ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF DENTAL RESEARCH

Fiscal Year 1973

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

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CRITIQUE SHEET

Please tear this sheet out of the Annual Report, FY 1973, write your comments or suggestions on the back, triple-fold with the address on the outside, staple and place in the Inter-office mailbox. We are anxious to make this Report of value to you and as accurate as possible. We would like to have your help.

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Office of Program Studies and Analysis
National Institute of Dental Research
Room 537, Westwood Building

U.S. NATIONAL INSTITUTE OF DENTAL RESEARCH

ANNUAL REPORT

July 1, 1972 - June 30, 1973

Compiled by

Office of Program Studies and Analysis

National Institute of Dental Research

National Institutes of Health

Bethesda, Maryland

Preface

The departure in the format of last year's Report, which we believe conveys more concisely and yet more meaningfully the total activities of the past year, is being followed again in this issue of the NIDR Annual Report - FY'73. It is organized in a manner which is calculated to increase its usefulness both to the community of science, whose primary concern is the substance of research, as well as to the various echelons of management, to whom information regarding the effective marshalling of fiscal and manpower resources is of prime interest.

Functionally, the principle alterations in presentation derive from:

----presentation of annual reports of the extramural categorical programs in a more uniform manner than in previous years. Progress in this direction was achieved by developing a more uniform format to be used by the programs and by assigning a single editor to coordinate the individual program reports with the report of the Associate Director for Extramural Programs.

----synthesis of intramural narrative progress reports into a format of presentation on a sectional rather than an individual basis, so as to state more clearly the scientific purposes, progress and research directions of these fundamental organizational units of the Institute.

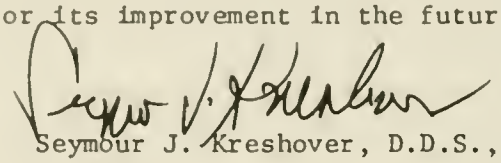
----expansion of the Notice of Research Project (PHS-166), normally completed at this time of the year for the Science Information Exchange, so as to include those items of management import previously presented as an integral part of the individual narrative project report. This alteration in the SIE report format was made feasible by operational changes undertaken by SIE in the way in which they store and retrieve information.

----identifying more distinctly those segments of the Institute's research undertaken through collaborative contract support, particularly in our recently expanded National Caries Program. As in the case of individual intramural research projects, the data from the conventional SIE report (PHS-1688) was adapted to this purpose in an expanded form.

----addition of a variety of indexes to assist the user in answering almost any question which might arise about intramural or contract research projects in force during the fiscal year.

----color coding of various sections of the overall report so as to make it more convenient to use.

The innovative nature of this effort, as in any new enterprise, increases its susceptibility to errors and oversights for which we apologize. However, the improvement to its internal usefulness and the favorable comment of users in general encourages us to continue in this direction. As usual, we invite your comments and suggestions for its improvement in the future.



Seymour J. Kreshover, D.D.S., M.D.
Director
National Institute of Dental Research

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REPORT OF THE DIRECTOR

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

July 1, 1972 - June 30, 1973

by

Seymour J. Kreshover

Pursuant to the request made by the Senate Committee on Appropriations in its report on FY 1973 appropriations, an evaluation of the Institute's plan for the optimum development of the Nation's dental research effort was made and forwarded to the Committee. The evaluation related to the criteria articulated in the general statement of the Committee's report, i.e., directing the Institute's resources at "those disease problems that are most important and widespread, where effective means for dealing with the problem are available, where new opportunities have emerged, and where the likely benefits justify the expected costs."

For the Committee report, five program areas were identified as high priority efforts: Caries, Periodontal and Soft Tissue Diseases, Craniofacial Anomalies, Restorative Materials, and Pain Control and Behavioral Studies. The research plans for each of these priority areas were delineated in terms of the current status, the opportunities for research progress, and plans for future activities.

The total NIH community of categorical Institutes has become increasingly involved with the planning and administration of research contracts. In FY 1973, the Institute directed its efforts toward refining contract review and award procedures in an effort to fully develop an efficient and accountable system.

The OMB decision to terminate NIH training grants and fellowships was made in January 1973. In accordance with the NIH general phase-out plan, training support for predoctoral, postdoctoral, and other forms of training activity has been projected through FY 1976--by that time the phase-out plan should be completed. NIDR maintains a strong sense of commitment to continued responsibility for assessing the research and academic manpower needs of dentistry. The Institute, therefore, plans to continue evaluation studies of past and present training efforts. Meanwhile, efforts will be made to take advantage of present training opportunities, however meager, and also to plan appropriate types of training mechanisms for the future.

During the year, Mr. John P. Patterson was named executive officer of the National Institute of Dental Research. Except for a 3-year stint with the Division of Extramural Research Programs of NIMH, Mr. Patterson has been at NIH since 1957. He has served in administrative posts with the CC, NIAID, and NCI. Mr. Patterson has received several awards for outstanding work.

A Data Processing Systems and Analysis Section was established, which focuses computer and data processing activities in the Office of the Director. An Administrative Management Section was also established in the Office of the Director, thereby providing comprehensive administrative and management support to the entire Institute with greater efficiency and economy.

As a result of the Institute's emphasis on expanding its clinical research programs, a new Laboratory of Oral Medicine was established and the existing Laboratory of Microbiology and Immunology was reorganized and expanded; each laboratory to include sectional activities in clinical fields. In parallel with these changes, the several previously operating clinical branches and sections have been reconstituted into two major program areas: 1) Neurophysiology and Anesthesiology, and 2) Clinical Investigations and Research Services.

