiology and Oral Disease Prevention Program to create the Science Transfer and Research Analysis Branch. The Cell Biology Section and the Molecular Biology Unit were established in the Laboratory of Developmental Biology and Anomalies, and the Bone Research Branch was created from the Mineralized Tissue Research Branch to more aptly reflect current or projected scientific programs. In another reorganization within the Intramural Research Program, functions and staff in the Laboratory of Oral Biology and Physiology, the Bone Research Branch, the Laboratory of Microbiology and Immunology, and the Clinical Investigations and Patient Care Branch were realigned.

Staffing and Recruitment

Staffing and recruitment activities received considerable attention during FY 1986. Recruitment activities for a Director, Epidemiology and Oral Disease Prevention Program, began early in 1986, but were halted due to Departmental cutbacks in SES positions. These activities were resumed in late summer, with the position advertised in Fall 1986.

Classification

The PMAS staff continued the DHHS requirement that each position be reviewed on a two-year cycle, and also carried out an intensive review of technical positions in the Extramural Program.

EEO Activities

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

Awards

The Institute continued to have an active employee incentive awards program. All staff were encouraged to submit nominees for awards so that the excellent quality of NIDR staff would be recognized. Again this year, NIDR SES/SSS staff were recognized for their contributions through performance bonuses.

Several staff members received recognition at the NIH level as well as through external awards, and some 35 other staff were recognized for their superior performance and special contributions at the Annual NIDR Awards Ceremony.

Policies and Procedures

An Operational Guideline describing personal property responsibilities and management was issued.

Professional Activities

During FY 1986, members of the PMAS participated in several trans-NIH activities such as the development of an automated personnel system, and were active in the International Personnel Management Association, a professional personnel society.

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's affirmative action and civil rights program are centered in the Institute's Equal Employment Opportunity Office. The Office serves as the principal source of information and advisor to the Institute Director and to top management on matters of equal employment opportunity, Affirmative Action/Federal Equal Opportunity Recruitment Programs, civil rights and contract compliance.

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

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.

This past fiscal year, the Committee sponsored two seminars on Radiation, Biological, and Chemical Safety (an area of major concern to NIDR staff who are not required to take safety courses) and on Positive Image for Support Staff. Moreover, forums were established for four guest speakers to discuss important educational opportunities for NIDR employees. In addition, the Committee nominated to the Director two outstanding employees for the NIH EEO Special Achievement Award.

Discrimination Complaints

The Institute had no informal or formal discrimination complaints filed in FY 1986. The EEO Manager and Counselor continued to provide, on an as needed basis, career counseling, guidance on job applications, training opportunities, and problem solving in supervisor/employee relations.

Minority Biomedical Research Support and Minority Access to Research

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 (MBRS) and Minority Access to Research Careers (MARC) Programs that relate to the overall mission of the Institute.

In Fiscal Year 1986, staff participated in the 14th Annual MBRS Symposium held in New Orleans, Louisiana. This Symposium provided an opportunity for staff to discuss with faculty and students research training opportunities at NIDR. As a result of their discussions, three MARC students and one MBRS student (as a part of their enrichment assignments) were provided an opportunity to work in the Institute's laboratories during the summer session. This was a new activity for the Institute.

Training

The EEO Office sponsored a two-day training session for the NIDR EEO Advisory Committee on the Federal equal employment opportunity laws and regulations which prohibit discrimination in employment.

Recruitment and Selection

In an effort to recruit more Hispanics, American Indians, and handicapped individuals into the biomedical sciences, the EEO Manager continues to identify and communicate with organizations and associations concerning our mission and activities. Another strategy for increasing awareness of NIDR activities and programs is the dissemination of information through other NIH EEO Managers participating in conferences such as the President's Committee on the Employment of the Handicapped and the National Congress of American Indian Conference.

Community Outreach

To enhance NIDR's relationship with the minority community, the EEO Office established two new programs--the NIDR Resource Collection and an outreach program with two public schools in the District of Columbia.

The NIDR Resource Collection is an initiative to supply three minority dental schools--Howard, Meharry, and Puerto Rico--with NIDR's surplus scientific books and publications. Since January 1986, our total effort is as follows: 80 journals and approximately 200 books have been contributed to the Resource Collection.

Two public schools in the District of Columbia, Ballou Senior High and Louis Charles Rabaut Junior High, have excellent science programs and numerous students interested in research and the life sciences. To increase their awareness of dental research and to provide encouragement in the early stages of career development, a seminar and two tours of NIDR laboratories were conducted for approximately 40 students and their science teachers.

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

Civil Rights

The EEO Manager serves as the Federal Contract Compliance Coordinator for the Institute. All contracting and project officers in the Institute have completed training on Contract Compliance and are presently administering 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 ASSOCIATE DIRECTOR
EPIDEMIOLOGY AND ORAL DISEASE PREVENTION PROGRAM
NATIONAL INSTITUTE OF DENTAL RESEARCH

This year was marked with the completion of a national epidemiologic survey of the oral health of U.S. adults and the beginning of the second national survey of oral health in U.S. schoolchildren. On the former, data analyses have begun and results will provide needed information on root caries, coronal caries and periodontal disease in adults. The latter survey shall permit the assessment of whether the downward trend in dental caries noted in the eight-year period before 1980 is continuing, or leveling. In addition, the children's oral health survey will provide national estimates of fluorosis, periodontal disease and soft tissue lesions. Program staff have participated in the planning of the oral health component of the National Health and Nutrition Examination Survey (NHANES) III.

Epidemiologic investigations of periodontal disease continue to evolve. A four-year longitudinal study of periodontal disease in Navajo Indian adolescents was initiated. Clinical and radiographic measurements of disease, together with the monitoring of suspected periodontal disease pathogens in sub-gingival plaque should elucidate the natural history of incipient periodontal disease in this age group. To pursue the question of whether signs of incipient disease in adolescents are indicative of high risk to chronic periodontitis in adults, a study was initiated to compare bone loss of 30-year-old individuals with that assessed from x-rays taken of these same individuals 15 years earlier.

Analysis of data from a 15-year study of a group of Sri Lanka tea-plantation workers with no access to dental care showed wide variation in the natural course of adult periodontitis. Three groups with different rates and severity of disease progression emerged.

A contract to determine the effectiveness of twice daily brushings with a 0.4% SnF_2 gel on periodontal health was initiated in January 1986. Approximately 550 adults are participating in one of three groups: those receiving the SnF_2 gel, those on a NaF gel and a placebo gel group.

A new method of summarizing data from cross-sectional studies on loss of periodontal attachment was developed. In addition, a new statistical technique for hypothesis-testing in clinical trials where attachment loss is the response variable and a method to analyze the level of intra- and inter-examiner consistency in diagnosing gingivitis in epidemiologic surveys were developed.

The development of a plan for a community-based periodontal diseases intervention program is under way. This program will be comprised of interventions directed towards the public and the health profession. A protocol to study the effects of self-assessment of gingival bleeding as a means to maintain and motivate appropriate oral health practices in adolescents has been developed and will be implemented in FY-1987.

The presence and role of suspected disease pathogens has received attention in several investigations. Efforts have continued in the development and production of anti-sera to major species of periodontal disease-associated

pathogens. A study of adults with and without periodontal disease will investigate immunologic, biochemical, behavioral as well as microbiologic assessments. Also the aforementioned longitudinal study of Navajo adolescents, the SnF_2 gel clinical trial, and the national survey of children's oral health have microbiologic components.

A significant portion of the Program's activities continue to focus upon the prevention of dental caries: new delivery systems, fluoride procedures, the combined use of fluorides and the use of fluorides in combination with sealants. The use of calcium diphosphate has been shown to increase the uptake of fluoride in tooth enamel. A clinical study to investigate the additive effects of fluorides in schoolchildren continues. Emphasis now is on the determination of the anti-caries effectiveness of various treatments on the permanent teeth and the extent of continued protection once treatments are terminated. The Nelson County, Virginia study since 1984 has involved the placement of sealants on selected newly erupted teeth. Incipient lesions, as well as the usual sound sites are part of the study design.

The relationship between water fluoride concentration levels and the prevalence of dental fluorosis and dental caries warrants current and reliable information. Findings from a study of the effect of severe dental fluorosis on the oral health of adults revealed that no adverse oral health effects were experienced in the study population. In comparing two communities, one with optimal fluoride levels and the other, with four times optimal, no important differences were detected between the adults in these communities in gingival bleeding, amount of plaque, loss of attachment, cervical abrasion, and temporomandibular disorders.

The shape and attachment mechanism of the intraoral controlled-release system are being refined. A range of therapeutic agents, in addition to fluoride, are being developed. A short-term trial of controlled-release tetracycline pellets in monkeys has produced favorable changes in the subgingival bacterial flora. The further development of the intraoral conntrolled-release system will provide a mechanism for the delivery of a wide range of therapeutic agents.

Studies of cariogenicity potential of foods and the assessment of non-sucrose sweetening agents continue to receive considerable attention. Regimens of agents and foods are being tested for their anticariogenic potential.

A new emphasis this year is on investigations of soft tissue lesions. Through the consultation of Dr. Jens Pindborg and other oral pathologists, several studies have been planned. Oral soft tissue lesions comprise a large part of the study of the natural history of oral infection of AIDS patients. Also in the national survey of oral health in children, the presence of soft tissue lesions will be documented. In the coming year a prospective study of soft tissue lesions in a VA population will be implemented.

Several studies have begun to investigate the relationship between the use of chewing tobacco and snuff among adolescents and their oral health status. A study of Navajo high school students reported high prevalence of use among the males (75%) as well as of the females (25.5%). Leukoplakia was found in 25.5% of the smokeless tobacco users at the site of tobacco placement. The national survey of oral health in U.S. schoolchildren also will assess the prevalence

of use and oral effects of smokeless tobacco. This study will include 45,000 elementary and high school children. In addition to these studies, Program staff in the course of the year served on committees for and contributed to the preparation of the oral health portion of the Surgeon General's Report of the Health Consequences of Using Smokeless Tobacco.

To address the control and treatment of Acquired Immune Deficiency Syndrome (AIDS), a multifactorial approach is needed. The Program is proceeding on several fronts. Preparations are under way for the investigation of the natural history of oral infection in AIDS patients. This should permit the documentation of the development and progression of oral disease and lead to better intervention and treatments. Available data on the current infectious disease knowledge and practices of health professionals, with particular attention to AIDS, are being analyzed. The information obtained will be used to plan and develop appropriate educational activities for the dental profession. Building upon the intraoral controlled-release system the use of antifungal agents for the treatment of Candidiasis, a common infection in AIDS patients, has been tested in vitro. This approach, once developed, would facilitate the treatment of this opportunistic infection.

The Program staff have been active in conference and training activities. Organized by the Acting Chief, Oral Disease Prevention Branch, the Program sponsored a workshop on Microbiological Diagnosis in Dental Caries and Periodontal Disease. Proceedings of this workshop will be forthcoming. At the request of the European Organization for Caries Research, the Epidemiology Branch Chief designed and conducted a course for young investigators entitled "Epidemiology:Principles and Applications" (July 5-8, 1986). The Epidemiology Branch Chief also announced and developed a training course on Clinical Diagnostic Methods. This latter course will be held December 8-11, 1986, and is geared towards state and local dental public health practitioners who are about to initiate epidemiologic surveys. Training on the conduct of clinical trials and epidemiologic surveys is being provided to Dr. Yang Shi from China who has been visiting since June 1986. In August, Dr. Steve Arthur completed his dental public health residency with the Field Studies Section.

There have been several administrative and programmatic changes in the course of the past year. A third branch was added on June 1, 1986 to the Program, entitled "Science Transfer and Research Analysis Branch (STRAB)," comprised of staff from the Office of Planning, Evaluation and Communications, NIDR. This branch expands the Program's mission beyond epidemiology and oral disease prevention. Specifically, the mission of STRAB entails the conduct of "activities designed to promote oral health by transferring the results of scientific research to other scientists, health care providers and the general public; and the conduct of research concerning the consequences of the changing patterns of oral diseases on economic, social and personal characteristics of the population and the profession."

In order to devote more time to the pursuit of epidemiologic research, Dr. James Carlos resigned from the position of Associate Director to become the Chief of the Epidemiology Branch. Dr. Carlos served as the Associate Director of the National Caries Program from 1971 to 1984 and for the past two years as the EODPP's Associate Director. Recruitment efforts for this position are under way.

Dr. Herschel Horowitz retired this year, leaving his position as Chief of the Clinical Trials Section. Dr. Horowitz served in this capacity (and in a similar one on the National Caries Program) since 1971. His contribution to the development and testing of a wide range of fluorides for public health applications are recognized worldwide.

The Program was fortunate to have benefited from several Visiting Scientists. Dr. Cyril Enwonwu from Nigeria developed state-of-the-science papers on mercurity toxicity and on AIDS. He now has joined the Meharry University faculty as Director, Center for Nutrition and Professor of Community Medical Care. Dr. Jukka Ainamo from Finland continues to work with Program staff in the development of clinical studies for the prevention of periodontal disease. Dr. Jens J. Pindborg from Denmark is providing training and guidance for the development of studies on oral soft tissue lesions. Dr. Bo Krasse from Sweden has provided leadership this past year as the Acting Chief of the Oral Disease Prevention Branch. This coming year the Program will continue to benefit from Drs. Ainamo and Pindborg and is joined by Dr. Richard Oliver from Minnesota and Dr. Leif Arne Heloe from Norway. The latter two will work in the area of disease measurement and clinically-defined need.

Report of the Epidemiology Branch

The primary new activities of the Epidemiology Branch during the year were in the areas of periodontal disease and oral soft tissue lesions.

In FY-1985, an epidemiologic study of Navajo Indian adolescents, conducted by staff of the Field Studies Section, showed a surprisingly high level of incipient, and in some cases, advanced, periodontitis. As a result, a sub-set of these children were enrolled in a 4-year longitudinal study in which clinical and radiographic measurements of disease are supplemented by monitoring of sub-gingival plaque for the presence and concentrations of suspected periodontal disease pathogens. An important question is whether signs of incipient disease in adolescence are indicative of high risk to chronic adult periodontitis. To further explore this possibility, a study was begun to compare the periodontal status of 30 year-olds with radiographic evidence of bone loss from x-rays taken of the same subjects at age 15. Clinical data for this study will be collected early in FY-1987.

A continuing problem in the epidemiologic study of periodontal disease is the lack of standard, widely accepted methods for summarizing and analyzing data. Staff of the Branch reported a new method of summarizing data on loss of periodontal attachment gathered in cross-sectional studies, as well as a new statistical technique for hypothesis-testing in clinical trials where attachment loss is the response variable. In addition, a method was developed for analyzing the level of intra- and inter-examiner consistency in diagnosing gingivitis in epidemiologic surveys.

Although it is established that periodontitis is a bacterially induced disease, the precise pathogens and their interaction with host defense mechanisms remain obscure. In an attempt to clarify this, the Branch initiated a collaborative study with the University of Texas, San Antonio, and the SUNY at Buffalo in which adults with and without periodontal disease will be compared on the basis of comprehensive microbiologic, immunologic, biochemical and behavioral evaluations.

It is clear that, despite the absence of personal oral hygiene or professional intervention, the natural course of adult periodontitis is widely different in different individuals. This was well illustrated by an analysis of data from a 15 year study of a group of tea-plantation workers in Sri Lanka who had no access to dental care. The results, which were reported in June, showed a distinct separation of these subjects into three groups with respect to the rate and severity of progression of the disease, and provided further rationale for attempts to develop screening methods to identify high-risk adolescents or young adults to facilitate the design of efficient prevention strategies.

Considerable concern has been expressed recently about the extensive and increasing use of snuff and chewing tobacco among U.S. adolescents. A study of Navajo high school students showed that 75% of males and, surprisingly, 49% of females were using these products, and that the prevalence of leukoplakia was 25.5% among smokeless tobacco users. Plans were developed to include a soft-tissue examination and questions about smokeless tobacco use in a national survey of the oral health of elementary and high school children

which will begin in October 1986. This survey is a repeat of a national study completed in 1980, and all Branch staff have been extensively involved in its design.

Contract-supported clinical trials were continued to determine the effectiveness of fluoride mouthrinsing in the prevention of root-surface caries in adults, and to determine whether fluoride supplements, given during pregnancy, can help prevent caries in the offspring. A third contract project was completed. This was a study of the cost-effectiveness of using non-dental personnel, given a special training course, to deliver a comprehensive program of caries prevention in elementary schools. Data from this research are currently being analyzed.

The National Survey of the Oral Health of U.S. Adults, begun in 1985, was completed in November. Staff of the Biometry Section are analyzing the data for initial reporting in October. The results are expected to provide benchmark estimates of the prevalence and geographic distribution of coronal caries, root-surface caries and periodontal disease in persons aged 21-70+, against which the impact of future preventive activities may be measured.

Two new studies were designed for implementation in early FY-1987. The first, to be done in collaboration with the Walter Reed Army Medical Center, will study the development and progression of oral disease in military personnel who are sero-positive to the HTLV-III virus, and those who have AIDS. A second study will involve long-term follow-up of several cohorts of children and adolescents in Bogalusa, Louisiana, to better document certain aspects of the natural history of caries and periodontal disease.

In a continuing attempt to better standardize methodology in oral epidemiology, the Branch announced a training course in Clinical Diagnostic Methods for epidemiologists from state and local dental public health agencies. The course is scheduled for December 8-10, 1986. The Branch Chief designed and directed a short course for young investigators titled Epidemiology: Principles and Applications. The course was given in Norway, July 5-8, 1986 in collaboration with the European Organization for Caries Research.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00387-03

PERIOD COVERED
October 1, 1985-September 30, 1986

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, Behtesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.35	.05	.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.)*

Potential associations between the levels of specific micro-organisms in saliva and the level of dental caries prevalence and incidence in a U.S. adosescent population are being investigated. Initial and interim findings indicate that there is a positive correlation between the level of S. mutans and Lactobacillus in saliva and the prevalence and incidence of dental caries in this study population. The initial caries prevalence for participants having high levels of bacteria in saliva were 1.5 DMF surfaces higher than that of those having low levels of bacteria in their saliva. There were 1.1 fewer new DMF surfaces that developed in subjects with low levels of bacteria in their saliva compared with those having high levels of bacteria in their saliva during the first half of the study.

GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00388-03

PERIOD COVERED
October 1, 1985-September 30, 1986

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:
.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.)*

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.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00402-02

PERIOD COVERED
.October 1, 1985-September 30, 1986

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:
.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.)*

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-00403-02

PERIOD COVERED
October 1, 1985-September 30, 1986

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:
4.0	1.0	3.0

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.)*

An epidemiologic survey of oral health in adults was implemented during FY-85. Dental exams were conducted on a cross-sectional sample, stratified by region and age, of approximately 20,000 employed adults 18 years of age and older and non-institutionalized seniors 65 years of age and over who attend multi-purpose senior centers. Exams were completed in March 1986, and processing of data was completed in August. Analysis of the prevalence of tooth loss, coronal caries, root surface caries and periodontal destruction is being assessed.

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-00409-02

PERIOD COVERED
October 1, 1985-September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Procedure for Evaluating the Reliability of a Gingivitis Index

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:
.01	.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.)*

A methodology is presented for assessing the reliability of an ordinally-scaled index and is illustrated by using data from a clinical trial in which gingival inflammation was assessed with the PMGI index, independently, by five examiners. One of the examiners was an experienced examiner, the others newly trained. All subjects were evaluated by each examiner initially and at the end of the study period. The reliability of the average score per subject, maximum score per subject, and the percentage of affected sites per person are estimated by the intraclass correlation coefficient. Procedures are presented that utilize various forms of the weighted kappa statistic for dissecting patterns in examiner agreement for specific sites, types of sites, all sites, and for the individual components and categories of the index. It is shown how these procedures can be useful for training and calibrating multiple examiners, who will be using such an index in a clinical study, so that adequate reliability levels can be realized.

Bibliographic Reference:

Kingman, A., "A procedure for evaluating the reliability of a gingivitis index," J Clin Periodontology 1986; 13: 385-391.

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-00418-01

PERIOD COVERED
October 1, 1985 to September 30, 1986

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:
.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.)*

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 significant deterioration for shallow sites.

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-00420-01

PERIOD COVERED
October 1, 1985-September 30, 1986

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:
.40	.40	

CHECK APPROPRIATE BOX(ES)

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

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

A national survey of the oral health of school children was designed to be implemented in 1986. A probability sample of approximately 45,000 school-aged children in kindergarten through twelfth grade will be selected. Dental exams for coronal caries, fluorosis, periodontal disease and presence of lesions of the oral soft tissues will be conducted during the 1986-87 school year. National and regional estimates of the presence of each disease and comparisons with the 1979-80 survey of children's oral health will be made.

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 00399-02

PERIOD COVERED
October 1, 1985-September 30, 1986

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

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

Carlos, James P., Chief, Epidemiology Branch — NIDR EODPP

Wolfe, Mary D., Epidemiologist — NIDR EODPP

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

LAB/BRANCH
Epidemiology Branch

SECTION
Field Studies

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
	.50	.55

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 purpose of this continuing study is to determine the prevalence and progression of gingivitis, epithelial attachment loss, and bone loss in a group of adolescents residing in the U.S.

The original cross-sectional study population consisted of approximately 600 Navajos, ages 14-19 years. Twenty-four posterior interproximal sites were examined on each subject. Gingivitis was assessed using a modification of the G.I. Loss of attachment was assessed using Ramfjord's technique. Bone loss was diagnosed from standardized bitewing radiographs.

Analyses indicated a high prevalence of disease: gingivitis (71%); attachment loss (89%); and bone loss (89%). The average number of sites in the mouth affected with the more advanced form of disease (attachment loss and bone loss) was also high: 32% of the sites had attachment loss, and 22% had bone loss.* A longitudinal study is in progress with the youngest subjects to investigate microbiologic, systemic and other factors that may contribute to the high prevalence of disease; 226 first and second year students were examined in February 1986, using the same clinical and radiographic techniques. In addition, subgingival plaque samples were obtained from mesio-buccal sites at all permanent molars and analyzed for A. actinomycetocomitans, B. gingivalis and B. intermedius.

*Wolfe, M. D. and Carlos, J. P. Incipient Periodontitis in Navajo Indian Adolescents. IADR Abstract # , 1986
Wolfe, M. D., Carlos, J. P. Periodontal Disease in Adolescents:Epidemiologic Findings in Navajo Indians. In. Press. Comm. Dent. and Oral Epidemiol.

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 00410-02

PERIOD COVERED
October 1, 1985-September 30, 1986

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)*

Loe, Harald, Director, NIDR — EODPP, NIDR

Morrison, Edith C., Senior Staff Fellow — EODPP, NIDR

Smith, Jacqueline I., Statistician — EODPP, NIDR

COOPERATING UNITS *(if any)*
Dr. Kenneth S. Kornman, Professor and Chairman, Department of Periodontics
Dr. Stanley C. Holt, Professor of Periodontics/Microbiology, University of Texas Dental School, San Antonio, Texas

LAB/BRANCH
Epidemiology

SECTION
Field Studies

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

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

Bacterialogic, immunologic, radiographic and genetic studies were carried out in January 1986 on 76 randomly selected male Sri Lanka tea laborers from three subpopulations with rapidly progressing, moderately progressing, and non-progressing periodontitis.

The specific aims of these studies will compare the presence and levels of selected putative periodontal pathogens, peripheral blood antibody titers, and evaluate the genetic determinants in the three distinct populations. In addition, tea laborers between the ages of 14-25 have been screened for a prospective study of the prevention of periodontal disease.

Two master data sets for Norwegian Surveys 1-5 were built in SAS during this period. Data bases for the Norwegian Study are:

LOE.NOR.AVO (All valid observations
LOE.NOR.IAS in all surveys.)

Two subpopulations were identified based on interproximal loss of attachment: (1) 42% with moderate disease and (2) 58% of the study group with loss of attachment not exceeding 2mm on any mesial surface at any survey.

Preliminary studies have determined the mean number of years to the onset of the loss of connective tissue related to the severity of inflammation and calculus deposition in both the Sri Lankan and Norwegian study groups. Future investigations will continue to focus on the stability of the gingival lesion, and factors which may influence the continuity or discontinuity of periodontitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00425-01

PERIOD COVERED

October 1, 1985-September 30, 1986

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	NIDR EB
Carlos, James P.	Chief, Epidemiology Branch	NIDR EB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

Field Studies

INSTITUTE AND LOCATION

NIDR, NIH

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.75	.25	.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.)*

Two hundred and twenty-six Navajo Indian children, aged 14-18, resident in a boarding school near Gallup, New Mexico, were interviewed to determine their use of chewing tobacco and snuff. Information was obtained on the types of products used, and the frequency and duration of use. A clinical examination was made of the oral soft tissues and leukoplakia recorded, using the diagnostic methods and grading described by Green and Polsen. Preliminary analyses indicate that 64.2% use smokeless tobacco (one of the highest levels of use yet reported in adolescents), and 25.5% of those subjects have leukoplakia. The data are now being further analyzed to investigate the effect of use of smokeless tobacco on periodontal health.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00044-16 EODP

PERIOD COVERED
October 1, 1985-September 30, 1986

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	NIDR, MSS
Molitoris, Eric	Microbiologist	NIDR, MSS

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:
1.85	1.25	.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.)*

Microbial strain data are being entered into a data bank to provide: data on specific organisms, identification 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, and ecological uses. The long term goal is to establish a world-wide data bank at a series of cooperating centers. The original bacterial system is being expanded to include the algae, yeasts, molds, protozoa, and hybridomas.

Genomic diversity as an indicator of stress has been applied to antibiotic resistance in bacteria isolated from animal populations (cattle, swine, chickens). Decrease in diversity is generally explained by non-specific increases in frequency of resistance.

GPO 814-818

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 Chemical and Physical Research

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00250-09 EODP

PERIOD COVERED
October 1, 1985-September 30, 1986

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

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

Walczak, Cynthia A.	Computer Scientist	NIDR, MSS
Krichevsky, Micah I.	Research Chemist	NIDR, MSS
Mercer, Paula	Computer Programmer	NIDR, MSS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology

SECTION
Microbial Systematics Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.42	2.12	.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.)*

Algorithms are being developed and tested for aiding in numberical 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 used 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.

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

Methods for interconversion between, and interrogation of, equivalent data bases with disparate formats (e.g., controlled vocabulary test versus numeric coding), are being developed as a necessary step in establishing an international Microbial Strain Data Network and for the Hybridoma Data Bank.

3C3 4/214

Annual Report of the Clinical Trials Section, National Institute of Dental Research

It has been clearly demonstrated that the prevalence of dental caries has declined in the United States in recent years. Further progress in caries prevention is likely to depend on the identification and development of more effective individual anticaries measures or combinations of measures that can produce substantial additive effects that are economically and logistically feasible for use in wide-scale programs. Clinical trials on new fluoride procedures, the combined use of fluorides, and the use of fluorides in combination with sealants continue to receive a significant portion of the research effort of the Clinical Trials Section. Increasing attention is also being given to the testing of procedures and agents that show potential for improving gingival and periodontal health. In view of concerns expressed in recent years regarding possible increases in the prevalence of dental fluorosis among schoolchildren in the U.S., the Section's research activities have included epidemiological assessments of the prevalence of this condition in various communities with different concentrations of fluoride in their drinking water.

During the past decade, clinical research has demonstrated that dental sealants are highly effective in preventing decay in occlusal surfaces and in buccal pits and lingual grooves of molars. Fluorides, in contrast, are most effective in controlling decay in the smooth surfaces of teeth. When used in combination, sealants and fluorides have the potential of virtually eliminating dental decay. To test this hypothesis, a sealant program was added in 1984 to a program of multiple fluoride administration ongoing in Nelson County, Virginia since 1972. Children in the County's schools ingest a fluoride tablet daily and rinse weekly with a fluoride solution; a fluoride dentifrice is provided for ad libitum use at home. An evaluation of the maximum effectiveness of the combined fluoride program in 1983 showed, for all surfaces, a 65% lower prevalence of dental caries compared with baseline findings. Mesiodistal (smooth) surfaces showed a particularly striking reduction of 90%; essentially all of the remaining decay occurred in pit and fissure tooth surfaces. Since 1984, sealants have been placed in selected newly erupted teeth of children in designated grades. Because skepticism exists about whether sealant placement in school programs can be accomplished cost-effectively, data on the longevity of the sealant material, number of children and sites treated, time required to carry out the procedure, salary of the operators and cost of the materials are being collected. As part of the study design, incipient lesions are being sealed along with the usual sound sites. Although there is a growing body of evidence to show that early lesions once sealed do not progress, many dentists still are reluctant to use pit and fissure sealants for fear of inadvertently covering an incipient lesion. Results of the study may help to establish further the safety of using this proven caries preventive measure.

Besides prompting an evaluation of sealants with fluorides, the Nelson County study has spurred interest in isolating the additive effects of fluorides. Because all study participants received all of the fluoride regimens in Nelson County, only the total effect could be evaluated. To determine the caries-preventive benefits specifically from daily fluoride supplements, weekly fluoride rinsing and the two methods combined, a long-term

study was initiated in 1981 in 20 elementary schools in Springfield, Ohio. The first follow-up examinations were made in 1983 on 1,154 participants who were in grades 2 and 3. Results from primary teeth showed that subjects in the combination treatment group had 33.2% and 18.9% fewer mean incremental dmfs compared with their cohorts in the rinse and tablet groups, respectively. The observed differences among the treatment groups approached statistical significance ($P = 0.06$). Because of exfoliation, participants at the next follow-up examination in 1986 will be too old (mostly 10 and 11 years of age) to assess caries experience validly in deciduous teeth. Thus, the encouraging two-year results represented the only opportunity to study the effectiveness of the treatments in the deciduous dentition of elementary schoolchildren. For permanent teeth, which were all newly erupted in 1983, incremental caries scores were too small to permit a valid evaluation of effectiveness. The procedures will be continued through grade eight and further examinations will be conducted to determine the anticaries effects in the permanent teeth following increasing years of exposure to the treatments. 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.

Recognizing the need for reliable and current information on the relation between water fluoride concentration and the prevalence of dental fluorosis and dental caries, the Clinical Trials Section conducted a cross-sectional survey in April 1980 of children, ages 8-16, in four areas of Illinois that had naturally-occurring fluoride in their drinking water at concentrations of 1, 2, 3 and 4 times the recommended optimum for their geographic area. In April 1982, a similar group of children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois.

Findings showed that the prevalence of caries was approximately 38 percent lower in the optimal fluoride area compared with the areas with negligible concentrations of fluoride in their water supplies. Mean caries scores in all three above-optimal fluoride areas were significantly lower than in the optimal area, but were not significantly different from one another. Caries-protection was somewhat compromised, however, among teeth diagnosed as having severe fluorosis, possibly as a result of food, debris or plaque becoming entrapped in the hypoplastic defects of severely fluorosed enamel. This effect was not present among teeth with lesser degrees of fluorosis. The prevalence of fluorosis was negligible in the nonfluoride area and characteristically low in the optimal fluoride area. Statistically significant increases in the prevalence and severity of fluorosis occurred in the above-optimal fluoride areas, with the condition being most pronounced in the 4-times optimal area. An additional finding was that first molars and incisors in children 8 to 10 years old were affected by more fluorosis than were the same teeth in children 13 to 16 years old. These teeth had been erupted for about five years longer in the older age group. At least two factors might have explained this difference. One was that abrasion or remineralization might have diminished the manifestations of fluorosis in these teeth in the older children. The other was that the younger group of children might have consumed greater amounts of fluoride during tooth development as a result of fluoride from multiple sources being more widely available to them.

The continued availability of the study sites in Illinois offered a unique opportunity to gather further information on some of the questions raised in the 1980 survey. Thus, in April 1985, the same communities were revisited and assessments of fluorosis and dental caries were made among children 8-10 and 13-15 years of age. Because the 13-15 year-olds are the same children who were 8-10 years old on the 1980 survey, the current fluorosis scores of incisors and first molars can be compared with the previous scores for the same teeth to determine whether any changes have occurred. Assessments in the new group of 8 to 10 year old children will reveal whether changes in the prevalence or degree of fluorosis have taken place in that age group during the five year period. Information of this type is needed for purposes of monitoring possible changes in dietary fluoride intake over time. Assessments of dental caries will indicate whether changes in the prevalence of caries have occurred during the five year period and, also, if the same patterns of caries prevalence have persisted by age group and by water-fluoride level. In addition to dental caries and fluorosis, the prevalence of gingivitis and dental calculus was assessed in all children in the 13-15 year age group. The latter assessments will help determine if a relationship exists between gingival health, degree of fluorosis, and water fluoride concentration. A report showing results of the 1985 survey and comparing findings with those of the 1980 survey is being prepared at the present time.

During the fiscal year, the Clinical Trials Section has collaborated with the Science Transfer and Research Analysis Branch in developing a plan for implementing and evaluating community intervention programs in various age groups in order to reduce the prevalence and severity of periodontal disease. One phase of the plan nearing the implementation stage involves a clinical trial that will be conducted in a group of teenagers. The study is designed to evaluate the long-term oral health outcome of using gingival bleeding rather than plaque as the focal point. The traditional approach to improving gingival health is the promotion of plaque control and plaque removal techniques. Through the use of a toothbrush and floss, the attention of the subject is focused on achieving a plaque free mouth. However, previous studies indicate that long-term changes in gingival health are not maintained and that gingival recession may occur when this method is utilized. The rationale of the proposed study is that, if bleeding is the condition to be eliminated or prevented, then the attention should be directed to cleaning those areas of the dentition where bleeding occurs. It is speculated that gingival health will improve and that gingival recession will be reduced as cleaning of the tooth is redirected from visible, accessible tooth surfaces to previously neglected areas. In addition to this particular clinical trial, other periodontal disease prevention programs are being developed for interventions in worksites and in multipurpose senior centers. The collaboration between the Clinical Trials Section and the Science Transfer and Research Analysis Branch will continue during the next fiscal year.

Contract-supported research activities in Fiscal Year 1986 consisted of the following:

In April, 1986, the final comprehensive report of contract N01-DE-32443, Effect of Severe Dental Fluorosis on the Oral Health of Adults, was received from the University of Michigan. Lifetime residents of two communities in New Mexico with optimal (Deming) and more than 4 x optimal (Lordsburg) fluoride

concentrations in their water supplies received a comprehensive oral health examination. Findings on 189 adults in Lordsburg showed a significantly higher prevalence and severity of dental fluorosis than did those of 187 adults examined in Deming. No important differences could be detected between the two communities in gingival bleeding, amount of plaque, loss of attachment, cervical abrasion, and temporomandibular disorders when regression analyses to account for confounding variables were performed on the data. The epidemiologic study showed that adults do not experience any adverse oral health effects from having been affected with advanced forms of dental fluorosis since childhood; the condition appears to be mainly one of cosmetic concern.

A 0.4% stannous fluoride gel has been accepted by the ADA Council on Dental therapeutics as effective in controlling dental decay and decalcification. However, the gel has not been accepted by the Council as effective in controlling periodontal disease. In January 1986, the University of Minnesota initiated clinical activities under contract N01-DE-52556, to determine the effectiveness of twice daily brushings with a 0.4% SnF_2 gel on periodontal health. A total of about 550 adults employed at several locations of a large business corporation in Minneapolis are participating in the study. Results of the SnF_2 group will be compared with those of comparable groups using either a NaF gel or a placebo gel. The study will be conducted for a period of 18 months with examinations at 6 month intervals. Clinical measurements along with microbiologic assessments, i.e., total number of plaque microorganisms and the proportion of specific microbial forms, are being made. Results of the first follow-up examination have been collected and are currently being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00070-14 EODP

PERIOD COVERED: October 1, 1985 to September 30, 1986 — 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.	Acting Chief, Clinical Trials Section	EODPP, NIDR
Nowjack-Raymer, Ruth	Clinical Trials Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Erazo, Barbara A.	Secretary (Typing)	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:
.57	.40	.17

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Baseline dental examinations were conducted in October 1972, of approximately 2200 children (grades 1-12). All participants in grades K-8 chew and ingest daily in school under teacher-supervision a sodium fluoride tablet containing 1 mg F, and rinse weekly with a 0.2% NaF solution. Fluoride-containing dentifrice and toothbrushes are distributed regularly to the children for use at home. Kindergarten classes were invited to participate in the program beginning in the 1976-77 school year. Children in the 7th and 8th grades in Nelson County's junior high school began to participate in the program in the fall of 1978 and 1979, respectively. Beginning in the Fall of 1980, high school students in Nelson County began to participate only in the tablet and dentifrice components of the program. For the period covered by this report, children in grades K-12 were participating. In January 1984, a sealant program was added to the ongoing fluoride program. Newly erupted permanent teeth of children in selected grades were sealed with a pit and fissure sealant. The program will run for five years. The combined preventive program has the potential of virtually eliminating dental caries in Nelson County.

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 00277-07 EODP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Dental caries and fluorosis in areas with different water fluoride concentrations

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
Nowjack-Raymer, Ruth	Clinical Trials Specialist	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR
Erazo, Barbara A.	Secretary (Typing)	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:
.08	.06	.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.)*

In April 1980, a cross-sectional survey was made of the prevalence of dental fluorosis and dental caries in seven communities in Illinois that had different concentrations of naturally-occurring fluoride in their public water supplies. The communities were grouped into four categories according to the approximate relation of their actual water-fluoride concentration to the recommended optimal fluoride concentration for the area (1, 2, 3 or 4 times optimal). The study population consisted of 807 school children, ages 8-16, who had lived continuously from birth in their respective communities and had always used the community supply as their primary drinking water source. Dental caries was assessed with the DMFS index and dental fluorosis was measured with Dean's Index and with a newly developed Tooth Surface Index of Fluorosis (TSIF). Fluorosis was assessed independently in each child by each Index. In addition, color photographs of the teeth of some children were taken to depict varying degrees of fluorosis. In April 1982, 316 children from four communities in Iowa with negligible concentrations of fluoride in their drinking water were examined for comparison with the children in Illinois. Two reports of findings based only on the Illinois data have been published. One presented basic findings on dental caries and dental fluorosis as measured with Dean's Index; the other presented findings based on the new Tooth Surface Index of Fluorosis. A third and final report, presenting the Iowa data along with supplementary findings from Illinois, has recently 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 DE 00310-06 EODP

PERIOD COVERED: October 1, 1985 to September 30, 1986

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
Erazo, Barbara A.	Secretary (Typing)	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	.71	.13

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Approximately 1700 kindergarten and first grade children were randomly assigned to one of the following groups:

Group I - Rinses once every week in school with a 0.2% sodium fluoride solution.

Group III - Ingests once a day in school a sodium fluoride tablet containing 1 mg of fluoride.

Group II - Carries out the regimens for both Group I and Group III.

The method of assignment resulted in three comparable groups, each containing about 560 children. Participants carry out their assigned treatments in classrooms under the supervision of a teacher. Treatments will be administered for a minimum of eight years. Baseline dental examinations were conducted in September 1981. The prescribed treatments were initiated shortly after the examinations were completed.

In September 1985, the fifth school year of treatments began in the classrooms. First follow-up dental examinations were made in October 1983 of 1,154 participants who were then in grades 2 and 3. Supplies have been ordered, schedules arranged and local personnel identified for the start-up of the sixth school year of treatments in September 1986.

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 00396-02 EODP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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
Erazo, Barbara A.	Secretary (Typing)	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:
.47	.41	.06

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 a follow-up to a previous survey carried out by the NIDR in April 1980 in four areas of Illinois that have fluorides naturally occurring in their drinking water supplies at concentrations of approximately one, two, three and four times that recommended as optimal for those areas. The same communities were revisited to examine children in two of the same age groups (8-10 and 13-15 years) that were evaluated in 1980. Study participants in the 8-to-10 year age group comprised all children of those ages who received parental consent to participate and whose residence 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 were conducted in April 1985 to assess the prevalence of dental caries and dental fluorosis in both age groups. In addition, the prevalence of gingivitis and dental calculus was assessed in all children in the 13-to-15 year age group.

ANNUAL REPORT OF LABORATORY METHODS SECTION

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Intraoral Fluoride Releasing Device (IFRD) developed under contract by the Southern Research Institute was designed to provide continual topical fluoride for periods of up to six months for the prevention of dental caries. The device has reduced the incidence of experimental dental caries in rats by more than 50% and has been used in humans for periods of up to six months without producing adverse effects. We are now involved in efforts to refine the shape and method of attachment of the device so that it will be more durable and easier to use in humans. Discussions have been initiated on the use of the device in a large-scale clinical trial in humans pending the delivery of the refined device/attachment system.

The same concept for slow-release has been used to develop systems for delivery of therapeutic agents. Intraoral controlled-release systems for antibiotics/antimicrobial and antifungal agents for the treatment of periodontal disease, AIDS-related oral diseases and other opportunistic oral mycotic infections have been fabricated. Prototype controlled-release tetracycline pellets (TCRPs) have been produced. A short-term trial of this device in monkeys produced favorable changes in the subgingival bacterial flora. The development and testing of this therapeutic approach will be pursued.

Dicalcium diphosphate (DCPD) has been shown to increase the uptake of fluoride in dental enamel. In a recently completed study of 44 volunteers aged 18-50 years, the participants rinsed with H_2O-SnF_2 or DCPD-SnF_2. The DCPD-SnF_2 treatments augmented fluoride uptake by enamel without interfering with the antiplaque effects of the SnF_2 treatments. In the evaluation of taste perceptions, women assigned a somewhat higher level of approval to the taste of DCPD that did men. Plaque-bound fluoride appeared comparable in the two groups.

There is interest among clinicians, epidemiologists and researchers to identify a simple method to estimate the resistance of tooth enamel to an acid challenge, i.e. to identify teeth at a high risk of developing caries. Members of the section have investigated a method termed the acid neutralization time (ANT). This approach determines the time required for the tooth to neutralize acid contained in a paper disc impregnated with a pH indicator. The method requires little equipment to perform and the acid challenge appears to remove a negligible amount of hard tissue. Pilot studies indicate that the demineralized area regains its initial appearance within one to three days. Of two indicators used, crystal violet and phenol red, the phenol red imparted a less noticeable transient discoloration.

Considerable time and effort has been involved in the development and production of antisera to the major species of bacteria associated with periodontal disease(s). In general, antisera produced were specific for representative strains of the immunizing species, while serotypes within a species cross-reacted. F. nucleatum was the only species where a common

antigen was not observed among immunizing strains. The antisera produced will prove useful in identifying periodontal bacteria in clinical specimens. Identification can be made without the difficulties of cultural isolation and characterization of these relatively fastidious bacteria.

The cariogenic potential of foods commonly consumed as "snacks" continues to receive considerable attention. Methods, feeding regimens and modified nutritionally adequate diets have been evaluated in our laboratories. Results indicate that a high degree of replicability was obtained in three separate trials of five "snack" foods. Although the actual caries scores varied from experiment to experiment, rank-ordering of the foods was identical in all three trials. This was true regardless of whether scores for the buccal-lingual-proximal surfaces, sulcal surfaces or the total caries scores were used for computing the Relative Caries Inducing Potential (RCIP).