In order to meet the FY 1974 personnel ceiling, reductions in staff positions had to begin in FY 1973. The National Institute of Dental Research, in common with the rest of the NIH and DHEW community, is required to operate under a set of restrictions governing the number of employees and the average grade of the civil service employees under the General Schedule pay system. These restrictions have impeded the Institute's ability to employ new investigators and supporting staff as program needs dictate and have necessitated the maintenance of additional records for control purposes and for the preparation of reports.

During the reporting period, the Institute continued its positive support of EEO and Upward Mobility programs. The EEO Committee of the Institute met on a regular basis to discuss problems of concern to NIDR employees, and through their EEO Counsellor/Council Member, received reports regarding EEO efforts on behalf of the NIH as a whole.

The personnel office, in conjunction with program leaders, surveyed all the positions in the Institute to determine their susceptibility to job restructuring with the result that during FY 1973, two employees were moved into restructured positions. In addition, all employees at the GS-7 level and below were interviewed, and data regarding skills and interests was obtained for inclusion in the NIH Opportunity Skills File. One NIDR employee was encouraged to apply, and was subsequently selected for the first STRIDE class.

The NIDR has also continued support for programs outside the Institute which provide opportunities for the economically disadvantaged, including the Summer Aid, Stay-in-School, and Federal Junior Fellowship Programs. Further, the Institute is training one employee under the Public Service Careers Program and has placed a STRIDE intern in a training position in one of the laboratories.

Both on-the-job and outside training continued to be provided for employees. The NIDR has sought to strengthen its overall programs through training in two broad categories: upward mobility, and scientific and technical experience. Upward mobility courses included orientation in the EEO philosophy, basic education courses, and courses leading to the development of

new skills. The scientific and technical area included emphasis on general biology, chemistry, and immunology as well as specific courses in radiation safety. A significant amount of course work has also been provided in computer science to enable Institute personnel to better utilize available hardware.

Activity directed toward increasing the number of women in grades GS-11 and above has resulted in two appointments and four promotions. Also worthy of note was that three women assumed leadership of sections during FY 1973.

Information Office

The Information Office contributes to carrying out the mission of NIDR by keeping the various publics informed about the activities of the Institute.

The impact of information functions is difficult to measure. However, recent studies have shown that publicity stimulated by the annual science writer seminars sponsored by the American Cancer Society generate at least $12 million a year in collections. Although NIDR Information Office efforts are not susceptible to such measurement, one evidence of impact is the growing volume of public inquiries, which has doubled over the previous year. Over 15,000 public inquiries were received this year, as contrasted with 7,600 in FY 1972.

The Information Office serves as the instrument whereby the Institute is responsive to the urgings of the Congress to let the taxpayer know what is being done with his tax money. A specific Congressional query during the year related to nutrition information for the public. Primarily through pamphlets prepared by the Information Office on nutrition in relation to oral disease, as well as other subjects, the Institute was able to make an affirmative response. Similarly, it has been responsive to consumer groups that seek practical information for the public on how to benefit from knowledge being developed by various Federal agencies.

At the House hearings for FY 1974 appropriations, the question was raised as to how NIDR is reaching the practicing dentist with the latest research findings. The Information Office contributes to this function through its various channels of communication, notably the monthly news service provided to dental journals. Regular use is made of these news items.

During the year, the Information Office was heavily involved in planning and organizing a conference to commemorate the 25th anniversary of NIDR, enlisting the support of the sponsoring organizations, and handling the administrative as well as informational aspects of the observance. Materials written by the Information Office include a major illustrated article for the July 1973 issue of the Journal of the American Dental Association; special speeches and tributes commemorating the anniversary; an anniversary brochure; 12 articles for a four-page special section in the NIH Record; and 17 press summaries.

Media contacts during the year on dental research or related subjects included U.S. News and World Report, U.S. Medicine, Changing Times, Business Week, Reader's Digest, Good Housekeeping, Woman's Day, Redbook, Chemical and Engineering News, Army Times, Scripps-Howard, the Washington Post, the New York Daily News, the Washington Star, and the Chicago Daily News.

Over 93,000 publications were distributed during the year. Recipients included such diverse groups as the Preventive Dentistry Workshop of Dutchess Community College, Poughkeepsie, N. Y.; The Columbia University School of Dentistry and Oral Surgery; Freedman's Hospital, Washington, D.C.; National Association Neighborhood Health Centers, New Orleans, Louisiana; American School Health Association, University of California; Health Information Centers, Memphis and Shelby Counties, Memphis, Tennessee; School for Health Care Services, USAF; Paramedical Occupational Center, Los Angeles, California; Department of Science Education, University of Georgia, Athens, Georgia; and the American Society of Geriatric Dentistry.

Exhibits were shown at six meetings: the American Dental Association, the Federation Dentaire Internationale, the National Association of Biology Teachers, the American Association for Health, Physical Education, and Recreation, and the National Science Teachers Association (two meetings). Information Office personnel arranged for all exhibit showings and staffed the latter four meetings. In addition, arrangements were made for an exhibit on canker sores to be developed for long-term display at NIDR's Dental Clinic in the Clinical Center.

Publications activity initiated by the Information Office included preparation of a new leaflet on "Dental Replants, Transplants, Implants," revision of the flyer on "Summer Opportunities for Dental Students" and updating of the list of graduate training opportunities which appears in the Journal of the American Dental Association. The leaflets on fellowships and training grants were reprinted.

"Tooth Care" was issued by the Department as the first in its HEW Consumer Information Series. NIDR and the Division of Dental Health were jointly involved both in drafting the pamphlet and in underwriting its production costs.

Substantial assistance was provided by the Information Office in readying papers presented at a symposium on "Comparative Immunology of the Oral Cavity" for book publication and in arranging for necessary artwork, clearances, etc.

The series of five colorcasts describing some of the research conducted and supported by NIDR, produced by the Information Office in cooperation with NBC-TV in Washington in 1970, were used in their entirety in one major city, and one segment was shown in three other cities. This makes a total of 11 major cities that have shown the series in its entirety since it was first introduced.

The film, "Laboratory of the Body," produced in 1969 by the American Dental Association under grant support and the close supervision of the Information Office, continued to enjoy great popularity during the year. Although intended for science-oriented high school students, it had also been shown to general audiences on television and is regularly used by several dental schools for showing to their students and in their recruitment efforts. During the year the film was shown to 622,300 persons. As of March 1973, the cumulative number of persons who have seen the film since its introduction was 2.6 million, exclusive of dental students and the television audience.

A major expenditure of Information Office staff time is on internal reports and the requirements of specialized audiences. Such internal reports include NIH weekly and monthly reports, HEW quarterly reports, research and program highlights, opening statements to Congress, special reports to Congress on particular segments of the Institute's program, the NIDR segment of the DHEW annual report, Presidential messages, and numerous other periodic and ad hoc reports.

There is growing interest by the dental profession in ceremonial messages from either the President or the Secretary of Health, Education, and Welfare. Nine such proclamations and other messages were prepared by the Information Office for various observances and meetings.

In summary, the Information Office this year answered 15,004 inquiries; distributed approximately 93,040 pieces of literature; exhibited at 6 meetings; prepared 144 press items, summaries, releases, features, and announcements, including a special article on dental research and your dental health for 139 house organs and 6 "Search for Health" columns; 81 internal reports; 2 speeches; 9 radio spot announcements; arranged 8 radio interviews and 1 television interview; prepared one new leaflet and revised one flyer and one article. The Office also arranged orientation programs and tours for approximately 51 visitors; processed 119 manuscripts and 76 abstracts as the NIDR clearance center; and prepared the NIDR component of the "NIH Annual Bibliography" and the "Professional Staff of NIDR" publications.

Office of Collaborative Research

In last year's annual report, this Office discussed the organizational and functional restructuring which it underwent in Fiscal Year 1972 in response to its added responsibilities. Professionalization of the Institute's contracting activities has thereby been largely achieved. Our efforts in Fiscal Year 1973 have been directed toward maturing and refining contract review and award procedures in an effort to fully develop a sophisticated contracting system.

This year, the OCR has convened ad hoc initial review committees in Bethesda to evaluate the technical merit of all proposals received in response to specific RFP's. Previously, initial reviews were conducted almost exclusively by mail ballot. This year only those projects involving less than $25,000 - 30,000 a year, or more sizable projects for which

only two or three proposals were received, were reviewed by mail. Furthermore, site visits to all those competitors in the zone of consideration were carried out when the project would cost more than $100,000 a year. The procedures for conducting initial reviews and evaluating the results of "competitive range" negotiations have been streamlined. Furthermore, on all proposed new contract actions this office now furnishes the secondary reviewers a complete accounting. For each proposed new procurement, the NIDR Contract Review Group is presented a five- or six-page general summary which discusses every step taken by the OCR from the time a "sources sought" announcement is published to and including those recommendations of a successful offeror. Technical merit summary sheets on each proposal in the zone of consideration, as well as cost analyses of each of these proposals, are likewise presented. The OCR has received favorable comments from NIH management and initial and secondary reviewers, as well as offerors themselves, as to the equitable, objective, and thorough nature of the Institute's review and award system.

During the year this Office also instituted internal policies to assure the timely receipt of technical progress reports as required by contract terms. These procedures entail follow-ups which enable each contract specialist to make certain that project officers furnish to this Office evaluations of the contractors' reports.

A considerable number of contract renewals as well as new procurements fall within the last quarter of the year. This Office has dispersed forthcoming renewals throughout the other three quarters of the year by writing contracts of six to eighteen months duration this year. Furthermore, the Institute has adopted a plan for systematically releasing RFP's much earlier in the year, thereby greatly reducing the possibility of new starts in the last quarter.

Finally, the NIDR this year received no unsolicited proposals. Only two sole source procurements were transacted.

Office of Program Studies and Analysis

Serving as the focal point within the Institute for program data and related information, this Office is committed to the ever-present task of collecting, processing and reporting on a wide variety of subject matters. The most frequent requests have to do with our own support of dental research--questions about the kind of research; who does it; where it is being done; how it is being supported; to what extent it is being supported; how long it has been supported; and what has been accomplished. Other requests are concerned with our research efforts as compared to those supported by other sponsors or to the total effort; the characteristics of the research environment; and comprehensive assessments of research manpower and training.

These data and the variety of reports compiled from them are crucial to the program planning activities of the Director, his senior management group, and the program leaders of the Institute. Over half of the requests for information

come from this sector; one-third come directly or indirectly from Congress, the Department of HEW, or some part of NIH; and the remainder from individuals or organizations outside the agency.

The magnitude of the task is so much greater than the resources available within the Office that it functions primarily as a "switching" station with much dependence on other data gathering and processing activities. It is for this reason that continual and effective liaison with the Division of Research Grants, Division of Physician and Health Professions Education, Division of Dental Health, National Library of Medicine, American Dental Association, American Association of Dental Schools, International Association of Dental Research, Association of American Medical Colleges, National Science Foundation, Science Information Exchange and similar organizations are so respectfully and carefully cultivated. The importance of this relationship is illustrated by the fact that over one-third of our information needs are obtained from sources outside of NIDR.

The majority of requests processed by this Office are of the ad hoc variety and have to do with our support of research by mechanism, disease category, performing institution, investigator, geographic location, numbers of awards, amounts of awards, and similar characteristics.