The section continues be interested and involved in assessing non-sucrose sweetening agents and dental caries in rats. Various regimens and combinations of agents and foods are in the process of being tested for their ability to prevent or limit the development of dental caries.

A short longitudinal collaborative study with Georgetown University has been completed. The population of 50 participants were observed over a period of approximately one year for soft tissue lesions, dental caries status, and gingival bleeding assessment. Data are being assessed for the parameters observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00112 13 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 chereclers or less. Title must fit on one line between the borders.)*
Preclinical screening of anticaries agents

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, leboratory, end institute effiliation)*

Shern, Roald J.	Principal Investigator	EODPP LMS NIDR
Kennedy, John B.	Biologist	EODPP LMS NIDR
Brunelle, Janet A.	Supvy Statistician	EODPP EB NIDR
Thomas, Sean H.	Clerk-Typist	EODPP LMS NIDR
Li, Shou-Hua	Statistician (Health)	EODPP EB 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 Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.91	0.37	0.54

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 spece provided.)*

The principal objectives of this project are the continuing efforts to identify antiplaque and anticaries agents suitable for short-term clinical investigation, as well as to develop methods for assessing the clinical potential for these agents.

The present study investigated a method of estimating the resistance of tooth enamel to an acid challenge when used in vitro or in vivo. This method, termed the acid neutralization time or ANT, measures the time required for the tooth to neutralize two microliters of acid contained in a paper disc impregnated with a pH Indicator. Findings from in vitro tests indicate that the method is simple, relatively reproducible and can detect the effects of fluoride treatments on enamel solubility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00408 02 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Short-term clinical trials of antiplaque agent NO1 DE 52484 CT 0600075

PRINCIPAL INVESTIGATOR *(List other professional personnel 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
Thomas, Sean H.	Clerk-Typist	EODPP LMS 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

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are: (1) to identify, adapt and pretest methods of measuring the bacterial and chemical composition of dental plaque and saliva; and, (2) to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries.

The present report supplements information provided previously (1985-1986) which noted that CPS applied orally enhances the ability of the teeth to acquire fluoride from rinses with SnF_2. Additionally it was noted the CPS treatments exhibited an acceptible taste and that the plaque bound fluoride and the clinical appearance of the oral hard and soft tissues were unaffected by CPS treatments.

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00416 01 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Production of Antisera to Bacteria Associated with Periodontal Disease

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

Little, Wayne A.	Microbiologist	EODPP LMS NIDR
Monell, Esteban	Bio Lab Tec (Animal)	EODPP LMS NIDR
Thomas, Sean H.	Clerk-Typist	EODPP LMS NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.71		0.71

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.)*

The purpose of this study was to produce antisera to the major species of bacteria associated with periodontal disease. These include representative strains of Bacteriodes gingivalis, B. intermedius, Haemophilus actinomycetemcomitans, Eikenella corrodens, Wolinella recta, Fusobacterium nucleatum and Capnocytophaga sp.

Indirect fluorescent antibody technique was used to titer rabbit antisera against immunizing strains and also against a battery of oral bacteria to test for cross-reactivity.

In general, antisera were specific for representative strains of the immunizing species, while serotypes within a species cross-reacted. F. nucleatum was the only species where a common antigen was not observed among immunizing strains.

The antisera produced in this study should prove useful in identifying periodontal bacteria in clinical specimens. Identification can be made without the difficulties of cultural isolation and characterization of these relatively fastidious bacteria.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00417 01 EODPP

PERIOD COVERED

October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 charecters 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
Monell-Torrens, E.	Bio Lab Tech (Animal)	EODPP LMS NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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.

Initial investigations focused on determining the feasiblity of utilizing biocompatible copolymers of hydroxyethyl methacrylate (MEMA) and methyl methacrylate (MMA) to produce a membrane-controlled delivery system for tetracyline that could be used for short-term intraoral therapy of periodontal disease. Prototype controlled-release tetracyline pellets (TCRP's) have been produced that are discs approximately 5 mm in diameter and 2 mm thick and release approximately 0.4 to 1 mg of tetracyline per day for up to 14 days. The TCRP is composed of an inner core or reservoir that consists of tetracycline mixed with a HEMA:MMA copolymer. The core is covered by a HEMA:MMA membrane that regulates the rate at which tetracycline is released from the pellet. The polymers used are also currently being used in a fluoride-releasing pellet that is being developed by the NIDR for providing topical fluoride for the prevention of dental decay. The polymers have proven to be very biocompatible.

A short-term trial of the TCRP in monkeys where the pellets were direct-bonded to anterior teeth produced favorable changes in the subgingival bacterial flora. Saliva samples collected during this experiment will be analyzed by HPLC to estimate intraoral tetracyline concentrations. Preliminary studies indicate that the copolymers also can be used to make controlled-release pellets for chlorhexidine and antifungal agents.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE0282-07 EODPP

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

COOPERATING UNITS *(if any)*

Southern Research Institute, Birmingham, AL 36255

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Laboratory Methods

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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.

Models of IFRD's with a shape and size suitable for attachment to a bicuspid tooth have been made and will be used for estimating production costs of IFRD's required for a clinical trial.

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 DE00405 02 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replicate screening of foodstuffs for potential cariogenicity

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

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Kingman, Albert	Statistician	EODPP EB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Thomas, Sean H.	Clerk-Typist	EODPP DPB NIDR
Zern Jr., Leidy D.	Animal Caretaker	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

A programmed feeding regimen was used to feed rats a cariogenic diet or one of five foodstuffs commonly consumed as "snack foods". A nutritionally adequate diet was presented as supplemental feedings to assure sufficient nutritional elements not supplied by the foodstuffs alone. Each foodstuff was replicated at least one time. The objective was to evaluate this regimen for wide scale use in the assessment of potential cariogenicity of various foods when compared to one of high cariogenicity.

The Relative Caries Inducing Potential (RCIP) was calculated separately using caries scores resulting from 1) buccal-lingual and proximal surfaces, 2) sulcal surfaces, and 3) the total caries scores. Although the value of the RCIP was different for each of the three surface scores, the rank ordering of the foods to sucrose (20%), in terms of cariogenic potential, was identical. The highest RCIP values were obtained when only the sulcal surface scores were compared and were: cookies 0.86, white bread 0.73, raisins 0.66, peanuts 0.48 and cheese 0.33.

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 DE00404 02 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

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

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

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Monell-Torrens, Esteban	Laboratory Technician	EODPP DPB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Thomas, Sean H.	Clerk-Typist	EODPP DPB NIDR
Zern Jr., Leidy D.	Animal Caretaker	EODPP DPB NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

Lylose is a sweetening agent developed in the U.K. by Tate and Lyle PLC. It is approximately 0.4 the sweetness of sucrose. Because of its potential use as a sucrose substitute by individual and industrial consumers, its cariogenic potential should be determined. Initially it was compared with two concentrations of sucrose using the rat animal model and a programmed feeding regimen.

Four groups (10/group) of rats were fed the following diets: Group 1 -Diet NIH 2000 (56 percent sucrose); Group 2 -Diet NIH 2114 (56 percent raw corn starch); Group 3 -Basic diet NIH 2000 with Lylose (56 percent) replacing sucrose; and Group 4 -Basic diet NIH 2000 containing 28 percent sucrose and 28 percent raw corn starch. The experiment was performed using a Konig-Hofer progammable feeding machine which delivered 17 meals/17 hour period of the assigned diets. An additional 12 meals of diet 2114 was provided all four groups as a supplement. All animals were initially infected with S. mutans 6715. Oral infection levels were determined twice weekly. After 56 experimental days the rats were sacrificed.

Considering caries scores for smooth surfaces only, those animals eating lylose had scores lower than the animals consuming 56 and 28 percent sucrose (p 0.03 and 0.01 respectively). Therefore, lylose may be considered a potential substitute for sucrose.

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 DE00407 02 EODPP

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Soft tissue lesions, tooth color variation and gingival bleeding in a population

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

Stiles, Horace M.	Chief LM Section	EODPP DPB NIDR
Brunelle, Janet A.	Chief B Section	EODPP EB NIDR
Gomez, Irma	Laboratory Technician	EODPP DPB NIDR
Thomas, Sean H.	Clerk-Typist	EODPP DPB NIDR

COOPERATING UNITS *(if any)*
Georgetown University Dental School, Washington, DC

LAB/BRANCH
Epidemiology and Oral Disease Prevention Program

SECTION
Laboratory Methods

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

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

Phase 1 clinical trials frequently involve 18-26 year-old individuals. Subjects are monitored frequently for the occurrence of soft tissue lesions (e.g. aphthous lesions), tooth staining and other manifestations in the oral cavity However, there is a paucity of data about these occurrences in a "normal" population, i.e. without any treatment or intervention.

Fifty otherwise healthy 18-26 year-old students at Georgetown University were examined six times over the period of approximately one year. At each examination a) any soft tissue lesions were noted and photographed; b) teeth were photographed; and c) bleeding index determined.

Data are presently being assimilated and various approaches to examination of the parameters involved in the study are being assessed.

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

ANNUAL REPORT OF THE BRANCH OF SCIENCE TRANSFER AND RESEARCH ANALYSIS, NATIONAL INSTITUTE OF DENTAL RESEARCH

The Science Transfer and Research Analysis Branch (STRAB) was established on June 1, 1986. Its current staff and functions were formed from the staff of the Office of Planning, Evaluation, and Communications (OPEC) in the Office of the Director, NIDR. The majority of staff activities during FY 1986 are addressed in the Annual Report of OPEC.

The 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 consequences of the changing patterns of oral diseases on economic, social and personal characteristics of the population and the profession.

It is well documented that the prevalence of dental caries is declining among most American children. Evidence is accumulating that this decline extends into young adult age groups. Much less is known about the prevalence of and trends in periodontal diseases. It is likely, however, that Americans in the future will retain more of their dentition longer than previous cohorts. Whether these changes will promote more or fewer dental services is currently a matter of considerable controversy. Most agree, however, that changes in disease patterns are likely to have profound consequences for both clinically-defined need and demand for dental services, and in turn, on the future economic, social, and professional characteristics of dentistry.

Research regarding the relations between the changing disease patterns, clinically-defined need, and demand for dental services is only beginning. An important impediment to this research in the past was the lack of a theoretical model that integrates disease and clinically-defined need into traditional theories of demand. Considerable effort has been expended during the previous year to develop such a theory. The theoretical model has now been developed and submitted to initial empirical test. During the coming year, development and empirical testing of the theory will continue. The concept of clinically-defined need will be refined and operational measures will be developed.

Several recent surveys have provided data that can be used to empirically test the new model relating need and demand. In addition, these databases will be exploited to further document the prevalence and trends in dental diseases and to begin to relate epidemiological measures of diseases to clinically-defined need. A survey conducted by the Research Triangle Institute is one source of data. Another is the soon-to-be-completed epidemiological survey of working adults being conducted by the Epidemiology Branch, E&ODPP, NIDR. A purchase order is being awarded to inventory all existing national databases and assess their analytical potential both singly and in combination. Further studies based on recommendations from the purchase order are anticipated. In addition, staff are participating in the planning of the third cycle of the Health and Nutrition Examination Survey (HANES III) that will be conducted by NCHS beginning in FY 1987.

While cross-sectional data can be valuable to explore these relations, panel studies offer a much stronger research design from which to address these issues. The feasibiltity of a panel of individuals in which disease, clinically-defined need and utilization can be followed over time within individuals will be actively explored.

Two internationally known scholars, Dr. Richard Oliver and Dr. Leif Arne Heloe, will spend the coming year as Visiting Scientists on the staff of STRAB. Both individuals are experienced in the area of disease measurement and clinically-defined need. They will lecture and conduct research on aspects of related issues.

As an extension of its science transfer and health promotion activities, staff of STRAB are developing a community-based periodontal diseases intervention program. The program will include demonstration projects for education of the public regarding periodontal diseases and improvement of periodontal health with preventive measures. To this end, the staff have been developing a plan based on a review of literature in general health promotion and in consultation with other BIDS and experts in the field. The final plan will be completed during the first half of FY 1987 and will include recommendations on specific measures to be used as well as groups to be targeted in the program. The final version of the plan will be reviewed by internal and external consultants and will serve as the framework for contracts and intramural projects in the future. One project which is planned for next year is a demonstration project of self-assessment of gingival bleeding as a means of motivating teenagers to maintain meaningful oral hygiene practices.

Three purchase orders have been awarded to analyze data about the current knowledge and practices of dental health professionals regarding the control of infectious disease transmission in dental settings. Particular attention will be paid to the knowledge and control of Acquired Immune Deficiency Syndrome. The information obtained from the analysis of these different data bases will be used to determine educational activities of STRAB in this area.

STRAB staff continue to develop dental health educational materials as well as foster health promotion and disease prevention activities. Plans for a free-loan exhibit aimed at increasing the public's awareness of periodontal disease have been developed and submitted for preliminary sketches. The six new exhibits should be completed early in the next fiscal year. Staff managed NIDR's scientific exhibits and responded to public inquiries at three national meetings: the National Association of School Nurses, American Dental Hygienists' Association, and the National Education Association. STRAB staff also arranged an all-day seminar featuring presentations by staff of NIDR for dental hygiene educators at the annual meeting of the American Dental Hygienists' Association. Five new posters in Spanish have been developed and will be available early in the next fiscal year. These posters are, for the most part, translations of English posters previously developed by STRAB staff. Three of these focus on fluoride and the remaining two emphasize the importance of sealants and fluorides. The leaflet, "Fluoride Tablets... A Healthier Smile for School Children," was updated and reprinted.

The members of STRAB have been active on several committees that deal with science transfer and health promotion. One member is responsible for evaluation of scientific program and membership approaches for the Federation Dentaire Internationale (FDi). This member is also President-Elect of Behavioral Sciences in Dental Research and organized and led a symposium on research methodology for

BSDR group at IADR. Two members are active in Working Group 3 of the Commission on Oral Health, Research and Epidemiology of the FDI. The Working Group is responsible for developing guidelines for dental associations on promoting oral health. A member of STRAB is the chairperson of, and another is a member of the American Association of Public Health Dentistry's Subcommittee on Sealants. The purpose of this subcommittee is to develop curricular guidelines for teaching the use of sealants to dental and dental hygiene students. One staff member is on the American Dental Association's National Advisory Committee on Fluoridation. One staff member initiated the formation of and served on a sealant task force which produced a guide to the use of pit and fissure sealants in public and private dental care settings.

STRAB staff continue activities in support of public health agencies in States and cities involved in the implementation, extension, or promotion of dental health programs using fluorides. In FY 1986, these activities have included provision of technical information for judicial and regulatory proceedings; performing liaison with other health 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. As part of the activities, a STRAB staff person is a member of the American Dental Assoication's National Advisory Committee on Fluoridation.

PRESENTATIONS
JUNE 1, - SEPTEMBER 30, 1986

HELEN GIFT

ALICE M. HOROWITZ

Preventing the Transmission of Infectious Diseases in Dental Settings: Educational Musts. Columbus Health Department and the Ohio State University, College of Dentistry, June 25, 1986, Columbus, Ohio.

Preventing the Transmission of Infectious Diseases in Dental Settings: Educational Musts. Cincinnati Health Department, June 26, 1986, Cincinnati.

Current Concepts in Preventive Dentistry. Alberta Dental Hygienists Association, August 1, 1986, Clear Lake, Alberta.

Implementing Dietary Fluoride regimes. Indiana Head Start and the University of Indiana College of Dentistry, August 6, 1986, Indianapolis.

Caries Prevention in Community Settings, South Carolina, Department of Health, August 21-22, 1986, Columbia, South Carolina.

INTRAMURAL RESEARCH PROGRAM

BONE RESEARCH BRANCH SUMMARY REPORT

The Bone Research Branch, now completing its third year of operation, 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 this past year, 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. Also this past year, three organizational changes occurred in the Branch. The nuclear magnetic resonance spectroscopy program, formerly in the Skeletal Biophysics Section, was separately recognized as the Protein Biophysics Unit, Dr. Dennis A. Torchia, Chief. The Skeletal Biophysics Section was renamed the Mineral Chemistry and Structure Section, Dr. E. David Eanes, Chief. Finally, Dr. A. Haridara Reddi's Bone Cell Biology Section was transferred to the Laboratory of Oral Biology and Physiology as part of an NIDR Intramural Program reorganization. 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. Study of such phenomena is 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.

In the past year, the Section's matrix biochemistry group, headed by Dr. Larry W. Fisher, focused on the precise characterization of defined bone matrix constituents. Five major noncollagenous proteins were purified to homogeneity from developing human bone. These molecules were unambiguously identified as distinct, primary gene products by a battery of biochemical analyses including amino-terminal protein sequence analysis. Further, the protein cores of two of these matrix constituents, small interstitial proteoglycans, were shown not only to be distinct from each other, but also to differ from analogous proteoglycans in other connective tissues. These data provided the first direct proof that clear differences in primary structure exist between what were previously believed to be almost identical matrix molecules in different connective tissues. Since it now appears probable that

at least one of these proteoglycans is derived from a single gene (see below) the data suggest that subtle, tissue-specific differences in transcription patterns (e.g., alternate splicing) may be selected to produce extracellular matrices of similar but not completely identical composition in the various connective tissues. Amino-terminal protein sequences also distinguished two bone sialoproteins as separate molecules, a heretofore unsuspected finding, and showed that one of these had identity with a newly discovered keratan sulfate proteoglycan found in abundance in rabbit, but not in human or bovine bone. Why this particular post-translational modification appears species-specific at first glance is not yet clear. In other tissue systems (e.g., articular cartilage), keratan sulfate enrichment on proteoglycan core proteins occurs with aging and/or disease processes. Human osteonectin was found to be almost identical in amino-terminal protein sequence to the bovine bone protein (only two conservative substitutions over the first 40 residues), but distinctly different in this regard from a recently described mouse analogue, synthesized at low levels by some non-bone tissues. The mouse protein does not appear to be retained in the non-bone extracellular matrices and differs from the human and bovine bone protein in other aspects (e.g., glycosylation and phosphorylation sites). It remains to be seen whether these differences represent species- or tissue-derived divergences. Since mouse bone is structurally different from bovine and human bone, these differences may have pronounced consequences for overall tissue architecture. Finally, a new, bone-derived short chain collagen was discovered this year. This protein, purified from bovine and human bone, has both collagenous and noncollagenous domains. The noncollagenous domain (M_r ~25,000) appears distinctly different in composition, charge and post-translational character from that of other short chain collagens described previously.

Studies in the Section's bone cell biochemistry program, headed by Dr. Pamela Gehron Robey, were aimed at the elucidation of mechanisms governing growth and differentiation of bone cells in culture. After immunoprecipitation of radiolabeled cultures with monospecific antisera and SDS gel electrophoresis, the following molecular species were identified as bone cell products in experiments this year; type I collagen (with no type III or V collagens present), fibronectin, osteonectin, bone proteoglycan (PGII), bone sialoprotein, osteocalcin, thrombospondin, alkaline phosphatase, beta-transforming growth factor (see below), and a short chain collagen. Preliminary pulse-chase analysis of the biosynthetic patterns exhibited by each of these proteins suggested that, in general, they differed in both compartmentalization and kinetics of secretion. In addition, the bone cells were shown to produce the following proteoglycans: a 660,000 M_r species containing chondroitin sulfate and a core protein of ~350,000; a 350,000 M_r species containing heparan sulfate and a core protein of ~100,000; and both 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 molecular species were incorporated into the extracellular matrix proper which could be induced to mineralize in a physiological manner (electron microscopic criteria) by manipulating culture conditions.

The active form of vitamin D, 1,25 $(OH)_2D_3$, was found to inhibit human bone cell growth while the lymphokine, IL-1, was mitogenic. Since both of these agents have been implicated as stimulators of bone resorption, it is interesting to note that they have opposing effects on bone-forming cells. At physiological doses of 1,25 $(OH)_2D_3$, osteonectin synthesis and mRNA levels

were unaffected, while at intermediate doses of this metabolite, osteonectin synthesis on a per cell basis was slightly increased. At higher doses of $1,25(OH)_2D_3$, osteonectin mRNA and biosynthesis were markedly decreased. These results were in contrast with the effects of this metabolite on collagen and osteocalcin synthesis which were elevated at most doses studied. Thus, the major effect of vitamin D_3 on human bone forming cells appears to inhibit their growth and disturb their normal biosynthetic pathways. The bone cells were also found to have message for, to actively secrete and also respond to the specific agent, beta-transforming growth factor (β-TGF). These studies, done in collaboration with Drs. Michael Sporn and Anita Roberts of the NCI, showed that this agent appears to be an autocrine or paracrine factor for bone cells. β-TGF was found to be a mitogen for the normal bone cell, but inhibited growth of two transformed, osteosarcoma cell lines. The normal bone cell had both high and intermediate affinity receptors for β-TGF and responded to its presence when only 1% of the receptors were occupied. These studies have important implications for bone cell metabolism in vivo and point toward eventual use of specific agents such as β-TGF to promote bone formation in pathological situations.

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. A full length cDNA for osteonectin was sequenced and the entire amino acid sequence for the protein then deduced. Osteonectin has a signal peptide of 17 residues and the secreted protein has an amino-terminal domain (52 amino acids in length) which only contains negatively charged amino acid residues (35% of the total). This region is thus presumed to be a likely calcium-binding domain for the protein. The remainder of the molecule is featured by a central, 84 amino acid long, cysteine-rich region (containing 11 of the molecule's total 15 cysteine residues) and areas of alternating positive and negative charge clusters. The carboxy-terminus of osteonectin (12 amino acids) is also highly charged (58% of all residues), complementing the amino-terminus of the protein. Osteonectin is extensively disulfide-bonded (7 internal bonds per 287 total residues) and the functional domains of the protein (apatite binding, collagen binding) would appear highly dependent on their three-dimensional arrangement. The osteonectin gene is approximately 30 kb in length. Approximately 90% of the molecule (starting from the 3' end of the DNA) is encoded by one-third of the gene, including 7 exons (150 bp) and six introns (1-2 kb). The remaining 10% (at the 5' end of the gene) is very complex, containing smaller exons (50 bp) and still larger introns (5-10 kb). Osteonectin mRNA levels were significantly higher (5 fold or greater) in cultured cells than in native, non-bone tissue and mRNA was detected in some fetal tissues known not to contain the protein. Thus, osteonectin expression (bone levels being generally 1000 fold greater than for any other tissue) appears both transcriptionally and translationally regulated.

Immunological analysis of recombinant core protein made by a cDNA encoding the small bone proteoglycan, PGII, showed that it shared antigenic epitopes with the small PGII-like proteoglycans of articular cartilage, skin and tendon. Further, substantial hybridization of the bone PGII cDNA was observed to mRNA's of similar length in articular cartilage, tendon, skin, smooth muscle and cornea, indicating a high degree of homology for these PGII species at the nucleic acid level as well. Gene copy analysis indicated that only one PGII gene is present per haploid genome. Thus, the variety of PGII-like molecules in the various connective tissues appear to be products of

a single gene. Since these may vary in both primary structure (see above) and glycosaminoglycan composition, the different tissue PGII species probably arise by variance in post-transcriptional and/or post-translational control. Like osteonectin, fibronectin and type I collagen, PGII transcription in chick embryo fibroblasts is sensitive to transformation with Rous sarcoma virus. This suggests that these matrix proteins may be under coordinate gene expression.

Proteoglycan Chemistry Section

Studies this year by this Section, Dr. Vincent C. Hascall, Chief, have shown that monoclonal antibody 1-C-6 recognizes a sequence epitope on cartilage proteoglycans in the hyaluronic acid-binding region (HA-BR) that is located on two distinct peptides. Thus, the HA-BR must contain at least two regions of polypeptide homology. This is analogous to similar findings for the link protein. Further, the results with monoclonal 5-C-4, which recognizes HA-BR and link protein equally well, indicate that these two polypeptides share common sequence epitopes and also have regions of homology. Identification of these regions may provide information useful for defining the structure of their binding sites for hyaluronic acid.

Procedures have been developed for immunizing mice with a mixed proteoglycan population and screening the resulting hybridomas for monoclonal antibodies which recognize individual highly purified, labeled proteoglycans. This has facilitated the identification of several monoclonals which recognize either the keratan sulfate-PG or the dermatan sulfate-PG from chick cornea and the dermatan sulfate-PGs or the heparan sulfate-PG synthesized by rat ovarian granulosa cells. These antibodies will be essential for identifying and characterizing the core protein precursors for each of these unique proteoglycans.

Previous work showed that bovine articular cartilage explants maintain steady state metabolism of proteoglycans for several weeks in culture. Studies this past year suggest that hyaluronic acid metabolism in these explants is also in steady state and that this glycosaminoglycan is turning over at a similar rate as for the proteoglycan. These results imply that the turnover of all components of the cartilage proteoglycan aggregate may be coordinately regulated by the chondrocytes.

The hydrophobic affinity chromatography procedure on octyl-sepharose has been refined to resolve several distinct proteoglycans, notably the corneal KS-PG and DS-PG species. This procedure has also proven useful for resolving the proteoglycans synthesized by bone cell cultures and for identifying the non-sulfated glycoprotein synthesized by human corneas from macular dystrophic patients in lieu of the normal KS-PG species. It is likely that this technique, which relies only on hydrophobic properties of the core proteins, will find wide applicability in further resolving the mixed proteoglycan populations usually isolated from tissues or cultures.

Protein Biophysics Unit

The focus of this Unit, Dr. Dennis A. Torchia, Chief, is to investigate the molecular structure and dynamics of proteins, nucleotides, and model compounds. The structural and dynamical information so obtained can be

correlated with function. Areas of present interest are 1) an intermediate filament, intracellular fibrous protein, mouse epidermal keratin subunit; 2) calcium binding proteins; and 3) model compounds for proteins and polynucleotides.

By incorporation of labelled amino acids into mouse epidermal intermediate filaments and subsequent measurement of nmr parameters of purified filaments, it was demonstrated that the amino and carboxy terminal portions are highly mobile while the interior region of the protein is more structured but still flexible. This correlates with structure predictions based on inspection of the amino acid sequence of intermediate filament protein (obtained by Dr. Peter Steinert of the NCI).

Incorporation of labelled amino acids also has been used in a multinuclear nmr study of the molecular dynamics and calcium-binding of staphylococcal nuclease. By recombinant DNA techniques, it was possible to purify, for the first time, large amounts of S. nuclease labelled with ^{13}C or ^{2}H at specific sites. Analysis of nmr spectra obtained with these samples reveals that (a) there are significant differences in the structure and motions of this protein depending on whether it is lyophilized, crystalline, or in solution; and (b) motions of amino acid residues in the interior of the protein molecule vary with their location.

Mineral Chemistry and Structure Section

The overall research goal of the Mineral Chemistry and Structure Section, Dr. E. David Eanes, Chief, is to acquire a more complete understanding of biomineralization mechanisms, with special emphases on the inorganic nucleation and growth processes which occur during the initial stages of mineral deposition in vertebrate hard tissue and on the physiological factors which initiate and regulate these processes. During the past year, work continued on the use of artificial lipid vesicles (liposomes) as in vitro models for studying membrane-controlled precipitation of calcium phosphate salts in physiological-like aqueous millieus. Transmission electron microscopic studies confirmed earlier chemical findings that ionophore-mediated Ca loading of PO_4-containing liposomes results in the precipitation of apatite within the aqueous interiors of the liposomes and, if the external aqueous milieu was metastable, outside the liposomes as well. Despite the observed closeness of the mineral-lipid association, the liposomes do not appear to actively initiate these precipitations. Previous chemical studies showed that the interior mineral formed spontaneously. Penetration of this mineral through the liposomal membrane covering was responsible for seeding the external mineral. Other liposome studies conducted during the past year made use of this relative unreactiveness of the lipid membranes to examine the effect membrane-anchored monoester phosphate groups had on calcification reactions. It was found that the inclusion of phosphatidic acid (PA) in the membrane envelope was ineffective in inducing the liposomes to initiate precipitation. In fact, as little as 5% PA totally inhibited penetrated intraliposomal mineral to initiate precipitation in metastable external solutions. This inhibition appears to be related to PA-mediated adsorption of liposomes onto the surfaces of the seed crystals limiting the access of reactant ions to these surfaces. Results such as these provide new insights into possible roles biomembrane regulatory factors may have in controlling mineral deposition in cartilage matrix vesicles in vivo.

Detailed characterizations (chemical, infrared, Raman, X-ray, EM, surface area, thermal analyses, and solution equilibrium pH) of ten previously synthesized, small crystal size hydroxyapatite preparations with surface areas of ~60 m^2/g, suitable for use as standards and controls for studies of tooth enamel have been completed. Consistent correlations between the combined chemical and physical data indicate a reliable compositional analyses. For example, these hydroxyapatite standards had hydroxide contents considered reliable within $\pm$ 5% of absolute and thus can utilized for infrared and Raman quantification of hydroxide content in tooth enamel. The crystal-surface/crystal-bulk ratios and small crystal-cross sections of these hydroxyapatite standards also facilitate infrared and Raman detection of inorganic surface reactions and ion diffusion in enamel crystals. In addition, two Raman bands at 1005 and 875 cm^{-1} in spectra of low Ca/P ratio synthetic apatite standards were assigned to stretching modes of HPO_4 ions; these bands should thus have application for detection and semi-quantification of HPO_4 ions in biological apatites.

Bone Cell Biology Section

This Section, Dr. A. Haridara Reddi, Chief, has continued its efforts to characterize bone inductive proteins. Methods described in detail in prior years' Summary Reports were used to purify bovine and human osteogenin. Bovine osteogenin, purified last year, has an apparent molecular weight of 22,000. The comparable human osteogenin fraction had three bands on silver stained SDS gels with a 22,000 M_r component. Among the variety of well-characterized growth factors tested (e.g, PDGF, EGF, FGF, CDGF, TGF-β), only osteogenin was found to have bone inductive potential *in vivo*. The highly purified osteogenin had no detectible transforming growth factor activity.

The cooperative interaction between the insoluble collagenous substratum and the soluble signal osteogenin operative in bone induction was also demonstrated. Substitution of the collagenous matrix by hydroxyapatite, β-tricalcium phosphate, glass beads, polymethylmethacrylate and polylactic acid were ineffective as substrata, indicating that the need for a collagenous substratum may be specific.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00012-24 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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)*

PI: Fowler, B. O. Research Chemist BRB NIDR

COOPERATING UNITS *(if any)*
Dr. J. Christofferson, Univ. of Copenhagen, Denmark

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:
1.10	.10	1.00

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.)*

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-quatitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-14 BRB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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)*

PI:	Fisher, L.W.	Staff Fellow	BRB NIDR
OTHERS:	Termine, J.D.	Chief	BRB NIDR
	Tuross, N.C.	NIH Postdoctoral Fellow	BRB NIDR
	Bolander, M.E.	Staff Fellow	LDBA NIDR
	Kinne, R.W.	Guest Researcher	BRB NIDR

COOPERATING UNITS *(if any)*

S.W. Whitson, SIU, School of Dentistry, Edwardsville, IL: S. Weinstein, U. Iowa Medical School; K. Vogel, Univ. of N. Mexico, Albuquerque, NM; F.A.Robey, FDA, Bethesda, Maryland

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.05	2.35	2.70

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

Analytical procedures (polyacrylamide gel electrophoresis, immunoblotting, specific dye-binding, RIA, ELISA, etc.) have been developed to quantitate the levels of bone specific noncollagenous proteins and a novel short chain collagen in (a) surgical specimens of bony tissue (osteonectin, bone sialoproteins I and II, bone proteoglycans I and II, and the short chain collagen, 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. Recent developments in monoclonal antibody production against osteonectin suggest that a family of related proteins similar to bone osteonectin may exist in soft tissues and in tissue culture.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00088-13 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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)*

PI:	Eanes, E.D.	Chief, Mineral Chemistry and Structure Section	BRB NIDR
OTHERS:	Heywood, B.R.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*
Dr. Ming Tung, American Dental Association Health Foundation Research Unit, 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:
3.10	1.90	1.20

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.)*

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 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.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00134-12 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters 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)*

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	Morales, T.I.	Staff Fellow	BRB NIDR
	Midura, R.	NIH Postdoctoral Fellow	BRB NIDR
	Xiaoming, Tien	Visiting Fellow	BRB NIDR
	Sayed, A. K.	Senior Staff Fellow	BRB NIDR
	McQuillan, D.J.	Visiting Fellow	BRB NIDR
	Glant, T.T.	Visiting Associate	BRB NIDR
	Correa, O.M	Visiting Associate	BRB NIDR

COOPERATING UNITS *(if any)* Dr. J. Kimura, Rush-Presbyterian-St. Lukes Med. Ctr.; Dr. L.S. Lohmander, Univ. Lund, Sweden; Dr. B. Caterson, U. WVA, Dr. S. Lamberg, Johns Hopkins Hosp.; Dr. J. Dingle, Strangeways Lab. Cambridge UK; Dr. J.Stevens, U. Ala. Birmingham; Dr. K. Vogel, U. NM, Alberquerque; Dr. C. Handley, Monash U. Australia

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:
8.30	7.20	1.10

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.)*

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) Metabolism of proteoglycans by rat ovarian granulosa cells; 4) Effects of bacterial endotoxins (lipopolysaccharides) and interleukin 1 on the regulation of proteoglycan metabolism in organ cultures of bovine articular cartilages; 5) Characterization of the keratan sulfate-proteoglycan and dermatan sulfate-proteoglycan in cornea.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z0 DE 00157-11 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters 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)*

PI:	Torchia, D.A.	Chief, Protein Biophysics Unit	BRB NIDR
OTHERS:	Hiyama, Y.	Visiting Associate	BRB NIDR
	Sparks, S.W.	Staff Fellow	BRB NIDR
	Mack, J.W.	NIH Postdoctoral Fellow	BRB NIDR

COOPERATING UNITS *(if any)* Dr. Paul E. Young, York College, SUNY, Jamaica, NY; Dr. Peter Steinert, DB, NCI; Dr. J.V. Silverton, LC, NHLBI; Dr. J.A. Gerlt, Dept. of Chemistry, Univ. of Maryland, Dr. J.S.Cohen, LP, NCI

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:
4.45	4.00	.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 purpose of this project is to investigate the molecular structure and dynamics of proteins, nucleotides 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 and polynucleotides.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00204-10 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Local and Systemic Regulation of Bone Development

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

PI:	Reddi, A.H.	Chief, Bone Cell Biology Section	BRB NIDR
OTHERS:	Muthukumaran, N.	Visiting Fellow	BRB NIDR
	Harrison, E.	Research Biologist	BRB NIDR
	Carrington, J.	Guest Researcher	BRB NIDR
	Prabhakar, B.	Senior Staff Fellow	LOM NIDR
	Howes, R.	Guest Researcher	LDBA NIDR

COOPERATING UNITS *(if any)*
Drs. J. Hollinger and D. Mark, USAIDR, U.S. Army; Dr. A. Roberts, NCI.

LAB/BRANCH
Bone Research Branch

SECTION
Bone Cell Biology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	3.20	1.55

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

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

The objective of this project is to investigate local and systemic control of bone development by extracellular matrix components. Projects currently under study are: 1) purification of bone inductive proteins from human and bovine bone; 2) specificity of osteogenin action and its modulation by other growth factors; 3) transforming growth factors and bone differentiation; 4) role of collagenous substratum in bone induction; 5) influence of aluminum on mineralization during matrix-induced bone development, and 6) influence of demineralized dentin matrix on transformation of muscle into cartilage.

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 00379-03 BRB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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

PI:	Young, M.F.	Staff Fellow	BRB NIDR
OTHERS:	Termine, J.D.	Chief	BRB NIDR
	Day, A.A.	Staff Fellow	BRB NIDR
	Findlay, D.M.	Visiting Fellow	BRB NIDR
	Robey, P.G.	Senior Staff Fellow	BRB NIDR
	Bolander, M.E.	Staff Fellow	LDBA NIDR

COOPERATING UNITS *(if any)*
H. Shimokawa, H., Tokyo Dental School, Tokyo, Japan

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:
4.80	3.45	1.35

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 spece provided.)*

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

The expression of bone and enamel matrix proteins have been studied by constructing recombinant cDNA libraries from ameloblast and 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 ameloblast tissue and in cultured bone cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00380-03 BRB

PERIOD COVERED
October 1, 1986 to September 30, 1986

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, end institute affiliation)*

PI:	Robey, P.G.	Senior Staff Fellow	BRB NIDR
OTHERS:	Termine, J.D.	Chief	BRB NIDR
	Beresford, J.N.	Visiting Fellow	BRB NIDR
	Heywood, B.R.	Visiting Fellow	BRB NIDR

COOPERATING UNITS *(if any)*
Drum, M.A., CIPCB, NIDR: Avioli, L.A., Jewish Hospital, St. Louis, MO; Roberts, A.B., LC, NCI

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:
2.23	1.39	.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.)*

Bone cells (human and bovine) have been utilized to 1) study the biosynthesis 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.

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

ANNUAL REPORT OF THE LABORATORY OF 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 a considerable focusing of 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 a strong center of clinical research has been built first 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 expanded. 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 and dental hygienists routinely participate in medical rounds and patient care discussions thus integrating oral health care concerns to total patient management.

The Section's staff are deeply involved in the continued 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 continued major effort, by Patient Care Section dentists and hygienists, to develop a cohesive clinical research program based on the unique patient problems seen at NIH. This effort has been tangibly aided by the close working relationship with the Clinical Investigations Section, as well as by collaborations with other branches at NIH. Two major protocol based study areas have been developed. 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 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.

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 one senior dental student served in an eight week program which emphasized providing dental care to medically compromised patients in a hospital environment.

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 protein synthesis, processing, routing and secretion; and (3) understanding the etiology of, and developing treatments for, specific salivary gland secretory dysfunctional states and associated oral disorders.

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 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 an improved acinar cell preparation and basolateral membrane vesicle preparations from rat and rabbit parotid glands. Studies have been directed primarily at transport mechanisms for Cl^-, Na^+, K^+ and Ca^{2+}. For example we have extended studies, initially reported last year, which demonstrated the existence, in intact cells, 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. To do this we followed ^{22}Na transport. Sodium uptake into vesicles was markedly dependent on K^+ and Cl^-, could be driven against a sodium gradient by a KCl gradient and showed a hyperbolic dependence on $[Na^+]$. Sodium uptake also showed a hyperbolic dependence on $[K^+]$ but a sigmoidal dependence on $[Cl^-]$. This demonstrates that Na^+ uptake across the basolateral membrane occurs via a single transport pathway consistent with a $Na^+:K^+:Cl^-$ stoichiometry of 1:1:2. The electroneutral nature of this process was confirmed by the lack of effect of membrane potential on Na^+ uptake. We have also employed a radiolabeled loop diuretic, [^3H]-bumetanide, as a structural and functional probe of the cotransporter. This ligand bound to vesicles in a Na^+, K^+ and Cl^- -dependent manner, exhibiting, under optimal conditions, a single binding site (K_d = 3μM) which appeared identical to the Na^+ transport site. These studies also gave evidence for two Cl^- binding sites, of high and low affinity.

The model proposed to describe the formation of primary saliva which incorporates the $Na^+/K^+/Cl^-$ cotransporter, requires the existence of an apical conductive pathway for Cl^-. This conductance would allow Cl^-, which had entered the cell via the cotransporter 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 evidence for such a Cl^- efflux channel and shown it to be dependent upon the concentration of the muscarinic-cholinergic agonist carbachol to which cells were exposed. Chloride efflux ($^{36}Cl^-$) from parotid acinar cells is rapid, blocked by the muscarinic antagonist atropine and by N-phenylanthranilic acid, a putative Cl^- channel blocker. We have also made considerable progress in attempts to obtain an enriched preparation of apical membranes to allow for more detailed studies of the Cl^- conductance mechanism.

The above studies on Na^+, K^+ and Cl^- fluxes have contributed enormously to our understanding of how saliva is formed, and accordingly how we can manage clinical problems related to salivary gland dysfunction. The unique capabilities of our Branch to meet the clinical problem with laboratory expertise is exemplified by studies performed this year which have capitalized on our intimate knowledge of ion fluxes. For years $^{99m}TcO_4$ has been in widespread clinical use in the diagnosis of salivary gland, and other tissue, dysfunctions. Yet the mechanism by which this radionuclide enters a cell is not established. Since $^{99m}TcO_4$ is considered a pseudohalide we speculated that the tracer is taken up by cells, substituting for Cl^-, via the $Na^+/K^+/Cl^-$ cotransporter. Using rat parotid acinar cells, we showed that $^{99m}TcO_4$ uptake is indeed Na^+, K^+ and Cl^- dependent and blocked by the loop diuretics furosemide and bumetanide. These findings provide strong evidence that $^{99m}TcO_4$ - uptake by tissues reflects the functional activity of the $Na^+/K^+/Cl^-$ cotransporter pathway.

It has long been known that Ca^{2+} plays a key role in stimulus - secretion coupling. Over the past few years we have made considerable progress in understanding Ca^{2+} handling mechanisms at the plasma membrane level. Our progress in this area has continued during this past year. We have identified the ATP-dependent Ca^{2+} pump as the primary mechanism for Ca^{2+} extrusion across the basolateral membrane out of the parotid acinar cell. This is a high affinity ($K_m \sim 60$-100nM) and high capacity ($V_{max} \sim 45$nmoles Ca^{2+}/min/mg protein) system, which requires Mg^{2+}, is sensitive to both a K^+ diffusion potential and a H^+ gradient,

inhibited by Cl^- replacement and inhibited by loop diuretics. Thus ATP-driven Ca^{2+} extrusion is electrogenic and likely can be modulated by any condition (hormonal, pathologic, pharmacologic) which can alter membrane potential or related ion fluxes. We previously have reported that aging rat parotid cells are defective in Ca^{2+} extrusion following α_1-adrenoreceptor stimuli. During this year we have utilized the aging paradigm to better understand receptor-coupled alterations in Ca^{2+} handling. First, through rigorous study of α_1-adrenoreceptor binding characteristics in intact cells, using a radiolabeled agonist as a ligand, we confirmed that alteration in Ca^{2+} fluxes observed in acinar cells from aging rats is indeed due to a post-α_1-adrenoreceptor deficit. No differences were detected in receptor characteristics between young and old rat tissue. Accordingly we next studied in detail ATP-dependent Ca^{2+} transport in basolateral membranes from young and old rat parotid cells. Initial rates of Ca^{2+} transport were reduced ~40% in vesicles from old rats. This was not due to increased Ca^{2+} permeability nor to secondary alterations in membrane permeability to K^+ or Cl^-. Kinetic analyses showed membranes from older rats had a higher Km Ca^{2+} (~50%) than those from younger counterparts (160nM vs 109nM) while no differences in Vmax were observed (45nmoles/min/mg protein). It would appear that this change in Ca^{2+} pump activity in great part accounts for the decreased Ca^{2+} efflux seen from parotid cells of aged rats.