While these represent the "bread-and-butter-type" of questions, the more challenging requests are illustrated by the following sample of response titles prepared in FY '73: List of National or Major Associations, Foundations, and Societies concerned with NIDR Special Emphasis Programs and their Counterparts in the State of Washington; Federal Inventory of Population Research; Analysis of Innovations--Diffusion of Innovation in Medicine; Newest Advancements in Prosthodontics; Citizen Participation Study; Projects to Develop, Test or Demonstrate New or Improved Health Services Methods of Technology; Scientific Research Policy Studies; Survey of NIH "Consortium Grant" Programs; Average Priority Scores and Approval Rate for Applications from U.S. Dental Schools; Number of People in U.S. with Maxillofacial Injuries and the Annual Cost; Issues for Analysis; and NIDR Research Relevant to NHLI.

The second most common form of information request is of the recurring type. Usually on an annual basis, these responses bear such titles as, Catalog of Federal Domestic Assistance; Health Evaluation Plan; NSF Annual Survey of Scientific and Technical Information Activities; NIDR Support of Nutrition Research; and Minority Grants Program Data. Compilation of the NIDR Annual Report and its abridgements are still other examples of the recurring project type.

Most of the questions received by the Office can be anticipated and are answered from data that is available from within the NIH structure. For purposes of accuracy, consistency and convenience, this information is collected regularly and published under one of four titles, NIDR Grants and Awards, Trainees and Fellows Supported by the NIDR, NIH Support to U.S. Dental Schools, and Dental Research in the United States and Canada. More and more of these data are being made available for current retrieval and processing by our own staff through computerized techniques. The same capability is being utilized to provide individualized information systems, the production of letters for mass mailing, address labels, and a variety of automated data processing services for this Office and a number of other NIDR program areas.

The Office is frequently involved in projects of such diverse nature that they are difficult to categorize but which, nevertheless, consume a significant amount of staff time and effort. For example, the Chief reviews and advises on the submission of technical reports to the National Technical Information Service; a major staff contribution was made to the series of six NIDR Training Program Directors Conferences that started in October; and, background material was developed for meetings of the AADS Committee on Dental Research Manpower. These, and other assignments are accepted as a matter of course and handled with the same care and consideration given to our more usual tasks.

Another major project in which we are currently involved is evaluative in nature. A contract for a questionnaire-type post-training survey has been awarded to Westat, Inc. and will be supported out of 1% set-aside funds. Originally designed to be an NIH-wide survey, only NCI and NIDR were sufficiently prepared to participate this fiscal year. This was due in part to early planning and data gathering by this Office. The report, which is due early in FY '74, is expected to give us the first reliable and the most complete information about the post-training experiences of our trainees and fellows.

The major problems encountered by the Office are more like irritants than obstacles. Physical moves, and we have had four in the last four years, are always accompanied by a period of inefficiency while files and records are being relocated and staff is adjusting to the new environment. Personnel vacancies as a result of transfers and illnesses added to the frustrations that accompanied an already limited work force, especially in the face of rather ambitious plans for expanded activities, e.g., the consolidated computerized control of all NIDR-supported research project information. Reorganization of program areas within the Institute contributed to the difficulty of reporting grants and awards data retroactively. And finally, the lack of good solid information on matters of relevance to NIDR but originating outside of its boundaries. For instance, the support of dental research by sponsors outside of the Federal government; the availability of and need for dental research investigators; or documentable evidence of dental research accomplishments. As previously stated, these hurdles represent challenges rather than insurmountable obstacles and they provide us the goals for the future.

Summary Report
of the Associate Director for National Caries Program
National Institute of Dental Research

The National Caries Program continued to expand its research and development activities in each of its major strategy areas of routes to caries prevention:

I. Methods to Attack the Microbiologic Agents
II. Methods to Protect the Tooth
III. Methods to Alter the Diet
IV. Methods to Improve Delivery and Acceptance of Caries Prevention Technics

A two-year study of the daily use of a mouthrinse of chlorhexidine glucanate was completed, in Aarhus, Denmark. The results demonstrate the feasibility of reducing the level of the oral flora by this method, without inducing undesirable overgrowth of any strain. The subjects experienced marked reductions in plaque accumulation and gingivitis; no caries developed. Plans were finalized to begin short-term clinical trials of approximately one dozen antibiotics and anti-microbials which have now undergone laboratory screening and successful animal testing.

Investigations continued in an attempt to improve understanding of the mechanism of action of fluoride compounds. Comparative study of children in six cities with and without fluoridated water, was unable to demonstrate a clear correlation between caries prevelence and level of fluoride in the outer enamel layers (as determined by abrasive biopsy), suggesting that the caries-preventing effect of fluoride may, in part, be exerted through an anti-plaque mechanism.

Amine fluoride compounds, which apparently possess anti-streptococcal properties, continue to look promising in short clinical trials. Accordingly, a two-year study of daily use of an amine fluoride mouthrinse and dentifrice was initiated under contract.

In an effort to develop information on the cost-effectiveness of currently available caries preventive methods, a large community demonstration program was begun in which topical fluoride, adhesive pit and fissure sealants and oral hygiene instruction are provided in combination to elementary school children.

Follow-up continued of approximately 4000 children participating in three long-term trials of a bis-GMA adhesive tooth sealant. Results thus far vary with the study site and personnel involved but are generally favorable. Best results have been seen in fluoridated Rochester, N. Y. where over 80% of the sealant material has been retained on the teeth one year after application.

Dr. Charles Donnelly, Chief, Caries Prevention and Research Branch, retired during the year and this position remains vacant. Dr. William Rogers assumed direction of the extramural activities of the Program, being appointed

Scientific Coordinator of Grants and Contracts in the Office of the Associate Director. Dr. Zora Griffo replaced Dr. Rogers as Chief, Caries Grant Programs Branch.

The Dental Caries Program Advisory Committee was formally constituted with 8 members appointed for overlapping terms. The Committee held its first meeting in March 1973.

Five workshop conferences were held during the year as a mechanism to provide program management with critical expert advice on program content and direction. Program areas examined included Topical Fluorides, Antimicrobials, Immunization, Trace Metals (2), and Physicochemical Aspects of Caries Initiation.

The entire Program staff assembled at Belmont Conference Center for three days in January 1973, to develop the Program Scientific Plan for FY 1974.

Contracts
National Caries Program
National Institute of Dental Research
Summary Statement

Contracts enable the National Caries Program to accomplish objectives such as answering a specific question, procuring a tangible item, or establishing process "know how" in a timely and efficient manner. The mechanism is used to obtain critically needed research in caries etiology, pathophysiology, and epidemiology and to develop and test agents, evaluate treatments, and analyze acceptance and utilization of new agents.

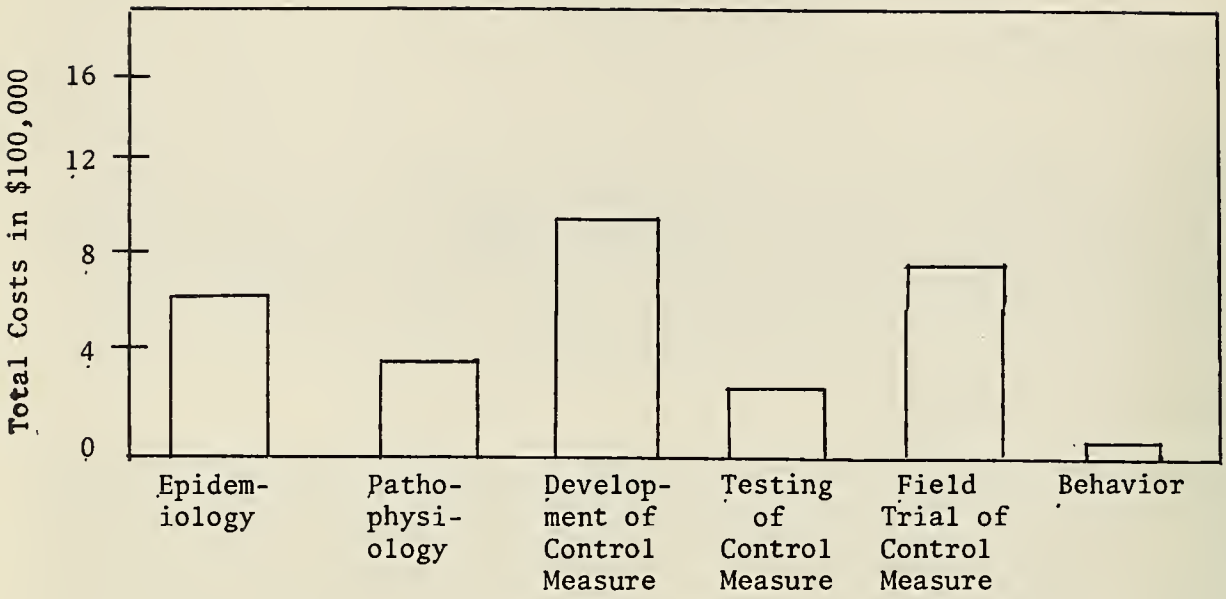
Administrative Activities

Comprehensive planning, centralized in the Office of the Associate Director, NCP, and involving extensive use of outside consultants, precedes formulation of all requests to initiate contracts. These plans, such as the 1974 "Blueprint" of grants, contracts, and direct operations, specify steps necessary to achieve program objectives, list priorities among objectives, and analyze time and resources required to carry out work. Subsequent to in-depth scientific review of proposals conducted by the Office of Collaborative Research and implementation of the contract, NCP project officers work on a week-to-week basis with the principal investigator to insure that progress is satisfactory. Progress is formally reported to them three times a year and renewal requests are closely appraised in two stages of review at the end of each contract period. Thus extensive staff involvement both in the initiation of the contract request and throughout the duration of the project insure that NCP objectives are achieved. The first NCP contracts were awarded two years ago. Some of these now are entering the stage of product delivery.

In FY 1973, the National Caries Program has supported 38 contracts, 5 interagency agreements, and one work order. Several more contracts, now in final stages of review, will be awarded before the end of the year. FY 1973 expenditures for contracts will be approximately $3.0 million, but numerous urgent program requirements are not supported at this funding level.

As shown below considerably more research is supported through contracts on development and evaluation of control measures for caries than on etiology and pathogenesis of the disease. Research on patient attitudes toward dental disease and acceptance of new agents is a component in several projects and reflects increasing NCP interest in the delivery of new agents to the public.

Contracts by Relation to Control Measure FY 1973 Funds



COMBATTING THE MICROBIAL AGENT

In FY 1973, the National Caries Program is supporting 14 contracts and 2 inter-agency agreements on microbial aspects of dental caries, including epidemiology, etiology, development of control measures, and clinical testing of control measures. Research presently is concentrated on smooth surface coronal caries; however, we are expanding research on caries of root surfaces. Dentists and oral health scientists have been aware that as teeth last longer caries of the root surface, usually associated with periodontal disease, is more frequently observed. The National Caries Program has contracted with Temple University to determine the prevalence of this form of caries. In one population examined by Temple University scientists 197 of some 500 subjects had at least one lesion. Many lesions noted clinically were not seen radiographically. Typically the incidence of root caries increased with age from approximately 13% in the 20-29 age bracket to 70% in that group of people over 60 years of age. Now that surveys show that root caries is both prevalent and severe, these scientists are examining the relationship of the disease to contributing factors, such as oral hygiene, gum recession, gingivitis, advanced periodontal disease, and coronal caries.