In addition to secreting water and electrolytes, parotid acinar cells secrete the majority (>80%) of parotid salivary proteins. As in the past our focus in this investigative area has been on β-adrenoreceptor regulation of secretory glycoprotein synthesis, processing, routing and release. Most importantly, during this past year, we have extended our observation, that hormone stimuli coupled to cyclic AMP can upregulate the N-linked protein glycosylation process, from rat parotid acinar cells to a wide variety of cell types including, human diploid fibroblasts, dispersed rat lacrimal cells, chinese hamster ovary (CHO) cells, C_6 glioma cells, bovine endothelial cells and rat submandibular ductal epithelial cells. This indeed appears to be a widespread phenomenon, with elevations in cyclic AMP resulting in 80-150% increases in the incorporation of [^{3}H] mannose, vs [^{14}C] leucine into cellular glycoproteins. Also we have supported our contention that this glycosylation change may be a result of a cyclic AMP-dependent phosphorylation event on key dolichol-linked glycosyltransferases. CHO cells with mutations in cyclic AMP dependent protein kinase expression were incapable of showing the cyclic AMP coupled enhancement of Man-P-Dol synthase activity (and an overall increase in glycosylation) as was seen with wild type CHO cells. Further study of this receptor-coupled modulation of protein processing came in studies employing an (1) aging paradigm and (2) 2 models of sympathetic denervation. In the former we showed that aged rat parotid cells have reduced basal levels of N-protein glycosylation and similarly reduced levels of Man-P-Dol synthase activity. Yet these cells have fully functional β-adrenoreceptors (known from our earlier work) and aging parotid cells show increased N-linked glycosylation, albeit at lower absolute levels, after treatment with a β-adrenoreceptor agonist. In the latter models we observed that denervation (we used both chemical and surgical models) resulted in changes in receptor characteristics which diminished the glycosylation response. However when the β-adrenoreceptor was bypassed, and glycosylation changes were induced by a cyclic AMP analogue, an excellent functional response was seen. We have also tried to further our understanding of the specific enzymatic steps involved in mediating the β-adrenoreceptor upregulation of N-linked glycosylation. This year we have examined the key dolichol cascade enzyme oligosaccharyltransferase. Activity of this enzyme, in microsomal membranes, was increased 2 fold when membranes from cells treated with a β-adrenoreceptor agonist were utilized. This was not a result of

increased levels of enzyme protein, or direct activation of the enzyme, but seemed mainly to reflect, more and better formed substrate (ie. glucose capped oligosaccharyl-PP-dolichol) available for the enzyme to use. We have also continued our studies which attempt to characterize N-linked oligosaccharides present on glycoproteins obtained from isoproterenol-treated cells. We have made use of several protein purification and characterization techniques in this endeavor including gel filtration chromatography, lectin affinity chromatography, SDS-PAGE, western blotting and "lectin staining". These studies suggest oligosaccharides present on glycoproteins from isoproterenol-treated cells are similar with respect to structure (ie. the proportion of complex to high mannose/hybrid forms) as seen on glycoproteins from control cells. The processing changes just occur more rapidly. Finally these studies also have been useful in extending our clinical perspectives. Using the chronic isoproterenol model to study functional parameters of salivary glycoproteins (bacterial aggregation and absorption to hydroxyapatite beads), we demonstrated that gland dysfunction can be the result of structural compositional changes in specific proteins, in the presence of perfectly normal salivary fluid secretion rates.

We have also made substantial progress in our efforts to utilize and to develop cell culture models for studying salivary physiology. During this reporting period we have utilized three established glandular epithelial cell lines. One, RSMTX, a transformed rat submandibular duct cell line which we have developed, and two, A253 and HBL-100, which are commercially available. A253 is derived from a human submandibular epidermoid carcinoma and HBL-100 from normal human mammary cells. In general, our initial approach was to determine if certain transport or biosynthetic events in these cells are modulated by hormonal or metabolic signals. We have found evidence for such control in all three cell lines. We have also tried to establish additional salivary epithelial cells in culture using primary explant techniques. We have obtained cultured epitheloid cells from a human parotid myoepithelioma and from normal bovine parotid gland, each of which have been subcultured several times. We have extensively examined culture conditions to determine the optimal methods for salivary epithelial cell growth. We have also been able to obtain primary cell outgrowths from a human parotid mixed tumor. These epithelial cells however have not survived a host of different subculturing methods.

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 280 patients. We have well established diagnostic approaches to the evaluation of patients with complaints suggestive of salivary gland dysfunction. Most patients seen have diminished gland secretory capacity. Approximately 60 such individuals were admitted as inpatients for intensive study this year. We are now involved in a third trial testing the efficacy of pilocarpine, a parasympathomimetic drug, in treating patients with gland hypofunction who have clear evidence of residual gland parenchymal tissue present. Patients receive the medication three times per day over a 6 month period; one month being a double-blind, randomly assigned placebo period. Initial results indicate no significant side effects associated with prolonged treatment and reports of subjective relief have been obtained from several patients. We have also focused considerable attention on subgroups of patients with salivary dysfunction that have a diagnosis of Sjogren's Syndrome, with or without associated connective tissue disease. We observed essentially uniform reductions in unstimulated salivary flow rates with these patients. However, importantly, we found that stimulated fluid secreting capabilities were quite variable; many individuals being able to produce stimulated flow rates within the normal range.

Similar results were found with respect to compositional values. These results clearly demonstrate the heterogeneity of these conditions and suggest that stimulated salivary function may be a poor indicator of the extent of salivary involvement in persons with autoimmune exocrinopathies. We have also recognized that clinical practitioners may find administering tests of salivary function awkward. We have therefore devoted significant effort towards determining what types of subjective information, conveyed by patients, is most useful as a diagnostic indicator of salivary gland functional ability. We found that subjective responses were most influenced by submandibular gland output and we have derived a series of questions which is very useful in distinguishing persons with true abnormalities in fluid secretion.

During this reporting period we have established two important, new clinical studies both of which are intimately related to our common theme of addressing problems in persons with compromised oral protective mechanisms. The first study involves patients with Acquired Immune Deficiency Syndrome (AIDS). These patients, like many debilitated or immunocompromised individuals, manifest oral candidal infections. In fact available data indicate that virtually all (>80%) high-risk persons who will develop AIDS have oral candidiasis. Candidal levels in the oral cavity are thought to be usually controlled by a group of salivary proteins with potent anti-fungal activity. These proteins, the histidine-rich proteins (HRP) are low molecular weight, highly cationic molecules. We have proposed to investigate the ability of salivary glands in patients with early stage AIDS to synthesize and secrete the HRP. Conceivably the levels of HRP in saliva may serve as a useful prognostic indicator for persons at risk to develop AIDS. We have prepared anti-sera to the HRP and have begun to develop an ELISA to quantitate salivary HRP levels. The second study is really not new, but it is the re-institution of a study at another categorical institute (NIA) through our collaboration. This inter-institute effort has restarted the oral physiology component of the Baltimore Longitudinal Study of Aging. This study is of normal physiology across the adult life span. Thus far we have evaluated 20 persons (a rate of ~100 persons/year), investigating submandibular/sublingual gland function (unstimulated; stimulated), general oral motor performance and oral sensory perception. For these studies we have revamped our previously used oral motor evaluation and attempted to design a more objective, cranial nerved based examination which is convenient and reproducible. Further we developed a broad spectrum test of oral sensory performance, examining taste, smell, temperature, tactile and textural sensory activities. We expect this study will provide us with a picture of normal oral physiology across the adult life span. These data will also serve as important control values for our many study patients with salivary gland dysfunction.

We continue to devote considerable effort to studying the oral phase of swallowing. We have clearly demonstrated that salivary gland dysfunction can be an etiologic agent in dysphagia. Patients who complain of dysphagia have 3-6 fold lower average flow rates of stimulated saliva than patients without such complaints. When the oral phase of swallowing is studied in such persons, approximately two fold increases in the duration of the oral phase of swallowing was noted for both dry and wet (with a water bolus) swallows. This supports our earlier suggestions of the important permissive role of saliva in initiating alimentary events. Studies on gustatory function have again been primarily related to evaluating patients displaying salivary gland dysfunction. Our previous work has suggested that normal salivary gland function is not necessary for normal gustatory function. This impression has been considerably substantiated. It is clear that although patients with impaired salivation may manifest impaired gustation, the

latter is not an obligate sequela of the former. In addition, this year, we initiated a longitudinal study of olfactory function in patients with early-diagnosed Alzheimer's Disease.

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 have made 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.

CLINICAL INVESTIGATIONS AND
PATIENT CARE BRANCH
PUBLICATIONS 1985-1986

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00212-10 CI

PERIOD COVERED
October 1, 1985 - September 30, 1986

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 M.	Research Psychologist	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf Clin I	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR

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:
1.5	1.1	.4

CHECK APPROPRIATE BOX(ES)

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

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

This project 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 and pleasantness for taste stimuli at higher, more commonly encountered levels of strength. These methods, applied to the study of possible 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.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00332-05 CI

PERIOD COVERED
October 1, 1985-September 30, 1986

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	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Drum, Margaret A.	Clinical Staff Dentist	CIPC	NIDR
Folio, John	Senior Staff Dentist	CIPC	NIDR
Atkinson, Jane C.	Dental Staff Fellow	CIPC	NIDR
Haller, Julie M.	Dental Hygienist	CIPC	NIDR
Harlow, Shelley A.	Dental Hygienist	CIPC	NIDR
Hughes, Christopher V.	Dental Staff Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*
Pediatric Branch, NCI; Inter-Institute Genetics Program, CC; Arthritis and Rheumatism Branch, NIADDK

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.05	1.6	1.45

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.)*

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

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 00336-05 CI

PERIOD COVERED
October 1, 1985 - September 30, 1986

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
Brown, Ashley	Guest Researcher	CIPC NIDR
He, Xinjun	Visiting Fellow	CIPC NIDR
Marmary, Yitzhak	Guest Researcher	CIPC NIDR
Roth, George S.	Research Chemist	LCMB NIA
Wellner, Robert B.	Sr. Staff Fellow	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:
4.2	3.5	.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 health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize primarily in vitro dispersed cell and membrane preparations of rat 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 has been employed as a useful model to study autonomic receptor control of Ca^{2+} handling in exocrine acinar cells. During this reporting period specific areas of study with these preparations include (1) characteristics of the ATP-dependent Ca^{2+} pump in parotid basolateral membranes and (2) mechanistic aspects of α-adrenoreceptor mobilization of cellular Ca^{2+}. We have also expanded our efforts into developing and utilizing cell culture to study exocrine secretory events. To this end we have (1) begun to characterize functional events, associated with secretion, in three established epithelial cell lines and (2) initiated primary cultures of epithelial cells from several types of salivary glands.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00337-05

PERIOD COVERED

October 1, 1985 - September 30, 1986

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
Gahl, William	Medical Officer	HGB	NIADDK
Helman, Joseph	Visiting Fellow	CIPC	NIDR
Lane, H. Clifford	Medical Officer	LIR	NIAID
Marmary, Itzak	Guest Worker	CIPC	NIDR
Sonies, Barbara C.	Speech Pathologist	RM	CC
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Yeh, Chih-ko	Visiting Associate	CIPC	NIDR

COOPERATING UNITS *(if any)*

RM, CC; DR, CC; HGB, NICHD; LIR, NIAID; Columbia University; SUNY, Buffalo; Boston University

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.9	2.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 syndrome, an autoimmune exocrinopathy, have been initiated. 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00362-04 CI

PERIOD COVERED
October 1, 1985-September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dental Development in Patients with Precocious Puberty

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	CIPC	NIDR
Li, Shou Hua	Statistician (Health)	EB	NIDR
Cutler, Gordon B., Jr.	Chief Developmental Endocrinology	DEB	NICHHD
Loriaux, D. Lynn	Clinical Director	DEB	NICHHD
Hench, Karen B.	Associate Investigator	CC	NICHHD

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch, NICHHD, NIH

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.15	0.35

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.)*

Attempts are being made to determine whether there are any correlations between skeletal age, chronological age, tooth and dental root formation in patients with precocious puberty. Oral examinations have been completed on 155 children and panographic radiographs obtained on 140. Using the most accurate available dental growth and development standards comparative studies are being conducted. These studies involve both cross-sectional and longitudinal evaluations. The NICHHD is a cooperating unit in the study and they have assigned their project number Z01 HD 00610.

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 00372-04 CI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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 I	CIPC NIDR
Banerjee, Dipak K.	Visiting Associate	CIPC NIDR
Ciardi, Joseph E.	Research Biochemist	LMI NIDR
Melvin, Jim E.	NRSA Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*

LMI, NIDR; M.D. Anderson Hospital; U. Texas - San Antonio

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.4	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Saliva contains a broad spectrum of proteins which are known to play a pivotal role in maintaining the integrity of the hard and soft oral tissues. Many of the proteins secreted from the parotid gland are glycoproteins and carry N-linked oligosaccharides. To study the mechanisms involved in synthesis, processing and secretion of N-linked secretory glycoproteins we have utilized in vitro cell and microsomal membrane preparations from rat parotid glands. We have previously demonstrated that β-adrenergic receptor stimulation increased protein N-glycosylation through a cAMP-mediated mechanism. This appears to be due to increased synthesis and utilization of oligosaccharide-PP-dolichol and enhanced activity of specific glycosyltransferases. We have also shown that β-adrenoreceptor stimulation modulates the rate of processing of N-linked oligosaccharides in a single high molecular weight (220kd) secretory glycoprotein. During the present reporting period we have 1) progressed in our studies on salivary protein-bacterial interaction after treatment with β-adrenergic drugs in rats in vivo; 2) examined the role of oligosaccharyltransferase in the enhancement of N-linked glycosylation seen in rat parotid acinar cells after β-adrenoreceptor stimulation; 3) observed similar increases in protein N-glycosylation after β-adrenergic receptor stimulation in human parotid acinar cells as in rat cells; 4) utilized two sympathetic denervation models, surgical and pharmacological (reserpine), to further examine β-receptor regulation of salivary gland function; 5) observed decreased (~30%) protein N-glycosylation with aging in rats; this change being associated with decreased (~50%) Man-P-Dol synthase activity; 6) demonstrated that cAMP enhanced glycosylation in many cell types other than parotid, including human foreskin fibroblasts, chinese hamster ovary (CHO) cells, bovine capillary endothelial cells and lacrimal gland acinar cells; 7) continued studies on the characterization of the four secretory glycoproteins (220, 38, 32, 17kd) exhibiting glycosylation changes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00395-02 CI

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Clinical Study of a Dentin Bonding Agent and Composite Resin in Posterior Teeth

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	CIPC NIDR
Folio, John	Senior Staff Dentist	CIPC NIDR
Guckes, Albert D.	Chief CODC	CODC CC
Moffa, Joseph P.	Chief Clinical Research Branch	Letterman Army Institute of Research

COOPERATING UNITS *(if any)*

Clinical Research Branch, U.S. Army Institute of Dental Research, Letterman Army Institute of Research, San Francisco, California

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.4	0.65

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.)*

This study is evaluating the clinical performance of a small particle bimodally filled hybrid proprietary composite resin and a dentin bonding system. This composite resin system is being employed for the restoration of class II carious lesions in posterior teeth. Its performance will be compared to that of a conventional amalgam. Fifty restorations of each of the two materials will constitute the study. Each restoration will be evaluated for clinical performance using a standardized method. Also, an attempt is being made to quantify any loss of material due to surface disintegration or wear using a recently developed scale and device.

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 00411-01 CI

PERIOD COVERED
October 1, 1985- September 30, 1986

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:
0.7	0.15	0.55

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.)*

These studies are evaluating the effectiveness of a formal orientation program designed to inform and motivate patients receiving head and neck radiation treatments regarding 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.

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 00412-01 CI

PERIOD COVERED
October 1, 1985- September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Maxillofacial Surgery and Implant-Prosthetic Reconstruction

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

Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Folio, John	Senior Staff Dentist	CIPC	NIDR
Wright, William E.	Senior Staff Dentist	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Helman, Joseph	Visiting Fellow	CIPC	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:
3.60	1.55	2.05

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.)*

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 radiographic, 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00415-01 CI

PERIOD COVERED: October 1, 1985 - September 30, 1986

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
George, Janet N.	Chemist	CIPC NIDR
Helman, Yossi	Visiting Fellow	CIPC NIDR
Kawaguchi, Mitsuru	Visiting Fellow	CIPC NIDR
Manganel, Michel	Visiting Fellow	CIPC NIDR
Melvin, James E.	NRSA Fellow	CIPC NIDR

COOPERATING UNITS *(if any)*: none

LAB/BRANCH: Clinical Investigations and Patient Care Branch

SECTION: Clinical Investigations Section

INSTITUTE AND LOCATION: NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	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.)*

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 phenomemon of primary fluid secretion by salivary acinar cells and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following.

(1) The transport of ions (Na^+, K^+, Cl^-, TcO_4^-), whose transmembrane and transepithelial movements are thought to be related to the process of primary salivary fluid secretion, was studied in vitro in a rat parotid acinar suspension and/or in isolated rabbit parotid basolateral membrane vesicles. These transport studies concentrated primarily on the properties of a basolateral Na/K/Cl cotransporter thought to be primarily responsible for producing the ion gradients which drive fluid secretion.

(2) The binding of a specific inhibitor of the Na/K/Cl cotransporter, [^{3}H]-bumetanide, to rabbit basolateral membrane vesicles was characterized.

(3) Procedures for isolating parotid acinar luminal membrane vesicles, and for improving existing basolateral membrane vesicle preparations and acinar suspensions were developed.

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

ANNUAL 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. Xiang-lin Qi, a Visiting Scientist from China, who is experienced in mathematical systems modeling techniques applied to simulation of visual responses to stimuli of diagnostic interest. Also new this year is Dr. Samuel Zeichner, a Staff Fellow, who replaces Dr. David Roberts. Dr. Zeichner is a board-certified dental radiologist with research interests and training in nuclear medicine and magnetic resonance imaging as well as being a DSB collaborator in the evaluation of diagnostic performance obtainable from existing radiographic modalities.

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 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.

Specific accomplishments in this area include the acquisition of outside support for concurrent procurement and testing of three different intraoral x-ray detectors from three different industrial sources, to provide more assurance that a practical system will evolve from the prototype now under development at NBS. Two of these detectors are at the cutting edge of current technology. One is based on the use of semiconductor random access memory chips as primary photon transducers capable of being accessed randomly. They are seen as an extension of central processing memory by the image-processing computer which is intrinsic to the x-ray system. Likewise, the other is grounded in semiconductor technology. It is based on the use of specially developed pin diodes arranged in a two-dimensional array to produce a real-time detector system. The third detector system being developed is less sophisticated. It 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. This detector will probably be used for early demonstration of system applicability in vivo, but represents a fall-back position when considered in the long run.

All three detector systems support the new design concept described last year which facilitates the potential for synthesis of any desired x-ray projection from the radiographic information acquired during a single automated scanning sequence. Essential to this design is an x-ray source capable of emitting pulses of radiation from eight or more foci distributed in a circular locus during a time interval short enough to preclude significant patient motion during exposure. To

this end, support for two different configurations has been mobilized. The first involves moving the focal spot around under computer control via electromagnetic deflection of the associated electron beam in a specially designed x-ray tube having an enlarged target anode. The other involves less development and hence is more fool-proof. It is comprised of eight conventional, commercially available x-ray tubes which are arranged circularly and fired sequentially like a Gatling gun.

Significant progress has been made in the development of substantive soft-ware for this project as well. Data from controlled studies now confirm that image subtractions produced from appropriately weighted projection data are compromised less by differences in projection geometry than comparable data produced using nearest-neighbor, control projections. In theory, this opens the door to image comparisons obtained from existing clinical records including the wealth of retrospectively produced periapical radiographs already residing in patient files.

Related experiments involve 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 only one component of the registration error created by changes in projection geometry is irreversible. This irreveresible component is determined by the spatial relationship existing between the x-ray source and the irradiated tissues of diagnostic interest. Experiments done in collaboration with investigators at Harvard University confirm 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. These findings complement the results reported last year which predicted that the relatively high speed and disparity control afforded by new filmless imaging systems of the types discussed above would preclude the need for fabricating custom stints for each different x-ray projection.

Other related software being developed by the DSB programming staff includes the implementation of a two-dimensional warping algorithm to correct 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.

A modality-based noninvasive sampling alternative which may improve diagnostic performance is exemplified by the spectroscopic analysis of hemoglobin through differential attenuation of tooth-scattered light using a narrow band-pass optical filter. Related collaborative efforts have been extended to optical techniques made possible with miniaturized fiberoptic

technology; one application being the measurement of oxygen tension in periodontal pockets. 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. Preliminary data confirm theoretical predictions that tiny amounts of blood can be detected reliably irrespective of the color of the light reaching the differential detector.

Particularly promising in terms of modality-specific applications is research based on the use of radiopharmaceuticals to predict loss of periodontal bone. Recent findings suggest 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 anticipates loss of supporting bone months before it can be detected radiographically. Application of magnetic resonance imaging technology to the same diagnostic task has been disappointing, but this modality has yielded a significant contribution to the problem of assessing noninvasively, soft articular structures associated with the temporomandibular joint.

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. Specific research is exemplified by theoretical models derived by DSB investigators to explain the degree to which noise from a variety of sources always limits the potential for improving diagnostic performance. Such noise may be derived from normal anatomical variations in the tissues of diagnostic interest, from the statistical uncertainty associated with the photons themselves, or from the variations associated with the the data sampling strategy.

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 automated isolation and quantification of metastatic cancer in computerized tomographic scans, 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 show that known lesion volumes measured invasively and those deduced noninvasively from digital analysis of contrast-corrected radiographs differ by amounts having a

standard error of approximately one mm^3. This work also complements results reported last year dealing with automated image registration and analysis. It demonstrates the increase in statistical power afforded by improved sampling techniques intrinsic to the method. Indeed, a statistically significant difference in the rate of healing of induced osseous lesions in two beagle dogs was found using digital subtraction of contrast corrected radiographs obtained from only three examinations distributed over a period of little more than two weeks.

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 the tissues of diagnostic interest. Recent efforts have focused on the development of an algorithm for detecting and delineating of areas in radiographs characterized by trabecular bone. The basic approach used involves the use of regional split and merge routines applied to data described with first order gray-level statistics in conjunction with quad-tree data management procedures. Preliminary results appear encouraging enough to warrant expanded development using second-order statistics expressed as co-occurrence matrices which will be considered as a next level of improvement.

Another area of continuing investigation involving research directed toward the development of improved systems concerns the role of disease prevalence in estimating cost-effectiveness of dental radiographic screening. The results of this work have shown conclusively that dental radiographic screening is not cost-effective. The significance of this work is that implementation of diagnostic selection guidelines based on these findings would reduce national health care expenditures and limit exposure of the population to radiation from unnecessary or nonproductive x-ray procedures. Also, these studies have generated the largest and most comprehensive data base related to rare intra-osseous lesions of the face and jaws from which could be extracted additional economic and epidemiologic information. Planned extensions of this work include the determination of typical costs associated with the diagnosis and treatment of selected dental diseases and developing high-yield selection criteria for dental radiographic examinations.

Conventional radiographic techniques for detecting intraosseous lesions (notwithstanding issues of prevalence) were also studied. Diagnostic accuracy obtainable from extra-oral panoramic and intra-oral periapical examinations was measured using an observer-performance paradigm. From a pool of 30,000 patient records, a sample of 194 was selected randomly. This sample was unique in that it was balanced between patients having biopsy-confirmed intraosseous pathology and those known to be lesion free for at least five years after x-ray examination using both modalities. Classical receiver operating characteristic (ROC) performance

analysis showed periapical radiography to be significantly better than panoramic type examinations in detecting intraosseous lesions. Moreover, neither was found to be particularly accurate. For specificities greater than 90% the data indicated sensitivities of no greater than 70% for either modality.

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 described last year likewise 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 relative to the development data base. It also demonstrated the observed mortality rate differences to be fully attributable to the differing quantitative levels of severity of illness measured at the admission day in the respective populations. Hence, application of this risk model as a factor in treatment decisions could result in considerable health care cost savings.

Considerable effort continues to be expended in the transfer of technology developed earlier by DSB. Perhaps the most widely spread example involves the use of a digital method to compare periapical radiographs obtained from patients suffering from periodontal disease who are undergoing a variety of controlled treatment regimes. The technique involves registration of successively exposed films using a computer-based projection system and subsequent subtraction of stored images after correcting for contrast differences attributable to incidental changes in the x-ray exposure and processing chemistry. Since last year, two algorithms developed by DSB staff for correcting contrast differences between radiographs and estimating the size of nodular lesions in tissues of diagnostic interest have been reprogrammed in the C language to facilitate application in other computer systems. These conversions are now being used by investigators at the Harvard School of Dental Medicine on an inexpensive microcomputer thus demonstrating the potential for practical implementation of digital radiographic analysis in routine clinical applications.

DSB was evaluated by the NIDR Board of Scientific Counselors on May 5th and 6th of this year. The preparation for this cyclic review process was more time-consuming than usual in light of administrative difficulties occasioned by the unexpected loss of the Branch's only secretary for a period of more than four months due to a job-related back injury. With extensive

cooperation by the entire DMS staff and generous support from the NIDR administrative office the review took place as planned, and the final report was complementary of Branch efforts over the five-years since the last evaluation by this group.

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. This is evidenced within NIH by collaborative application for two different U. S. patents based on technology developed over the last year, by DSB's representation as a coordinating member of the NIH Wide Image Processing Group, and by its leadership role in the development of an NIDR sponsored workshop to be held on September 29-October 1 of this year which will focus on the impact of technology on diagnostic and therapeutic opportunities in dentistry.

Publications:

Pollack, M.M., Ruttimann, U.E., Glass, N.L., and Yeh, T.S.: Monitoring patients in pediatric intensive care. Accepted for publication in Pediatrics.

Ruttimann, U.E., Albert, A., Pollack, M., Glass, N.L.: "Development of a Dynamic Assessment of Severity of Illness in Pediatric Intensive Care", Critical Care Medicine, Vol. 14, No. 3, pp. 211-111.

Ruttimann, U.E., and Webber, R.L.: "Fast Median Filtering by Logical Operations for Implementation on General-Purpose Image Processors". Accepted for publication in Optical Engineering, Sept. '86.

Ruttimann, U.E., Webber, R.L., and Saffer, A.: "Calibrated Volume Determination of Localized Bone Lesions by Subtraction Radiography". Accepted for Publication in MEDINFO "86.

Ruttimann, U.E., Webber, R.L., and Schmidt, E.: "A Robust Digital Method for Film Contrast Correction in Subtraction Radiography". Accepted for publication in J of Periodont Res.

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". Accepted for publication in Proc. SPIE., MEDICINE XIV, 1986.

van der Stelt, P.F., Ruttimann, U.E., and Webber, R. L.: "Restoration and Enhancement of Tomosynthetic Images and its Applications in Dentistry". Proc. Ninth Annual Symp. on Computer Applications in Medical Care, Nov. 1985, pp. 673-677.

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". Accepted for publication in Journal of IADMFR.

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.

Webber, R.L.: Computers in Dental Radiology: A Scenario for the Future. JADA, Vol. 111, September 1985, pp. 419-424.

Zeichner, S.J., Ruttimann, U.E., Reiskin, A.B., and Webber, R.L.: "Prevalence of Intra-Osseous Lesions of the Face and Jaws with Reference to Cost-Effectiveness of Dental Radiographic Screening". Submitted for Publication in Journal of Dental Research.

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. in press, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00065-15 DS

PERIOD COVERED
October 1, 1985 - September 30, 1986

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.	Senior Staff Fellow	DS	NIDR
van der Stelt, Paul F.	Visiting Fellow	DS	NIDR
Zeichner, Samuel J.	Senior Staff Fellow	DS	NIDR

COOPERATING UNITS *(if any)*
Childrens' Hospital National Medical Center
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:
2.90	1.95	0.95

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 spece 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 focused 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 occuring over long intervals of time, and combined in ways permitting tomosynthetic display of specific slices of the teeth and jaws.

An all-electronic prototype is being fabricated which couples an x-ray source capable of moving its focal spot to different positions in a circular orbit under the control of a digital computer. This process takes less than a second to produce eight discrete pulsed exposures which are recorded in real time using one of three different video-based detector systems currently under development; a pin-diode array, a solid-state chip based on D-RAM technology, and a tiny image-intensified optical system fiber-optically coupled two a miniaturized video camera.

Significant progress has been made in the development of related soft-ware. Data from controlled studies now confirm that image subtractions produced from appropriately weighted projection data are compromised less by differences in projection geometry than comparable data produced using nearest-neighbor, control projections. In theory, this opens the door to image comparisons obtained from existing clinical records including the wealth of retrospectively produced periapical radiographs already residing in patient files.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00211-10 DS

PERIOD COVERED
October 1, 1985 - September 30, 1986

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.	Senior Staff Fellow	DS	NIDR
van der Stelt, Paul F.	Visiting Fellow	DS	NIDR

COOPERATING UNITS *(if any)*
Radiation Physics Group, National Bureau of Standards

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Behtesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.58	0.95	0.63

CHECK APPROPRIATE BOX(ES)

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

SUMMARY OF WORK *(Use standard unreducad type. Do not excaed tha 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 diseases. Initial efforts have been made in an approach of combining the economics of quad-tree image characterization with the split-and-merge procedure for image segmentation. An algorithm used to detect lesion boundaries has been improved. The new approach makes use of a nonparametric test for change of distribution based on the Mann-Whitney statistic. This change makes the algorithm considerably more robust in the presence of large image noise. Another improvement developed this year permits absolute quantification of lesion volume expressed in cubic millimeters of an equivalent to homogeneous bone. The standard error associated with this process was measured in a controlled study and found to be approximately 1.3 mm^3. 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 00373-04 DS

PERIOD COVERED
October 1, 1985 - September 30, 1986

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 Investigetor.) (Name, title, leboratory, end institute affilietion)*
Webber, Richard L. Chief, Diagnostic Systems DS NIDR

COOPERATING UNITS *(if any)*
Harvard School of Dental Medicine, Boston, MA.
Diagnostic Radiology Department, Clinical Center, NIH
Biological Engineering and Instrumentation Branch, NIH

LAB/BRANCH
Diagnostic Systems Branch

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.68	0.40	0.28

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

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

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 magnetic resonance imaging (MRI), nuclear medicine (^{99m}Tc-MDP), fiber-optic systems making use of visible light (hemoglobin-specific differential spectrum analysis and micro-fluorescent optical transducers).

MRI was found to be particularly useful in imaging the capsular tissues of the temporomandibular joint including the meniscus. Also seen to advantage with this tool were periodic vascular changes in the nasal mucosae known as the nasal cycle and spontaneous salivary secretions. Preliminary data from two beagle dogs suggest that differential uptake of ^{99m}Tc-MDP predicted changes in the dynamics of wound healing in periodontal bone even before lesions were induced surgically in these animals. In vitro data obtained using a prototype filtered fiber-optical system designed to detect the presence of oxygenated blood in the pulp chamber of vital teeth confirm theoretical predictions that tiny amounts of fresh blood can be detected reliably irrespective of the angulation of the fiber-optic probe or the color of the light reaching the differential detector. Fiberoptics are also being investigated as means for measuring the oxygen tension of periodontal tissues in vivo. This technology makes use of chromic differences, induced in a dye sensitive to the presence of oxygen, to monitor the concentration of this gas in tissues of diagnostic interest. such as plaque situated deep in a periodontal pocket.

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

ANNUAL REPORT OF THE LABORATORY OF DEVELOPMENTAL BIOLOGY AND ANOMALIES

NATIONAL INSTITUTE OF DENTAL RESEARCH 1985 - 1986

Changes have occurred in the internal organization of LDBA. Two new organizations have been created within the Laboratory, the Cell Biology Section and the Molecular Biology Unit. The founding of the Cell Biology Section with Dr. Hynda Kleinman as Chief recognizes the important and productive program that she has developed and led the past several years. The Molecular Biology Unit with Dr. Yoshihiko Yamada as Chief has made notable progress in cloning various connective tissue proteins, determining their structure, and studying their regulation. Molecular biology is now a major theme in LDBA.

Dr. Mark Bolander, formerly a Staff Fellow in LDBA, has been invited by the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the National Institute of Diabetes, Digestive and Kidney Disease and the Academy of Orthopedic Surgeons to establish an Orthopedic Research Unit at the NIH. By agreement, the Orthopedic Research Unit will be housed in Bldg. 30 in LDBA space for two years. LDBA will also contribute support services and some personnel costs to the Orthopedic Research Unit during this period. Current collaborations of the Orthopedic Research Unit with NIDR staff will continue while new activities are undertaken with scientists in the other areas. A copy of the annual report of the Orthopedic Research Unit is attached to the LDBA Annual Report.

Since the role of cell-matrix interactions is basic to understanding cancer metastasis, LDBA has been invited to participate in research supported by the Breast Cancer Study Group of the National Cancer Institute. This will expand our long standing interest in tumor cell biology and a productive collaboration with NCI scientists and help to support postdoctoral fellows in LDBA studying the invasive activity of tumor cells.

Dr. Elliott Schiffmann, a senior member of LDBA, has transferred to the Laboratory of Pathology in the National Cancer Institute.

Summary of research activities

Considerable progress has been made during the past year in applying molecular biology to the study of connective tissue and its diseases. These studies emphasize cartilage and basement membrane proteins due to their critical roles in development. Cartilage and basement membrane matrices are composed of distinct sets of matrix proteins. Cartilage proteins, including collagen II, cartilage proteoglycan and link have been cloned in LDBA. Studies are underway on the regulation of collagen II promoter by the transfer of this gene into various cultured cells and into fertilized mouse eggs. These studies show preferential expression of this promoter in developing mesenchymal cells and that both positive and negative factors are regulating the expression of this gene in different cells. Studies on the gene structure of the link protein are well underway. Unexpectedly, we have found that there are two distinct link protein mRNAs which arise by alternative splicing. Recombinant DNA technology is ideally suited for elucidating the structure of proteoglycans and studies on cartilage- and on basement membrane-specific proteoglycans are progressing well. Much of the structure of laminin and of

collagen IV has also been elucidated by cloning these proteins. In addition, changes in these components in development and in disease are being assessed.

Research in LDBA has also taken the direction of identifying cell surface receptors which bind specifically to matrix molecules such as laminin, collagen and proteoglycan. Two such receptors, the laminin receptor and anchorin, a collagen receptor from mesenchymal cells, have been cloned and are under study. A possible molecular mechanism by which laminin encourages nerve regeneration may involve distinct receptors for laminin, with one promoting cell attachment while a second receptor mediates axon formation. Also the region on the laminin molecule which binds to the laminin receptor has been identified through the use of synthetic peptides which were prepared based on the amino acid sequence of laminin as deduced from nucleotide sequence data. The active peptide is homologous to portions of EGF and is able to promote epithelial cell attachment and migration. Such active synthetic peptides might have medical and biological uses.

A patent has been submitted by the U.S. Government on the reconstituted basement membrane (termed matrigel) developed in this Laboratory. Sufficient demand exists for this material that it is being produced and distributed by commercial organizations. Additionally, tubes prepared from this material have been found to support the regeneration of nerves. A "use patent" has been applied for by Boston's Children's Hospital and LDBA scientists. Clinicians are interested in using constructs of reconstituted basement membrane as guides for nerve regeneration.

Much attention in LDBA is being directed toward the study of tumor cell invasiveness. The ability to pass across basement membranes is highly correlated with the metastatic activity of tumor cells, presumably because the basement membranes form a barrier to the invasion and passage of most normal cells. A simple in vitro assay using "matrigel" as a reconstituted basement membrane has been designed to assess invasiveness. The invasiveness of tumor cells appears to be a defined phenotype involving an increased expression of laminin receptors and the production of enzymes which degrade basement membranes. Studies are in progress with several different cell types to define the molecular events which regulate the metastatic phenotype. For example, estrogen induces breast cancer cells to become invasive and metastatic, while withdrawal of estrogen causes breast cancer cells to lose their invasive activity. Possible ways of interfering with the invasiveness of malignant tumor cells are under study.

SPECIFIC RESEARCH ACCOMPLISHMENTS

STRUCTURE, FUNCTION AND GENETICS OF BASEMENT MEMBRANE MATRIX

Biological activity of matrix components

Biological studies on the effects of a basement membrane matrix (matrigel) on cell and organ growth and differentiation in vitro and in vivo are continuing. This matrix contains a variety of basement membrane components (laminin, collagen IV, entactin-nidogen, and heparan sulfate proteoglycan) and has the appearance in the electron microscope of thin sheets resembling the lamina densa zone of basement membrane. Matrigel has a variety of effects on cultured cells. For example, isolated pancreatic cells, when cultured in this material, form acinar-like structures with abundant secretory vesicles which abut on a lumen. Glomerular cells in matrigel form aggregate structures resembling glomeruli while kidney tubular cells form tubules. Endothelial cells aggregate to form capillary-like structures within hours of plating single cells in matrigel.

Matrigel has also been shown to promote nerve growth and myelination in vitro. Peripheral nerve and spinal ganglia cells send out long axons in its presence. In vivo, it promotes optic and sciatic nerve growth and spinal cord regeneration particularly when used inside a nerve guide tube or when the nerve guide is fabricated out of the matrigel. Under these conditions, nerve regeneration occurs faster and over greater distances than has been achieved previously. Matrigel has been prepared from human placenta and future collaborative studies will investigate its regenerative activity on spinal cord.

Matrigel also induces substantial differences in the morphology and behavior of malignant versus nonmalignant cells. Metastatic cells rapidly adhere, spread and penetrate into the matrix whereas non-metastatic cells lack these activities and grow in small clumps. Such differences have been observed with many human tumor cell lines and the invasive activity detected with this system is correlated with the cells in vivo metastatic activity. The matrigel assay for invasiveness provides a reliable and rapid method for estimating the metastatic potential of tumor cells.

Characterization of cellular receptors for laminin and collagen

Cells bind to the extracellular matrix via specific cell surface receptors. We are using biochemical and molecular approaches to define receptors for laminin and for collagen IV. A membrane glycoprotein (M_r=67,000) has been described by several laboratories as the laminin receptor on muscle, on tumor cells and on macrophages. Since laminin appears to have separate sites which promote cell attachment and neurite process (axon) formation, we investigated the laminin binding components on membranes isolated from a neuroblastoma x glioma hybrid (NG108-15) cell line. This hybrid cell line has a number of characteristics of neuronal cells including the ability to secrete acetylcholine and the ability to form synapses with muscle cells. Like other neuronal cells, the NG108-15 cells produce axonal-like processes when plated onto laminin. Such processes form in the presence of cycloheximide, vinblastine, and cytochalasin. Thus, it is likely that neither protein

synthesis, microtubules or microfilaments are responsible for process formation. Additionally, we have found that we can distinguish between the portions of laminin required for attachment and for process formation using antibodies to laminin with different specificities. Presumably the neuronal cells have an additional receptor for laminin that is specific for process formation. Future studies will be directed at defining the site on laminin responsible for neurite outgrowth using approaches similar to those used to define the cell attachment site (see below).

We have also begun to define the cell surface receptors for laminin on neuroblastoma x glioma cells. The (Mr=67,000) laminin receptor described by others is present on these cells. Two additional laminin binding proteins (M_r~=110,000 and 180,000) are also present on these cells. These may be neuron-specific receptors. Future studies will focus on characterizing the latter two receptors by immunological and molecular approaches.

Multi domain structure of laminin subunits.

Laminin is the major glycoprotein in basement membrane and is composed of three chains, designated A (400 KD), B1 (230KD), and B2 (220KD). Laminin has been found to exert diverse biological activities including stimulating epithelial cell growth, migration, and proliferation. Laminin alters cell morphology, including promoting neurite outgrowth. Laminin also binds to other basement membrane components and specific membrane protein receptors. Its complex structure plus difficulty in separating its subunits have hampered the detailed characterization of laminin. Therefore cloning has proven to be very helpful in elucidating the primary structure of the laminin chains.

We have constructed full length cDNA for the murine laminin B1, and B2 chains by a series of primer extensions. We have also isolated overlapping cDNA clones which encode most of the A chain. Analysis of the deduced amino acid sequence reveals that the B1 chain has a number of distinct domains (Fig. 1). Domains I and II are rich in α-helix. Domain α appears to interrupt the α-helical structure and is lacking from the B2 chain. It contains 6 cysteines, and several glycines and prolines but is only 33 amino acids in length. Domains III and V contain a large number of cysteines and could form rod-like structures. A most striking feature of these domains is a homologous stretch of about 50 amino acids repeated many times in both chains. A portion of the repeat in Domain III shows sequence homology to EGF and TGFα. Domains IV and VI likely form globular structures. A portion of the structure of the B2 chain is similar to that of the B1 chain. Notably, both B1 and B2 chains contain the same length of the heptad repeats with regular positioning of hydrophobic amino acids at the C terminus resulting in a coiled-coil structure. This region of the chains is probably important in stabilizing subunit interaction in laminin by forming a double-helical stem. The amino acid sequences in the N-terminal globular domain and the two cysteine-rich domains are highly conserved between the B1 and B2 chains. The high level of the conservation suggests that these domains in the B1 and B2 chains may have a common function. Surprisingly, the structure of other regions are not well conserved between the B1 and B2 chains.

A laminin domain responsible for cell attachment and migration

Studies are in progress using synthetic peptides derived from cDNA sequence

data to localize active sites on the B1 chain of laminin which mediate cell attachment, growth, migration, neurite outgrowth, and type IV collagen binding. Synthetic peptide of 20 amino acids to each structural domain (I-VI) on laminin was synthesized. Antibodies to each of the peptides were raised by immunization with peptide-albumin conjugates and were found to cross react with a laminin B chain. The antibodies and peptides were tested for their effects on cell attachment. Antibody to a peptide conjugate from domain III inhibited cell attachment to laminin although the peptide itself did not support cell attachment. These observations suggested that a nearby sequence was involved in cell attachment and that antibody to the peptide blocked attachment for steric reasons. Synthetic peptides were prepared from adjacent sequences. One of these peptides (CDPGYIGSR) was found to stimulate cell attachment in the range of 10-50 μg/ml when coated on plastic and to inhibit cell attachment to laminin substrates presumably by competing with the intact laminin molecule for binding to the cell receptor. This peptide promotes the chemotaxis of tumor cells as does laminin itself. The possibility that this peptide interacts with the laminin receptor is indicated by experiments which show that the peptide causes the elution of the laminin receptor (M_r = 67,000) from laminin affinity columns. These studies suggest that this synthetic peptide encompasses sequences in the B1 chain that support cell attachment and migration. Future studies will attempt to define the minimum sequence of amino acids necessary for activity.

Gene structure and expression of laminin.

We have isolated the laminin B1 chain gene. The gene is 65 Kb in size and contains 36 exons. Its transcription initiation sites were determined and about 1 Kb of the 5′ flanking region of the gene was characterized by DNA sequencing. We have begun to construct recombinant plasmids which contain various lengths of the promoter linked to the CAT gene to examine which sequences are involved in regulating the expression of the laminin B1 chain gene.

We have examined the methylation status of three segments of the B1 chain gene in 7 different types of tissues and cell lines with different levels of synthesis. We have found that only the 5′ end of the gene is hypomethylated in tissues which synthesize laminin. In contrast, the rest of the gene is methylated in every tissue examined. Our results suggest that the expression of the B1 chain gene is inversely correlated with methylation of the gene.