Though strains of Actinomyces naeslundii and A. viscosus are recognized to induce periodontal lesions and root surface caries in gnotobiotic rats, the organisms responsible for human root caries are not uniquely known. To obtain this information dental scientists conducting epidemiologic studies need to be able to identify and enumerate with ease plaque bacterial populations associated with root caries. It is known that about one-third of the cultivable microorganisms from gingival deposits are gram positive facultative or anaerobic filament-forming bacteria of genera Actinomyces, Rothia, Lactobacillus, Arachnia,

Bifidobacterium, Corynebacterium, and Bacterionema. The National Caries Program has entered into an interagency agreement with the Center for Disease Control in Atlanta, Georgia, to develop fluorescent antibody reagents which can be used to identify and possibly enumerate these organisms in plaque, in slide smears, and on agar plates. The contractor will provide us with information on the optimal conditions for application of these conjugates in the laboratory and on large-scale production of the conjugates. It is relevant to point out that not only are these organisms possibly important to root caries and periodontal disease, but with injury to mucous membranes some of these organisms can spread throughout the body and cause classical actinomycosis of various organs.

Two similar projects support research on S. mutans, which considerable evidence suggests is a chief agent for coronal caries. Again, no practical method currently is available to study the infectious process in humans. Therefore, the National Caries Program, both for use in its intramural program laboratory studies and for use by the scientific community at large, is supporting a contract with Huntingdon Research Laboratory and an interagency agreement with the Center for Disease Control, Atlanta, Georgia, to produce fluorescent antibody conjugates. Widespread availability of standardized conjugates for each of the strains of S. mutans will promote consistency in identification of these strains by scientists throughout the country.

NCP scientists believe earlier detection of caries lesions would: (1) decrease the time taken for epidemiologic studies, (2) allow studies of remineralization and other natural defense mechanisms for early caries, and (3) aid in studies on effects of foods and food additives on caries. Supported by a contract with the American Dental Association, a scientists is evaluating the clinical use of natural tooth fluorescence in detecting incipient caries lesions. To validate that decreased fluorescence indicates incipient caries, it is necessary to show by microscopic and biochemical techniques that indeed caries processes are evident and that clinical and radiographically demonstrable lesions develop later at these sites. In studies of enamel areas of decreased natural fluorescence the American Dental Association scientist finds through use of the scanning electron microscope that the surface appears more porous and overlaid by rod-shaped microorganisms in a thin web that coats the enamel.

Evidence has come to light in the last year or two that the ability of microorganisms to adhere to enamel and to mucosal cell surfaces is a major factor in pathogenicity and is reflected in epidemiologic findings. To further the study of these relationships the National Caries Program has contracted with the University of Minnesota to develop an assay for adherence of microorganisms to enamel. The assay measures the attachment of radio-labeled microorganisms to granular hydroxyapatite. With it, scientists will be able to assay chemotherapeutic agents that might block adhesion as well as study the adhesion mechanism and its relationship to disease. The National Caries Program also has contracted with the University of Florida to confirm the existence of and describe natural factors that block adhesion and cell-to-cell agglutination. Under this contract human saliva is being surveyed for ability to inhibit the adhesion of strains of S. mutans to smooth surfaces

and for correlations of adhesion inhibition to caries incidence and past dental history. Thus far the principal investigator on this contract finds that only caries-free persons have saliva which inhibits adhesion greater than 30%. Where adhesion inhibition is less pronounced some caries-free and high-caries persons are represented. The investigator thinks the adhesion-inhibitory material in saliva is an antibody to dextran sucrase, an extracellular enzyme of S. mutans.

The etiology and pathophysiology of human caries is particularly difficult to study because many months are required for clinical manifestation of the lesion. A model for studying human caries in shorter periods is suggested by the rampant caries reported to follow X-radiation of the head and neck. Currently the National Caries Program is supporting contracts with the University of Texas at Houston and the Cancer Research Center, Columbia, Missouri, to examine this model for studies of caries etiology. Since salivary gland function largely disappears after irradiation, the condition potentially provides new ways of looking at etiologic interrelationships of saliva, cariogenic microorganisms, and nutrients. The investigator at the University of Texas reports that within two to three weeks following X-radiation, xerostomia (dryness of the mouth) develops and is accompanied by increases in S. mutans, in lactobacilli, and in yeast, and decreases in S. sanguis, neisseria, and other organisms. In an average of seven months, 11 patients suffering from xerostomia demonstrated 195 new caries lesions. Conversely in 7 patients treated with a topical fluoride gel, there were only two new caries surfaces after 5 1/2 months. It is hoped that other control measures in addition to fluoride can be quickly evaluated in this model.

The National Caries Program currently is supporting 7 contracts to develop and test control measures such as immunization, techniques to disperse plaque, and new antimicrobials specific for cariogenic microorganisms. Many dental scientists believe that elimination of dental plaque can effectively reduce the incidence of human caries. To test this hypothesis, we are supporting a contract with Beckman Instruments, Inc., to produce enzyme systems which specifically degrade the adhesive dextran-like polymers produced by human cariogenic streptococci. Beckman Instruments has developed techniques for obtaining high yields of human streptococcal polymers to be used in enrichment and screening of fungal dextranases. Some of these dextranase preparations now are being employed by NCP laboratory staff in animal tests to decrease plaque. This contract also has provided fundamental information on the structure of dextrans. For instance, dextran from S. mutans, HS7, has been found to be highly branched with 25% of the glucose residues involved in alpha 1-3-6 branch points. 31% of the glucose linkages in this molecule are alpha 1-3 with insignificant amounts of alpha 1-4, or alpha 1-2.

Cariogenic microorganisms are critically dependent upon sucrose and other food carbohydrates for energy as well as for polysaccaride synthesis. Interference with special mechanisms transporting monosaccharides into the cell could provide a means of controlling S. mutans. Therefore the Program has contracted with the University of Minnesota to investigate unique features of the membrane associated phosphotransferase sugar uptake system of oral cariogenic bacteria and to analyze for antimicrobials which could inhibit this system.