The DNAase I sensitivity of chromatin containing the B1 chain gene was examined in F9 cells. Our results show that several sites which are hypersensitive to DNAase I appear in the promoter and in the first intron when the cells are induced to produce laminin by treatment with retinoic acid. We conclude that the changes in the DNAase I sensitivity are correlated with the expression of this gene and that the first intron could participate in the regulation of the gene.

Using cDNA clones for the B1, B2, and A chains of laminin and a cDNA clone for the α1 chain of type IV collagen, we measured steady state levels of mRNA for these basement membrane proteins in differentiating F9 cells and in various normal tissues. Since the increases observed for each of these mRNA species was proportionate to the others during differentiation of F9 cells, it is likely that the expression of these basement membrane components is

coordinately regulated in these cells. Significant variations in the levels of mRNA for the laminin B1, B2, and A chains relative to the level of mRNA for the α1(IV) collagen chain were observed in different tissues. The highest levels of B2 chain mRNA were found in heart, whereas B1 chain mRNA was highest in kidney. In the heart, the level of B2 chain mRNA was one third of that found in the kidney. The level of the A chain mRNA was surprisingly low in all tissues examined. These results suggest that genes for the laminin B1, B2 and A chains and for α1(IV) collagen chain are not coordinately expressed in these tissues and that isoforms of laminin may exist which differ in chain composition.

Particularly striking are the alterations of mRNA levels in mice homozygous for the cpk gene. Such animals show an enormous increase in kidney size after birth, with the formation of multiple cysts resulting in the death of the animals after 4 weeks. This mutant represents an interesting model for human polycystic kidney disease. A 5-10 fold increase in laminin B1 and B2 chain and α1(IV) mRNA synthesis is found in the animals with little or no A chain mRNA.

Thickened basement membranes are observed in renal glomeruli, tubules, in capillaries and around nerves in diabetic individuals. It is likely that the accumulation of basement membrane causes the premature degeneration of these tissues in the diabetic. We are investigating various animal models of diabetes including ECM viral induction in mice and streptozotocin induction in rats. We have found a 2-3 fold increase in the steady-state in RNA level for basement membrane proteins in the diabetic kidneys of these animals. The mRNA levels for laminin B1 and B2 chain and the α1(IV) chain are increased within 14 days of the onset of diabetes and are further increased with insulin therapy. In contrast, tight metabolic control restored the mRNA level toward normal. Such data implicate the interaction of environmental factors with the metabolic defects occurring in diabetes as causing the increase in basement membrane.

Genes for type IV collagen

We previously isolated four overlapping clones from mouse genomic libraries which encode about 80% of the α1(IV) collagen chain. We have isolated an additional genomic clone which extends some 10 Kb from the previous clones. We have sequenced two exons in this clone and found them to code for the N-terminal domain of the molecule.

We have isolated a cDNA clone for the human α2(IV) collagen chain. The deduced amino acid sequence of this 2.1 Kb clone shows that it encodes approximately 450 helical residues and the entire carboxyl terminal globular domain. The triple-helical region has a number of interesting features including additional and larger non-helical interruptions than found in corresponding locations of the human α1(IV) chain. Two of these interruptions are flanked by RGD sequences and could be important for cell attachment to type IV collagen. This clone has been used to localize the gene on chromosome 13 by hybridization with somatic cell hybrid DNA and by *in situ* hybridization. The data suggest that the human α1(IV) and α2(IV) genes are organized in a cluster, an organization unique for the collagen multigene family.

Basement membrane proteoglycan

Previously, we isolated and characterized a heparan sulfate proteoglycan from the basement membrane produced by EHS tumor cells. This proteoglycan consists of a M_r=400,000 core protein containing 3-4 heparan sulfate side chains. We have now used proteolytic digestion to establish the domain structure of its core protein. Trypsin digestion releases a large (M_r=200,000) globular peptide that lacks heparan sulfate side chains. Further digestion of this fragment releases M_r=44,000 and 46,000 fragments. These fragments increase in apparent molecular weight after reduction indicating that their conformation is maintained by disulfide bonds. Antibodies raised against these two fragments do not cross react indicating that they are immunologically distinct, despite similarities in size and disulfide bonding. These observations indicate that the core protein consists of a trypsin sensitive region (M_r=200,000) containing the heparan sulfate side chains and a trypsin resistant region (M_r=200,000) that contains 2 subdomains (M_r=44,000 and 46,000). Electron microscopic examination of the proteoglycan confirms the clustering of the glycosaminoglycan side chains at one end of the molecule and the globular structure of the core protein. Partial amino acid sequences have been obtained from the M_r = 44,000 and 46,000 fragments and oligonucleotides corresponding to these sequences have been synthesized and are being used to obtain cDNA clones to this region of the core protein.

Antibodies raised against the basement membrane proteoglycan from the EHS tumor react with all basement membranes in native tissues, indicating the presence of a immunologically similar component in all basement membranes. These antibodies precipitate the precursor protein (M_r=400,000) of the proteoglycan from EHS cells in culture, as well as from a wide variety of cultured cells including glomerular endothelial cells and other tumor cells. Our recent studies show that basement membrane proteoglycans from other sources have immunological and structural similarities but are not identical. For example, the glomerular heparan sulfate proteoglycan appears to be smaller and to lack one of the domains present in the proteoglycan from the tumor.

Progress has been made in isolating molecular clones to the basement membrane proteoglycan core protein. cDNA prepared from randomly primed EHS mRNA was inserted into the λGT11 expression vector and the resulting library was screened with affinity purified antibodies to the proteoglycan core protein. Two immunopositive clones of 313 and 228 base pairs were obtained. Both clones hybridize to the same 11 kb mRNA, the size expected for the M_r=400,000 core protein. Furthermore, this mRNA appeared in F9 cells after induction with retinoic acid. The clones have extensive homology in nucleotide sequence and the deduced amino acid sequence also showed a high degree of homology. Antibodies to the fusion protein of the 313 base clone react with the core protein of the proteoglycan but only with certain peptides in digests of the proteoglycan and not with the M_r=200,000 trypsin fragment. This suggests that the clones encode for related regions present in the heparan sulfate binding domain of the core protein.

Basement membranes and the invasive activity of tumor cells

The ability to degrade and pass across basement membranes signals the presence of malignant cells in a tumor and the potential of the cells to metastasize. Previous work has shown that the invasive cells bind avidly to basement

membranes via laminin and secrete degradative enzymes that attack basement membrane. These activities allow metastatic cells to penetrate through basement membranes into normal tissues where they may form new lesions. The possibility that metastasis is not a random process was suggested by the observation that tumor cells were attracted to factors in the organs to which they metastasize.

To facilitate studies on the invasive behavior of cells, we have used a reconstituted basement membrane as an in vitro barrier to the passage of cells. Cells from malignant tumors are able to cross the barrier while benign tumor cells and normal cells do not. We have found an excellent correlation between metastatic activity in vivo and in vitro invasiveness in human, as well as in animal tumor cells.

Invasiveness in vitro and metastatic activity in vivo are reversibly inducible in breast cancer cells. When breast cancer cells were studied, it was noted that cells grown in the absence of estrogen were not invasive, while cells exposed to estrogen showed a dose-dependent increase in invasiveness. Estrogen increased the number of laminin receptors, the binding of the cell to laminin and presumably the ability of the cells to degrade basement membranes. Invasiveness was lost when estrogen was removed and this change was accompanied by a loss of laminin receptors. Transformation of the cells by the ras oncogene also made the cells invasive but no longer dependent on estrogen. Further work in this system will attempt to determine whether diffusible factors, such as growth factors mediate invasiveness and to identify enzymes used by the cells in crossing the basement membrane. Drugs will be assessed for their ability to inhibit the invasiveness of tumor cells and the conversion of cells from benign to malignant status.

Nature and invasiveness of Kaposi sarcoma cells

Our colleagues in the Division of Virology, Center for Drugs and Biologics, Food and Drug Administration (C. Mitchell, R. Seeman, D. Wierenga, I. Levenbook, R. Dunlap, M. Lundquist and G.V. Quinnan) have developed a method for establishing cell cultures from Kaposi sarcoma lesions using microdissection of tumor tissue from AIDS patients and subsequent culture of the cells derived from this tissue in a selective culture media. Cells isolated in this fashion have some immunological markers similar to normal endothelial cells but show anchorage independent growth in soft agar, as expected for tumor cells. The Kaposi cells were able to invade through a reconstituted basement membrane in vitro (see above), whereas normal endothelial cells and fibroblasts were not able to invade this barrier. The data are consistent with the identification of the Kaposi tumor cells as transformed endothelial cells. Future work will attempt to obtain better cultures of Kaposi sarcoma cells from these tumors and also be directed toward determining their origin and the factors involved in their growth in AIDS patients.

STRUCTURE AND FUNCTION OF CARTILAGE

Alternative splicing of rat link protein gene.

Link protein stabilizes the interaction of hyaluronic acid and proteoglycan. We previously isolated a cDNA clone encoding two thirds of rat link protein

and demonstrated that there are four distinct RNA transcripts of varying size for link protein. Now, we have constructed a cDNA library by the extension of a specific oligonucleotide primer to a link protein sequence to obtain clones for the complete coding sequence (Fig. 2). Two cDNA clones were isolated with identical 5′ and 3′ sequences, but which differ by the insertion of a 159 bp internal segment in the larger clone. Concurrently work on the link protein gene lead to the isolation of DNA nearly encompassing the whole gene. The inserted sequence present in the larger cDNA clone was found to correspond to a single exon in the link protein gene. Our data suggest that alternative splicing allows a single gene to encode at least two different forms of link protein, one having a molecular weight of 38,000 and the other a molecular weight of 43,000. This heterogeneity may have biological significance in the function of this protein.

Structure of the cartilage proteoglycan core.

We have isolated overlapping cDNA clones which span about 5.5 Kb encoding some two thirds of the rat cartilage proteoglycan core. The sequencing of 3.2 Kb of the 3′ portion of the clones DNA is complete. Protein sequence homology searches of the NBRF Data Bank uncovered a strong homology between the chicken asialoglycoprotein receptor (hepatic lectin) and a cysteine-rich domain at one end of the proteoglycan. The diagram of the structure of this region of the molecule (Fig. 3) shows the location of lectin-like domain and cluster of 82 ser-gly sequences sites to which chondroitin sulfate chains attach. There is a consensus sequence in which the ser-gly sequences occur, i.e. SGXXSGXXXX. Further, the ser-gly rich region has a number of homologous repeats about 30 amino acids in length suggesting that this domain evolved by multiple duplications of a single genetic unit. Prior studies on the proteoglycan have shown that its N-terminal domain binds to hyaluronic acid. Since the C-terminal domain of the proteoglycan shows homology to a lectin, it is possible that in the cartilage matrix both ends of the proteoglycan are associated with other matrix components.

Differential expression of type II and type I collagen promoters.

We have studied the expression of the α1(II) and α2(I) collagen promoters by DNA transfection into chick limb bud mesenchymal cells, chondrocytes and embryonic fibroblasts using promoter-chloramphenicol acetyl transferase (CAT) reporter plasmids. The advantage of such constructs is that this enzyme can be easily assayed and its expression is controlled by whatever promoter is attached to the CAT DNA. The α1(II) collagen chain promoter is expressed at very high levels when transfected into limb bud mesenchymal cells which have chondrogenic potential. The region of this promoter which increases the level of CAT expression in these cells is located between -380 and -1000 bp from the transcription initiation site of the α1(II) cartilage collagen gene. However, when the same construct is transfected into differentiated chondrocytes, only a low level of expression is observed. A high level of expression of the α2(I) collagen chain promoter is observed in mesenchymal cells and in fibroblasts, but a low level of expression occurs in chondrocytes. Such observations suggest that the expression of these promoters is under both positive and negative controls, probably by nuclear proteins binding to distinct sequences in these promoters.

Permanent chondrocyte lines were established in this Laboratory by infecting

fetal chondrocytes from rat and quail with a retrovirus expressing either the myc or raff oncogenes. The α1(II) collagen chain promoter-CAT construct has been introduced into this cell line in an integrated form. The chondrocytes express this construct and transcription decreases after treatment of the cells with retinoic acid. We will analyze the expression of this promoter bearing various deletions in cells treated with vitamin A derivatives to help determine through which DNA sequences their actions are mediated. Such studies should help to define the molecular action of these teratogens.

Chondrogenesis and gene expression

As stated above the entire amino acid sequence for link protein, the matrix component responsible for stabilizing the association of cartilage proteoglycan with hyaluronic acid has been determined from cDNA clones. We have now found that the link protein is produced concomitantly with cartilage proteoglycan and collagen II as limb bud cells differentiate into chondrocytes. Further, chondrocytes lose the ability to produce link protein when treated with retinoic acid. These results clearly indicate that the link protein gene is coregulated with other cartilage genes.

Cloning anchorin, a collagen binding protein.

Anchorin is an integral membrane protein, present on the surface of both chondrocytes and fibroblasts which binds to collagen. This receptor may mediate cell adhesion and/or be involved in regulating collagen synthesis. We have isolated anchorin clones from an expression cDNA library of chick sterna RNA. One of the clones which has an insert of 800 bp was sequenced and identified as coding for anchorin based on a comparison of its deduced amino acid sequence with that of the protein. The sequence contains a presumptive transmembrane segment of about 20 hydrophobic amino acids at the carboxy terminus. Northern analysis showed that the clone hybridized to a 1.7 Kb mRNA present in sterna, calvaria and crop as well as in fibroblasts. The level of anchorin mRNA was increased more than three-fold in chick embryo fibroblasts after RSV transformation.

Correction of genetic defects of cartilage in culture

The cartilage matrix deficiency (cmd/cmd) chondrocytes have been shown by our previous work to produce an abnormal matrix lacking its characteristic proteoglycan when grown in culture. Furthermore, they have a reduced and uneven deposition of type II collagen in their matrix in vitro compared to normal chondrocytes. When the cartilage proteoglycan was added to the culture, it was incorporated into the matrix produced by cmd/cmd cells and they responded by correcting the abnormal and reduced distribution of type II collagen. This suggests that the intact cartilage proteoglycan plays an important role in the assembly of matrix and in the regulation of chondrocyte biosynthetic activity. The chondrocytes of cmd/cmd in normal media make fibronectin but when the media is supplemented by cartilage proteoglycan they do not. Studies of other chondrodystrophic mutants in culture are in progress to see if, for example, the growth hormone deficient mice hcp/hcp dwarf or the type II collagen deficient Dmm/Dmm and cho/cho behave in the same way as the cmd/cmd. Also cross correction studies by media from different mutant chondrocytes will be carried out using the high density spot culture technique in collaboration with Dr. Koji Kimata, Nagoya.

Chondronectin

Monoclonal antibodies to chondronectin, a chondrocyte cell attachment factor present in cartilage, vitreous, and serum, have been used to characterize the levels of this molecule in forming cartilage and in serum. Chondronectin is rapidly lost along with type II collagen and the cartilage proteoglycan after vitamin A treatment of cultured chondrocytes. Serum levels of chondronectin increase with age and in certain patients with myositis ossificans. Thus, serum levels of chondronectin may reflect the activity of the cell in cartilage and show alterations in disease states.

PRELIMINARY STUDIES ON TRANSGENIC MICE

The new major effort in transgenic mice is beginning to produce positive results after a long set up time. This project requires timed mating of inbred selected strain females as donors of fertilized eggs which are removed and incubated prior to DNA injection. DNA constructs are injected directly into the male pronucleus of the zygote and then zygotes are implanted surgically in the uterus of matched, timed mated, pseudopregnant females of a phenotypically different strain who have been produced by mating with reproductively active but sterile vasectomized males. The incorporation of the injected DNA is determined by measurement of either these DNA species in the progeny or the product of some highly specific and sensitive biochemical marker gene included in the DNA. Offspring containing the construct are then mated to produce a new genetic line containing the construct either in homozygous or in heterozygous form and the phenotypic aspects of construct function are monitored in either adult, newborn or embryo animals by timed mating of the line in question just as if it was a line containing a mutant gene.

Two constructs are currently being used in transgenic experiments. Both use chloramphenicol acetyl transferase (CAT), a bacterial gene, as a marker. Each is introduced into the male pronucleus in about 400 copies per zygote. We are using C57Bl/6J, CBA/J, and FVB/N strain donor mice and NIH-outbred Swiss mice as foster mothers. The structure of the constructs are as follows:

1. BSCAT pSV2 CAT vector, SV40 promoter is replaced by rat type II collagen promoter (1.8 Kb).

2. pB9000 pSV2 CAT vector, SV40 promoter is replaced by mouse laminin B1 chain promoter (9.0 Kb).

At the present time, we have 33 new lines of BSCAT transfected mice produced in NIDR in the FVB/N background. Two lines were produced earlier by Dr. Westphal (NICHD). There is some preliminary evidence for changes in activity with age with very young animals exhibiting more activity than older ones. We are breeding to get homozygous expression but have not yet produced proven homozygous animals.

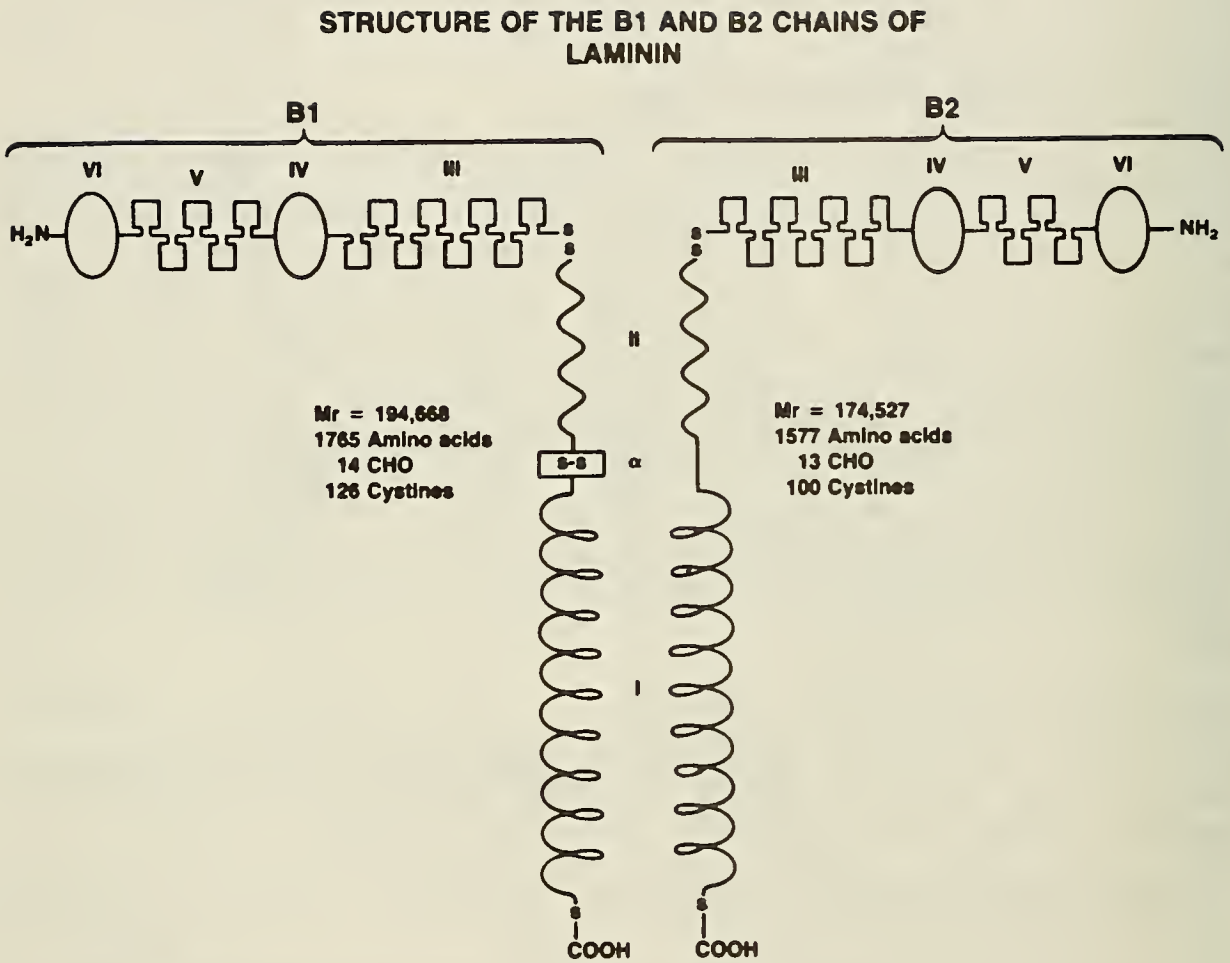

Fig. 1 Comparative structure of the B1 and B2 chains of laminin as deduced from nucleotide data. These chains have very similar domains. The B2 chain is somewhat smaller.

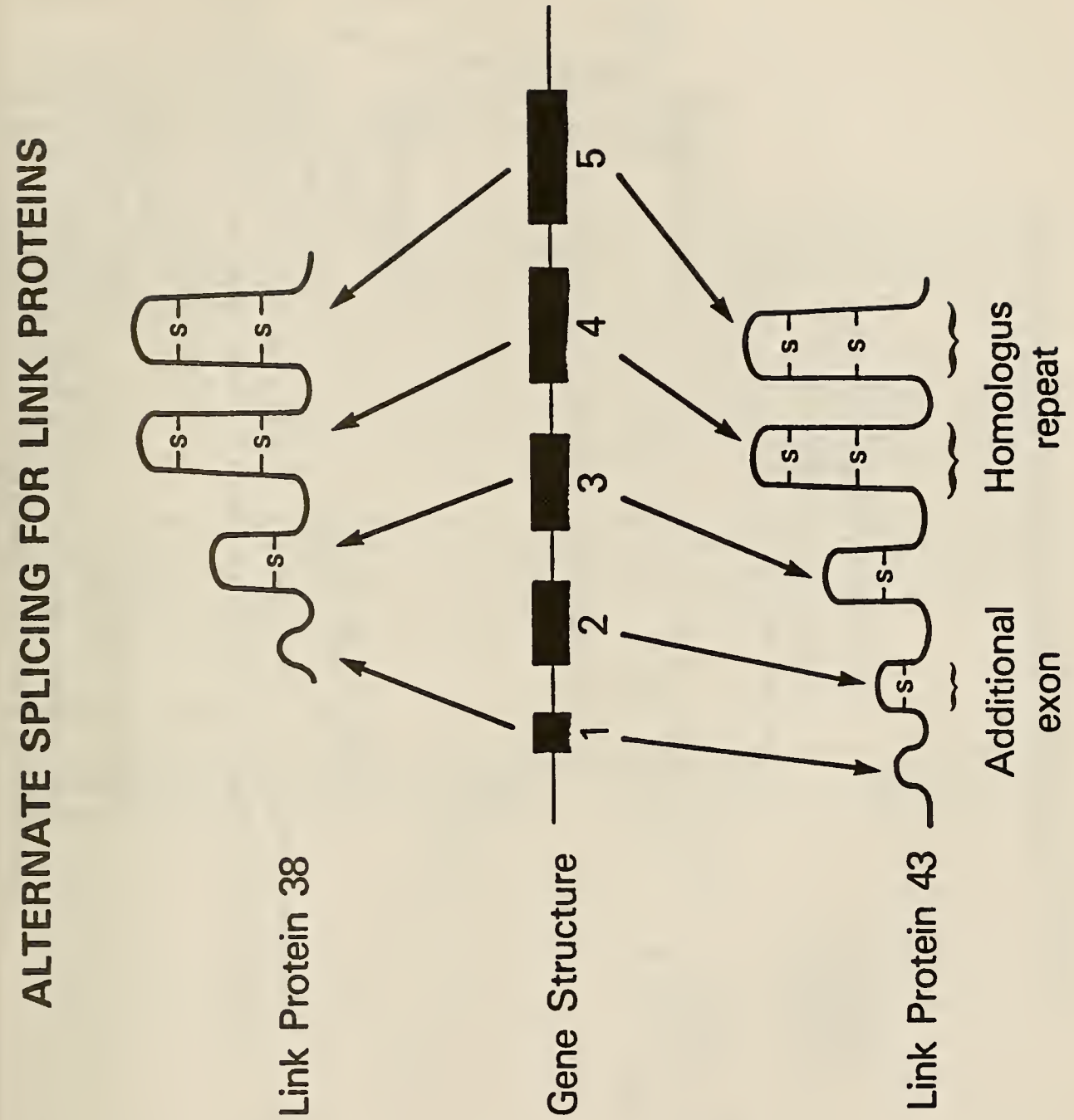

Fig. 2 Alternate splicing discovered in the cartilage link protein. Exon 2 is present in the larger link protein mRNA. Preliminary data suggest that link protein 43 is a minor constituent of cartilage. Other tissues have not yet been examined.

Fig. 3 Analysis of part of the structure of the cartilage proteoglycan. The carboxyl terminal globule is a cysteine-rich region with lectin homologies. Adjacent to this region is a serine-glycine reach region believed to be the site of attachment of chondroitin sulfate chains in the intact proteoglycan. The ser-gly repeats are not contiguous but are regularly spaced.

ORTHOPAEDIC RESEARCH UNIT ANNUAL REPORT

Professional personnel:
Mark E. Bolander, M.D. - Senior Staff Fellow
Gabor Nemeth, M.D., Guest Worker - Starting 5/1/86
Lyman S. Smith, M.D., Guest Worker - Until 6/20/86

At the invitation of the NIAMS and the NIDDK, and with significant financial support provided by the American Academy of Orthopaedic Surgeons and the Orthopaedic Research and Education Foundation, an Orthopaedic Research Unit was established at the NIH on July 1, 1986. This unit is currently located in the Laboratory of Developmental Biology and Anomalies (LDBA), NIDR, and receives collaborative and operational support from that Laboratory. The Orthopaedic Research Unit is part of the Structural Biology Section of the Laboratory of Cellular and Developmental Biology, NIDDK. The unit director reports to Dr. Alasdair Steven, Section Head, and Dr. Robert Simpson, Laboratory Chief in the Laboratory of Cellular and Developmental Biology.

Mark E. Bolander has been appointed director of the Orthopaedic Research Unit. Dr. Bolander is a board eligible orthopaedic surgeon who was a fellow in the LDBA for two years prior to establishing the Orthopaedic Research Unit. His current research interests are the regulation of non-collagenous proteins in bone and gene expression in fracture healing.

Summary of Work

Investigations are currently underway on the regulation of gene expression in fracture healing and on the structure of osteonectin, a non-collagenous glycoprotein found primarily in bone.

SPECIFIC RESEARCH ACCOMPLISHMENTS

Cloning and sequencing a cDNA for osteonectin: (in collaboration with John Termine and Marion Young in the Bone Research Branch)

Osteonectin, a bone glycoprotein, was originally described in a collaboration of LDBA with Dr. John Termine (BRB, NIDR). It represents approximately 15% of the non collagenous proteins of bone and binds to calcium to hydroxy apatite, and to collagen and thus may have a role in the mineralization process. It is also greatly reduced in animal models for osteogenesis imperfecta (a brittle bone disease) and in some human patients. We have used molecular biology and monoclonal antibodies to characterize this protein.

A 2.1 Kb cDNA for bovine osteonectin has been isolated from a bovine osteoblast cDNA library and sequenced. Confirmation of this clone as coding for osteonectin was made by comparison with the known amino acid sequence for osteonectin. Analysis of the 305 amino acid residue sequence deduced from this nucleotide sequence has shown that osteonectin has at least three and possibly four structural domains in addition to a 17 amino acid signal peptide. The first domain is a very acidic region, some 55 amino acid residues long, that is probably a high affinity calcium binding site. This domain contains 2 homologous repeats. The second domain is a sequence of 85 amino acids that contains 11 cysteine residues and two possible glycosylation sites. This is followed by a hydrophilic region some 30 amino acid residues long. The remaining 96 amino acid residues appear unremarkable except for the presence of four cysteine residues. Based on this analysis, we have proposed a structural model for osteonectin, illustrated in the attached figure.

Development of an RIA for osteonectin: Several monoclonal anti-osteonectin antibodies have been developed which recognize unique parts of the osteonectin molecule. These antibodies react with osteonectin from many different species suggesting that the structure is highly conserved. The development of an RIA using these antibodies will greatly assist both laboratory and clinical investigations into the synthesis and function of osteonectin in normal and diseased bone.

Gene expression in fracture healing: Gene expression in healing bone can be determined by Northern analysis of RNA extracted from fracture callus. Using a rat femur fracture model, we have extracted RNA from the fracture callus at regular intervals after injury. Complimentary DNA probes have been used to evaluate the expression of extracellular matrix protein genes, including collagen types I, II, III and X, osteonectin, and fibronectin, in fracture repair. This investigation should allow us to define the temporal sequence of significant molecular events in fracture healing. By extending this study to evaluate fracture healing in rat models of diabetes mellitus and osteopetrosis, we hope to demonstrate how the normal healing process is altered in these diseases.

Quantitative digital subtraction radiography in the assessment of experimental fractures: (in collaboration with Richard Webber and Samuel Zeichner, Diagnostic Systems Branch)

The evaluation of experimental fractures healing is hampered by inadequate research tools which can be used to quantitatively monitor the progression of fracture healing. This project investigates the ability of subtraction radiography techniques to quantify changes in bone density that occur during the process of bone formation or bone resorption.

Cadaveric bone specimens (phalanges and femoral head) were serially decalcified, and extracted calcium was determined by atomic absorption spectroscopy and gravimetric methods. Radiographs obtained after decalcification were converted to digital images. Sequential images of each specimen were gamma corrected and digitally subtracted using an algorithm developed in the Diagnostic Systems Branch. Changes in mineral content will be calculated from bone density and calcium standards, then compared to the spectrophotometric and gravimetric determinations of calcium loss. If in vitro tests are successful, studies of experimental fractures will be performed.

Delayed fracture healing is a problem of great magnitude. Accurate measurements of experimental fracture healing by image subtraction will offer a significant means to assess the healing progress and to follow the effects of therapy in the laboratory setting. Such a tool could aid in the assessment of how bone healing is regulated physiologically and how it can be manipulated pharmacologically.

Direction of future work:

Future studies will include the following projects:

1. Determination of serum levels of osteonectin in growth, development, aging, and bone diseases using a monoclonal RIA.
2. Recent papers have shown homologies between osteonectin and SPARC, a protein synthesized by the parietal endoderm in mice. The possible role of these proteins in the various tissues will be investigated.
3. Evaluation of fracture healing for the expression of oncogenes, heat shock protein genes, and growth factors. Attention will be given to evaluating the influence of growth factors on fracture repair.
4. Investigation of the incorporation of osteonectin into the extracellular matrix, and determination of the sequences that function as collagen binding and calcium binding domains. This information would be important in clarifying the function of osteonectin in normal bone mineralization, and in understanding the abnormalities in osteonectin seen in osteogenesis imperfecta and osteopetrosis.

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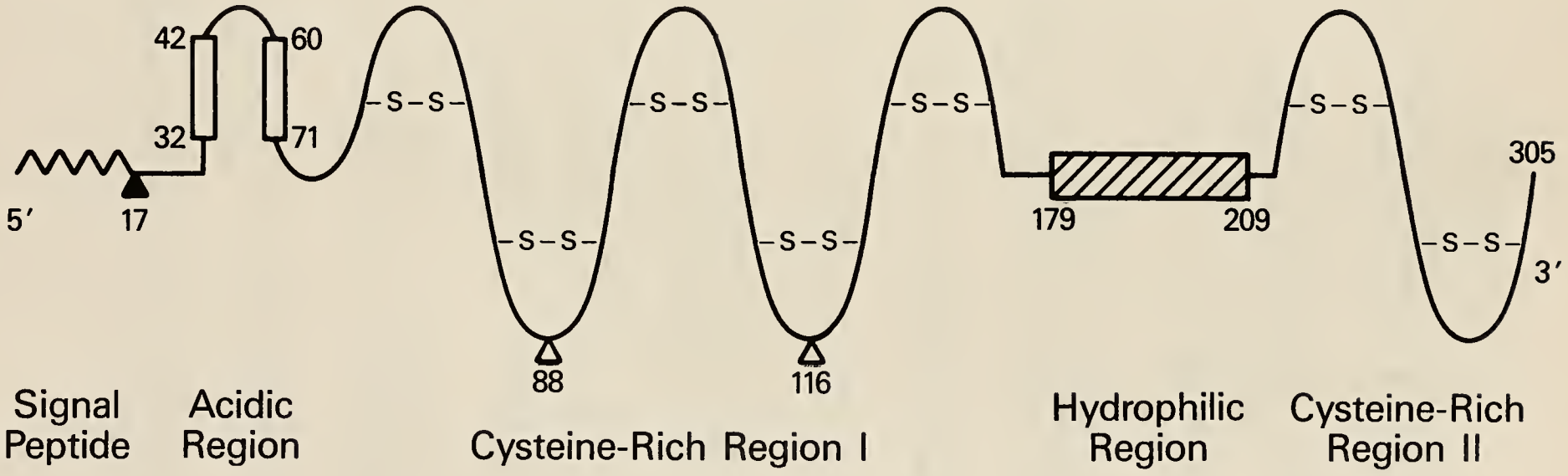
2. Young, M.F., Bolander, M.E., Day, A., Ramis, C.I., Robey, P.G., Yamada, Y., and Termine, J.T.: Osteonectin mRNA: distribution in normal and transformed cells. Nucleic Acids Research, 14:4483-4497, 1986.

3. Bolander, M.E., Fisher, L.W., Robey, P.G., Prabhakar, B.S., and Termine, J.T.: Monoclonal antibodies against osteonectin recognize different epitopes, submitted, Calc. Tiss. Int., 1986.

4. Bolander, M.E., Young, M.F., and Yamada, Y.: Molecular cloning and characterization of osteonectin cDNA: homology with a SPARC, a protein secreted by mouse parietal endoderm. In preparation.

Model of Osteonectin Structure



▲ Cleavage site of signal peptide
△ Potential glycosylation sites
□ Internal homologies
▨ Hydrophilic region

PUBLICATIONS OF 1986

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00009-25 DB

PERIOD COVERED
October 1, 1985 - September 30, 1986

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. Dev. Bio. & Anomalies	DB NIDR
Albini, Adriana	Visiting Associate	DB NIDR
Thompson, Erik	Guest Researcher	DB NIDR
Reich, Reuven	Guest Researcher	DB NIDR
Ebihara, Isao	Visiting Fellow	DB NIDR
Killen, Paul	Staff Fellow	DB NIDR
Iwamota, Yukihide	Visiting Associate	DB NIDR
Laurie, Gordon	Visiting Associate	DB NIDR

COOPERATING UNITS *(if any)*
Max Planck Institute for Biochemistry, W. Germany; McGill University, Canada; State University of New York at Buffalo; M.D. Anderson Hospital; VA Hospital, San Francisco; Upjohn Co., Kalamazoo; Food and Drug Adm.; NCI.

LAB/BRANCH
Laboratory of Developmental Biology and Anomalies

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece 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: Z01 DE 00024-20 DB

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental processes in genetically controlled malformations

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

Brown, Kenneth S.	Medical Director	DB NIDR
Campbell, Marlissa	Postdoctoral Fellow	DB NIDR
Harne, Leslie C.	Bio Lab Technician (Animal)	DB 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

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☐ (a1) Minors
☐ (a2) Interviews

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

We are using mice with genetic defects in craniofacial development or differences in susceptibility to teratogens as models to study the mechanisms involved in craniofacial malformation. Mutations that cause cleft lip, cleft palate, malocclusion and exencephaly have been found to have chondrocytes that produce altered cartilage matrix. Several different genetic defects in matrix biochemistry result in similar malformation syndromes. Other mutant genes reduce or render defective essential cellular processes such as keratinization or other types of cell differentiation with resultant malformation. These mouse genetic test systems are produced using uniform, highly inbred, genetically defined strains and timed mating resulting in control of stages of development for tests of target tissues at critical periods of susceptibility in vivo or in vitro. Using DNA constructs of genes regulating the synthesis of molecules in cartilage or basement membrane with attached signal markers we are producing transgenic mice for the study of the role of these regulatory DNA segments in tissue and time specificity of gene action. The DNA fragments are incorporated into one cell embryos and expression is monitored for their incorporated DNA in the offspring of the transgenic individuals. Organ or organ primordia such as limb buds or cells from embryos of appropriate genetic type and developmental stage are labeled in vivo or in vitro with isotopic precursors for specific structural molecules or signal markers for transgenic DNA in order to determine the mechanisms of gene action in development and the mechanisms of teratogenesis.

These methods have proven successful in identification molecular defects in cartilage matrix and other specific molecular errors that result in malformation. We are continuing to use this approach to determine the molecular vocubulary of development.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00025-20

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, Yoshihiko	Chief, MBI	DB NIDR
Doege, Kurt	Postdoctoral Fellow	DB NIDR
Sasaki, Makoto	Visiting Associate	DB NIDR
Tomoyuki, Myashito	Visiting Fellow	DB NIDR
Rhodes, Craig	Biologist	DB NIDR
Selmin, Ornella	Visiting Fellow	DB NIDR
Iwamoto, Yukihide	Visiting Associate	DB NIDR
Horton, Walter	Staff Fellow	DB NIDR
Martin, George	Chief	DB NIDR
Fernandez, Pilar	Visiting Fellow	DB NIDR

(Continued)

COOPERATING UNITS *(if any)*

Max-Planck Institut fur Biochemie; Johns Hopkins University; NCI; Roswell Park Memorial Institute; Sagami Chemical Institute; Columbia University; Stanford University, FDA; NICHD

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

☐ (a1) Minors

☐ (a2) Interviews

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

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.

Nunez, Anne Marie	Visiting Associate	DB NIDR
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
Abramczuk, Jan	Visiting Scientist	DB NIDR
Brown, Kenneth	Medical Director	DB NIDR
Hassell, John	Research Biologist	DB NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00149-12 DB

PERIOD COVERED: October 1, 1985 - September 30, 1986

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, Yoshihiki	Chief, MBU, LDBA	DB NIDR
Horton, Walter	Postdoctoral Fellow	DB NIDR
Noonan, Douglas	Postdoctoral Fellow	DB NIDR
Doege, Curt	Postdoctoral Fellow	DB NIDR
Leyshon, Webster	Biologist	DB NIDR
Horigan, Elizabeth	Biologist	DB NIDR
Mosley, General	Biological Lab Technician	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

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

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

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

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 proteins of proteoglycans from cartilage and basement membrane. Basement membrane proteoglycans from glomerular and EHS tissue have been found to be immunologically similar. Peptide mapping of these proteoglycans show them to contain a immunologically similar domain of M_r=45,000 but the glomerular proteoglycan lacks a M_r=44,000 domain found in the EHS proteoglycan. Genetic clones to both the cartilage and basement membrane proteoglycan have been obtained by screening expressing vectors with antibodies to their core proteins. We have sequenced nearly 2/3 of the cartilage proteoglycans core protein and discovered a globular domain at the carboxyl terminus that has extensive homology with lectin binding proteins and a serine-glycine rich domain, for glycosaminoglycan attachment, that contains SGXXXXSGXX as a consensus spacing. Immunological evidence indicate our clones to the basement membrane are from the heparan sulfate attachment region.

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. An immortalized chondrocyte line has been developed for this purpose by infecting fetal rat costal chondrocytes with the myc oncogene. 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 for type II procollagen, cartilage proteoglycan core protein, and link protein while stimulating the synthesis of type III collagen and fibronectin. Furthermore, these alterations in protein synthesis retinoic acid acts to change the phenotype of the chondrocytes, possibly by affecting transcriptional activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-10

PERIOD COVERED

October 1, 1985 - September 30, 1986

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
Luckenbill-Edds, L.	Guest Researcher	DB, NIDR
Graf, J.O.	Postdoctoral Fellow	DB, NIDR
Kitten, G.T.	Postdoctoral Fellow (NRSA)	DB, NIDR
Cannon, F.B.	Biologist	DB, NIDR
Horn, V.	Postdoctoral Fellow (NRSA)	DB, NIDR
Ogle, R.C.	Guest Researcher	DB, NIDR

COOPERATING UNITS *(if any)* NINCDS, NCI, NHLBI, NIH; Max Planck Inst. Biochem., Munich; TX Tech, Lubbock TX; Univ. of Montreal and McGill Univ., Canada; Georgetown Univ.; Harvard Univ., MA; CNRS, Paris; Upjohn Co., Kalamazoo, MI; Univ. of British Columbia, Canada; Helitrex, Princeton, NJ; Univ. of Minnesota; Univ. of WI

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

SECTION

Cell Biology Section

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are studying the function of extracellular matrix components and their molecular interactions with cell surfaces. The Cell-substratum attachment glycoprotein laminin is being studied at the molecular level with synthetic peptides and with their corresponding antibodies to define the active domains for cell attachment, growth, migration, and type IV collagen binding. Our data have identified an active site for cell attachment and for cell migration. This region has homology to epidermal growth factor and likely is important in indicating cell-matrix interactions in development and in regeneration. Cell-matrix interactions are regulated by cell surface matrix receptors. Using molecular and biochemical approaches, we are identifiying and characterizing these components on fibroblastic and on neuronal cells. Our data define how cells interact with cell attachment proteins and with collagens. These and other studies indicate that specific cell surface receptors bind to matrix components and thus control the biological responses of the cells to the matrix.

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 00275-08 DB

PERIOD COVERED

October 1, 1985 - September 30, 1986

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. Dev. Biol. & Anomalies	DB NIDR
Brown, Kenneth S.	Medical Director	DB NIDR

COOPERATING UNITS *(if any)*

University of Minnesota; NCI, NIH

LAB/BRANCH

Laboratory of Developmental Biology and Anomalies

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.10	.10	

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 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.

ANNUAL REPORT OF THE LABORATORY OF MICROBIOLOGY AND IMMUNOLOGY
NATIONAL INSTITUTE OF DENTAL RESEARCH
October 1, 1985 to September 30, 1986

The Laboratory of Microbiology and Immunology performs fundamental research in oral microbial ecology, physiology and pathogenesis as well as in areas of immunology that relate to the role of lymphocytes, macrophages, basophils and their mediators in the development of acute and chronic inflammatory responses. Over the course of the past several years, our investigations have become more dependent upon the application of molecular biological approaches to the solution of complex problems. This is readily apparent in our studies on 1) Bacterial surface structures and specific adherence to eucaryotic and procaryotic cell receptors; 2) Development of genetic exchange systems in lactic acid bacteria; 3) Cloning and characterization of bacterial metabolic enzymes; 4) The regulation of cytokine synthesis by lymphocytes and macrophages and, 5) Cloning of receptors on basophils that are involved in IgE-mediated histamine release. Our achievements in these and other projects, which are documented below, can be attributed to the interdisciplinary nature of the Laboratory, to our numerous collaborations with outside institutions and to the successes of our staff in developing and applying newer technologies in their research programs.

The Microbiology Section focusses on microbial ecology, biochemistry and molecular biology. The program seeks to explain physiological and pathological traits of oral microorganisms in molecular terms. The underlying theme of this program is founded on the premise that new knowledge accruing from fundamental inquiries will establish a basis for the formulation of rational approaches to the control or eventual eradication of oral diseases having a microbial etiology.

The Section's ecology program continues its investigations on intergeneric coaggregation reactions 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. Past studies were devoted to conducting extensive surveys of oral bacteria that were designed to assess the scope and specificity of the coaggregation reactions and to test various models that were proposed to account for the diverse types of interactions observed. We are now concentrating on the isolation and chemical characterization of certain cell surface lectins that mediate some of the coaggregation reactions.

We reported last year that certain members of the oral microbial flora act as coaggregation bridges that serve as a link between other non-coaggregating pairs of bacteria. Thus, Actinomyces israelii PK14 and Streptococcus sanguis 34 are unable to coaggregate with one another, but each can coaggregate with Bacteroides loescheii PK1295. Different lectin-like surface molecules on B. loescheii are involved in these interactions, since the coaggregation of this organism with S. sanguis is reversible by lactose whereas the coaggregation with A. israelii is not. Recently, three classes of coaggregation defective mutants of B. loeschii have been obtained. One class lost the ability to coaggregate with S. sanguis but retained its ability to coaggregate with A. israelii. A second class lost the ability to coaggregate with A. israelii but retained its ability to coaggregate with S. sanguis. The third class of mutants lost its ability to coaggregate with both organisms and this class of mutants was devoid of fimbriae. This suggests that the different molecules responsible for

mediating these coaggregations are associated with the fimbriae, but it is not known whether the molecules are located on a single fimbrial structure or whether B. loeschii has two different types of fimbriae. In collaboration with the Clinical Immunology Section, purified fimbriae from wild type B. loeschii have been used to prepare monoclonal antibodies as an aid to identifying the lectins involved in the coaggregation reactions with A. israelii and S. sanguis. One class of monoclonal antibody blocked only coaggregation with A. israelii, while a second class prevented interaction only with S. sanguis. Western blot analyses of wild type B. loescheii fimbrial proteins with the class one monoclonal antibodies revealed a 75 Kd protein while use of the class two antibodies identified a 46 Kd protein. The various coaggregation mutants and the monoclonal antibodies are now being used to confirm the role of these two proteins in the bridging coaggregation reactions.

It may also be noted briefly that Capnocytophaga ochracea is another example of a bridging organism that participates in coaggregation reactions with different strains of A. israelii and S. sanguis. Our preliminary studies have provided two interesting results. First, the surface lectins on C. ochracea involved in these coaggregations are different from those on B. loescheii. The reaction between C. ochracea and A. israelii PK16 was inhibited by N-acetylated amino sugars while the coaggregation between C. ochracea and S. sanguis H1 was blocked by rhamnose. Second, the surface lectins on C ochracea are located on the outer membrane rather than on fimbriae. A strategy similar to that described for B. loescheii is now being employed to characterize the coaggregation lectins from C. ochracea.

Our biochemical studies have centered on two fundamental aspects of microbial physiology: 1) carbohydrate transport, metabolism, and regulation, and 2) mechanisms of protein turnover.

We continue to investigate the biochemical basis for the bacteriostatic action of the non-metabolizable glucose analogue, 2-deoxy-D-glucose (2DG) and 2-fluoro-D-glucose (2FG) in Streptococcus lactis. It was previously reported that these analogues initiated a futile cycle involving first their phosphorylation and transport into the cell by the phosphotransferase system followed by their intracellular dephosphorylation and export as the free sugars. Repetition of this cycle depletes the cell of energy (ATP) and accounts for the bacteriostatic action of 2DG and 2FG. We have recently examined the effect of these sugar analogues on the intercellular pool of amino acids. These studies have led to the discovery of two previously unknown amino acids in S. lactis. One is N^5-(1-carboxyethyl-ornithine [N^5-(1-CE)] and the other is N^6-(1-carboxyethyl)-lysine. These amino acids were isolated from S. lactis, purified by thin layer chromatography and their molecular structures determined by ^1H and ^{13}C-NMR spectroscopy. In collaboration with Dr. Henry Fales (NHLBI), N^5-(1-CE) has also been synthesized chemically from both poly-S-ornithine and (2S)-N^2-carbobenzyloxy-ornithine. The physical and chemical properties of the amino acid isolated from S. lactis were identical to the synthetic compound and the bis-N-trifluoroacetyl-di-n-butyl esters of the natural and synthetic compounds gave identical GC-MS spectra. We are now investigating the possible physiological roles of these unusual amino acids. Very preliminary evidence suggests that N^5-(1-CE) may have a function in regulating the arginine biosynthetic pathway.

In another study, we are investigating the transport and metabolism of glucose in the obligate anaerobe, Fusobacterium nucleatum. Glucose uptake by this organism has been shown to be tightly and specifically coupled to the metabolism of lysine or glutamate. Moreover, greater than 80% of the glucose transported is converted to glycogen. We are currently investigating the nature of the obligatory link between lysine (or glutamate) metabolism and the uptake of glucose and its incorporation into glycogen.

Our studies on protein turnover continue to focus on the mechanism of oxidative inactivation and subsequent proteolytic degradation of a fructosyltransferase (FT) from Streptococcus salivarius. We reported previously the development of an in vitro FT inactivation system that allowed us to unequivocally identify one of two NADH oxidases present in this organism as the enzyme responsible for the copper ion-dependent, oxidative inactivation of FT. This NADH oxidase has now been partially purified and shown to produce both H_2O_2 and superoxide anion in a ratio of 2:1 respectively. Results obtained from H_2O_2 and oxygen radical scavenger studies have provided some insight into the mechanism by which NADH oxidase catalyzes FT inactivation. A role for H_2O_2 was indicated by the fact that catalase protected against FT inactivation. Inactivation, however, is not due simply to a direct interaction of H_2O_2 with the enzyme because neither H_2O_2 alone nor in combination with Cu^{2+} had any effect on FT activity. It appears rather that H_2O_2 acts in concert with one or more species of oxygen radicals and Cu^{2+} to effect the inactivation. In this regard, superoxide and hydroxyl radical scavengers also afforded protection against inactivation. Our results suggest that FT inactivation in S. salivarius may occur in a site-specific manner by a transition-metal-catalyzed Haber-Weiss reaction. According to this mechanism, FT first binds Cu^{2+} at or near the catalytic site. The bound Cu^{2+} is then reduced by superoxide and subsequently reoxidized by H_2O_2 (products of NADH oxidase) with the generation locally of hydroxyl radicals. The hydroxyl radical is considered most likely responsible for the inactivation of FT, since neither H_2O_2 nor superoxide alone had any effect on FT activity. The data also suggest that the oxidative inactivation of FT represents a 'marking' step that identifies the enzyme as a substrate for subsequent proteolytic degradation.

Our molecular biology program has made significant advances in two areas: 1) the use of recombinant DNA techniques to identify and chemically characterize surface structures on oral bacteria that mediate specific coaggregation or adherence reactions; and 2) the study of lactose metabolic plasmids.

Actinomyces viscosus has been shown to possess two types of fimbriae. Type 1 fimbriae possess an adhesin that is responsible for the adherence of the organism to the tooth surface. Type 2 fimbriae contain lectin-like molecule that mediate a coaggregation reaction with S. sanguis. We reported last year that the A. viscosus gene encoding for a 59 Kd subunit of the coaggregation lectin was cloned in E. coli. Subcloning has now allowed the isolation of the entire gene on a 1.9 kbp BamH I-Pst I fragment and M13 Sanger dideoxy sequencing of the gene will now be undertaken. The 59 Kd polypeptide has also been purified to homogeniety. In collaboration with the Humoral Immunity Section, a structural and conformational analysis of the cloned gene product is being conducted and these results are summarized in that Section's report.

We continue to use molecular biological approaches for studying lactose metabolic genes in Lactobacillus casei and this work complements our biochemical

studies on carbohydrate transport and metabolism in the lactic acid bacteria. The immediate objective of these studies is to resolve structure-function relationships among the various components of the lactose-phosphoenopyruvate (PEP) phosphotransferase system (PTS). It was reported last year that certain genes encoding for lactose metabolism in L. casei are carried on a plasmid. These include the genes that code for phospho-β-galactosidase, lactose-PTS Factor III (III^{lac}), and lactose-PTS Factor II. The L. casei lactose-PTS factor III has been purified to homogeneity and used to prepare anti-III^{lac} antibodies in rabbits. The anti-III^{lac} IgG was then used to screen a library of E. coli clones containing L. casei plasmid DNA restriction fragments. Clones producing L. casei III^{lac} were identified and the cloned protein has been shown to be identical to the III^{lac} produced by L. casei. The III^{lac} gene has now been subcloned on an 850 bp DNA fragment and M13 dideoxy sequencing has been started. Once the structure of III^{lac} has been determined we will be able to modify it by site-specific mutations and then correlate these modifications to structure-function relationships with other components of the lactose PTS. In this regard, the development of a liposome-mediated DNA exchange system for the lactobacilli that was reported last year should provide us with a mechanism for introducing these specifically modified genes back into the original host.

The program of the Humoral Immunity Section encompasses the definition of molecular events involved in the attachment and colonization of different oral surfaces by microorganisms and the resultant induction of inflammation of oral tissues. Investigations have focused on the identification, characterization and purification of microbial surface structures that mediate adherence as well as the complementary receptors for these adhesins on tooth, epithelial, other microbial and phagocytic cell surfaces. The response of the host to oral infection is multifaceted. Whereas antimicrobial antibodies, complement and phagocytic cells may all participate in the control of the oral flora, the interaction of bacteria with these humoral and cellular host components generates a number of inflammatory mediators which may contribute to tissue destruction. Current emphasis is also directed towards delineating these processes.

The previous identification and isolation of two types of fimbriae (type 1 and type 2) on certain oral actinomyces have provided a basis for investigating the potential function of these surface structures in a variety of processes. These fimbrial components influence the site of colonization in the oral cavity by different bacterial strains. Actinomyces viscosus T14V, an organism possessing both type 1 and type 2 fimbriae, is found predominantly on the tooth surface. This interaction is mediated by the type 1 fimbriae. In collaborative studies with Dr. William Clark it has been shown that monospecific antibodies against the type 1 but not the type 2 fimbriae, react with strains of A. viscosus and A. naeslundii that adsorb strongly to saliva coated hydroxyapatite (SHA), an in vitro model of the acquired pellicle, and inhibit bacterial attachment to this matrix whereas the antibodies do not bind to strains which adsorb poorly to SHA. In addition, mutants of A. viscosus T14V which lack type 1 fimbriae do not attach to SHA. The type 1 fimbriae react with the acidic proline rich proteins found in saliva and in the acquired salivary pellicle. A. viscosus T14V and a mutant with only type 1 fimbriae adsorb to proline rich protein coated hydroxyapatite beads and cause aggregation of proline rich protein coated latex beads while mutants with only type 2 fimbriae or those lacking fimbriae were non-adherent.

Strains such as A. naeslundii WVU45, which possess only the type 2 fimbriae, are commonly associated with epithelial surfaces. This property can now be

attributed to the interaction of the type 2 fimbrial lectin with a carbohydrate containing receptor on epithelial cells. The initial event in this process appears to be the production of sialidase by the actinomyces, an enzyme that exposes receptors for the bacteria on the mammalian cells. A mutant of A. naeslundii WVU45 and mutants of A. viscosus T14V which lack the type 2 fimbriae fail to attach to the epithelial cells and saccharides containing β-linked Gal and GalNAc inhibit adherence. Certain plant lectins also inhibit attachment and these have proven to be useful probes for the identification and purification of the epithelial cell receptors for the actinomyces lectin. The lectin from Bauhinia purpurea, which binds to Gal and GalNAc, and the peanut agglutinin, which is specific for Galβ3GalNAc, are similar in reactivity to the actinomyces lectin and detect a band of 160 Kd on sialidase treated Western blots of epithelial cell extracts separated by SDS-PAGE. An IgM monoclonal antibody specific for Galβ3GalNAc also detects this band. This 160 Kd component is a cell surface sialoprotein which can be surface and metabolically labeled. Recent evidence also indicates that glycolipids may serve as receptors for the actinomyces lectin. Radiolabeled A. naeslundii WVU45 bound directly to the gangliosides GM1, GD1b and asialo GM1 but not to GM2 or asialo GM2 separated by thin layer chromatography. Following treatment of the TLC plates with sialidase, the bacteria also bound to GD1a and GT1b. These gangliosides all contain terminal Galβ3GalNAc. In addition, the bacterial lectin recognized globoside but not globotriaosylceramide or the Forssman glycolipid. Interestingly, globoside contains terminal GalNAcβ3Gal. Attachment of the parent bacterial strain to these gangliosides and to globoside is inhibited by lactose and the mutant of A. naeslundii WVU54 lacking type 2 fimbriae fails to adhere to any of these glycolipids. Purification of these epithelial cell glycoprotein and glycolipid receptors for the type 2 fimbriae is in progress utilizing appropriate lectin affinity columns and lipid separation techniques.

Whereas the fimbrial adhesins confer specific attachment properties on the actinomyces resulting in colonization, current studies also demonstrate that the type 2 fimbriae are instrumental in initiating the destruction of these bacteria by polymorphonuclear leukocytes. This process is undoubtedly accompanied by the release of the lysosomal constituents of the phagocytic cells which can lead to inflammation of the surrounding oral tissues. Parent strains of A. viscosus T14V and A. naeslundii WVU45 and a mutant of A. viscosus T14V possessing only type 2 fimbriae are rapidly killed by human polymorphonuclear leukocytes and the number of bacteria destroyed is markedly enhanced by sialidase. In contrast, bacterial mutants lacking type 2 fimbriae are resistant. Bacterial killing is inhibited by β-methylgalactoside and lactose but not by cellobiose or α-methylgalactoside. Electron microscopic examination strongly suggests that the bactericidal activity can be attributed to phagocytosis. Current studies are directed at characterizing and purifying the receptor for the actinomyces lectin on polymorphonuclear leukocytes utilizing strategies similar to those employed in investigations concerning the properties of the epithelial cell receptor. The peanut agglutinin and the lectins from Bauhinia purpurea and Ricinus communis, all of which inhibit phagocytosis, detect a band of approximately 100 Kd on sialidase treated Western blots of extracts of polymorphonuclear leukocytes separated by SDS-PAGE. Partial purification of this receptor has been achieved by chromatography on lectin affinity columns.

The type 2 fimbrial lectin of the actinomyces also recognizes a carbohydrate receptor on certain strains of oral streptococci. Through interactions such as these, microbial communities are probably established on oral surfaces, a

postulate which has been strengthened by examination of plaque samples utilizing different fluorophore conjugated monospecific antibodies to the type 2 fimbriae and its receptor on Streptococcus sanguis 34. Studies in our laboratory have identified a single antigen on S. sanguis 34 which serves as the receptor for the actinomyces lectin. Dr. Floyd McIntire has purified this component and determined that this antigen is composed of a repeating phosphodiester linked hexasaccharide containing GalNAc as well as GalNAcβ3Gal. Clearly emerging from studies concerning this receptor for the fimbrial lectin and the receptors on epithelial cells and polymorphonuclear leukocytes is the definition of a series of specific interactions of the type 2 fimbriae which may result in bacterial colonization, accumulation of plaque or destruction of the bacteria.

While these functional properties of the actinomyces fimbriae have been identified and the receptors for them at least partially characterized, relatively little is known concerning the structure of these adhesins. Structural information is being obtained by two approaches. One utilizes monoclonal antibodies to map the epitopes of the type 1 and type 2 fimbriae of A. viscosus T14V. Binding studies have identified antibodies reactive with the same or different epitopes and complement fixation by a single antibody or pairs of monoclonal antibodies is providing data regarding the spacing of the epitopes. Complement activation by IgG antibodies is strictly dependent on the bridging of two appropriately spaced antibodies by a single molecule of the Clq subcomponent of the first component of complement. Although single antibodies to the type 1 fimbriae activate complement, the effect is enhanced by the combination of certain antibodies reactive with two different epitopes indicating that these epitopes are closer than an epitope which is present once in each subunit. In contrast to the results obtained with the type 1 fimbriae, individual monoclonal antibodies to the type 2 fimbriae are very inefficient activators of the complement sequence. Thus, although as stated below, the molecular weights of the subunits of the type 1 and type 2 fimbriae are nearly identical, the type 2 may be more elongated than the type 1. The anti-type 2 antibodies, however, effectively activate complement when a second antibody is included. Studies have been initiated to precisely determine the distance between these epitopes by energy transfer between pairs of fluorescein and rhodamine conjugated Fab fragments of the monoclonal antibodies.

The second approach to determining the structure of these fimbriae is cloning of the genes in Escherichia coli. The gene encoding the 65 Kd type 1 fimbrial subunit has been localized on a 1.9 kb fragment using pUC13 as an expression vector. Subclones containing the 1.4 kb fragment obtained by Sal I digestion of the type 1 gene express the N terminal 47 Kd region of the protein, while subclones carrying the 0.5 kb C terminal portion of this gene do not produce an immunoreactive product. The intact type 1 subunit as well as the 47 Kd terminal polypeptide react with each of 5 monoclonal antibodies directed against three distinct type 1 epitopes and affinity columns prepared with these antibodies have proven to be particularly useful for purification of the cloned protein. Rabbit antibody has been prepared against the cloned type 1 subunit. It reacts in Western blotting with partially dissociated type 1 fimbriae to give a pattern that is indistinguishable from that obtained with anti-fimbrial antibody. The isolated subunit exists as a monomer or possibly a dimer and exhibits no tendency to aggregate or self assemble. It is not yet known whether the subunit possesses functional activities like those of fimbriae. Collaborative studies with the Microbiology Section have resulted in cloning of the gene for the subunit of the type 2 fimbriae. The gene encoding the 59 Kd

type 2 fimbrial subunit has been identified on a 2.5 kb fragment and plasmids which carry fragments of the gene express the N terminal 22 Kd region or the C terminal 35 Kd region of the subunit. Of nine monoclonal antibodies produced against different type 2 epitopes, eight react with the cloned 59 Kd protein in ELISA and of these, one reacts with the N terminal 22 Kd region and two with the C terminal 35 Kd region. The reaction of five different anti-type 2 antibodies with the intact subunit but not with either fragment suggests that the subunit conformation depends on the presence of both fragments. This suggestion is consistent with the finding that the intact subunit is a heat modifiable protein whereas the N and C terminal fragments are not.

In addition to fimbrial mediated attachment, oral microbes can adhere to other microbes and host cells by antibody and complement dependent mechanisms. Polyvalent antibodies against A. viscosus T14V or its mutant lacking both types of fimbriae and antibodies monospecific for each of the two types of fimbriae are all extremely effective opsonins for phagocytosis. The potential enhancement of phagocytosis by deposition of the C3b fragment of the third component of complement (C3) on the cell wall or on each of the two types of fimbriae is currently under investigation.

Studies of the effects of C3 and its cleavage products on Candida albicans have also been initiated. The hyphal form of this pathogenic organism possesses a receptor(s) for C3bi and its further degradation product, C3d. The activation of complement by C. albicans presumably results in the covalent binding of C3b to the cell wall and cleavage products of this C3b would then be available for attachment to the C3bi or C3d receptors of other organisms belonging to the same species. This interaction may provide a mechanism by which the population of candida is increased at a particular site. The C3d receptor has been partially characterized and purified. It appears to be a mannoprotein. However, the mannose does not appear to be directly involved in recognition of the C3 fragments. The receptor is destroyed by heat, trypsin, pronase and dithiothreitol. Sequential ion exchange chromatography and elution from C3d affinity columns of candida extracts have yielded a fraction which has receptor activity and is composed of one major and two minor bands on SDS-PAGE. Monoclonal antibodies against C. albicans have been raised which inhibit the recognition of C3d by this receptor and will be used in its further characterization and comparison with other eukaryotic C3d receptors.

The Cellular Immunology Section continues its investigations into the physiological and biochemical events regulating normal mononuclear cell function, with a new emphasis on the molecular genetics of these responses. Many of these studies have provided the basis for analyzing mononuclear cell deficiencies in patients with the acquired immunodeficiency syndrome (AIDS). Based on the original observations by members of the Section which indicated that mononuclear phagocytes obtained from these patients, in addition to their T lymphocytes, were functionally deficient, the Cellular Immunology Section was awarded a supplemental budget to enable expanded studies characterizing these defects and their therapeutic implications.

The acquired immunodeficiency syndrome is a world-wide epidemic and the National Institutes of Health is actively involved in the treatment of the disease and is encouraging basic research to characterize the disease and formulate effective therapies. The HTLV-III virus has been detected in the saliva of AIDS patients, thus implicating a possible oral route of transmission.

Consequently, the NIDR is taking an active role in basic research to define the cellular and molecular aspects of the disease. Human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), the etiologic agent for the acquired immunodeficiency syndrome (AIDS), and cytomegalovirus (CMV), a DNA virus in the herpesvirus group which causes severe organ pathology in AIDS patients, induce immunosuppression *in vitro* and *in vivo*. The mechanism of this immunosuppression has been the subject of recent investigations but remains poorly understood. Therefore, to further our understanding of how the immune system responds to these viruses, members of the Cellular Immunology Section are investigating the effect of HTLV-III/LAV and CMV on monocytes. These studies have revealed that several monocyte functions are abnormal in AIDS patients which likely contribute to the widespread dissemination and uncontrolled growth of opportunistic infections in these patients. Defective chemotaxis to inflammatory stimuli may play an important role in the depressed inflammatory reaction to foreign antigens, such as the near absence of granulomatous reactions to *Mycobacterium avium intracellulare*, in AIDS patients. Monocytes from AIDS patients also exhibit depressed cytotoxicity *in vitro* for certain protozoa reflecting their acquisition of certain life-threatening organisms, particularly protozoa. Furthermore, an assay was recently established to evaluate cytotoxicity for *Candida albicans*, the cause of severe and often incapacitating oral cavity disease in AIDS patients, to evaluate the AIDS monocyte reactivity to this organism. In addition to this deficient microbicidal activity of AIDS monocytes, the Section has demonstrated that the inability of monocytes to present antigen and to act as accessory cells for T lymphocytes is likely a contributing factor to the suppressed antigen response characteristic of AIDS. In this regard, monocytes from AIDS patients exhibit defective production of the immune inflammatory mediator, IL-1. In order to determine the nature of the IL-1 defect at the molecular level, mRNA isolated from control and LPS-stimulated monocytes from AIDS patients and normal volunteers was screened by slot blot and Northern blot hybridization for IL-1 sequences using radiolabeled synthetic oligonucleotides. Varying amounts of the 1.8 kilobase IL-1β message were observed in the RNA samples. The amount of specific mRNA detected in AIDS samples was often comparable within 2-4 fold of normal controls; however, in some AIDS patients, the level of IL-1 mRNA remained unchanged upon LPS stimulation. This spontaneous transcription of the IL-1β gene without LPS stimulation suggests that the cells had been stimulated *in vivo*, possibly a result of opportunistic infections. Our results suggest that the defect in IL-1 production by monocytes of AIDS patients must occur post-transcriptionally either in the processing of the RNA to the mature message or later during translation, assembly, or secretion of the IL-1 protein. Future studies will focus on these later events to characterize the IL-1 defect. In an extension of these studies we had the opportunity to examine monocyte function in homozygous twin brothers, one a homosexual with AIDS and the other a healthy heterosexual. By replacing the AIDS twin's monocytes with those of his healthy brother, the depressed lymphocyte proliferative responses in the AIDS brother could be partially restored, documenting the significant impact of the abnormal monocytes to the patient's immunosuppression.

Equally important are our preliminary *in vitro* data in which we have infected normal monocytes with HTLV-III/LAV and find that they also have depressed accessory cell function, suggesting that the monocytes are a direct target of the retrovirus which causes impaired function. *In situ* hybridization techniques have allowed us to detect not only viral RNA but also RNA for monokines produced by monocytes. By combining this technology with cytochemical

staining, the mononuclear cells from AIDS patients can be further assessed in terms of the presence of HTLV-III RNA and various cytokine and oncogene RNA. The recent availability of the IL-1α and β cDNA probes will greatly simplify these studies and in addition, allow us to assess the processing of the IL-1 transcript by S1 nuclease or RNase protection experiments. These studies should provide conclusive evidence of the involvement of the monocyte in HTLV-III infections.

Studies of the effect of CMV on monocytes have been equally revealing. Specifically, we have shown that normal monocytes can be infected in vitro with laboratory and clinical isolates of CMV and following infection exhibit a variety of depressed effector cell functions. CMV causes increased spontaneous release of oxygen reactive intermediates which, in contrast to uninfected monocytes, could not be augmented further in vitro, indicating CMV-induced refractoriness to additional stimuli. CMV infection also may impair antigen presentation by monocytes contributing to the host's acquisition of additional opportunistic infections during CMV infection. Thus, the Section has shown that human monocytes are a target for both HTLV-III and CMV and that infection of this cell results in depressed effector and accessory cell functions. This documentation of the role of viral-infected monocytes has important implications for understanding the mechanism of immunosuppression in vitro and devising appropriate immunomodulation/antiviral therapies.

Significant new observations have also been made in understanding normal monocyte physiology. Monocyte migration into an inflammatory site is critical to the outcome of an inflammatory lesion, yet the mechanisms of recruitment are not clearly defined. In collaborative studies with Dr. Michael Sporn and colleagues of NCI we have documented a role for transforming growth factor beta (TGFβ) in monocyte recruitment. Transforming growth factor beta (TGFβ) is a 25,000 Kd peptide originally defined by its ability to induce transformation of nonneoplastic cells in culture. More recently, however, TGFβ has also been shown to be a product of hemopoietic cells including platelets and lymphocytes and may play a role in wound healing. Injection of TGFβ subcutaneously in newborn mice induced the rapid formation of granulation tissue similar to that which occurs in tissue repair. The rapidity of these events and the deposition of collagen matrix suggested a direct effect on the recruitment of inflammatory cells and/or fibroblasts. The predominance of monocyte-macrophages within hours after the injection of TGFβ suggested that TGFβ might serve as a chemoattractant to recruit circulating monocytes to the injection site. Monocytes from healthy volunteers were consistently found to migrate in an in vitro chemotaxis assay to TGFβ (0.1-1.0 pg/ml). The ability of monocytes to respond to the minute concentrations of a diffusing concentration gradient of TGFβ suggested a highly sensitive and selective mode of monocyte recruitment. Since monocyte chemotaxis is considered to be dependent upon the interaction of a chemotactic ligand with a specific cell surface receptor, studies were initiated to evaluate peripheral blood monocyte receptor expression for TGFβ. Incubation of monocytes purified by CCE with increasing concentrations of biologically active ^{125}I-TGFβ demonstrated saturable binding for TGFβ. Scatchard analysis of the binding data showed a high affinity binding site with a dissociation constant equal to 1-10 pM. This high affinity receptor is consistent with the low concentrations of TGF necessary to initiate a chemotactic response.

The TGFβ-induced accumulation of monocytes in vivo is accompanied by an increase in the local connective tissue fibroblast population. The addition of TGFβ to purified monocytes in culture resulted in the appearance of biologic

activity in the monocyte supernatants which stimulated fibroblast proliferation as quantitated by incorporation of $TdR^{3}H$ in dermal fibroblast monolayers *in vitro*. The ability of TGFβ to override the normal lag phase required for cell recruitment and matrix synthesis may have important therapeutic implications in individuals with impaired wound healing.

Additional programs involving members of the Clinical Neurosciences Branch, NIMH, have revealed that human monocytes have receptors for and respond chemotactically to several neuropeptides. In addition to the presence of neuropeptide receptors, we have also been able to demonstrate that human aveolar macrophages store and secrete the neuropeptide, bombesin. Neuropeptide synthesis may therefore be a general feature of various immune cell populations. Neuropeptides with chemotactic activity include β-endorphin and other opiates, substance P, bombesin, and benzodiazepines. The presence of opiate chemotactic receptors has been demonstrated through ligand binding experiments. In concert with these binding studies, preliminary biochemical characterization of the opiate receptors through chemical cross-linking of a radiolabeled ligand to its receptor is in progress. Our results reveal that the opiate recognition molecule is a 110 Kd protein with an isoelectric point of 4.4. Identical molecules are present in rat brain tissue, human macrophages, and lymphocytes. Pharmacologic specificity can be demonstrated through the use of various agonists and antagonists of the opiate receptor. These compounds are exceedingly potent since activity can be detected at 10^{-14} M. The presence of multiple, diverse, neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

Of potential importance in the regulation of monocyte function is our new work on interleukin 2 receptors on peripheral blood monocytes. Activation of monocytes results in the expression of interleukin 2 (IL2) receptors which are absent on resting monocytes. LPS induces maximal IL2 receptor expression within 12 hr as determined by flow microfluorometry whereas γIFN-induced IL-2 receptor expression required 48 hr. The appearance of IL2 receptors occurred in parallel with an increased density of class II major histocompatibility complex products (HLA-DR). To understand the genetic basis for IL2 receptor expression, we used a cDNA probe encoding the human IL2 receptor to evaluate IL2 receptor gene expression in resting and activated monocytes. By 4 hr after LPS stimulation, IL2 receptor mRNA species of 3500 and 1500 bases appeared, reaching peak levels by 12 hr. The LPS-activated monocyte IL2 receptor protein expressed on the cell surface followed within a few hours after the detection of IL2 receptor mRNA. We conclude that monocyte activation stimulates gene expression of IL2 receptors which, in association with increased HLA-DR expression, may have an important immunoregulatory function.

Another major focus in this Section involves the characterization of immune-mediated modulation of connective tissue. Identification of the mechanisms whereby the inflammatory process modulates connective tissue metabolism may facilitate the development of new therapeutic modalities for controlling these pathologic events. With this objective in mind, studies have focused on defining the pathways by which inflammatory cells regulate connective tissue metabolism. The mononuclear cells which comprise the cellular constituents of chronic inflammatory lesions have been shown to generate soluble

factors which provide signals between lymphocytes, monocytes and the connective tissue target cells. The mononuclear cell dependence of connective tissue pathology in erosive arthritis and in fibrotic lesions has been definitively demonstrated in an experimental animal model. In this rodent model, systemic administration of group A streptococcal cell walls (SCW) results in the development of hepatic granulomas and chronic erosive polyarthritis. These chronic lesions have been shown to be composed primarily of T lymphocytes of the helper/inducer subset and Ia+ macrophages with fewer mast cells, eosinophils and plasma cells. Lymphocytes isolated from the inflamed tissues constitutively released lymphokines including IL-2, CSF, LDCF and IL-3 providing a mechanism for enhanced recruitment and activation of additional inflammatory cells. CSF and γIFN stimulate a variety of macrophage activities including enzyme release, phagocytosis, cytotoxicity, and monokine synthesis. The macrophages clearly serve as scavengers removing debris within the lesion, but they also play an important role as accessory cells in regulating immune processes and make major contributions to the reparative phase as well.

The SCW arthritis and granuloma model also provides an opportunity to explore the locus of antiinflammatory steroid action in chronic inflammation. Treatment of the animals with methylprednisolone at 1,15 and 30 mg/kg daily for 3 days beginning on the day of SCW injection and at 3 day intervals thereafter for 4 weeks resulted in a marked effect on these lesions. The development of liver granulomas was blocked in a dose dependent manner as was the development of erosive polyarthritis in the joints. The development of both of the lesions was blocked in the acute phase of the disease preventing the exudative and cellular recruitment phases of the inflammatory response. These data are consistent with other studies suggesting that the primary locus of the corticosteroid effect in wound healing is on the inflammatory response when administered early enough. Since the acute and chronic lesions appear to be mediated by different mechanisms, it will be of interest to detemine whether there is differential susceptibility to the effects of steroid administration.

In earlier in vitro studies, we implicated a molecular link between inflammatory mononuclear cells and alterations in fibroblast growth and function. We have extended these observations in the SCW animal model in which we document the T cell dependence of fibrosis. The development of granulomas composed primarily of lymphocytes and macrophages was associated with the recruitment and proliferation of connective tissue cells. Furthermore, this expanded population of fibroblasts generated a collagenous structure consisting primarily of types I and III collagen around the granuloma leading to the formation of fibrotic nodules throughout the livers of the treated animals. Intact granulomas as well as mononuclear cells derived from the granulomas spontaneously elaborated a soluble factor(s) that stimulated fibroblast proliferation. Physiocochemical analysis revealed that the primary granuloma-derived peak of fibroblast growth activity corresponds to an apparent molecular weight of 40,000 which is consistent with a previously described T lymphocyte-derived fibroblast activating factor (FAF) in human and guinea pig. During this past year, efforts have continued in the purification of human FAF. FAF has been characterized as a 40,000 Kd, pI 5-5.2 protein which conserves its biological activity after exposure to pH changes ranging from 2 to 9, and temperature of 56°C for 1 hr and 90°C for 3'. This protein is sensitive to treatment with reducing agents. Western blotting is in progress to exclude the co-identify of FAF with other cytokines using specific antibodies toward different cytokines. FAF mRNA has been translated by Xenopus Laevi oocytes

injected with mRNA isolated from concanavalin A (Con A) stimulated T cells. The oocytes translate a product(s) that has FAF activity in the fibroblast proliferation assay and that is physicochemically indistinguishable from the original FAF obtained from cultured normal human T lymphocytes. Consequently, these studies are an important step in the molecular characterization, sequencing and cloning of this mediator.

In addition to regulation of fibroblast proliferation, studies are also in progress to examine how mononuclear cell products influence collagen production. Electrophoretic analysis of radiolabeled products of normal human fibroblasts reveals an increase of collagen production after stimulation with Il-1 and FAF, and a decrease after treatment with γIFN. mRNA samples are also being screened with α(I), α(II), II and III collagen probes after fibroblast stimulation and by Northern blotting analysis it appears that FAF is a potent inducer of mRNA for collagen α(I). A cDNA probe for human collagenase has recently become available which will enable simultaneous evaluation of the differential effect of cytokines on collagenase and collagen production. These studies of the expression of collagen/collagenase genes in fibroblasts will be extended in situ to the hepatic granulomas and inflamed synovias.

An important advance in defining the cellular requirements for these pathophysiologic events was made when it was determined that in the absence of functional T lymphocytes (athymic rats), injection of SCW does not trigger lymphokine production and the arthritis and organized granulomas do not develop in the animals. Furthermore, a specific inhibitor of T cell function, the fungal metabolite cyclosporin A, was shown to be effective in interrupting the sequence of events culminating in pathologic tissue changes in this model. If the animals were treated with cyclosporin A beginning at the time of SCW injection, the development of synovitis and destructive changes in the joint and the fibrotic granuloma lesions in the liver were prevented. The CSA dependent immunosuppression seems to be mediated by inhibition of the production of T lymphocyte-derived lymphokines including IL-2, IL-3, γInterferon (γ-IFN), and FAF. Since cyclosporin A appears to be a specific T cell inhibitor, blocking lymphokine synthesis at the transcriptional level, these studies provide direct evidence that the inflammation and subsequent connective tissue changes are T cell-mediated. Further exploration of these regulatory mechanisms has been facilitated by the receipt of an Arthritis Foundation Research Grant by members of the Cellular Immunology Section. In addition, our cyclosporin A studies in the rat have prompted the use of this drug in the treatment of severe, refractory human rheumatoid arthritis in ongoing collaborative efforts with the Arthritis and Rheumatism Branch, NIAMS, NIH.

In related studies, we examined whether CsA also inhibits production of GM-CSF by activated T lymphocytes. Although recent studies have shown that CsA inhibits the gene expression of some mitogen or antigen-induced lymphokines, this inhibitory activity of CsA appears to be selective and does not inhibit the expression of all inducible cytokine genes. The proliferation and differentiation of granulocyte/macrophage (GM) progenitor cells into mature granulocytes and macrophages in the bone marrow or in inflammatory lesions is regulated by the glycoproteins designated colony-stimulating-factor (CSF) including G-CSF and interleukin-3 (IL-3), also known as multi-CSF Our data show that while CsA completely inhibited the production of IL-3 and IL-2, it was ineffective in blocking GM-CSF production. In the presence of CsA, no IL-2 or IL-3 activity could be detected in the supernatants of a TPA-stimulated EL-4

thymoma T cell line. Anion exchange chromatography which separates IL-3 and GM-CSF confirmed the dissociation between GM-CSF and IL-3. Additonal support for the differential inhibitory effect of CsA was obtained at the molecular level. cDNA probes specific for murine GM-CSF and IL-2 were used to study the effect of CsA on GM-CSF and IL-2 gene expression. Northern analysis revealed GM-CSF and IL-2 mRNA within 8 hours after stimulation with TPA. However, in the presence of 0.5 µg/ml of CsA, no IL-2 mRNA was seen while GM-CSF mRNA was clearly detected. These data document the selectivity of CsA inhibition and indicate that the the gene expression of GM-CSF and IL-3 are not directly linked. These findings have potential significance not only in defining the molecular regulation of leukocyte maturation, but may also be therapeutically relevant since CsA is commonly used in organ transplantation and its use may not preclude CSF dependent repopulation of leukocytes.

Once activated by the mononuclear cell products, the synovial fibroblasts as well as activated macrophages produce the enzyme collagenase. Earlier work from this laboratory demonstrated that activated human monocytes produce this key enzyme through a prostaglandin-dependent mechanism. Since inflammatory lesions contain many biological mediators which may influence monocyte activation, current research efforts encompass exploration of the influence of these inflammatory molecules on PGE_2 and collagenase production. Since we have shown that human monocytes have specific receptors for IFN, the potential role of this immunomodulator on monocytes was initially evaluated. The addition of 10-1000 units/ml IFN-γ to monocyte cultures had no effect on either PGE_2 or collagenase synthesis. However, when γIFN was added together with con A, a dose dependent inhibition in both PGE_2 and collagenase production was evident. The inhibition of collagenase by γIFN could be reversed by the addition of exogenous PGE_2 or dβcAMP which demonstrates that IFN-γ regulation of collagenase production occurs through the arachidonic acid dependent pathway. In contrast to IFN-γ, another immunomodulator, recombinant human GM-CSF, independently stimulated both PGE_2 and collagenase synthesis by monocytes. The preliminary data suggest that GM-CSF may serve as an activating signal for monocyte enzyme synthesis and secretion which is consistent with in vivo observations. Furthermore, these observations can now be tested in situ to evaluate this possibility. The ratio of IFN-γ to GM-CSF may be very important in determining the extent of monocyte collagenase production at an inflammatory site.

The Clinical Immunology Section continues studies on the mechanisms involved in the secretion of inflammatory mediators from mast cells or basophils. The release of mediators from mast cells/basophils is an excellent model for studying immunological and biochemical aspects of cell secretion. These cells secrete two types of mediators, the preformed mediators (e.g. histamine, serotonin) and the secondary mediators which are synthesized following cell activation (e.g. products of arachidonic acid metabolism such as the leukotrienes). This Section has developed cultured rat basophilic leukemia cell lines which are very useful for studies of the biochemical events which occur during histamine release. The cells divide rapidly, grow attached to plastic and have surface IgG and IgE receptors similar to normal basophils. They contain histamine and serotonin, and although obtained from a tumor they have many similarities to normal mast cells or basophils. The cells are activated by crosslinking of the immunoglobulin receptors to release mediators such as histamine, serotonin, prostaglandins and other arachidonic acid products. The availability of large numbers of cultured rat basophilic leukemia cells allows direct biochemical study of the events which occur in cells following activation. Variants of the rat basophilic leukemia

cells have also been obtained which 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 variants of the rat basophilic leukemia cell line and also the biochemical changes which occur during the release of mediators.

The rat basophilic leukemia cell line (RBL-2H3) has high affinity IgE receptors ($Fc_\epsilon R$) on its surface. Four monoclonal antibodies were produced that inhibit IgE binding to the high affinity IgE receptor ($Fc_\epsilon R$) on rat basophilic leukemia cells. The four monoclonal antibodies (mAb) fall into two groups. The first group was comprised of 3 antibodies (mAb BC4, mAb CD3, and mAb CA5) that reacted with the $Fc_\epsilon R$ at epitopes close or identical to the IgE-binding site. With ^{125}I-labeled antibodies there was reciprocal cross-inhibition between the antibodies and IgE. The antibodies activated both RBL-2H3 cells and normal rat mast cells for histamine release. The 3 antibodies immunoprecipitated the α, β, and γ components of the receptor. The number of radiolabeled Fab fragments of 2 of these antibodies bound per cell was similar or equal to the number of IgE receptors. In contrast, the mAb BC4 Fab bound to twice the number of IgE receptor sites. Therefore, the portion of the $Fc_\epsilon R$ exposed on the cell surface must have two identical epitopes and an axis of symmetry. There 3 monoclonal antibodies recognize different but closely related epitopes in the IgE-binding region of the $Fc_\epsilon R$. The fourth monoclonal antibody (mAb AA4) had different characteristics. In cross-inhibition studies, IgE and the other 3 monoclonals did not inhibit the binding of this ^{125}I-labeled monoclonal antibody. The number of molecules of this antibody bound per cell was approximately 14-fold greater than the $Fc_\epsilon R$ number. This monoclonal antibody caused the inhibition of histamine release and it appears to bind to several cell components. Cloned variant cell lines with changes in IgE receptor number or mAb binding were selected after mutagenesis of the RBL-2H3 cells. Cloned lines with fewer than 10,000 $Fc_\epsilon R$ on their surface could release histamine following IgE-mediated stimulation. Using ^{125}I-labeled IgE or mAb, the binding characteristics of the cell lines were studied. The binding of mAb BC4 and IgE is highly correlated in these cell lines ($r = 0.94$) but mAb AA4 and IgE binding are poorly correlated ($r = 0.30$). Four cell lines (A4A1, A4C1, C4A2 and C5C6) were selected for further binding studies with ^{125}I-labeled mAb (BC4, CA5, CD3 and AA4). Two of these cell lines have a reduced number of IgE receptors (73 to 89% decrease) with parallel reduction in mAb BC4, mAb CA5 and mAb CD3 binding. All four cell lines have a low number of mAb AA4 binding sites (78 to 98% decrease). Therefore, there is independent variation of the AA4 epitope compared to the sites to which the other mAb's and IgE bind. Similarly, binding of ^{125}I-labeled dimeric IgG could not be correlated with IgE binding in these 4 cell lines. In the A4A1 cell line, K_1 for IgE binding was measured and found to be essentially identical to that of RBL-2H3. Furthermore, Scatchard plots of IgE binding to A4A1 and RBL-2H3 were parallel. Therefore, the low IgE receptor number is not due to differences in binding affinity. Inhibition of ^{125}I-IgE binding by mAb AA4 was studied in 2 cloned lines with reduced AA4 binding but with an IgE receptor number equivalent to RBL-2H3. In these 2 cell lines the inhibition curves were similar to the curve with RBL-2H3 cells. These data indicate that receptor-associated mAb AA4 binding sites are not preferentially lost on these variant cell lines. Therefore, variant cell lines have been selected which have a decreased number of IgE receptor or mAb AA4 binding sites.