Major characteristics of the glucose transport system of S. mutans have been worked out, and studies are in progress on the fructose transport system.

On laboratory shelves in pharmaceutical houses there are known to be many antimicrobial agents which have potential value against caries. To identify such agents the National Caries Program has contracted with the Medical College of Georgia to solicit medicinal agents from pharmaceutical houses and assay these for inhibition of bacterial plaque formation, acid production, and growth. Screening is against S. mutans, salivarius, sanguis, and mitis, S. aureus, L. caseii, albicans, O. viscosus, and A. israelii. In the first year of this contract 21 compounds were tested, while 56 new compounds are undergoing tests in the second year. Thus far 7 agents have exhibited desirable properties both in vivo and in vitro, and 5 agents look suitable for clinical testing.

The National Caries Program has awarded two contracts for research to establish the feasibility of caries controlled through immunization. At Forsyth Dental Center a scientist is evaluating different adjuvants and sites of injection to determine the vaccination system eliciting most rapid onset of high-titered salivary antibodies. Both salivary immunoglobulins and salivary antibody-forming cells induced by vaccination are being examined. In three experiments which have been completed in pathogen-free rats the scientist reports that salivary IgA antibodies to mutans were consistently elicited and that immunized animals subsequently challenged with S. mutans developed less caries and had lower levels of the microorganism than did non-immunizing animals. At Southern Illinois University an investigator is using glucosyl and fructosyl extracellular enzymes of cariogenic streptococci as a basis for vaccines. The purified enzymes, glucosyl and fructosyl transferases (plaque polysaccharide forming enzymes) and glycosidic hydrolases (enzymes that split complex carbohydrates) are inoculated to rats or monkeys in the area of the submaxillary gland. Subsequent development of salivary antibody titers and protection from caries is followed in these experiments. In his first progress report, this investigator reports vaccination with glycosidic hydrolase appears to provide some caries protection in monkeys.

During FY 1973 the National Caries Program has contracted with the Royal Dental College, Aarhus, Denmark, for a clinical trial of chlorhexidine, an agent attracting considerable attention for possible control of caries. In this study dental and medical students rinse their mouths daily with a 0.2% solution of chlorhexidine for 1 minute. Shifts in plaque microflora, differences in plaque and caries incidence, and possible deleterious side effects of daily long-term use of the agent are being carefully monitored. Early results from the Aarhus study show that the numbers of tooth surfaces covered with plaque and the amount of gingivitis decreased after chlorhexidine treatment, and that plaque samples have a 50% reduction in anaerobes, aerobes, and streptococci. Staining of the teeth or gingiva by chlorhexidine was not found to be a problem, and no other adverse reactions were observed.

INCREASING THE RESISTANCE OF THE TOOTH

It is commonly held that the caries-inhibitory effect of direct applications of fluoride to teeth increases with the amount of fluoride that becomes associated with the enamel surface and the depth of fluoride penetration. These relationships need to be examined in detail and related to mechanisms of fluoride anti-caries action and to techniques increasing fluoride uptake by enamel. To obtain this information, we are contracting for clinical trials of topical agents including evaluation of anti-caries activity and periodic determination of residual enamel fluoride.

Through contracts during FY 1973, the National Caries Program has supported four clinical trials on efficacy and mechanism of different topical fluoride treatments and supported two projects to analyze the fluoride content of enamel samples. Stickney Township in Illinois is carrying out a study to determine whether the low caries rate in children receiving municpally fluoridated water can be reduced further by topical fluoride treatment. The treatment involves a 15-minute exposure to fluoride-containing gel one time each day for 25 days and produced very high levels of enamel F. Two years later the fluoride level of the teeth in the treated group had dropped, but remained more than 3 times that of the control group, and clinical examinations showed that teeth had received considerable protection above that provided by municipal fluoridation. Two studies of simple caries control for children are being carried out by a Forsyth Dental Center investigator. One involves a semiannual topical treatment of dilute H_3PO_4 followed by 0.62 Molar NH_4F. These solutions are swabbed on the teeth by a dental hygienist. The other involves supervised, daily 1-minute mouth rinses with a small amount of 0.5% NH_4F. The fourth study, conducted in Puerto Rico by University of Miami scientists, utilizes an amine fluoride-containing gel applied to the teeth under the direction of a school nurse. The gel is applied to the teeth for 10 minutes each day for 5 days with a fitted mouthpiece.

To analyze enamel samples derived from above studies and from research carried out directly by NCP scientists, a contract is maintained with the Kendall Company, Barrington, Illinois. Each year this firm carries out several thousand fluoride, calcium, and phosphate analyses for NCP. Through an interagency agreement, a second fluoride analytical service has been established at the Naval Dental Research Institute at Great Lakes Naval Base. This service will analyze enamel biopsies provided by the dental component of the National Nutrition Survey.

Polymer films also appear promising in protecting the tooth surface, particularly in the pit and fissure areas. Thus the Program has contracted for three longitudinal studies under conditions resembling possible eventual public health use of these agents. The studies are being carried out in Rochester, New York (Health Research, Inc.), the Virgin Islands (University of Puerto Rico), and Washington, D. C. (Howard University). In each study an ultraviolet polymerized sealant is used. Some of the questions under evaluation are the amount

of training that is necessary to apply the sealant properly, the anti-caries efficacy of treatment, and retention of the sealant. Data from these studies now entering their third year show that differences in technique of application cause large differences in sealant retention and that some sites are considerably more difficult to seal than others.