Many cellular functions are modulated by phosphorylation of proteins. The IgE - or ionophore-mediated activation of rat basophilic leukemia cells (RBL-2H3) was accompanied by the prominent and rapid phosphorylation of a 92,000 Kd cellular component. The phosphorylation occurred prior to histamine release and correlated with the extent of secretion from the cells. Although the phosphorylation required the presence of Ca^{2+} in the medium, it was not inhibited by La^{3+} which blocks the stimulated entry of Ca^{2+}. Phosphorylation occurred in the absence of histamine or arachidonic acid release in 20 variant cloned cell lines with various defects in histamine release. Phosphorylation was also observed in variants which were defective in the antigen stimulated $^{45}Ca^{2+}$ uptake by the cells. Therefore, the stimulation of Ca^{2+} dependent kinase is an early event in the activation of the rat basophilic leukemia cells. In a further series of investigations, the cells were stimulated either through the IgE receptor or with the calcium ionophore A23187, and the total cellular proteins were analyzed by two dimensional gel electrophoresis. The pattern of changes in ^{32}P incorporation into polypeptides was similar following either antigen - or calcium ionophore-stimulation. At least 14 polypeptides demonstrated increased ^{32}P incorporation after activation with either antigen or ionophore. Twelve additional phosphorylated peptides were detected in stimulated cells that could not be seen in the absence of activation. There was one polypeptide that dephosphorylated after stimulation. Time course experiments revealed a similar overall pattern of phosphorylation with both secretagogues. Only 2 polypeptides (26 Kd and 92 Kd) failed to phosphorylate in the absence of extracellular Ca^{2+}. These results demonstrate the phosphorylation of a large number of polypeptides during the secretory event. It also indicates that the phosphorylation of most of these occurred in the absence of extracellular Ca^{2+} and preceded the rise in cytoplasmic Ca^{2+} concentration. There was a significant increase in a methionine-containing polypeptide 3 min after stimulation of the cells for histamine release. The apparent molecular weight of this polypeptide was 61 Kd and it had an approximate isoelectric point of 6.6. This increase was present after both IgE - and calcium-ionophore activation of the cells. This 61 Kd polypeptide might be related to the histamine secretory process.

Histamine secretion from rat basophilic leukemia cells (RBL-2H3) is accompanied by the release of arachidonic acid from cellular phospholipids, due to the activation of phospholipase enzymes. We observed the intracellular activation of phospholipase A_2 (PLA_2) during histamine release. The amount of PLA_2 activity in cell homogenates was dependent on the concentration of secretagogue used to activate the cells and was present in homogenates prepared 30 sec after cell stimulation and reached maximum between 5 and 10 min. The PLA_2 activation preceded histamine release, and occurred in the absence of Ca^{2+} in the extracellular medium, which completely inhibited release of arachidonic acid and histamine. However, the activity of the enzyme required Ca^{2+}. The PLA_2 activity in the homogenates and the extent of cell stimulation for histamine release were maximal at the same concentration of antigen, and both were blocked by the addition of monovalent hapten. The enzyme in the homogenates was capable of cleaving arachidonic acid from different phospholipids. These results demonstrate the activation of PLA_2 enzyme(s) in cellular homogentaes during the secretory process. The resulting production of lysophospholipids could play a critical role in histamine release from cells.

The source of arachidonic acid released after IgE or calcium ionophore A23187 stimulation has also been investigated. The 48-hour culture of the cells with [^{14}C]arachidonic acid resulted in labeling of the phospholipids to constant

specific activity. After IgE stimulation, the cellular [^{14}C]arachidonate released was predominantly from phosphatidylinositol (PI)/phosphatidylserine (PS) (66%), less from phosphatidylethanolamine (PE) (26%), and minimally from phosphatidylcholine (PC). In contrast, after ionophore stimulation the [^{14}C]arachidonate, was mostly from PE (55%) followed by about equal amounts from PS/PI and PC (24% and 20%, respectively). Therefore, the source of the released arachidonic acid depends on the stimulus. In contrast, the results are different when the cells are cultured for only 2 hours with [^{14}C]arachidonic acid. The stimulation of the cells with IgE or ionophore resulted in the release of the [^{14}C]arachidonate from PC (81% and 96%, respectively). This suggests the presence of several pools of phospholipids that are labeled at different rates and have variable proximity and/or accessibility to the phospholipase(s) enzyme(s) activated during cell secretion.

The IgE mediated histamine release from RBL-2H3 cells is associated with an increase in cytosol Ca^{2+} levels (Ca^{2+} signal) and substantial hydrolysis of membrane ionositol phospholipids due to phospholipase C activation. Several clones of the RBL-2H3 cell line showed varied responses to antigen that ranged in extent from undetectable (BUDR 1A3, 2B1 and 1B3) to about 80% of those in 2H3 cells (TG 2B6). The initial rate of response in the partially responsive clones was similar to that of 2H3 cells but the maximal response was reached earlier. In most of these clones, as in the 2H3 cells the Ca^{2+} signal and hydrolysis of the phospholipids were correlated. However, TG 1B3, which showed very little Ca^{2+} signal, still showed modest phospholipid hydrolysis and histamine release. Phospholipase C activity towards all inositol phospholipids was present in extracts and membranes of all the clones tested. Moreover, activity in the nonresponsive clones was 3 to 5 times higher than that in 2H3 cells. Studies with phorbol ester and Ca^{2+} ionophore also indicated the presence of protein kinase C activity in the 1A3 and 1B3 clones. These data point to no obvious defect in the genetic expression of enzymes involved in the inositol phospholipid cascade system in the nonresponsive clones but they may point to defects in the mechanism of activation of phospholipase C. The culture of cells with dexamethasone inhibited IgE- and ionophore- mediated histamine release. Prolonged exposure to the drug was required with maximal effect observed in 8-15 hrs. Dexamethasone blocked the IgE-mediated Ca-influx, the release of arachidonic acid, and the receptor-mediated phosphatidylinositol breakdown. Therefore, exposure of the cells to dexamethasone results in the inhibition of both phospholipase A_2 and phospholipase C pathways of arachidonic acid generation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE-0007-26 LMI

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Studies on the Regulation of Carbohydrate Metabolism in Oral Bacteria.

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

Wittenberger, Charles L.	Chief, Microbiology Section	LMI, NIDR
Schoen, Roberta A.	Biologist	LMI, NIDR

COOPERATING UNITS *(if any)*

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:
2.99	1.00	1.99

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.)*

One aspect of this investigation involves attempts to delineate the biochemical reactions involved in the inactivation and proteolytic degradation of a cell-associated fructosyltransferase (FT) produced by Streptococcus salivarius. We are focusing on the FT inactivation step because this reaction appears to 'mark' the enzyme for subsequent proteolysis. In an in vitro system, it has been demonstrated that a partially purified NADH oxidase is responsible for catalyzing a copper-dependent oxidative inactivation of FT. The reaction products of the NADH oxidase are hydrogen peroxide and superoxide anion. A second NADH oxidase has also been isolated that produces only water and this enzyme is completely ineffective in the in vitro inactivation of FT. Both superoxide and hydrogen perioxide are required in addition to copper for FT inactivation. Our results suggest that FT inactivation by these two dioxygen reduction products may occur in a site-specific manner by a metal-catalyzed Haber-Weiss reaction. We suggest that FT first binds cupric ions at or near the catalytic site. The bound cupric ions could then be reduced by superoxide anions and subsequently reoxidized by hydrogen peroxide with the generation locally of hydroxyl radicals. Hydroxyl radicals are most likely involved in the inactivation, since neither hydrogen peroxide nor superoxide anions alone have any effect on FT activity.

A second aspect of our current studies deals with the mechanism of activation of streptococcal lactate dehydrogenases (LDHs) by fructose 1,6-biophosphate (FBP). We showed previously that FBP mediates a conformational change in the enzyme which results in a marked increase in its affinity for substrate and coenzyme. We now find that FBP also affects the quaternary structure of these LDHs. A partially purified LDH from S. salivarius exists as a tetramer in the presence of FBP. When FBP was removed from the enzyme, it rapidly lost activity and was converted to a diamer. Addition of FBP back to the inactive diamer resulted in a restoration of activity that was accompanied by a return to the tetrameric form.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00034-18 LMI

PERIOD COVERED

October 1, 1985 to September 30, 1986

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	NIDR LMI
Hook, William A.	Research Microbiologist	NIDR LMI
Berenstein, Elsa H.	Microbiologist	NIDR LMI
Wu, Minjie	Visiting Fellow	NIDR LMI
Kitani, Seiichi	Visiting Fellow	NIDR LMI
Sublett, Suzanne	Microbiologist	NIDR LMI
Ogle, Rebecca	Biologist	NIDR LMI

COOPERATING UNITS *(if any)*

NHLBI, 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, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.00	3.00	3.00

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.)*

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 Ca^{2+} ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of 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 00042-16

PERIOD COVERED

October 1, 1985 - September 30, 1986

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 Lab 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, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.90	1.00	2.90

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project seeks to use genetic, biochemical and physiological approaches to investigate the pathogenicity of oral bacteria. The three specific areas of investigation are: 1) molecular cloning and characterization of genes encoding surface structures that mediate attachment of oral microbes, 2) characterization of plasmid-coded and chromosomal metabolic genes from oral bacteria and 3) development of systems for genetic exchange in oral bacteria. A gene encoding the structural subunit of the Type 1 fimbriae of Actinomyces viscosus was cloned into Escherichia coli using pUC13 as an expression vector. The coding sequences were subcloned into pUC13 on a 1.9 Kbp Pst I-BamH I fragment; the clone expresses the complete 65 Kdal fimbrial subunit. The gene encoding the synthesis of the Type 2 fimbriae was subcloned on a 2.5 Kbp Sma I fragment. Other subclones of both genes expressed truncated proteins corresponding to the N and C termini of the fimbrial antigens. M13 dideoxy sequencing of both fimbrial genes is in progress. Purification schemes for both cloned proteins have been developed. The plasmid coded β-galactosidase of L. casei was subcloned into E. coli using the plasmid expression vector pKK223-3. The 5.2 Kbp Hind III fragment also encoded sequences homologous to ISL1, a putative transposon. The β-galactosidase was purified to homogeneity and shown to be composed of two 70 Kdal and two 42 Kdal subunits. Factor III of the lactose PEP:PTS was cloned from pLZ64 into coli. M13 sequencing of β-galactosidase and Factor III is in progress. High frequency transformation of L. casei protoplasts was obtained using the β-galactosidase plasmid, pLZ15, encapsulated in liposomes. Transformants were detected as blue colonies on X-gal plates; unaltered pLZ15 supercoiled DNA was isolated from transformants.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00043-16 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Physiological and Genetic Studies on Pathogenic Oral Microoganisms

PRINCIPAL INVESTIGATOR *(List other professional 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)*

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:
2.00	.90	1.10

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

☐ (a1) Minors

☐ (a2) Interviews

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

To gain insight in the molecular architecture of Actinomyces viscosus fimbriae, recombinant DNA clones expressing genes for fimbrial proteins were sought. One plasmid (pAV1402) was identified in a cosmid gene library prepared in Escherichia coli from A. viscosus T14V DNA that expressed an antigen that reacted with polyclonal antibody against type 2 fimbriae. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that pAV1402 expressed an immunoreactive 59 kDa protein (p59) that comigrated with a component of partially dissociated type 2 fimbriae. Fimbriae labeled by radioiodination showed virtually the same pattern as fimbriae stained with several type 2 specific monoclonal antibodies after SDS-PAGE. These monoclonal antibodies also reacted with p59, which indicates that the structural gene for p59 (fimA) encodes a major repeating subunit of type 2 fimbriae.

Mapping and shotgun subcloning experiments with BamHI showed that fimA was contained within the 9.7-kb HindIII B fragment of pAV1402. This fragment was subcloned into pUC13. Expression of p59 was strongly orientation dependent, which indicates that the putative Actinomyces promoter for fimA has little or no activity in E. coli. Further mapping revealed that fimA expression from pAV1402 was depended upon the nearby P2 promoter of tet in the vector. Subsequent subcloning of the 9.7 kb HindIII fragment resulted in the identification of a 2.5 kb SmaI fragment that expressed p59. To raise expression, the SmaI fragment was cloned into the expression vector pKK223-3 to yield clone AV3463. p59 can be purified to homogeneity from IPTG-induced cultures of AV3463 by a series of steps including sonification, ultracentrifugation, DEAE-chromatography, gel filtration, and affinity chromatography.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00046-15 LMI

PERIOD COVERED

October 1, 1986 - September 30, 1986

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
Feldman, Gerald	NRC Research Associateship	LMI, NIDR
Hunt, Denise	Microbiologist	LMI, NIDR
Wahl, Larry	Research Microbiologist	LMI, NIDR
McCartney-Francis, Nancy	Microbiologist	LMI, NIDR
Dougherty, Susanne	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

R. Wilder, M.D., ARB, NIAMS; D. Yocum, M.D., ARB, NIAMS; I. Katona, M.D., USUHS; M. Sporn, LC, NCI; A. Roberts, LC, NCI; L. Wakefield, LC, NCI; A. Hand, LOP, NIDR

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:
5.67	1.95	3.72

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 monocyte chemotaxis to inflammatory stimuli. A newly identified chemotactic ligand is transforming growth factor beta (TGFβ), a 25,000 Mr peptide originally defined by its ability to induce transformation of nonneoplastic cells in culture. More recently, TGFβ has been shown to be a product of mononuclear cells and to induce the rapid formation of granulation tissue similar to that seen in tissue repair when injected subcutaneously. Our studies indicate that the mechanism of granulation tissue formation may be the consequence of TGFβ stimulation of monocyte directed migration at concentrations of 0.1-1.0 pg/ml. In additional studies, monocytes were shown to possess specific cell surface receptors which bind 125-I-TGFβ. At higher concentrations, TGFβ stimulates monocytes to generate growth factors which may account for the fibroproliferative and fibrogenic response associated with TGFβ injection.

Additional studies defining activation and regulation of monocyte function have revealed that activation of macrophages induces IL2 receptor gene expression. This IL2 receptor expression may have an important immunoregulatory function. In ongoing studies, we have demonstrated that once recruited and activated, mononuclear cells generate fibroblast activating factors which stimulate fibroblast proliferation. In an experimental animal model the injection of bacterial cell walls induces hepatic granuloma formation which progresses to fibrotic lesions. The fibrosis is T cell dependent and does not occur in athymic animals or animals treated with the T cell inhibitor, cyclosporin A. These studies provide insight into the cellular and molecular mechanisms regulating immune-mediated alterations in connective tissue metabolism.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00061-15 LMI

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Complement Activation and Inflammation

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

Sandberg, Ann L.	Chief, Humoral Immunity Section	LMI, NIDR
Mudrick, Linda L.	Microbiologist	LMI, NIDR
Cisar, John O.	Research Microbiologist	LMI, NIDR
Kurashima Chieri	Visiting Associate	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. Richard Calderone	Georgetown Univ.
Dr. A. E. Vatter	Univ. of Colorado

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.33	1.00	1.33

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 interactions of the oral actinomyces and Candida albicans with polymorphonuclear leukocytes (PMNs) and the complement system have been further defined. Actinomyces viscosus T14V and A. naeslundii WVU45 are destroyed by human polymorphonuclear leukocytes. Electron microscopy indicates that this process is attributable to phagocytosis. The lectin associated with the type 2 fimbriae of the actinomyces initiates the bactericidal activity by binding to carbohydrate containing receptors on the PMNs following exposure of these receptors by sialidase, an enzyme produced by the actinomyces. Mutants of A. viscosus T14V and A. naeslundii WVU45 lacking type 2 fimbriae are not phagocytosed. Bactericidal activity is inhibited by lactose and β-methylgalactoside but not by cellobiose or α-methylgalactoside. Antibodies reactive with the actinomyces type 1 or type 2 fimbriae as well as the cell wall stimulate phagocytosis in the absence of neuraminidase. Two antisera, one raised against the parent strain of A. viscosus T14V and one against the mutant lacking fimbriae are dependent on complement activation with the resultant deposition of the C3b/C3bi fragments of the third component of complement (C3) for optimal phagocytosis. Receptors for C3bi and a further degradation product of C3 (C3d) are present on the hyphal form of Candida albicans. This receptor, which has been identified by rosetting with C3d coated erythrocytes, is a mannosylated protein and has been partially purified by ion exchange and C3d affinity chromatography. One major and two minor bands are present in the acid eluate from the affinity column. Candida carrying C3bi or C3d may attach to the complementary receptors on other organisms with the resultant enhancement of the yeast population at a specific site. Studies concerning the cooperative effects of monoclonal antibodies in complement activation to define the relationship between the epitopes of the type 1 or the type 2 fimbriae of actinomyces are being extended. The distances between these epitopes are being measured by energy transfer between pairs of fluorophore conjugated Fab fragments of the monoclonal antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00216-10 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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)*

Wahl, Larry M.	Research Biologist	LMI, NIDR
Wahl, Sharon M.	Research Microbiologist	LMI, NIDR
Hojo, Hiroshi	Guest Worker	Sendi, Japan
Hojo, Sachiko	Guest Worker	Sendi, Japan
Lampel, LeRoy	Bio Lab Technician	LMI, NIDR
Kreutz, Marta	Chemist	LMI, NIDR

COOPERATING UNITS *(if any)*

I. Katona, M.D., USUHS, K. Eckels, M.D., Walter Reed, S. Klicks, Walter Reed, J. Sundeen, NCI, J. Cossman, 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, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.60	1.80	.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.)*

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 concentrated on the effect of biologically defined molecules such as recombinant interferon-γ (rIFN-γ) and recombinant granulocyte-monocyte colony stimulating factor (rGM-CSF) on the production of PGE2 and collagenase by monocytes. While the addition of rIFN-γ alone to monocytes had no effect on PGE_2 and collagenase synthesis, when added to Con A stimulated monocytes rIFN-γ caused a dose dependent inhibition of both products. Collagenase synthesis was restored in rIFN-γ treated cultures by the addition of exogenous PGE_2 which indicated that IFN-γ regulated collagenase production by its effect on arachidonic acid metabolism. In contrast to IFN-γ, our preliminary findings with rGM-CSF indicate that this molecule stimulates PGE_2 and collagenase synthesis by human monocytes.

Studies on the role of the immune system in bone resorption have focussed on our previous observation that spleen cells from osteopetrotic (op) rats have reduced proliferative capacity to mitogens when compared to normal littermates. Our recent experiments have shown that while IL-1 levels of spleen cell cultures from normal and op rats were similar, the IL2 levels of Con A or PHA stimulated spleen cells from op rats were decreased by 70%. However, the inability of op spleen cells to proliferate to Con A could not be overcome by the addition of normal spleen cells supernatants, purified IL 2 or arachidonic acid metabolite inhibitors. Based on monoclonal staining, similar percentages of suppressor and helper T cells were found in normal and op spleens. Thus, the immunosuppression in the op rats may be the consequence of decreased ability to produce IL2, a more efficient suppressor T cell population and/or non-T cell populations which suppress proliferation. Furthermore, these defects in immunologic responses may contribute to the inability of the op rats to regulate bone metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00254-09 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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	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:
3.30	2.30	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 spece provided.)*

Studies are continuing to define the fimbrial adhesins of Actinomyces viscosus, an oral bacterium that colonizes tooth surfaces, forms dental plaque and contributes to the initiation of gingivitis and periodontal diseases. The type 1 fimbriae of Actinomyces mediate bacterial adsorption to saliva treated hydroxyapatite, an in vitro model of the tooth surface. The host receptors recognized in this interaction appear to be the acidic proline rich proteins found in saliva and as constituents of the acquired salivary pellicle. The structural gene for a 65 kilodalton type 1 fimbrial subunit has been localized on a 1.9 kilobase fragment of Actinomyces DNA using recombinant DNA technology. The encoded protein reacts with several monoclonal antibodies against type 1 fimbriae and has been purified using an affinity column prepared with one of these. Rabbit antibody against the cloned fimbrial subunit also reacts with Actinomyces type 1 fimbriae. Type 2 fimbriae account for the cell-associated lectin activity detected by the lactose sensitive coaggregation of Actinomyces with strains of Streptococcus sanguis. Interactions of this type appear to be involved in interbacterial adherence and the formation of specific microbial communities within dental plaque. The gene for a type 2 fimbrial subunit has been localized on a 2.5 kilobase fragment of Actinomyces DNA. It encodes a 59 kilodalton protein which has been purified and reacts with several specific monoclonal antibodies directed against type 2 fimbriae. The involvement of the cloned type 1 and type 2 fimbrial subunits in receptor binding by each type of fimbriae is being examined. These studies have begun to provide a structural basis for understanding the mechanisms of oral microbial adherence.

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 DE00273-08 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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 professionel personnel below the Principal Investigator.) (Neme, title, leboretory, end institute affiliation)*

Kolenbrander, Paul	Research Microbiologist	LMI, NIDR
London, Jack	Research Microbiologist	LMI, NIDR
Donkersloot, Jacob	Research Microbiologist	LMI, NIDR
Andersen, Roxanna	Microbiologist	LMI, NIDR
Weiss, Ervin	Fogerty Visiting Fellow	LMI, NIDR
Hughes, Christopher	Dental Staff Fellow	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. A.L. Delisle, University of Maryland, School of Dentistry Baltimore, MD
Dr. L.V. Holdeman, VPI and SU, Blacksburg, VA.
Dr. C. Tylenda, University of Pittsburgh, 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:
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 stenderd unreduced type. Do not exceed the space provided.)*

As additional information regarding cell-to-cell recognitions among oral bacteria is discovered, it is becoming increasing 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. Both the actinomyces and streptococci, which are thought to be early colonizers of the tooth surface, and the gram-negative bacteria, capnocytophaga and bacteroides, are being studied. First, coaggregation-defective mutants were isolated and antisera to the respective parent strains were prepared. The antisera were absorbed with whole cells of mutants and the adsorbed antisera were used to detect surface proteins in the parent that were missing in the mutants. Monoclonal antibodies to the proteins of Bacteroides loescheii PK1295 were prepared and subsequently used to indicate that a 75kD and 46kD protein were involved in mediating coaggregation with Streptococcus sanguis and Actinomyces israelii.

Five bacteriophage from sewage and eight phage from human dental plaque, all of which infect A. viscosus, were compared. The five sewage phage and several human oral phage were indistinguishable morphologically. Two other human oral phage were much larger and very different by restriction endonuclease analysis of their respective DNA molecules.

Coaggregation between A. naeslundii PK606 and various streptococcal reagent strains that represent the six streptococcal coaggregation groups has been used as a model to study the relationship of these coaggregation mechanisms to those observed between other oral bacteria and these streptococcal reagent strains. Coaggregation-defective mutants of PK606 exhibit properties that are consistent with the model that four basic kinds of surface structures mediate coaggregation between the actinomyces and 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 00290-7 LMI

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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:
3.00	1.00	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 specificty. Hybridomas have been produced against the Fc$_\varepsilon$ receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

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 DE00341-05 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Regulation of Sugar Transport and Metabolism in Lactic Acid Bacteria

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

Jack Thompson	Visiting Scientist	LMI, NIDR
Jack London	Research Microbiologist	LMI, NIDR
Sally Z. Hausman	Microbiologist	LMI, NIDR
Michael A Curtis	Visiting Associate	LMI, NIDR
Stanley Robrish	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Henry Fales	LCH NMLBI
Stephen P. Miller	LCM, NMLBI

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:
1.45	.95	.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.)*

Two new amino acids have been discovered, isolated and purified from cells of Streptococcus lactis: N(5)-(1-carboxyethyl)ornithine and N(6)-(1-carboxyethyl) lysine. Confirmation of structure has been obtained by 1-H and 13-C nuclear magnetic resonance spectroscopy (NMR), mass spectroscopy and by chemical synthesis. Probable routes of in vivo synthesis of the two novel compounds, have been elucidated by radiotracer procedures and by thin-layer fluorography. The first detailed studies of sugar transport by the oral anaerobic microorganisms Peptostreptococcus anaerobius (Gram-positive) and Fusobacterium nucleatum (Gram-negative) have been conducted. Glucose transport by these bacteria is dependent upon the presence of specific amino acids in the extracellular environment. The biochemical basis for this unique amino acid dependency, and the modes of energy coupling to sugar accumulation have been established. The specificity of the glucose carrier in F. nucleatum has been determined by use of structural glucose analogs, and glycogen has been found to represent the major product of glucose uptake by the cell. These findings, obtained by use of [14-C]glucose and chemical analysis, have been elegantly confirmed by ultrastructural and morphological studies involving electron- and phase-contrast microscopy.

PHS 6040 (Rev 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00374-04

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Generation of GM-CSF by short-term cultures of murine bone marrow

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

Pluznik, Dov H.	Visiting Scientist	LMI, NIDR
Bickel, Matthias	Visiting Fellow	LMI, NIDR
Tsuda, Hiroyuki	Visiting Scientist	LMI, NIDR
Weedon, Lynda	Biologist	LMI, NIDR
Mergenhagen, Stephan E.	Chief, Laboratory of Microbiology and Immunology	LMI, NIDR

COOPERATING UNITS *(if any)*

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:

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 present report describes studies on: a) control mechanisms of GM-CSF production by EL-4 thymomoa T cells as compared to that of other lymphokines, and b) the regulation of differatiation of myeloid cells by G-CSF. EL-4 cells when stiumlated by mitogens produce several lymphokines like IL-2, IL-3 and GM-CSF. Cyclosporin A (CsA) inhibits the production of IL-2 and IL-3 but not that of GM-CSF. Supernatants from mitogen stimulated EL-4 cells were fractionated by anion exchange chromatography and in the absence of CsA two peaks of activity, representing GM-CSF and IL-3 were identified. In contrast, only a single peak of activity identified as GM-CSF was detected in the presence of CsA. In additional experiments, Northern blots of poly(A+)RNA isolated from mitogen stimulated EL-4 cells in the presence and absence of CsA were hybridized with GM-CSF and IL-2 cDNA probes. Expression of the GM-CSF gene was detected independent of CsA while the expression of the IL-2 gene was inhibited by CsA. These data suggest a different control mechanism for GM-CSF production than that for IL-2 and IL-3. G-CSF induces differentiation of Ml murine myeloid leukemia cells into mature granulocytes and macrophages and also causes an accumulation of the cells in the G_1 phase of the cell cycle. We examined therefore, whether synchronization of Ml cells in G_1 could have an effect on G-CSF-induced differentiation as quantitated by expression of Fc receptors (FcR) and lysozyme activity. Cells were arrested in early G_1 by density inhibition in the absence of serum and in late G_1 by aphidicolin. Cells synchronized in early G_1, when stimulated with G-CSF, showed an enhanced expression of FcR and lysozyme activity. Eighty percent of the cells expressed FcR 18 hours after addition of G-CSF, while in exponentially growing cells this percentage was reached 72 hours after addition of G-CSF. Cells synchronized in late G_1 did not show enhanced expression of differentiation markers. These results imply that with respect to G-CSF induced differentiation, G_1 phase can be dissected into an early permissive and a later non-permissive stage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00381-03 LMI

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters 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
Thornton, Angela	Federal Junior Fellow	NIDR CIPCB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	1.00	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.)*

Adherence of bacteria to saliva-coated surfaces, saliva-induced aggregation of bacteria and interactions between different genera of oral bacteria (coaggregation, interbacterial adherence) are considered important in the formation and persistence of dental plaque.

Changes in parotid saliva proteins after chronic isoproterenol treatment of rats have been associated with substantial decreases in the saliva-induced aggregation and in the adherence to saliva-coated hydroxyapatite of strains of Streptococcus mutans and Streptococcus sanguis. In contrast the aggregation and adherence of Actinomyces viscosus T14V were unaffected. Of the streptococcal strains tested only strains of S. mutans subspecies rattus were aggregated by saliva from isoproterenol- treated rats. Results of experiments that measured salivary protein adsorbed to hydroxyapatite and to bacterial cells suggest a role for an acidic proline-rich protein of Mr 40K in adherence.

Lactose-sensitive coaggregation between Propionibacterium acnes PK93 and Streptococcus sanguis DL1 cells bound to saliva-coated hydroxyapatite (SHA) took place at bacterial concentrations between those found in human saliva and 1000-fold higher. Over this range of cell concentrations P. acnes did not adhere to SHA. Adherence to SHA of heat-treated DL1 or S. sanguis 34 (neither of which form coaggregates with PK93) did not increase the binding of P. acnes to the SHA. These results and the inhibition of the SHA-associated coaggregation by lactose or N-acetylgalactosamine support the concept that bacteria unable to attach to saliva-coated surfaces can become established in plaque via lectin-mediated interactions with primary colonizers of the tooth surface.

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 00382-03

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Growth and Interaction of Oral Microorganisms

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

Robrish, Stanley A.	Research Microbiologist	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:
1.20	1.20	

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 sequence of use of amino acids and glucose has been investigated in pure culture studies using Fusobacterium nucleatum. Preferential use of glutamate and lysine before glucose has been documented in both continuous and batch culture for F. nucleatum. A fraction of the glucose was used at all dilution rates in continuous culture while all the glutamate and lysine were used at low dilution rates when the amino acids are limiting. When glucose and glutamate were added to a depleted medium in batch culture, no glucose was used until exhaustion of the glutamate. Incremental additions of glutamate to a batch culture of F. nucleatum, whose medium was depleted of glutamate but not glucose, resulted in growth and glucose use only following addition and use of the glutamate.

Glutamate dependent glucose utilization has also been shown with washed suspensions of F. nucleatum. Anaerobiasis was also necessary for glucose transport and of 18 amino acids tested, only glutamic acid, lysine, and histidine supported the transport of glucose. Studies with other hexoses and hexose analogues have shown that only glucose and galactose are transported in this manner. Both glucose and galactose were transported into a stable form in the cells which was not extractable with hot water but which could be extracted with hot alkali and precipitated with ethanol. This product appears to be glycogen when obtained from glucose grown cells. The nature of the polymer resulting from galactose use is unknown and under investigation.

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 00385-03

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Lectin Dependent Adherence of Actinomyces to Human Epithelial Cells

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

Brennan, Michael	Senior Staff Fellow	LMI, NIDR
John Cisar	Microbiologist	LMI, NIDR
Richard Joralmon	Natl. Res. Serv. Awardee	LMI, NIDR
Ann L. Sandberg	Chief, Humoral Immunity Section	LMI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Humoral Immunity Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.20	2.10	.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 Gal/GalNAc reactive lectin associated with the type 2 fimbriae of the oral actinomyces mediates the adherence of these bacteria to a number of mammalian cells including erythrocytes, epithelial cells and polymorphonuclear leukocytes. On eukaryotic cells the galactose-containing receptors for the actinomyces lectin appears to be masked by sialic acid since bacterial attachment to these cells occurs only after treatment with sialidase, an enzyme secreted by the actinomyces. A 160 Kd cell surface sialoprotein on a human oral epithelial (KB) cell line has been identified as a putative receptor for the actinomyces. Analysis using Gal/GalNAc specific plant lectins and a monoclonal antibody specific for Galβ3GalNAc indicates that the 160 Kd glycoprotein contains this carbohydrate sequence which is a potent inhibitor of actinomyces lectin mediated interactions. Certain glycolipids also contain the sequence Galβ3GalNAc and we have recently shown that the actinomyces bind to gangliosides containing this sequence and to globoside which contains a terminal GalNAcβ3Gal. Our studies show that both glycoproteins and glycolipids may serve as receptors for the actinomyces lectin and studies are in progress to define and purify the glycoconjugates on eukaryotic cells which interact with the actinomyces.

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 DE00386-03

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Role of Neuropeptides in Immune and Inflammatory Reactions

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

Michael R. Ruff Microbiologist LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. C. Pert, Clinical Neuroscience Branch, NIMH

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:
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.)*

Neuropeptides, small signal peptides largely known for their role as transmitters of nerve impulses in the brain which mediate mood and emotion, have now been shown to regulate immune system function. Our work reveals that human monocytes have receptors and will respond chemotactically to numerous neuropeptides. Neuropeptides which we have reported on include β-endorphin and other opiates, substance P and bombesin. We have shown that a major class of psychoactive drugs, the benzodiazepines, are also potent chemoattractants. In this case we have directly demonstrated the presence of chemotactic receptors through ligand binding experiments. The presence of multiple, diverse, neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

In addition to the presence of neuropeptide receptors we have also been able to demonstrate that human aveolar macrophages store and secrete the neuropeptide bombesin. Neuropeptide synthesis may therefore be a general feature of various immune cell populations. Although speculative, such results are consistant with the possibility of a multi-directional communication network via neuropeptides and their receptors. The purpose of such a network would be to link the body's cellular defense and repair systems with the nervous and endocrine systems and thereby integrate the internal millien of the entire organism.

Additional work has suggested that a major cause of human cancer, small cell lung carcinoma, may not, as previously thought, arise from lung epithelium but originates from hemopoietic cells when the normal macrophage mediated repair of lung tissue is deranged by continouous heavy smoking. This work represents a major conceptual shift in the understanding of this disease as well as other cancers in which there exists an in flammatory component and suggests novel strategies for disease control with clinical implications.

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, 1985 - September 30, 1986

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

Effect of HTLV-III and CMV on Monocyte/Macrophage Function

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

Smith, Phillip D., M.D.	Senior Staff Fellow	LMI, NIDR
Wahl, Sharon M., Ph.D.	Section Chief	LMI, NIDR
Wahl, Larry M., Ph.D.	Microbiologist	LMI, NIDR
Francis, Nancy, Ph.D.	Senior Staff Fellow	LMI, NIDR
Midura, Sharon B.	Microbiologist	LMI, NIDR
Allen, Janice B.	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Thomas Folks, Ph.D.	LIR, NIAID
Howard E. Gendelman, M.D.	LIR, NIAID
Scott Koenig, M.D., Ph.D.	LIR, NIAID

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.60	1.30	1.30

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), the etiologic agent in the acquired immunodeficiency syndrome (AIDS), and cytomegalovirus, which frequently causes life-threatening organ pathology in AIDS patients, induce impaired T cell function in vivo. However, whether macrophage dysfunction contributes to this immunosuppression is unknown. Therefore, the goal of this project has been to determine whether these viruses are capable of infecting monocytes and whether infected monocytes exhibit impaired effector and/or antigen-presenting function. The results obtained thus far indicate that both HTLV-III/LAV and CMV can infect human monocytes. In addition, virus-infected monocytes appear to have impaired cell functions including chemotaxis, interleukin-1 production, cytotoxicity and accessory cell activity. In addition, preliminary results indicate that HTLV-III/LAV (in vivo) and CMV (in vitro) induce maximal but suboptimal monocyte activation as determined by surface antigen expression and oxygen reactive intermediate generation. These findings strongly implicate virally induced impairment of monocyte function as contributing to the immunosuppression caused by HTLV-III/LAV and CMV.

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 00397-02 LMI

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Molecular mechanisms of fibroblast activation

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

Agelli, Maria	Visiting Fellow	LMI, NIDR
Wahl, Sharon M.	Chief, Cellular Immunology Section	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. M. Sobel, NCI

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

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

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

Previous studies in this laboratory have focused on the role of lymphocyte and monocyte products in modulating fibroblast growth and function. Unregulated production of these cytokines in certain chronic inflammatory lesions may be associated with pathologic disruption of normal tissue architecture as occurs in scleroderma and rheumatoid arthritis. In order to modulate these pathologic events it is necesary to define the mechanisms responsible. Therefore, our aim has been to study the molecular mechanisms of cytokine stimulation of fibroblast proliferation and collagen synthesis. The T cell derived fibroblast activating factor (FAF) has been characterized physicochemically and furthermore, messenger RNA for FAF obtained from activated T lymphocytes has been successfully translated in the xenopus oocyte translation system. The oocyte translation product is biologically and structurally indistinguishable from the FAF produced by the T cells. In additional studies, mononuclear cell-derived cytokine regulation of collagen synthesis has been studied. The protein synthetic data indicate that collagen production can be up- or down- regulated by different cytokines. FAF and IL1 appear to increase collagen production while γIFN inhibits synthesis. These studies are being extended to a molecular analysis of collagen synthesis using cDNA probes for pro α(I), pro α2(II) and (III) collagen chains in order to determine whether cytokine regulation is pre- or post-translational. If immunomodulators can be identified which significantly influence collagen synthesis, they may provide a target for modulating potentially pathologic sequlae in chronic inflammatory lesions.

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 00424-01 LMI

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

COOPERATING UNITS *(if any)*
Ildy M. Katona, M.D. Rhem. Sect. Dept. of Ped. Med., USUHS
F. Edward Hibert School of Medicine Bethesda, MD

LAB/BRANCH
Microbiology and Immunology

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.90	.90	1.00

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

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

This laboratory is involved in studies to describe the role of mononuclear cells in the inflammatory process and in various disease states. Experiments have been designed to study cytokine gene expression in normal and viral (HTLV-III) - infected human monocytes. Previous studies have demonstrated that monocytes from patients suffering from acquired immunodeficiency syndrome (AIDS) are defective in their ability to produce interleukin 1 (IL-1), a cytokine responsible for activating T lymphocytes. Our experiments indicate that the IL-1 gene is transcribed in lipopolysaccharide-stimulated monocytes from AIDS patients; the level of transcription varies between patients as compared to normal controls. In a few patients, high levels of IL-1 mRNA are detectable in unstimulated monocytes, indicating a spontaneous production of IL-1 message. In situ hybridization techniques are being used to expand these findings. Further studies will address post-transcriptional events which could account for the decreased IL-1 protein activity in AIDS monocytes. The functional role of the IL-2 receptor on the cell surface of activated monocytes is being assessed. Preliminary evidence suggests that IL-2 inhibits transcription of the IL-1β gene in activated monocytes. Whether this inhibition acts through the IL-2 receptor remains to be proven.

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- 0427-20 LMI

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Role of membrane associated molecules in the metabolism and ecology of oral bacteria

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

London, Jack	Microbiologist	LMI, NIDR
Tempro, Paulette	NRSA	LMI, NIDR
Weiss, Ervin	Visiting Fellow	LMI, NIDR
Kolenbrander, Paul	Microbiologist	LMI, NIDR
Hausman, Sally	Microbiologist	LMI, NIDR

COOPERATING UNITS *(if any)*

Dr. Harold Neimark, Downstate Medical Center, SUNY, New York, N.Y.
Dr. Roger Celesk, Miles Laboratories, New Haven, Conn.
Dr. Angelika Kagermeier, Poliklinik for Zahnerhaltung, Erlangen, West Germany

LAB/BRANCH

Laboratory of Microbiology and Immunology

SECTION

Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.90	1.40	.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.)*

The three neotype species of Capnocytophaga can be identified and classified by the specificity of the surface adhesins which are responsible for their coaggregation to various gram positive oral bacteria. The adhesin activities on the surface of Capnocytophaga ochracea were distinguished by sugar inhibition studies. Coaggregation between C. ochracea and strains of (1) Streptococcus sanguis, (2) Actinomyces viscosus - Actinomyces naeslundii and (3) Actinomyces israelii were inhibited by low concentrations of rhamnose (1mM), high concentrations of rhamnose (10mM) or combinations of rhamnose and sialic acid, respectively. Capnocytophaga sputigena coaggregates with the same strains of the A. viscosus - A. naeslundii cluster and A. israelii group as C. ochracea; these interactions are inhibited by high concentrations of rhamnose (10mM) or rhamnose and sialic acid, respectively. C. gingivalis coaggregates only with strains of A. israelii in a sialic acid sensitive interaction. Naturally occurring coaggregation defective mutants of C. ochracea were isolated which paralleled the phenotypes of C. sputigena and C., gingivalis, that is, the respective coaggregation patterns of these mutants and their sugar inhibition profiles corresponded perfectly with the two neotype species. The reciprocity exhibited by antisera prepared against C. ochracea or C. gingivalis in blocking coaggregation of both gliders with their respective partners indicates that the adhesins on these two organisms are not only functionally homologous, but that they may actually share a degree of structural homology as well.

Ribitol-5-P dehydrogenase was purified to homogeneity from ribitol-grown cells of Lactobacillus casei C116 and compared to the xylitol-5-P dehydrogenase from L. casei C183. This key component of the ribitol metabolizing pathway was found to be physically and kinetically distinct from the xylitol-5-P dehydroagenase. No immunological homology could be demonstrated between the two enzymes.

ANNUAL REPORT OF THE LABORATORY OF ORAL BIOLOGY AND PHYSIOLOGY

NATIONAL INSTITUTE OF DENTAL RESEARCH

The Laboratory of Oral Biology and Physiology conducts basic research on the structure and function of secretory cells and tissues, and on specific enzyme-catalyzed posttranslational protein modifications. The Laboratory is divided into two research groups: the Experimental Morphology Section, which employs structural, biochemical and immunological approaches to investigate the secretory process in the salivary glands and pancreas; and the Enzyme Chemistry Section, which focuses on the mechanisms of action and physiological significance of the transglutaminases and their products, and the formation, fate and function of the amino acid hypusine and the protein in which it occurs. Steady progress has been made during the past year on all ongoing research projects. Highlights of the Laboratory's efforts are presented below.

Experimental Morphology Section

Significant advances have been made during the past year in the development and application of immunocytochemical labeling procedures for the localization of specific proteins at the ultrastructural level. Employing the protein A-gold method on thin sections of plastic-embedded tissue, the distribution of both cellular and secretory proteins has been studied in different tissues. An interesting and suprising finding has come from the use of antibodies to the regulatory (R) subunits of cyclic AMP-dependent protein kinase. Cyclic AMP-dependent protein kinase is an important intracellular enzyme, which is activated by increased levels of cyclic AMP and serves to phosphorylate specific substrate proteins involved in a variety of cellular functions. In the rat parotid gland, cyclic AMP-dependent protein kinase is believed to be involved in regulation of exocytosis, but its exact role remains to be determined. Using polyclonal antibodies specific for type I and type II R subunits (obtained from R.A. Jungmann, Northwestern University) and monclonal antibodies specific for the type II R subunit (raised in collaboration with R.P. Siraganian, LMI, NIDR), both subunits were detected in the nuclei and cytoplasm of parotid acinar cells. In addition, predominantly RII, but also RI, were found in the content of the acinar secretory granules. This localization corroborates our previous finding that cyclic AMP-binding proteins are present in saliva, and establishes the acinar secretory granules as their source. When the monoclonal antibody was used to label other secretory tissues, it was found that the granules of several different exocrine and endocrine cells also contained the R subunit. Photoaffinity labeling of secretory fluids produced by some of these tissues confirmed that cyclic AMP-binding proteins are secreted by these cells.

Thus, the regulatory subunits of cyclic AMP-dependent protein kinase appear to be packaged and secreted by several different cells. It is unclear at present why this intracellular regulatory protein should be secreted, or what its function may be in the extracellular environment. An important question relates to the mechanism of synthesis of a protein which appears to function intracelluarly, but is also segregated and secreted along with other "typical" secretory proteins.

In other novel studies, the activity and distribution of cyclic AMP-dependent protein kinase was examined in salivary glands and cardiac muscle from rats flown on the NASA Space-Lab 3 mission. Altered endogenous protein phosphorylation, kinase activity and photoaffinity labeling of R subunits were observed in the parotid and sublingual glands. In the heart, endogenous protein phosphorylation was increased, while type II R subunits were decreased in the particulate fractions of males, but not females. Similar changes have been observed during simulated weightlessness and during chronic exposure to increased gravity. These studies may help in understanding the cardiac deconditioning observed during prolonged weightlessness.

Immunogold labeling has been used to study the localization and distribution of several secretory proteins in the salivary glands. Lingual lipase, a digestive enzyme produced by the serous lingual glands, was localized to the secretory granules of acinar cells of the gland, as well as the demilune cells of the lingual mucous glands. Two secretory proteins (B_1 and D) from the neonatal rat submandibular gland were found to be present in acinar and/or duct cells of several glands of the head and neck in both young and adult animals. Antibodies to B_1 (obtained from W.D. Ball, Howard University) and to amylase were used to show that after 24-72 hours starvation, secretory proteins are degraded in lysosomes of parotid acinar cells. These antibodies were also used to demonstrate that in normal rats, and especially in streptozotocin-diabetic rats, acinar secretory proteins are endocytosed from the lumen by cells of the intercalated and striated ducts. These findings substantiate the long-standing but previously unproved assumption that the duct cells of salivary glands are involved in salivary protein reabsorption.

Other studies in the section focused on the growth and function of exocrine gland acinar cells in vitro. In previous work, conditions for the growth of acinar cells in monolayer culture were developed. The differentiated characteristics of these cells have been examined this year. The cells maintain a population of secretory granules and endoplasmic reticulum cisternae, and form tight junctions with each other. Synthesis of specific proteins is maintained, albeit at a reduced level, and secretion of these proteins can be induced by treatment with secretagogues. This work establishes this in vitro system as a useful model for studies of exocrine cell function and regulation. In one such study, in vitro preparations of parotid acinar cells are being used to characterize the lysosomal system of the cell and its role in endocytosis. Two populations of lysosomes, which differ in their morphology and enzyme content, have been separated on Percoll gradients, One population, which corresponds to the basal lysosomes described several years ago, is labeled with exogenous tracers and thus appears to be involved in the initial stages of endocytosis, while the other population remains unlabeled. These initial studies suggest that the basal lysosomes may also function in receptor-mediated endocytosis, an important but little-studied process in exocrine cells.

Enzyme Chemistry Section

During the past year, work on elucidating the function and the mechanisms of biosynthesis and regulation of the unusual amino acid,

hypusine, and the single protein in which it occurs has continued. Through double labeling experiments it has been determined that hypusine formation from spermidine and lysine occurs through oxidative cleavage of spermidine producing an aldehyde which forms an enzyme-bound imine intermediate with lysine of the protein eIF-4D. Several inhibitors of various steps in hypusine formation, including inhibitors of spermidine synthesis and synthetic peptides resembling the structure around hypusine in eIF-4D, are being utilized to study the regulation of hypusine synthesis. New experiments are underway to determine the catabolic events associated with hypusine metabolism. Finally, significant advances have been made in molecular biology studies of the hypusine-containing protein. The gene for eIF-4D has been cloned successfully and its nucleotide sequence is now being determined.

Studies on transglutaminases have focused on basic aspects of their enzymology, substrates and products, as well as their functions in normal and pathological conditions. Synthetic glutamine peptides have been used to examine substrate specificity of the transglutaminases, and the results suggest that individual enzymes may have individual specificities. New information on the catabolism of γ-glutamylamines, the products of transglutaminase action, has shown that the enzyme γ-glutamyltranspeptidase is responsible for the degradation of γ-glutamylamines in the kidney, rather than γ-glutamylamine cyclotransferase, as previously thought.

The list of physiological substrates of the various transglutaminases continues to grow, and now includes polyamine-containing proteins in the liver, mast cell proteins to which histamine is covalently bound, the aminopropeptide of type III collagen, apolipoprotein B and VLDL, LDL and HDL, and certain proteins of saliva, particularly one of the proline-rich glycoproteins. In most cases, the functions of these transglutaminase catalyzed modifications are unknown, although it can be speculated that many of them involve regulatory aspects of membrane, cytoskeletal or extracellular matrix functions. For example, the incorporation of α_2-plasmin inhibitor into crosslinked fibrin by factor XIII reduces the degradation of fibrin clots by neutrophil proteases. In the oral cavity, transglutaminases are abundant, being secreted by the major salivary glands and derived from desquamating epithelial cells of the oral mucosa. Transglutaminase-mediated modifications of the proline-rich glycoprotein or other salivary proteins may contribute to the formation of the acquired enamel pellicle or to the layer of salivary protein bound to mucosal surfaces. Both of these adherent protein layers play significant roles in oral homeostatic processes and in the protection of oral tissues.

Reorganization Of The Laboratory

With the resignation of Dr. Arthur Hand as laboratory chief, the Laboratory of Oral Biology and Physiology will be under new leadership during the coming year. Dr. John Folk, Chief of the Enzyme Chemistry Section, will serve as Acting Chief of the laboratory. Reassignment and relocation of research groups are currently underway, and several renovations to the laboratories are planned for the next year. Personnel in the Experimental Morphology Section have been transferred to the Clinical Investigations and Patient Care Branch, and to the Laboratory of Microbiology and Immunology, and will be relocated in Building 10. The

Bone Cell Biology Section of the Bone Research Branch has become part of the Laboratory of Oral Biology and Physiology. The electron microscope and photography facility will remain under Dr. Hand's supervision as an Institute resource. These changes in the Laboratory are anticipated to better align current interests within the Institute and provide opportunity for the development of new research initiatives.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00001-34 LOBP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHERS:	Thacher, S.	Staff Fellow	LOBP NIDR
	Martinet, N.	Guest Researcher	LOBP NIDR
	Beninati, S.	Visiting Associate	LOBP NIDR
	Piacentini, M.	Guest Researcher	LOBP NIDR

COOPERATING UNITS *(if any)* Dr. B. Gamen, Dept. Chem., Baker Lab., Cornell Univ., Ithaca, NY: 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:
3.72	3.41	0.31

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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 00028-19 LOBP

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters 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)*

PI:	Hand, A.R.	Chief, LOBP	LOBP NIDR
OTHERS:	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
	Moreira, J.E.	Guest Researcher	LOBP NIDR
	Lotti, L.V.	Visiting Fellow	LOBP NIDR

COOPERATING UNITS *(if any)*

Ball, W.D., Dept. of Anatomy, Howard University, Washington, D.C.

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.23	1.92	1.31

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Basic mechanisms of the secretory process are studied in cells of the rat pancreas, salivary and lacrimal glands. 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) experimental pathology and lysosome function in salivary glands; (3) structure and permeability properties of junctional complexes in rat salivary glands; and (4) the role of salivary duct cells in protein reabsorption.

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 00049-15 LOBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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:	Cocuzzi, E.T.	Visiting Fellow	LOBP NIDR
	Kim, H.C.	Guest Researcher	LOBP NIDR
	Uchino, R.	Visiting Fellow	FDA

COOPERATING UNITS *(if any)*
Dr. M. Galanakis, SUNY, Stony Brook, NY; Dr. A. Janoff, SUNY, Stony Brook, NY: Dr. M. Lewis, DRS, NIH; Dr. F. Carmassi, Pisa University, Pisa, Italy

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:
2.92	2.81	0.11

CHECK APPROPRIATE BOX(ES)
☐ (a) 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 "temporary tissue matrix" (fibrin or fibrin-connective tissue) during tissue or bone fracture repair and in the modulation of specific cellular processes. The cross-linking of α2-plasmin inhibitor to fibrin plays a major role in stabilization of the "temporary tissue matrix" which is vital for the initial phase of cell migration and proliferation during tissue repair. A number of substances such as oxygen metabolites, sulfhydryls, and albumin in the plasma and tissue fluid can affect the catalytic activity of factor XIIIa and transglutaminases. We find that lipids and neutral detergents also play an important role in the modulation of factor XIIIa activity. Lipoproteins are shown to be capable of cross-linking in the presence of factor XIIIa and cellular transglutaminase. A possible role of transglutaminase in oral physiology is being investigated. The ductal saliva from rat parotid and submandibular glands following isoproterenol stimulation contains both transglutaminase and proteins that are modified or crosslinked by transglutaminase. Transglutaminase in whole saliva is also found to be derived from the epithelium of the oral cavity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00199-10 LOBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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	LOBP NIDR
OTHERS:	Hart, T.	Visiting Fellow	LOBP NIDR
	Kleinman, H.	Research Chemist	LOBP NIDR
	Zhang, W.	Visiting Fellow	LOP NEI

COOPERATING UNITS *(if any)*
Dr. A. Robbins, GB, NIADDK

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.31	2.38	1.93

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.)*

Secretory and endocytic processes in several cell types are currently under investigation. Cell dissociation and short term culture (up to 1 month) methods have been established for rat exorbital lacrimal, parotid and pancreatic acinar cells. These cultures 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.

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 00285-07 LOBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclic AMP-dependent Protein Kinase: Subunit Distribution in Mammalian Cells

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

PI:	Mednieks, M.I.	Senior Staff Fellow	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Cheng, L.F.	Visiting Fellow	LOBP NIDR
	Siraganian, R.P.	Chief, Clinical Immunology	LMI NIDR

COOPERATING UNITS *(if any)* R.A. Jungmann, Northwestern Univ., Chicago, IL; D. Kelley-Geraghty, Northwestern Univ., Chicago, IL; A. Fine, NY Vet. Admin. Hospital, New York; D.E. Philpott, NASA Ames Res. Ctr., Moffett Field, CA; R. Grinland, NASA Ames Res. Ctr., Moffett Field, CA; K. Catt, ERRB, NICHD, Bethesda, MD.

LAB/BRANCH
Laboratory of Oral Biology and Physiology

SECTION
Experimental Morphology Section

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.46	1.13	1.33

CHECK APPROPRIATE BOX(ES)

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

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

Cellular events resulting from hormonal, pharmacologic and environmental stimuli and which are mediated via the action of cyclic AMP-dependent protein kinase (ATP: protein phosphotransferase, E.C. 2.7.1.37) were studied in various tissues. Photoaffinity labeling of protein kinase regulatory subunits was employed in determining stimulus-dependent redistribution of isoenzymes in subcellular fractions. Intracellular localization of protein kinase regulatory subunits was established using EM immunogold labeling on thin sections and quantitated by morphometric procedures. Distribution of the regulatory subunits of type II protein kinase in stimulated parotid and other secretory cell types was determined by the EM immunogold method using monoclonal antibodies to type II regulatory subunit. We have previously shown that protein kinase regulatory subunits are components of saliva. Their presence was directly demonstrated in secretory granules of parotid acinar cells, in cells of the seminal vesicles, exocrine and endocrine pancreatic cells, and in pituitary and intestinal endocrine cells. These findings were verified by the presence of 8-azido-cyclic AMP-labeled proteins (R subunits or their proteolysis products) in the secretory fluids of salivary glands, seminal vesicles and the pancreas. It appears, therefore, that protein kinase regulatory subunits are secretory proteins in addition to serving their intracellular roles.

Immunogold localization was employed to determine the effects of stimulation with FSH on regulatory subunit distribution in ovarian granulosa cells. Photoaffinity labeling was used to determine the distribution of isoenzymes in rat heart muscle from animals flown in the NASA Space Lab-3 and in monkey and rat cardiac tissues from simulated reduced gravity or increased gravity experiments. Gingival tissues were used to test the effects of wound healing on protein kinase activity and distribution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00311-06 LOBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

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:	Folk, J.E.	Chief, Enzyme Chemistry Section	LOBP NIDR
OTHERS:	Park, M.H.	Senior Staff Fellow	LOBP NIDR
	Beninati, S.	Visiting Associate	LOBP NIDR

COOPERATING UNITS *(if any)*
Dr. H.L. Cooper, NIC
Dr. B. Ganem, Dept. Chemistry, Baker Lab., Cornell University, Ithaca, NY
Dr. D.T. Chuang, Cleveland Veterans Administration Medical Center, Cleveland, OH

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:
2.81	2.00	0.81

CHECK APPROPRIATE BOX(ES)
☐ (a) 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 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.

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 00376-03 LOBP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Secretion, Purification, Antibody Production of Enzymes from Lingual Serous Glands

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

PI:	Field, R.B.	Staff Fellow	LOBP NIDR
OTHERS:	Hand, A.R.	Chief, LOBP	LOBP NIDR
	Chung, S.I.	Research Chemist	LOBP NIDR
	Dromy, R.	Visiting Fellow	LOBP NIDR

COOPERATING UNITS *(if any)*

Mr. Robert A. Boykins, FDS, Division of Biochemistry and Biophysics, Bethesda, Maryland

LAB/BRANCH

Laboratory of Oral Biology and Physiology

SECTION

Experimental Morphology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.78	2.22	0.56

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 order to investigate the secretory process in the lingual serous (von Ebner's) gland of the rat tongue, we are studying the effects of cholinergic and β-adrenergic agonists and antagonists and the adenylate cyclase activator, forskolin, on the secretion of lingual lipase and amylase. The amount of secretion is determined by in vitro incubation of the dissected glands with the agonists. The tissue and medium are assayed for lipase and amylase activity and portions of the tissue are taken for light and electron microscopy.

Lingual lipase and amylase were purified from von Ebner's gland. Antibodies to the purified lingual lipase were prepared and used to localize the enzymes by fluorescence and protein A-gold techniques.

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

ANNUAL REPORT OF THE LABORATORY OF ORAL MEDICINE,
NATIONAL INSTITUTE OF DENTAL RESEARCH

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

Studies have continued on the projects described in last year's annual report and new projects have been initiated. Our work on a vaccine to Herpes Simplex Virus (HSV) showed that the recombinant vaccinia-HSV vaccine protected animals for well over a year against lethal HSV infection, but protection against a latent infection was not quite as good as at four months after vaccination. As reported last year, a model for studying the reactivation of HSV in humans was developed using UV light. A double blind clinical study has now been initiated and the effect of drugs such as acyclovir on preventing UV-induced reactivation is being evaluated. Over the last year we have succeeded in establishing in our laboratory the techiniques for making transgenic mice. A major thrust will be put into this area over the next year. We have also been successful in constructing expression vectors and have introduced genes (e.g., HSV) into different continuous cell lines. We now plan to "engineer" other cells with genes coding for autoantigens. In addition, we have developed a new method for making human monoclonal antibodies of predetermined specificity. This involves the binding of biotinylated antigens to B lymphocytes with specific receptors for those antigens, isolating the B lymphocytes to which the biotinylated antigens bound by fluorescein activated cell-sorting and immortalizing these cells with Epstein-Barr Virus so that they continuously make monoclonal antibodies of the predetermined specificity. This method may prove useful to other investigators.

Over the last year a number of new techniques have been introduced into the laboratory (see below) and are making it possible to do experiments that were undreamed just a short time ago. These rapid changes in technology mean that we must be constantly retraining people in our laboratory and recruiting new people who have had some exposure to these latest techniques. Over the next year we will try to broaden our base of experience and recruit people to work on 1) the preparation of transgenic mice, 2) the construction of expression vectors to engineer eukaryotic cells and 3) the preparation of human monoclonal antibodies of predetermined specificity.

Since last year, a number of new techniques were introduced into the laboratory and existing ones modified. Specific techniques include: (1) biotinylation of cloned DNA probes for in situ hybridization; (2) expression of specific viral genes in cells by co-transfection techniques; (3) nucleic acid gel electroelution blotting; (4) lambda gt11 cDNA cloning; (5) quantitation of mRNA with SP6 and SP7 RNA polymerase generated riboprobes (6) establishment of transgenic mice by means of nuclear microinjection of early embryos; (7) microinjection of somatic cells in vitro; (8) use of biotinylated EBV in binding studies to human peripheral blood mononuclear cells in single and double fluorescence studies by flow cytometry; (9) preparation of pure human B lymphocytes by Percoll(R) gradient and/or "passive rosetting"; (10) sorting of antigen specific B lymphocytes from whole human peripheral blood B cells by biotinylated antigen and FITC-avidin flow cytometry. Selection of B lymphocytes bearing surface immunoglobulin of different isotypes by anti-IgG, IgA or IgM fluorescent probes; (11) production of human monoclonal antibodies by fusion of immortalized EBV-B blasts with F3B6, a human-mouse non-secretor heterohybrid fusion partner; (12) preparation of mRNA from hybridoma lines utilizing the lithium chloride method; (13) primer extension of hybridoma mRNA for cDNA synthesis; (14) sequencing of the cDNA copies of hybridoma mRNA using Maxam-Gilbert sequencing methods; (15) slot blots and Northern blots of cytoplasmic RNA isolated from lysates of hybridoma cells; (16) biotinylated enzymes as probes of macrophage subpopulations and function; (17) southwestern blotting analysis of DNA binding proteins; (18) size exclusion HPLC coupled with SDS-PAGE-Western blotting-immunostaining for purification of autoantigens; (19) preparation and labelling of cDNA probes for a number of lymphotropic viruses including HTLV-I, II, III, EBV and CMV; (20) in situ hybridization; (21) restriction endonuclease analysis of clinical isolates of HSV; (22) development of a nasal challenge model for the study of HSV infection in the mouse; (23) microinjection of primary pancreatic islet cell cultures with oncogene-containing plasmids in attempts to immortalize beta cells; (24) construction of genomic libraries using the new lambda vectors EMBL3 and EMBL4; (25) transfection with the eukaryotic cloning vector pSV2gpt and selection of stable transformants with mycophenolic acid containing HAT medium; (26) Southern blot hybridization with RNA probes generated SP64 and T7 RNA polymerases; (27) analysis of the transcriptional status of genes using riboprobes which will allow detection of ribosomal RNA at the pg 0.1 level; (28) sib selection protocol for isolation of cloned genes.

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) expression of glycoprotein B gene of HSV-1 (City of Hope Medical Center, Duarte, California); (3) studies on spontaneous lymphocyte transformation (NIAMDS); (4) evaluation of cross-reactivity between viruses and normal cells (Scripps Clinic and Research Foundation, La Jolla, California); (5) lymphocytic choriomeningitis virus-induced thyroiditis (Scripps Clinic and Research Foundation, La Jolla, California); (6) slow viruses and diabetes (NINCDS); (7) long-term complications of EMC virus-induced diabetes (LDBA, NIDR); (8) identification of myositis and SLE autoantigens (NIDDKD); (9) production of transgenic mice carrying constructs of collagen II and laminin gene regulatory sequences (LDBA, NIDR); (10) characterization of lymphocyte subsets by monoclonal antibodies (The Wistar Institute of Anatomy and Biology); (11) characterization of antibodies to human thyroglobulin and microsomes (Mount Sinai School of Medicine); (12) preparation of human monoclonal antibodies (Cetus Corporation, Palo Alto, California); (13) binding studies of autoantibodies and sticky IgM's (NCI); (14) T cell receptor beta chain polymorphism in IDDM patients and correlations with the HLA DR types (Mount Sinai School of Medicine and University of Texas Health Center); (15) determination of the structure of antigens recognized by human or rat monoclonal autoantibodies (University of Pennsylvania and NIDDKD); (16) viruses and autoimmunity (Mount Sinai Hospital, New York); (17) studies on cross reactivity of anti-coxsackievirus antibodies with myocardium (Johns Hopkins); (18) studies on coxsackievirus involvement in diabetes (St. Thomas's Hospital, London); (19) antigenic variants of coxsackievirus (University of Commonwealth of Virginia, Richmond); (20) probing for HTLV genomes in autoimmune diseases (NCI); (21) monoclonal antibodies to osteonectin and osteogenin (BRB, NIDR). (22) vaccinia recombinants and immunity to herpes simplex virus (NIAID). (23) UV induced reactivation of HSV and treatment (NIAID); (24) investigation of role of DNase I hypersensitive regions of chromatin in chemical carcinogenesis (LEP, NCI); (25) investigation of the effect of cloned oncogenes on the growth and transformation of primary mouse keratinocytes (LEP, NCI); (26) molecular cloning of Alzheimer amyloid gene (LCNSS, NINCDS); (27) cloning and characterization of autoantigens of Graves disease by immunoassay of lambda gt11 cDNA library from human thyroid carcinoma (LBM, NIDDKD).

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

I. HERPES SIMPLEX VIRUS AND OTHER PERSISTENT INFECTIONS

1. One of the most exciting developments this past year concerns a new area of research in our laboratory - transgenic mice. We microinjected mouse embryos with the glycoprotein D gene of herpes simplex virus (HSV gD) and showed that the resulting animals carried HSV gD sequences. The molecular biology and possible pathogenic role of these HSV sequences will be studied over the next year.

2. Expression of Herpes Simplex Virus gD glycoprotein in tissue culture cells. The gene coding for HSV gD, one of the major viral surface glycoproteins, has been placed under the regulation of two different enhancer-promotor sequences; one is the early region of SV40 virus, used for generalized expression in cells of all lineages; the other derives from the rat insulin I gene, and is used for tissue-specific expression in cells of pancreatic beta-cell origin. Using eukaryotic expression vectors these constructs have been introcuded into NIH/3T3 cells and RINm5f cells (a rat insulinoma cell line). Expression of gD on the surface of transfected cells and stable integration of the viral gene has been achieved. These results open up a number of avenues of research into the molecular biology and immunology of HSV infections.

3. Herpes Simplex latency in the central nervous system of mice. One of the major open questions in the molecular mechanisms of HSV latency is the identification of latency-associated genes, i.e., viral genes continuously expressed during latency. Characterization of such genes, should they prove to exist, would provide new insight into the mechanisms of establishment of latency, on the one hand, and therapy of the latent infection, on the other. Using cDNA probes from trigeminal ganglion RNA of HSV-infected mice we have identified a gene or subset of genes transcribed into mRNA throughout the latent phase of the infection; in fact, these gene(s) are expressed throughout the life time of latently infected mice. The gene(s) map in the UL region of the UL-IRL boundary of the viral genome, is a region largely uncharacterized at the transcriptional level. We are presently cloning cDNA copies of these transcripts to further characterize their expression at the molecular level.

4. A vaccine against HSV. During the last year we have continued our studies on the efficacy in mice of a recombinant vaccinia virus vaccine expressing the herpes simplex (HSV) type 1 glycoprotein D gene. In humans, primary infection with herpes simplex virus is often followed by the establishment of latent infection in the sensory ganglia of the affected dermatome. Because these latent infections can periodically reactivate to cause recurrent disease, vaccines are needed that can protect against both primary and latent HSV infections. As

noted previously, infectious vaccinina virus recombinants that contain and express the gene for HSV type 1 glycoprotein D were constructed by colleagues in NIAID. Mice immunized with this recombinant virus were protected against lethal challenge with both HSV type 1 and type 2. The majority of immunized mice were also protected against the development of latency in the trigeminal ganglia when challenged with HSV-1 by the lip route. Over the past year, we have demonstrated that substantial but somewhat decreased protection persists beyond one year post vaccination with the recombinant virus vaccine. Booster doses of the same gD recombinant result in increased levels of neutralizing antibody and improved protection against lethal intraperitoneal challenge with HSV-1. Previous vaccination with another vaccinia recombinant (vaccinia hepatitis or vaccinia flu) prior to vaccination with vaccinia gD results in decreased levels of neutralizing antibody and decreased protection against lethal HSV challenge. The effect of booster doses of vaccinia gD recombinant on protection against the development of latent infection is now under study.

5. We have developed a manipulable model of reactivation of HSV in humans. Using ultraviolet light to induce a "sunburn" as a stimulus for reactivation, we found that on 60% of the occasions patients who were exposed to UV light in an area of frequent recurrences developed a recurrence at the site of UV-exposure. Using this model we are now conducting a double blinded study to examine the ability of the antiviral agent acyclovir to block UV light-induced reactivation of HSV.

6. Mice infected with lactic dehydrogenase virus (LDV) develop a lifelong five- to ten-fold increase in plasma lactate dehydrogenase (LDH). The increase in enzyme level is due to impairment of enzyme clearance. Since its discovery in the early 1960's, LDV has remained unique in being the only model where the elevation of an enzyme is due to impairment of its clearance. Over the last year, we showed that mice injected with silica also develop an increase in plasma LDH and that the enzyme elevation is due to impairment of clearance. Examination of the factors that regulate enzyme levels in normal mice showed that, whereas there was no difference in resting enzyme levels among inbred strains, when mice were stressed by the administration of an enzyme load, certain of the inbred strains cleared the enzyme rapidly (Balb/cAnN) and others (B10.D2/nSnNJ) slowly. Moreover, in B10.D2/nSnNJ mice, enzyme clearcance was age-related. When the rapid clearers and slow clearers were injected with LDV and/or silica, LDH levels were substantially higher in the slow enzyme clearers. It is concluded that both genetic and environmental factors influence the clearance of LDH and that impairment of enzyme clearance may be a more important factor than previously suspected in regulating enzyme levels in disease states.

II. AUTOIMMUNITY

A) Triggers of Autoimmunity

1. Autoantibodies are found in a number of important human diseases. What initiates the autoimmune response is not known. One of the possibilities is that a viral infection could trigger an autoimmune response by acting on effector cells of the immune system. Previous studies in our and other laboratories showed that human B lymphocytes immortalized in vitro by infection with Epstein-Barr virus (EBV) make autoantibodies. Over the last year we have isolated spontaneously proliferating human B lymphocytes from peripheral blood of autoimmune patients and normal controls. This outgrowth appears to be caused by latent Epstein-Barr virus (EBV) infection. Most of the cell lines which were derived secreted immunoglobulins and about one-third produced autoantibodies when screened by immunofluorescence against a bank of eight normal organs. Many of the autoantibodies reacted with antigens in more than one organ, and most commonly reacted with smooth muscle and epithelium. All of the autoantibodies except one were of the IgM class.

This work supports the argument that transformation of cells by EBV is an explanation for the transient autoantibodies seen in diseases such as infectious mononucleosis. Since the majority of the adult population carry EBV in a latent form, immunoregulatory abnormalities resulting in the proliferation of EBV-transformed cells could lead to the production of autoantibodies. This might explain the autoantibodies seen in patients on immunosuppressive drugs and in patients with diseases such as acquired immunodeficiency syndrome and malaria. EBV thus appears to be a good candidate to explain at least the transient appearance of autoantibodies associated with certain immunoregulatory abnormalities.

2. "Molecular mimicry" may be another mechanism by which viruses trigger an autoimmune response. The idea is that antibodies raised against certain viral antigens may react with normal host cell antigens. Earlier we showed, using over 600 monoclonal antiviral antibodies made against 11 different viruses, that approximately 4% cross-reacted with cells in specific organs. Over the last year, we screened over 200 of these monoclonal antibodies against human T-cell surface antigens. Six of these monoclonal antibodies reacted with human T-cell surface antigens and all six were directed against measles virus. This might be one of the explanations for the immunological abnormalities associated with measles virus infection.

3. MOR-h1 is a human multiple organ-reactive (MOR) monoclonal autoantibody (Ab1) that reacts with human growth hormone (hGH) and a 35 kD protein found in anterior pituitary, thyroid, stomach, and pancreas. 4E6 is a mouse monoclonal anti-idiotypic antibody (Ab2) that

reacts with the paratope of MOR-h1 and is ligand inhibitable. Both of these antibodies were described in detail in past Annual Reports. This last year we made an antibody to 4E6 (Ab3) in rabbits. This antibody is of the IgG class. By competitive inhibition and immunofluorescence experiments using Ab3, the 4E6 paratope (Ab2) is found to have a conformational resemblance to an epitope on hGH and the 35 kD protein. This raises the possibility that antibodies made in response to certain anti-idiotypic antibodies may be one of the mechanisms for triggering an autoimmune response. If this turns out to be the case it is conceivable that clonal proliferation of a single anti-idiotypic antibody could trigger an autoimmune cascade.

B) Expression of Autoantigens and Properties of Autoantibodies

1. Making human monoclonal antibodies. As described last year, selection of antigen specific B lymphocytes by FACS and their immortalization by EBV has allowed for the preparation of human momoclonal IgM antibodies with predetermined specificity from peripheral blood B cells of healthy subjects. In addition, sorting of IgG lymphocytes, immortalization by EBV and their selection in limiting dilution have now allowed for the preparation of IgG with predetermined specificity. This development should have broad application.

2. Studies on EBV-lymphocytes interaction. Biotinylation of purified EBV has allowed for characterization of its reactivity with human peripheral blood mononuclear cells. It has been established that EBV binds only to B cells, not T cells, NK cells, dendritic cells or monocytes. All B cells bind and are infected by EBV. The optimal target of EBV is constituted by a resting B cell. Activated B cells bind EBV and become immortalized with an efficiency similar to that of resting B cells. In contrast, EBV cannot bind to and immortalize activated and proliferating B cells. This approach provided visual evidence of EBV binding to B cells and likely constitutes a general model for studying the interaction between other viruses and the surface of target cells.

3. Identification of autoantigens. To investigate the autoimmune pathogenesis of spontaneously occuring diabetes mellitus in BB rats, spleen cells of newly diagnosed dibetic BB rats were fused with mouse myeloma cells. Hybridomas were screened by indirect immunofluorescence and by ^{51}chromium release assays using the RINm5F rat insulinoma cell line. One clone, E5C2, produced an IgM(k) antibody which was cytotoxic for RINm5F cells, but not for other rat cell lines nor for primary rat islet cells. However, treatment of primary rat islet cells with neuraminidase exposed this surface antigen and rendered the cell susceptible to complement-mediated lysis by E5C2. E5C2 also bound to normal monkey and human islets, after neuraminidase treatment. Using immunostaining of glycolipids separated by thin-layer chromatography, hapten inhibition assays with defined carbohydrates, and Western blots, the antigens recognized by E5C2 on RINm5F cells were identified as

glycoproteins with molecular weight of 60 and 68 kilodaltons. The antibody recognizes a carbohydrate antigen containing the sequence Galβ1-4GlcNAc-R, which on RINm5F cells is predominantly hidden by covalently bound sialic acid. These studies raise the possibility that hidden antigenic determinants on islet cells exposed by a variety of means may be the target for autoimmune attack.

III. DIABETES AND ONCOGENES

1. The isolation of transforming genes from human beta cell tumors (i.e., insulinomas) has never been reported. Since beta cells are terminally differentiated endocrine cells, it is of particular interest to determine whether a certain type of oncogene is associated with the proliferation of this normally non-dividing cell type. Transforming genes from a variety of human tumors and tumor cell lines have been detected with the classical 3T3 cell transformation assay. Last year transfection of 3T3 cells with genomic DNA from two human insulinomas failed to produce transformed foci containing human DNA sequences. However, using a more sensitive bioassay, the same two insulinoma DNAs induced 3T3 cell tumors in nude mice following cotransfection with pSV2-neo and selection for neo positive colonies. Since relatively large amounts of human donor DNA were integrated in the 3T3 cell tumors, a second round of co-transfection and tumorigenesis was required to segregate the excess human DNA from the putative transforming sequences. All secondary tumors from the human insulinomas show hybridization with the BLUR 8 probe for human repeated sequences. Genomic libraries of secondary transformants of one of the insulinomas were constructed in the lambda vector EMBL3. Five lambda clones which contain human DNA were isolated by filter hybridization to total human genomic DNA and the BLUR 8 probe. These phage are currently being tested for tumorigenicity with the nude mouse assay to determine whether any of them contain the complete active oncogene.

2. In a search for genes essential in beta cell function we have focused on the superoxide desmutase (SOD) gene. This enzyme is thought to protect cells against free-radical damage which is thought to occur in diabetes. To study the regulation of SOD gene expression and eventually manipulate at will this gene under a variety of experimental conditions, we have isolated and sequenced cDNA clones of the rat Cu/Zn dependent SOD, and have used these clones to map the rat genomic sequences coding for this enzyme. Rat Cu/Zn SOD is a single copy gene which is transcribed into an mRNA species of approximately 800 bases. The coding sequences of hunman and rat SOD are 83% homologous, although the extent of homology drops to 66% in the 3'-untranslated region. The predicted rat SOD amino acid sequence is very similar to that of other eukaryotic SODs, showing 70% homology with the SODs of other mammals. Sequence conservation is particularly high in domains believed to be of

functional importance. We have tentatively mapped a DNase hypersensitive site to an intron within the gene. We also plan to study its transcriptional status in insulin-producing cells.

3. Virus induced diabetes; slow viruses. It has been known for some time that lesions in certain areas of the brain also can result in hyperglycemia. This raised the possibility that viral infections of the central nervous system might directly or indirectly cause glucose abnormalities. In order to investigate this possibility, we inoculated a slow virus, scrapie, intracerebrally into six-week old female Golden syrian hamsters. In addition to the subacute fatal spongiform encephalopathy with characteristic vacuolation in the brain, the infected animals showed abnormal glucose tolerance tests. Radioimmunoassays revealed a significantly reduced release of insulin in scrapie-infected animals as compared to controls. There were no apparent histopathological changes in the islets of Langerhans in the infected animals. Indirect immunofluorescence showed no discernible differences in the content of insulin, glucagon and somatostatin between infected and control animals. These studies suggest that damage to the central nervous system is responsible for the induction of diabetes in the scrapie-infected animals.

4. An exhaustive review of the literature on the immunological abnormalities associated with insulin-dependent diabetes mellitus (IDDM) was conducted over the last year and a review article written. The review brought out several not well recognized points. First, autoantibodies to a wide variety of autoantigens, besides pancreas-associated ones, are found in IDDM. In addition, abnormalities of cell-mediated immunity covering a broad spectrum of reactivities are present. Second, many of the humoral and cell-mediated immunological abnormalities are transient and revert back to normal once the diagnosis of IDDM is made and treatment with insulin begins. Third, in experiments with animal models of IDDM, treatment with insulin can restore a number of the immunological abnormalities. Moreover, in vitro insulin can show powerful immunopotentiating properties. Thus, an argument was constructed suggesting that some of the immunological abnormalities seen at diagnosis of IDDM may result from early fluctuations of insulin levels or aberrant regulation of insulin secretion which may occur before clinical diabetes is apparent.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00 421-01

PERIOD COVERED

October 1, 1985 - September 30, 1986

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)*

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Salata, Kalman	Staff Fellow	LOM, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Viral Diseases, NIAID
Laboratory of Clinical Investigation, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.89	2.13	2.76

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Ongoing studies in mice with a recombinant vaccinia virus vaccine for herpes simplex (HSV) have shown that substantial but somewhat reduced protection against both lethal and latent infection with HSV-1 persists for greater than one year post vaccination with a single vaccination of the vaccinia-herpes recombinant. Mice boosted with a second vaccination of the recombinant demonstrated increased neutralizing antibody to HSV and increased protection against lethal infection with HSV-1.

We have developed a manipulable model of ultraviolet (UV) light-induced reactivation of HSV in humans. Using this model, we have initiated a double blinded protocol to investigate the ability of the antiviral agent acyclovir to block UV induced reactivation of HSV in patients with frequently recurrent nongenital HSV.

One of the major open questions in the molecular biology of HSV latency is the identification of viral genes responsible for maintenance of the latent state of HSV in host ganglia. Using c-DNA probes made from trigeminal ganglia RNA of mice latently infected with HSV we have identified a viral gene or subset of genes which appear to be transcribed throughout the latent phase of infection. This gene(s) maps in the UL region of the UL-IRP boundary of the viral genome.

Impairment of enzyme clearance may be a more important factor than previously suspected in regulating enzyme levels in disease states. Studies in mice conducted over the past year have shown that alteration of the function of the reticuloendothelial system whether due to environmental or genetic causes yields changes in the clearance of the enzyme lactic dehydrogenase (LDH) and consequent changes in serum levels of LDH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-00422-01

PERIOD COVERED
October 1, 1985 - September 30, 1986

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	J. Srinivasappa	Visiting Scientist	LOM,NIDR
A.L. Notkins	Medical Director	LOM,NIDR			
J. Abramczuk	Visiting Scientist	LOM,NIDR	O. Tachiwaki	Visiting Associate	LOM,NIDR
D.W. Drell	Staff Fellow	LOM,NIDR			
M.I. Lerman	Expert	LOM,NIDR			
P.R. McClintock	Guest Researcher	LOM,NIDR			
E.L. Oates	Staff Fellow	LOM,NIDR			
B. Prabhakar	Sr. Staff Fellow	LOM,NIDR			

COOPERATING UNITS *(if any)*
Scripps Clinic and Research Foundation, La Jolla, CA
NINCDS and NIDDK, NIH, Bethesda, MD
Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Laboratory of Oral Medicine, NIDR

SECTION

INSTITUTE AND LOCATION
NIDR, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.50	5.03	5.47

CHECK APPROPRIATE BOX(ES)
☐ (a) 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 year emphasis has been shifted towards molecular biological studies with several major lines of investigation being pursued to identify, 1) autoantibodies in insulin-dependent diabetes mellitus (IDDM), 2) activated oncogenes in human insulinomas and other endocrine diseases, and 3) genes essential for beta-cell function. Recombinant DNA expression libraries of cDNA from a rat insulinoma cell line have been screened with autoimmune serum from IDDM patients and several clones coding for potential autoantigens have been isolated. Our attempts to establish human beta-cell lines by transfection and microinjection with oncogenes have met with preliminary, success. These cell lines will be invaluable for assessing the immunological status of IDDM patients. We are well into the characterization of one possibly two activated oncogenes from human insulinomas. DNA sequences responsible for tumorigenesis have been isolated after several rounds of tumor formation in nude mice and seem to be unrelated to any known oncogene. Nucleotide sequence analyses and tumorigenesis will be used to determine whether we have isolated a new oncogene, associated with tumors of beta-cell origin. We have initiated a second project to isolate transforming genes from other endocrine diseases, such as thyroid carcinoma and multiple endocrine neoplasia. In a search for genes essential in beta-cell function we have focused on the superoxide dismutase (SOD) gene, responsible for oxygen detoxification. This gene is involved in several forms of chemically-induced diabetes and is overexpressed in beta-cells. We have cloned, isolated and sequenced cDNA copies of rat SOD mRNA, and we are presently studying its transcriptional status in insulin-producing cells. Finally, we are developing a gene therapy model of intervention in hormone deficiency diseases. We have constructed recombinant plasmids that carry the rat insulin I gene under the regulation of the MMTV LTR and are studying glucocorticoid-modulation of insulin expression in lymphocytes transfected with this construct.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-423-01

PERIOD COVERED
October 1, 1985 - September 30, 1986

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. Prabhakar	Sr. Staff Fellow	LOM,NIDR	M. Nakamura	Visiting Fellow	LOM,NIDR
P. Casali	Visiting Scientist	LOM,NIDR	E.L. Oates	Staff Fellow	LOM,NIDR
A.L. Notkins	Medical Director	LOM,NIDR	A. Puga	Expert	LOM,NIDR
G.P. Allaway	Visiting Fellow	LOM,NIDR	K.F. Salata	Staff Fellow	LOM,NIDR
J. Abramczuk	Visiting Scientist	LOM,NIDR	M. Shibata	Visiting Fellow	LOM,NIDR
S. Burastero	Visiting Fellow	LOM,NIDR	Y. Uchigata	Visiting Fellow	LOM,NIDR
A.B. Hartman	Staff Fellow	LOM,NIDR	J. Srinivasappa	Visiting Associate	LOM,NIDR
M.I. Lerman	Expert	LOM,NIDR			

COOPERATING UNITS *(if any)*
Mount Sinai School of Medicine, New York, NY
Cetus Co., Palo Alto, CA
NIDDK, NIH, Bethesda, MD

LAB/BRANCH
Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
17.27	10.94	6.33

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

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

Using biotinylated self antigens (e.g., thyroglobulin insulin, etc.) and fluoresceine activated cell sorter human B lymphocytes that bind to these antigens have been selected. These cells have been immortalized using Epstein-Barr virus into monoclonal antibody producing cell lines. These cell lines have been stabilized by fusing them with human myeloma cells. To determine the potential human B cell repertoire against self antigens by limiting dilution analysis we have determined the frequencies of B cells producing IgM, IgG and IgA antibodies to thyroglobulin, insulin, ssDNA and the Fc fragments of IgG.

Monoclonal anti-idiotypic (anti-Id) antibodies have been made against some of the human monoclonal autoantibodies. One of these anti-ids when inoculated into rabbits induced an anti-anti-Id antibody. These anti-anti-Ids showed specificities similar to that of the original human monoclonal autoantibody and suggested that an anti-Id can trigger a cascade of autoimmune responses.

Peripheral blood lymphocytes obtained from both normals and patients with autoimmune disorders when cultured give raise to spontaneously proliferating lymphocytes that produce autoantibodies. Epstein-Barr virus antigens are found in these cells and are thought to be responsible for the transformation. These studies very strongly suggest that the EBV infected B cells may be responsible for the autoantibodies found in patients who are on immunosuppressive therapy, with AIDS and other diseases in which the immune system is perturbed.

PHS 6040 (Rev. 1/84) GPO 904-917

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, behavorial and psychophysical techniques to study neural function as it relates to the processing of sensory signals about tissue-damaging stimulation. 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 acute and chronic 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 pain transmission. In addition, we have utilized models of tissue injury and inflammation, and a new model of demyelination, to examine changes in neural function associated with inflammation and nerve pathology. In our correlative studies of behavior and neural function in awake monkey, we have begun to look at the role of the cerebral cortex in pain transmission in the normal state. Our animal studies provide a conceptual framework for human studies on mechanisms of acute postsurgical pain and chronic pain conditions.

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, and the Clinical Center.

Investigators in the Branch have received considerable recognition for their research accomplishments. Dr. Dionne was appointed Editor of Anesthesia Progress and also edited the proceedings of an NIH Consensus Conference on "Anesthesia and Sedation in the Dental Office". He also was the organizer and co-editor of an NIDR workshop on dental anxiety. Dr. Ruda was appointed to the Scientific Program Committee of the Society of Neuroscience and the International Association for the Study of Pain, Dr. Gracely received similar recognition by the American Pain Society. Dr. Bennett was appointed Associate Editor of the journal, Pain. Dr. Dubner was nominated for President of the American Pain Society and is Scientific Program Chairman for the 1987 meeting of the International Association for the Study of Pain. He also was the recipient of the 1985 Carl Schlack Award of the Association of Military Surgeons for outstanding contributions in dental education and research. A number of investigators were invited to participate in the triennial meeting of the International Union of Physiological Sciences held in Vancouver, but were not able to attend because of restrictions on international travel.

Research accomplishments of the Branch are presented in more detail below.

Neurochemistry of Nociceptive Pathways in Normal and Pathological States

With the use of immunocytochemical methods and retrograde markers, we continue to define 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 elucidated the role of a newly discovered neuropeptide in the dorsal horn, calcitonin gene-related peptide (CGRP). CGRP is a 32 amino acid peptide that is found in highest density in those spinal dorsal horn laminae associated with nociception. CGRP may be the first dorsal horn neuropeptide whose terminal arborizations arise exclusively from primary afferent axons. A dense band of CGRP immunoreactivity was observed in dorsal horn laminae I and IIa, while in lamina V, CGRP appeared as dense patches of discrete axonal strands. Ultrastructurally, the CGRP axonal terminals were similar in all of these laminae and were either dome-shaped endings or large endings associated with glomeruli. The endings contained oval agranular vesicles and a few dense core vesicles. These endings were found to synapse on spines, large caliber dendritic shafts and cell somata. Of considerable interest was the finding that CGRP coexisted with substance P in many primary afferent axons. Experiments were designed to take advantage of this finding in order to demonstrate the distribution of substance P primary afferents as opposed to the presence of substance P in dorsal horn neurons and extrinsic axons. Our observations suggest that small diameter nociceptive primary afferent axons which contain substance P terminate in laminae I, II and V of the spinal dorsal horn. The co-localization of CGRP with substance P suggests that this newly-discovered neuropeptide may play some role in the transmission of nociceptive information.

Studies on the neurochemistry of brain stem raphe neurons projecting to the dorsal horn continued this year. Over 1400 neurons in the nucleus raphe magnus and pallidus were examined. In both nuclei, serotonin-containing somata and proximal dendrites were contacted by dopamine beta hydroxylase, substance P, enkephalin or serotonin immunoreactive axonal varicosities. However, differences in the frequency and location of contacts were observed. These data suggest that there are different patterns of interaction between these chemical messengers and serotonin-containing neurons in the brain stem. Since these same chemical mediators also are present in the dorsal horn, interactions at both the spinal and brain stem levels ultimately determine which information is transmitted to higher brain centers related to the experience of pain.

Immunocytochemical methods in combination with retrograde tracer methods were used to examine the presence of neuropeptides in long ascending somatosensory pathways. Separate populations of cells contributing to the spinoreticular tract or the spinomesencephalic tract were identified as containing enkephalin (ENK), dynorphin (DYN),

cholesystokinin (CCK) or vasoactive intestinal polypeptide (VIP) immunoreactive material. The highest yield of double-labelling occurred with CCK where up to 30% of the intrinsic CCK cells were also part of the spinoreticular tract. They were found only at the border between lamina VII and the central canal region. Unlike the CCK neurons, double-labelled VIP neurons were mostly found in the lateral spinal nucleus. ENK- and DYN- containing double-labelled neurons were predominantly localized near the central canal. ENK neurons double-labelled from the spinomesencephalic tract were identified in laminae I, V, VII and in the central canal region, whereas the DYN-containing neurons were only found in the lateral part of lamina V. These experiments provide some of the most complete and earliest findings that neuropeptides in the dorsal horn are found in long projection pathways in addition to their localization in local circuit neurons.

This year we have initiated a number of studies on the effects of peripheral tissue injury and nerve damage on the expression of pain and on nervous system changes associated with the injury. A model of chronic peripheral inflammation in rats has been developed to study the relationship between inflammation and the spinal cord content of opioid peptides (enkephalin and dynorphin). DYN content increased during inflammation and the changes in DYN content paralleled the increase in edema and hyperalgesia. Treatment with indomethicin reduced the edema and hyperalgesia and also reduced the increase in DYN content. These findings suggest that the increase in DYN is related to afferent input coming from the inflamed limb. The increase in DYN peptide in the dorsal horn was accompanied by an increase in dynorphin messenger RNA measured in nitrocellulose blots probed with labelled cDNA probes for DYN and ENK. There was a nine-fold increase in dorsal horn DYN message in contrast to only a modest increase (50 to 100%) in the ENK message. These increases in gene transcription suggest that there is an increase in DYN and ENK synthesis associated with inflammation of the limb and that there is a differential activation of opioid peptide systems in the dorsal horn. The increase in message also indicates that cell bodies in the dorsal horn are the source of this increased synthesis. In an effort to localize this source of DYN and ENK increase, immunocytochemical studies were initiated. At the height of the inflammation, rats were treated with colchicine to intensify peptide levels in cell somata. There was a striking increase in the number of opioid-staining neurons in lamina V. A somewhat less intense increase was seen in laminae I and II. These increases were seen for DYN and ENK, although ENK immunoreactive neurons exhibited the smallest increase. These immunocytochemical findings confirm the radioimmunoassay and mRNA data and localize the increases in opioid peptides associated with inflammation to specific laminae--those known to contain neurons responsive to noxious inputs.

In other experiments we examined the effect of transection of the sciatic nerve in cat on the neuronal activity in dorsal horn lamina I. There is a definite somatotopic organization of cells in this region of the lumbar dorsal horn in normal animals: toe receptive fields occupy the medial 2/3s of lamina I with cells with receptive fields on the foot

and ankle concentrated more laterally. Cells with receptive fields on the leg and thigh occupy only the most lateral one or two electrode penetrations. Acute transection of the sciatic nerve resulted in almost a complete loss of receptive fields of lamina I neurons. Thus, cells that ordinarily responded to noxious inputs applied to their peripheral receptive fields were totally denervated. In contrast, in animals in which the transection was performed 20 to 82 days earlier, there was a dramatic alteration in somatotopy: the frequency of lamina I neurons with leg and thigh receptive fields increased, their response characteristics were irregular in comparison to normal animals, and the mediolateral distribution of cells with leg and thigh receptive fields was shifted medially in the lamina. Immunocytochemical staining of lumbar dorsal horn from animals with sciatic neuromata revealed changes in the content and distribution of neuropeptide-containing fibers. There were decreases in the staining patterns for substance P, CCK, somatostatin and CGRP in the superficial dorsal horn. Neither serotonin nor enkephalin exhibited a change in staining pattern on the lesioned side of the spinal cord. These experiments on the neurochemical and electrophysiologicl reorganization of the dorsal horn following chronic peripheral nerve injury may provide insights into the mechanisms of dysesthesia and allodynia following peripheral nerve transection in humans.

To further examine the effects of denervation on neuronal activity and behavior, we have developed a model of hyperalgesia and allodynia in rat that involves demyelination of the sciatic nerve. This is the first model of peripheral neuropathy in animals that appears to adequately mimic neuropathic pain in humans. The experimental neuropathy is produced in rats by placing loose ligatures around the common sciatic nerve. The ligation produces a profound hyperalgesia that is present no later than day 5 and lasts for about 3 months when it gradually diminishes and is replaced by a persistent hypoalgesia. The hyperalgesia is determined with a radiant heat thermal device developed in our laboratory. The rats remove their paws from the heat and the latency of this paw flick is markedly reduced following the ligation. Multiunit EMG recordings confirmed that the response threshold is lowered and the response duration is increased after ligation. Compound action potential recordings have showed that there is an abnormal condition in the large, myelinated axons during the hyperalgesic state. The abnormalities appear to mimic that seen in demyelinating diseases and the presence of severe demyelination has been confirmed histologically. Preliminary results with immunocytochemical techniques suggest that CGRP and substance P are depleted from spinal lamina V. Fluoride-resistant acid phosphatase also is depleted in lamina II. These neurochemical changes are present 10-30 days postoperatively. This model shows great promise for the future understanding of the effects of painful peripheral neuropathy, a condition that is extremely resistant to treatment in humans. We thus have the opportunity to investigate the mechanisms that produce such pain dysfunctions and to investigate the efficacy of potentially useful therapies.

This year we have also developed an acute carrageenin model of inflammation that mimics acute pain produced by tissue damage resulting

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). This animal model has been utilized to correlate behavioral changes with neurochemical changes in plasma and tissue associated with the inflammation. Behavioral changes have been assayed with the radiant heat thermal device discussed earlier and by the use of the standard Randall-Selitto mechanical pressure test. The thermal device has greater bioassay sensitivity and less population variability than the mechanical pressure device. With both measures, however, we have found that the hyperalgesia is carrageenin dose-dependent and is blocked by indomethicin and morphine. Significant increases in circulating bradykinin occur during the hyperalgesia. A parallel increase in bradykinin occurs in the subcutaneous perfusate from the inflamed paws of anesthetized animals. Preliminary evidence suggests that this circulating bradykinin may play a role in stimulating the secretion of beta-endorphin, another opioid peptide, from the pituitary. Thus, our findings this year implicate products of all three opioid prohormones, prodynorphin, proenkephalin, and proopiomelanocortin, in neurohumoral mechanisms of pain and inflammation.

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. We determined that a particular class of neuron in the dorsal horn, the wide-dynamic-range type, participated in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. We also learned that opiate drugs such as morphine can act directly at the dorsal horn level to attenuate the perceived intensity of such stimuli. This year we are continuing to examine the relationship between opioid peptide action in the dorsal horn and the response properties of the neurons. We also have initiated studies of the role of the cerebral cortex in pain by studying neuronal activity in cerebral cortex areas 3b,1 and 2 while the monkeys perform the same thermal discrimination task. As a prelude to such studies, we examined the properties of neurons in the face area of the cerebral cortex in anesthetized animals. To date 23 cortical neurons have been isolated that responded to noxious stimuli applied to the face. Most were of the wide-dynamic-range type and possessed receptive fields restricted to one division of the trigeminal system. The responses of these neurons were graded by changes in noxious heat stimulus intensity. However, we also found that interstimulus interval had a profound effect on the responses of these neurons. With short interstimulus intervals, the response to a second temperature increase in the noxious heat range was a positively accelerating function for temperatures up to 47°C. With longer interstimulus intervals, the response to these second temperature increases was not systematically related to stimulus intensity. If interstimulus interval has the same effect in awake, behaving monkeys, we may be able to utilize this finding to examine the role of cerebral cortex neurons in the sensory discriminative aspects of pain. Initial studies in humans have shown that reaction times or detection latencies are

inversely related to the intensity of second temperature increases in the noxious heat range. Subjects made magnitude estimates of these same stimuli and the magnitude of sensation was also linearly related to the intensity of the stimulus. These findings are of considerable importance because they indicate that detection latencies reflect the magnitude of perceived intensity and thus can be used as an indirect measure of the perceived intensity in nonhuman primates.

We have continued to examine the interneuronal circuits in the dorsal horn responsible for the segmental modulation of nociceptive transmission. Neurons belonging to the principal ascending projection pathways exhibit inhibitory postsynaptic potentials (IPSPs) produced by input from low-threshold A-beta mechanoreceptive afferents. The amplitude of these IPSPs decreases in a step-like manner as the frequency of stimulation is increased from 2-30 Hz. This phenomenon was observed in all three classes of projection neurons studied (spinothalamic tract, spinocervical tract and dorsal column postsynaptic tract) and in both low-threshold mechanoreceptive and wide-dynamic-range neurons. In previous studies we identified a population of small interneurons in lamina III with the same characteristics as these IPSPs: excitatory drive only from A-beta afferents and an inability to follow repetitive stimulation in the 2-30 Hz range. Intracellular staining of these neurons showed that their axons arborized and emitted many boutons in laminae III-IV, where the dendritic arbor of the projection neurons mentioned above also are located. Thus, it appears likely that these interneurons are inhibitory in nature and produce the IPSP found on the projection neurons. These findings may be of clinical relevance since this inhibitory circuit may be at least partly responsible for the ameliorating effects of pain therapies such as transcutaneous nerve stimulation and dorsal column stimulation.

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 was developed last year that provides temporal information about the effects of analgesic agents on the intensity of painful stimuli. The method is modeled after the classical staircase psychophysical procedure and the subjects report pain levels in units of stimulus energy, thus avoiding many of the biases of other psychophysical methods. This year the sensitivity of the method was assessed further in a study examining the effects of dose. Subjects received varying doses of fentanyl, a short acting opiate, before oral surgical procedures. Fentanyl produced significant analgesia peaking at 11 minutes post infusion. Analgesia was greater with both increases in temperature and fentanyl dose. The method was sensitive enough to distinguish between three doses of fentanyl. Thus, this procedure should provide a reliable measure of both endogenous and exogenous opioid sensitivity.

The method has also been used to assess the effectiveness of hypnotic analgesia. Subjects could be divided into two groups based on the effectiveness of hypnotic suggestions for analgesia as assessed by two methods. "Cold pressor responders" only showed analgesia with the cold pressor method, which is subject to considerable subject bias. "Consistent responders" showed analgesia with both the cold pressor method and the relatively bias-free interactive staircase method. The demonstration of hypnotic analgesia with this new method provides some of the strongest evidence to date that hypnotic analgesia includes an actual perceptual change in addition to response changes resulting from the "demand characteristics" of the experiment.

Assessment and Treatment of Chronic Pain

We are continuing to evaluate the effects of narcotic analgesics and electrical brain stimulation on clinical and experimental pain in a group of chronic pain patients, some of whom received chronic brain electrode implants for pain relief. These electrodes are placed in brain pathways where they are presumed to activate descending, opiate-related, pain-suppressing systems. Five patients participated in this study this year. All five participated in a preoperative evaluation, and three received an implanted electrode and participated in a postoperative evaluation. Results are similar to those found previously. Morphine significantly reduced the magnitude of clinical pain in the preoperative assessments and this effect was reversed by naloxone. In contrast, the analgesia produced by brain stimulation in the postoperative evaluation was no greater than that observed after sham stimulation. This analgesia was not reversed by the narcotic antagonist naloxone.

The use of deep brain stimulation to control human pain evolved from the findings in animals that electrical stimulation of peri-aqueductal gray sites activated a descending analgesic system mediated by endogenous opioid-like compounds. The human brain stimulation procedure is assumed also to activate a descending opioid system. The previous findings that the stimulation-produced analgesia does not show an opioid time course and is not reversed by a narcotic antagonist suggest that the analgesia is not produced by an opioid mechanism.

Some of our newer clinical trial studies evaluate mechanisms of pain and new treatment plans for deafferentation pain. Diabetic neuropathy pain and shingles pain are chronic deafferentation pain syndromes associated with sensory loss, hyperalgesia and dysesthetic sensations. We are evaluating the efficacy of tricyclic antidepressant drugs in the treatment of these painful syndromes. These agents are thought to increase the availability of serotonin or norepinephrine levels in the brain and may act by influencing activity in descending pain-suppressing pathways. Last year we completed a study of the effectiveness of this treatment in diabetic neuropathy pain and found that amitriptyline reduced pain independent of its effects on depression. We are now in the process of completing a study comparing amitriptyline to placebo or lorazepam. An interim analysis was

performed after 29 patients and amitriptyline was superior to lorazepam with a trend towards superiority of amitriptyline to placebo. It would appear that the effects of amitriptyline are at best modest in this condition. Lorazepam, a benzodiazepine, appears to be without effect, contradicting anecdotal reports of its effectiveness.

About 5% of patients with AIDS develop a painful neuropathy usually involving the feet and hands, and associated with burning dysesthesias. Most of the patients with AIDS-related neuropathic pain, however, have advanced disease and long-term controlled studies with them are not feasible. Therapy, therefore, needs to be based on conclusions drawn from patients with similar pain syndromes. We now have three studies of the treatment of neuropathic pain that are open to AIDS patients, but also include patients with diabetic neuropathy, phantom limb pain, shingles pain, and traumatic neuralgia. We are also examining the frequency of pain and discomfort in NIH AIDS patients, using a 4-page questionnaire and a brief physician or nurse interview. Sixteen patients have completed the questionnaire and pain was slight or moderate in all but one patient. One patient was taking analgesic medications. No patients had painful neuropathy but one had postherpetic neuralgia and had taken part in our treatment study.

We have also investigated the effect of amitriptyline in the management of chronic orofacial pain resistant to other forms of treatment. Our findings indicate that pain was significantly reduced in comparison to placebo for both high and low doses of amitriptyline. Amitriptyline reduced depression scores in the depressed patients but not in non-depressed patients as compared to placebo. Thus, pain reduction in the non-depressed group was not associated with a parallel change in mood.

Myofascial pain dysfunction (MPD) of the face and neck has been attributed to a variety of causes, but most individuals feel that the pain and dysfunction result from masticatory muscle spasm which in turn causes ischemic muscle pain. 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. An interim analysis of the first 28 patients suggests efficacy of the active drug as compared to placebo. We also have initiated a tension headache study in which non-drug therapy (home physical therapy and an exercise program) are compared to therapy with ibuprofen and diazepam.

Mechanisms and Treatment of Acute Pain Produced by Tissue Injury and Inflammation

These studies of pain mechanisms and the development of new methods of acute pain control involve the use of an oral surgery model of post-surgical pain following removal of impacted third molar teeth. A major finding from last year was that intravenous administration of corticotropin releasing factor (CRF) increased circulating levels of beta-endorphin (B-END), via activation of the entire pituitary-adrenal axis, and significantly reduced patient reports of post-operative pain. To determine whether the analgesic effect was due to pituitary secretory

activity, a second study was conducted in which the effect of inhibiting the pituitary-adrenal axis was evaluated for post-operative pain. Patients were administered three doses of dexamethasone or placebo. Dexamethasone was given at doses below those capable of producing clinically detectable anti-inflammatory effects. Intravenous administration of dexamethasone resulted in a suppression of the pituitary-adrenal axis and a dose-related hyperalgesia. Thus, activation of the pituitary-adrenal axis suppresses post-operative pain, while inhibition of the pituitary-adrenal axis enhances post-operative pain.

Parallel animal studies determined whether secretion of pituitary B-END subserved the analgesia resulting from administration of CRF. These studies used the rat hot plate test. The pituitary B-END hypothesis was supported by observations that CRF analgesia was blocked by 1) opiate antagonists, 2) surgical removal of the pituitary gland, 3) dexamethasone pre-treatment, and 4) passive immunization with anti-endorphin antisera. These findings indicate that secretion of pituitary B-END results in analgesia in both clinical and animal models of pain.

We also use the oral surgery model to evaluate novel agents, methods of administration, and drug combinations in comparison to standard analgesic therapy. A new line of investigation has been initiated in the past year to evaluate the analgesic interaction of proglumide, a relatively specific antagonist of the putative neurotransmitter cholecystokinin, and morphine. A dose-response study will determine the optimal dose of proglumide to administer in combination with morphine. Subsequent studies will assess the relative potency of morphine alone in comparison to morphine plus proglumide and the interaction of proglumide with endogenous pain suppressing systems activated during surgical stress. Interim analysis of the proglumide study suggests that both 0.5 mg and 5.0 mg doses of proglumide potentiate analgesia produced by 4 mg of morphine. The 5.0 mg dose of proglumide plus 4 mg of morphine results in analgesia comparable to 8.0 mg of morphine. Parallel animal studies using the rat hot plate model have also demonstrated proglumide potentiation of morphine analgesia. The results of these investigations suggest that novel non-opioid drugs can result in significant analgesia with a minimum of centrally-mediated side effects such as drowsiness, dizziness and nausea.

PUBLICATIONS

NEUROBIOLOGY AND ANESTHESIOLOGY BRANCH

October 1, 1985 - September 30, 1986

Dionne, R.A.: Suppression of dental pain by preoperative administration of flurbiprofen. Amer. J. Med. 80 (Suppl. 3A): 41-49, 1986.

Dionne, R.A.: Drug Interactions and Adverse Effects. In: Dionne, R.A. and Laskin, D.L. (Eds.), Anesthesia and Sedation in the Dental Office, Elsevier, New York, 1986, pp. 57-65.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-18 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Design and Computer Interfacing of Neurophysiologic Instrumentation

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

Brown, Frederick J. Electronic Engineer (Instru) NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology & 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 spece 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.

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 00132-12 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986 CT 006102

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
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Joris, Jean	Visiting Fellow	NA NIDR
Schmidt, Elizabeth A.	Clinical Nurse	NA NIDR
Flores, Christopher	Biologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

Goldstein, David G., NHLBI, NE

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.35	.40

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project 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 stimulates 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.

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 00133-12 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986 CT 0060101

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:
1.45	.45	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 spece 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 scaling method was used to assess the relation between analgesia time course and fentanyl dose. Fentanyl produced analgesia peaking at 11 min post infusion that was greater with increasing stimulus temperature and fentanyl dose. Rate of analgesia onset was not dose dependent.

A second study using the interactive scaling method showed that the method was sensitive to changes in response range but not to increases in overall stimulus intensity.

Reliability of the alternative forms of the Descriptor Differential Scale was reassessed in a cross-validation study. Preliminary results show reliabilities ($r = .8$) similar to those found in the first study.

A study of hypnotic analgesia showed that the cold-pressor pain measure, which is very vulnerable to response bias, shows significantly greater analgesia than the inteactive scaling method. Thus, previous results may reflect partially response changes unrelated to analgesia.

A study showed that hypertensive subjects are relatively pain-insensitive, suggesting that hypertension may prove to be a maladaptive attenuation of stress-related distress.

An initial study of pediatric pain showed that systematic physician ratings of patient pain can be used to continuously adjust the rate of continuous morphine infusions to provide adequate pain control with minimal side-effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00246-09 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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 professionel personnel below the Principel Investigator.) (Name, title, laboratory, and institute effiliation)*

Singer, Elyse J.	Medical Staff Fellow	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Schmidt, Elizabeth A.	Clinical Nurse	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:
1.45	1.00	.45

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.)*

The objective of this study is to evaluate the efficacy of ibuprofen and diazepam alone and in combination, versus a placebo, in the short-term management of patients with the myofascial pain-dysfunction syndrome (MPD). Pain and mandibular function are assessed over a washout period (2 weeks) and treatment period (four weeks). Beta-endorphin levels and cortisol levels are being assessed from blood samples of these patients.

The headache study looks at a similar population group with myogenic headache who have pain on a daily or near-daily basis plus muscle tenderness to palpation and limited range of motion in the cervical spine (due to spasm). The study compares non-drug therapy (exercise, home physical therapy) versus a drug combination (ibuprofen/diazepam) and a placebo drug. All arms of the study are balanced to give each group equal time and physician, and family attention. Pain, quantitative muscle tenderness (measured with a hand-held "algometer"), range of motion, and side effect data are collected by an independent, blinded evaluator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00276-08 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Brain Stimulation Analgesia in the Control of Chronic Pain

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

Gracely, Richard H.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Max, Mitchell B.	Neurologist	NA NIDR

COOPERATING UNITS *(if any)*

Young, Ronald, UCLA, Los Angeles, California
Smoller, Bruce, Psychiatrist, Bethesda, Maryland

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Clinical Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	.70	.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 spece 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. Participants in this study will be (1) chronic pain patients receiving surgically implanted stimulating electrodes for pain control; (2) chronic pain patients maintained on traditional narcotic analgesics who will not receive implanted stimulating electrodes; and (3) healthy normal volunteers. 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 nonsurgical 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.

Results from five patients this year support previous findings from this project. Morphine significantly decreased the magnitude of low back pain and this effect was reversed by naloxone. The effect of deep brain stimulation was no greater than that observed after sham stimulation and this effect was not reversed by naloxone. These results suggest that clinical analgesia observed with this method is not opioid mediated and either is less potent than morphine or does not follow the time course of a narcotic analgesic.

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 00286-07 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

CT 0060133

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

Evaluation of Oral Analgesics for Ambulatory Patients

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

Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Schmidt, Elizabeth A.	Clinical Nurse	NA NIDR
Lavigne, Gilles	Guest Researcher	NA NIDR
Troullos, Emmanuel	Dental Staff Fellow	CIPC 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:
1.60	1.25	.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.)*

The project consists of a series of clinical trials using the oral surgery model to evaluate novel agents, methods of administration and drug combinations in comparison to standard analgesic therapy. Previous studies demonstrated the superiority of an investigational analgesic, flurbiprofen, in comparison to acetaminophen and oxycodone plus acetaminophen, and that etidocaine, a long-acting local anesthetic, suppresses postoperative pain. A factorial study demonstrated that the combination of flurbiprofen and etidocaine results in independent and additive effects to markedly suppress pain in comparison to standard therapy while resulting in fewer side effects. A current investigation is evaluating the ability of proglumide, an anatagonist of cholecystokinin, to potentiate morphine analgesia in the oral surgery model. Interim data analysis suggests that 5.0 mg of proglumide plus 4.0 mg of morphine sulfate results in analgesia comparable to 8.0 mg of morphine sulfate. The analgesic specificity of drugs acting at opiate receptor subtypes is being evaluated in a clinical trial of spiradoline, a structurally unique kappa receptor agonist. The interaction of proglumide and spiradoline with endogenous pain inhibitory substances such as plasma beta-endorphin is also being evaluated in these latter studies. These investigations may contribute to the development of novel opioid analgesics with enhanced efficacy but without the liabilities of traditional opioids such as morphine.

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 00288-07 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one lina 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 T.	Research Biologist	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Humphrey, Emma L.	Biol. Lab. Tech. (Elec. Mic.)	NA NIDR
Cohen, Leslie V.	Biologist	NA NIDR
Tashiro, Takashi	Visiting Fellow	NA NIDR
Pretel, R. Stephanie	Postdoctoral Fellow	NA NIDR
Iadarola, Michael J.	Senior Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.95	2.80	2.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The neural circuitry of the dorsal horn of the spinal cord forms the basis for the mechanisms of pain and analgesia. Our lab has made significant inroads in understanding the neuronal connectivity which subserves these sensory phenomena through experiments involving multiple markers to identify interactions between neural elements.

The anatomical features of calcitonin-gene-related peptide (CGRP), a novel peptide which may arise exclusively from primary afferent axons, was examined. Dense CGRP immunoreactivity was observed in the superficial dorsal horn and in lamina V. Ultrastructurally CGRP varicosities appeared either as central endings of glomeruli or dome-shaped. They contained mainly oval granular vesicles and formed asymmetrical synapses on dendrites and cell bodies. Mainly unmyelinated axons contained CGRP, suggesting an important role in pain transmission.

Coexistence of CGRP and substance P (SP) in axons in the dorsal horn was examined using immunologically distinct multiple fluorescent markers. The density of coexistent axons was greatest in laminae I, IIa and V. Since dorsal root ganglia neurons are the only likely source of the coexistent axons, our data suggest that SP primary afferent axons, which are important in nociception, terminate in these laminae.

A second series of experiments attempted to quantify norepinephrine, enkephalin, substance P and serotonin inputs to serotonin raphe neurons. Serotonin neurons in the caudal raphe nuclei received a differential number of contacts both with respect to the neurochemical they contained and the location of contacts.

A third series of experiments identified the location of opioid dorsal horn neurons which responded to chronic inflammation of the limb. Neurons in laminae I, II and V increased their level of opioid peptide as assayed by immunocytochemistry. Opioid neurons in other laminae appeared to respond less to this noxious input.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-07 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 chrecters 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 professionel personnel below the Principel Investigator.) (Name, title, leboratory, and institute affiliation)*

Dubner, Ronald	Chief, NAB	NA NIDR
Anton, Fernand	Visiting Fellow	NA NIDR
Maixner, William	PRAT 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:
1.35	.85	.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.)*

We examined the effects of morphine 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^{o}C from a previous noxious heat level of 46^{o}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. Morphine (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. These effects were opiate receptor-mediated since they were antagonized by systemically-administered naloxone (0.5 mg/kg, im.) given 40 minutes after the microinjection of morphine. There were no effects of morphine 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 opiates on the perceived intensity of noxious heat stimuli at the earliest central relay pathway transmitting noxious information.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-05 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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
Maixner, William	PRAT 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:
.75	.35	.40

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project correlates behavioral responses with neural responses of thalamic projection and non-projection neurons in the medullary dorsal horn (trigeminal nucleus caudalis) produced by noxious thermal stimuli in the behaving monkey. Medullary dorsal horn neurons encode thermal discriminative information used by the monkey to perform a thermal detection task. Many medullary dorsal horn neurons encode thermal intensity in a manner which allows the detection of small changes in noxious temperatures. The role of dorsal horn wide-dynamic-range (WDR) and nociceptive-specific (NS) neurons in the encoding of the perceived intensity of noxious stimuli was determined while monkeys detected near-threshold changes in the intensity of noxious heat stimuli. Behavioral detection latencies were a reliable measure of the perceived intensity of these stimuli. There was a significant correlation between behavioral detection latency and neuronal discharge of WDR, but not NS neurons. In addition, WDR neurons exhibited greater activity on correctly-detected versus non-detected trials, whereas NS neurons did not. We conclude that WDR neurons are involved in the encoding process by which monkeys perceive the intensity of noxious heat stimuli near detection threshold. Some thermally sensitive neurons also respond to other stimuli used by the monkey for the successful completion of the task. This task-related activity occurs in characteristic patterns of excitation and/or inhibition and some neurons which exhibit such activity project to the thalamus. The task-related responses exhibited by some of these neurons may modulate sensory activity and thereby influence the perception of and response to oral-facial pain.

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 00366-04 NA

PERIOD COVERED
October 1, 1985 - September 30, 1986

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
Gracely, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Kishorekuma, Raganna	Medical Staff Fellow	NA NIDR

COOPERATING UNITS *(if any)*
Schafer, Susan, CC
Chang, Alfred, NCI
Epstein, Todd, CC

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Clinical Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	1.30	.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.)*

The purpose of this work is to elucidate the principles of treatment of chronic pain syndromes that are considered resistant to conventional treatment, such as pain caused by nerve injury and by advanced cancer. There is a particular emphasis on pain caused by peripheral neuropathies. Such as syndrome occurs in about 5% of patients with AIDS.

We focus on pain in patients with painful neuropathy and post-herpetic neuralgia. A study showing amitriptyline to relieve diabetic neuropathy pain in both depressed and non-depressed patients was completed. Thirty-eight of a projected 40 patients with postherpetic neuralgia have completed a crossover study of amitriptyline, lorazepam, and placebo. An interim analysis at n=29 showed a trend towards relief with amitriptyline. Two further studies examining the mechanism of relief of neuropathic pain by antidepressants will begin soon. Amitriptyline potentiates the action of both serotonin and norepinephrine at central synapses. We will examine fluoxetine which is specific for serotonin, and desipramine, which is relatively specific for norepinephrine.

We will also collaborate with CC Anesthesiology and NHLBI in a study of catecholamine kinetics in the vascular system of patients with reflex sympathetic dystrophy, to see how alterations in catecholamine release affect their 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 00377-03 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Responses of Primate SI Cortical Neurons to 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
Laylon, Lynette L.	Postdoctoral Fellow	NA NIDR
Chudler, Eric H.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	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:
3.60	3.00	.60

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This study examines the psychological and neurophysiological basis of the human's ability to detect small temperature changes superimposed upon noxious levels of thermal stimulation. In the first part of the study we examined the relationship between the reaction time to small temperature changes and the ability of humans to estimate the magnitude of the pain sensation. These data show that there is a close correspondence between reaction time and the magnitude estimate of the pain sensation. In addition, even when the reaction time has plateaued and further increases in stimulus intensity do not produce faster reaction times, there are still increases in the magnitude estimates of thermal pain.

The responses of cortical nociceptive neurons to small temperature changes superimposed upon noxious levels of thermal stimulation were examined. Manipulations that produce changes in the intensity of pain sensation in humans also produced concomitant changes in the discharge of cortical nociceptive neurons. Therefore, we conclude that cortical nociceptive neurons may participate in the sensory-discriminative aspects of pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00378-03 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Amitriptyline in the Relief of Orofacial Pain, Depression and Sleep Disorders

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

Dionne, Raymond A.	Research Pharmacologist	NA NIDR
Singer, Elyse J.	Medical Staff Fellow	NA NIDR
Schmidt, Elizabeth A.	Clinical Nurse	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

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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:
.70	.60	.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 project is evaluating the analgesic efficacy of amitriptyline at two doses in comparison to placebo for the management of chronic orofacial pain; to determine an optimal dose to be used; and to determine the mechanism of action of antidepressants in pain management. A double-blind incomplete cross-over design of drug administration is used and each subject is administered in two four-week periods, separated by a two week washout, one of the following: placebo, high dose amitriptyline, or low dose amitriptyline. Both high dose (up to 150 mg) and low dose (10-30 mg) of amitriptyline result in significant pain relief in comparison to placebo. Amitriptyline reduced depression scores in the depressed patients but not in non-depressed patients, providing evidence that its analgesic effect is not associated with a parallel change in mood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00394-02 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Physiology and Morphology of Dorsal Horn Projection Neurons

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

Hylden, Janice L.K.	Staff Fellow	NA NIDR
Nahin, Richard L.	Postdoctoral Fellow	NA NIDR
Bennett, Gary J.	Research Biologist	NA NIDR
Ruda, Maryann T.	Research Biologist	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:
3.10	2.40	.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.)*

In the present research project, we have employed a combination of physiological and immunocytochemical approaches to the study of somatosensory systems. Two major groups of experiments are described.

The activity of lumbar spinal dorsal horn lamina I neurons with afferent drive from the sciatic nerve was studied in intact cats and in cats with acute sciatic nerve transection or chronic sciatic nerve transection with neuroma formation. The majority of neurons recorded ipsilateral to a neuroma had no receptive field; those that did respond to peripheral stimuli had irregular response properties in comparison to the cells recorded in control animals. In addition, the characteristic somatotopy of lamina I cells was not observed in some cats with neuromata--indicating that some cells had altered receptive fields following chronic nerve transection. Immunocytochemical examination of spinal cord tissue from cats with neuromata revealed a decrease in the staining of substance P, somatostatin, cholesystokinin (CCK) and calcitonin gene-related peptide-containing fibers ipsilateral to the neuroma.

The neuropeptide content of long ascending somatosensory projection neurons was examined in the rat and cat. Separate populations of retrogradely labeled rat spinoreticular neurons were found to contain CCK, vasoactive intestinal polypeptide, dynorphin (DYN) or enkephalin (ENK)-like material. The highest yield of double-labeled cells occurred for CCK; up to 30% of intrinsic CCK neurons also contained the retrograde marker, decreasing to 2-5% for ENK-labeled cells. Retrogradely labeled spinomesencephalic neurons were found to contain ENK (cells located in laminae I, V, VII and X) and DYN (cells located in lateral lamina V).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-01 NA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

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	1.40	.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.)*

An experimental neuropathy of peripheral somatic nerve was produced in rats by placing loose ligatures around the common sciatic nerve in one hindleg. Pain sensation was measured by evoking the nocifensive flexion reflex with radiant heat directed to the plantar hindpaw. The latency of the reflex (paw-flick) was significantly shorter on the operated side at 5 days postoperatively and remained so for about 3 months. The amplitude and duration of the paw-flick from the operated side were also abnormal, being unusually large and prolonged. These data demonstrate that the neuropathy has produced the state of hyperalgesia. Other behavioral signs suggest that allodynia (the evocation of pain by normally innocuous stimuli) is also present. Thus, the animals habitually hold the effected leg in a slightly flexed position while standing, lying down, or sleeping; thereby avoiding touching the effected foot. Multiunit EMG recordings from the relevant flexor muscles confirmed that the reflex had a lower threshold and an abnormally long duration. Testing the pain threshold during the first 5 days postoperatively was complicated by the fact that the threshold for neurogenic inflammation was lowered. This phenomenon suggests that there is an abnormality in unmyelinated nociceptor function because these fibers are known to mediate neurogenic inflammation. Compound action potential (CAP) recordings from the effected nerves at various times during the presence of hyperalgesia showed grossly abnormal nerve conduction. Preliminary examination of the nerves after osmication confirmed that the nerves were severely demyelinated. Preliminary work indicates that there are also changes in the central nervous system. Fluoride resistant acid phosphatase is greatly reduced in lamina II. Substance P and calcitonin gene-related peptide are depleted in lamina V.

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 00414-01 NA

PERIOD COVERED
October 1, 1985 - September 30, 1986

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

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	.60	.90

CHECK APPROPRIATE BOX(ES)

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

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

This project is investigating the role of CNS peptide-containing neurons in sensory processing. A model of chronic peripheral inflammation has been developed in order to understand the relationship between spinal cord opioid-containing neurons (enkephalin and dynorphin) and abnormal afferent input due to inflammatory processes. Spinal cord dynorphin neurons exhibit a three-fold increase in peptide content at the peak of inflammation; in contrast, little effect is seen for the enkephalin-containing neurons. In order to examine further the physiological implications of the dynorphin peptide increase we have measured the levels of the mRNAs coding for the dynorphin and enkephalin peptide precursor proteins.

RNA blots were prepared from poly A^+ enriched RNA isolated from rat dorsal spinal cord under control and inflammation conditions. The dynorphin mRNA was found to undergo a nine-fold increase after inflammation. This result has been reproduced twice. In contrast, the enkephalin mRNA is increased to a much smaller extent (50-100% over control). Preliminary time course results indicate that the dynorphin mRNA is increased as early as day two of inflammation. The changes in dynorphin peptides and mRNA parallel one another and also parallel the development of a marked hyperalgesia to both a thermal stimulus and a mechanical stimulus.

These data suggest that there is a mild activation of spinal cord enkephalin neurons and a marked activation of spinal cord dynorphin neurons since the synthesis of both message and peptide are increased. Thus, spinal dynorphin neurons may play a unique role in modulating inflammatory pain. The significance of these studies is that they provide a model in which to study the in vivo regulation of opioid neurons and a new framework from which to evaluate the role of multiple spinal opioid neurons in the control of chronic pain as encountered in arthritis, injury and possibly cancer. Further elucidation of the pivotal role of the spinal dynorphin system may provide a new avenue for the pharmacotherapy of the chronic pain state as well as 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 00426-01 NA

PERIOD COVERED
October 1, 1985 - September 30, 1986

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:
2.20	1.40	.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.)*

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 unusually 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

INDEXES

NATIONAL INSTITUTE OF DENTAL RESEARCH
ANNUAL REPORT - FISCAL YEAR 1986

INDEX BY PROJECT NUMBER

Z01	DE00362-03	ROBERTS, MICHAEL W.	119
Z01	DE00366-03	MAX, MITCHELL B.	270
Z01	DE00372-03	KOUSVELARI, ELENI E.	120
Z01	DE00373-03	WEBBER, RICHARD L.	135
Z01	DE00374-03	PLUZNIK, DOV H.	204
Z01	DE00376-02	FIELD, RUTH B.	227
Z01	DE00377-02	KENSHALO, DANIEL R.	271
Z01	DE00378-02	DIONNE, RAYMOND A.	272
Z01	DE00379-02	YOUNG, MARIAN F.	103
Z01	DE00380-02	ROBEY, PAMELA G.	104
Z01	DE00381-02	CIARDI, JOSEPH E.	205
Z01	DE00382-02	ROBRISH, STANLEY A.	206
Z01	DE00385-02	BRENNAN, MICHAEL J.	207
Z01	DE00386-02	RUFF, MICHAEL F.	208
Z01	DE00387-02	KINGMAN, ALBERT	47
Z01	DE00388-02	KINGMAN, ALBERT	48
Z01	DE00392-02	SMITH, PHILLIP D.	209
Z01	DE00394-01	HYLDEN, JANICE L.	273
Z01	DE00395-01	ROBERTS, MICHAEL W.	121
Z01	DE00396-01	DRISCOLL, WILLIAM S.	70
Z01	DE00397-01	AGELLI, MARIA	210
Z01	DE00399-01	CARLOS, JAMES P.	54
Z01	DE00402-01	LI, SHOU HUA	49
Z01	DE00403-01	BRUNELLE, JANET A.	50
Z01	DE00404-01	STILES, HORACE M.	79
Z01	DE00405-01	STILES, HORACE M.	78
Z01	DE00406-01	STILES, HORACE M.	80
Z01	DE00407-01	STILES, HORACE M.	80
Z01	DE00408-01	SHERN, ROALD J.	74
Z01	DE00409-01	KINGMAN, ALBERT	51
Z01	DE00410-01	LOE, HARALD	55
Z01	DE00411-01	WRIGHT, WILLIAM E.	122
Z01	DE00412-01	BRAHIM, JAIME S.	123
Z01	DE00413-01	BENNETT, GARY J.	274
Z01	DE00414-01	IADAROLA, MICHAEL J.	275
Z01	DE00415-01	TURNER, ROY JAMES	124
Z01	DE00416-01	LITTLE, WAYNE A.	75
Z01	DE00417-01	MIRTH, DALE B.	76
Z01	DE00418-01	KING, ALBERT	52
Z01	DE00420-01	BRUNELLE, J. A.	53
Z01	DE00421-01	ROONEY, JAMES F.	243
Z01	DE00422-01	PUGA, A.	244
Z01	DE00423-01	PRABHAKAR, B.	245
Z01	DE00424-01	MCCARTNEY-FRANCIS, NANCY	211
Z01	DE00425-01	WOLFE, MARY D.	56
Z01	DE00426-01	BENNETT, GARY J.	276
Z01	DE00427-01	LONDON, JACK	212

NATIONAL INSTITUTE OF DENTAL RESEARCH
ANNUAL REPORT - FISCAL YEAR 1986

INDEX BY PRINCIPAL INVESTIGATOR

Z01	DE00427-01	LONDON, JACK	212
Z01	DE00009-24	MARTIN, GEORGE R.	163
Z01	DE00275-07	MARTIN, GEORGE R.	169
Z01	DE00281-06	MIRTH, DALE B.	77
Z01	DE00285-06	MEDNIEKS, MAIJA I.	225
Z01	DE00366-03	MAX, MITCHELL B.	270
Z01	DE00417-01	MIRTH, DALE B.	76
Z01	DE00424-01	MCCARTNEY-FRANCIS, NANCY	211
Z01	DE00199-09	OLIVER, CONSTANCE	224
Z01	DE00374-03	PLUZNIK, DOV H.	204
Z01	DE00422-01	PUGA, A.	244
Z01	DE00423-01	PRABHAKAR, B.	245
Z01	DE00204-08	REDDI, A. HARIDARA	102
Z01	DE00288-06	RUDA, MARYANN T.	267
Z01	DE00332-04	ROBERTS, MICHAEL W.	116
Z01	DE00362-03	ROBERTS, MICHAEL W.	119
Z01	DE00380-02	ROBEY, PAMELA G.	104
Z01	DE00382-02	ROBRISH, STANLEY A.	206
Z01	DE00386-02	RUFF, MICHAEL F.	208
Z01	DE00395-01	ROBERTS, MICHAEL W.	121
Z01	DE00421-01	ROONEY, JAMES F.	243
Z01	DE00034-17	SIRAGANIAN, REUBEN P.	194
Z01	DE00061-12	SANDBERG, ANN L.	198
Z01	DE00112-12	SHERN, ROALD J.	73
Z01	DE00246-08	SINGER, ELYSE J.	264
Z01	DE00290-06	SIRAGANIAN, REUBEN P.	202
Z01	DE00392-02	SMITH, PHILLIP D.	209
Z01	DE00404-01	STILES, HORACE M.	79
Z01	DE00405-01	STILES, HORACE M.	78
Z01	DE00406-01	STILES, HORACE M.	80
Z01	DE00407-01	STILES, HORACE M.	80
Z01	DE00408-01	SHERN, ROALD J.	74
Z01	DE00157-10	TORCHIA, DENNIS A.	101
Z01	DE00341-04	THOMPSON, JOHN	203
Z01	DE00415-01	TURNER, ROY JAMES	124
Z01	DE00007-25	WITTENBERGER, CHARLES L.	193
Z01	DE00046-14	WAHL, SHARON M.	197
Z01	DE00065-14	WEBBER, RICHARD L.	133
Z01	DE00211-09	WEBBER, RICHARD L.	104
Z01	DE00212-09	WEIFFENBACH, JAMES M.	115
Z01	DE00216-09	WAHL, LARRY M.	199
Z01	DE00250-08	WALCZAK, CYNTHIA A.	61
Z01	DE00373-03	WEBBER, RICHARD L.	135
Z01	DE00411-01	WRIGHT, WILLIAM E.	122
Z01	DE00425-01	WOLFE, MARY D.	56
Z01	DE00025-19	YAMADA, YOSHIHIKO	165
Z01	DE00379-02	YOUNG, MARIAN F.	103

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