MODIFYING THE DIET

Caries control through decreased consumption of sucrose is made difficult because of public predilection for sweet foods, patterns of snack food consumption, and agricultural, food processing, and marketing patterns based on sucrose. The National Caries Program has committed appreciable resources to solve problems in this complex area. Through twelve contracts, we are investigating new sweeteners (both carbohydrate and noncarbohydrate), developing new formulae and processes to lower the sucrose content of high-sucrose foods, evaluating food characteristics that magnify the cariogenicity of snacks, and developing dietary additives to make sucrose less cariogenic in foods.

At the University of Pennsylvania a noncarbohydrate sweetener isolated from berries of a tropical plant is being studied. This remarkable substance, several thousand times more sweet than sucrose, is a protein. Scientists at the University are gathering information that would establish whether it is feasible to use this sweetener in foods and that possibly would lead to the synthesis of simpler sweet-tasting polypeptides. Development of this sweetener has assumed more importance and urgency with recent Food and Drug Administration rulings that restrict use of other noncarbohydrate sweeteners such as saccharin and cyclamates. At the University of Alabama a scientist is attempting to reduce cariogenicity of sweet foods by substituting carbohydrates such as fructose, glucose, corn syrup solids, lactose, lactulose, and sorbitol for sucrose. At the Research and Development Center of Foremost Foods, Inc., and the J. F. Bell Laboratories of General Mills, Inc., food scientists are formulating and testing new low-sucrose foods containing other carbohydrates which would be competitive in the marketplace. Typically, the sucrose content of these snack foods would be 15% of customary. Some foods being modified are candies, jellies, confections, soft drinks, frosting mixes for cakes, puddings, cookies, and cereals. Many of the sucrose-substituted and nonsubstituted foods are being tested for relative cariogenicity through contracts with the University of Alabama at Birmingham, Harvard University, and Eastman Dental Center. In these tests rats are allowed access to the foods in a pattern simulating human snacking of high-sucrose foods. Thus information on probable cariogenicity in the human can be obtained, and additives to inhibit caries can be evaluated. In addition we have contracted with Eastman Dental Center to develop *in vitro* tests for rapid evaluation of caries potential of foods and with the University of Alabama in Birmingham to develop a test for cariogenicity of foods in the human mouth. In this novel approach problems caused by differences in individual tooth resistance are obviated by using uniform, small slabs of bovine enamel attached to prosthetic devices in the mouths of volunteers. The slab can be removed for close examination of its surface to observe the effects of different diets or anti-caries agents.

The National Caries Program has contracted for actual clinical trials of food cariogenicity at the University of Minnesota. In these trials student

volunteers receive only experimentally prepared diets, and habits of oral hygiene are closely controlled, in a hospital environment. Plaque samples are harvested at frequent intervals and analyzed for microbiological and biochemical changes. Trimetaphosphate, an effective cariostatic agent in animal tests but not yet found effective in human tests, will be reevaluated in the University of Minnesota study. Studies of trimetaphosphate also are being carried out through contract with the University of Alabama in Birmingham. In the latter trial trimetaphosphate is incorporated into gum, which is dispensed and chewed several times a day for 10 minutes.

The Program also is attempting to distinguish through epidemiologic studies natural, caries-limiting factors which might provide the basis for new control measures. To identify these factors an accepted practice is to compare a low caries population with one which resembles it closely except for wide differences in caries incidence. Among populations which have been identified as useful in such studies are sets of villages in Colombia, South America, and Papua-New Guinea. In the latter area large differences in caries have been noted in villages located along the Sepik River. It is believed that minerals in silt deposited in the gardens during floods may be responsible for low caries rates and may exert their effect according to different farming practices. To establish the nature of the factor which protects against caries, we have contracted with the World Health Organization to conduct oral examination of the villagers, collect and analyze samples of food, water, and soil. A similar study, supported by contract with Forsyth Dental Center, is under way in Columbia, South America, where two nearby villages show striking differences in caries prevalence. Areas of low caries prevalence not associated with fluoride also are known in the United States. The National Caries Program has contracted with Eastman Dental Center to determine the extent to which trace element content of teeth might account for these rates. Scientists at Eastman Dental Center have arranged for over 100 dentists in low caries control areas to collect specific teeth from residents. These teeth and other samples are then analyzed at Eastman Dental Center.

DEVELOPING IMPROVED DELIVERY SYSTEMS FOR CARIES

As seen in the above sections, the National Caries Program has a strong research and development program on caries control measures. In parallel, the National Caries Program is developing another to insure acceptance and utilization of newly developed agents and treatment plans. Many factors affect utilization, such as perceived health status and benefits, costs to individuals in public health programs, and ease and attractiveness of use. Much depends on the type and level of information which the public receives about new control measures. Currently we have contracted for three studies to evaluate the benefit and cost of specific treatment regimens in school children. Educational programs to encourage greater concern for tooth care are included in the protocols. One of these was initiated in the beginning of 1972 in fifth and sixth grade school children at the Navajo Indian Reservation in Arizona. This study will determine the effectiveness and cost-benefit ratio of a treatment combining application of two sealants to the pit and fissure area of the molars and repeated fluoride applications. The Program also has contracted with the University of Michigan and the State University of New York for studies to determine if school age caries can be eliminated through use of

all anti-caries treatments now known to be effective. In both studies children already receive fluoridated water and some oral hygiene instruction and prophylaxis. Though differing in detail, each study is evaluating the added benefits of periodic application of adhesive sealants to protect occlusal surfaces and acidulated phosphofluoride treatment to protect smooth surfaces. In addition, a behavioral motivation program of oral hygiene instruction and family encouragement is being tested through the contract with the State University of New York. These large trials in Ypsilanti, Michigan, and Buffalo, New York, are just finishing their first year and will require several further years for completion.

The National Caries Program has also contracted with the University of Minnesota for research to develop a prognostic test for caries. Such tests conceivably could strongly motivate individuals to improve their oral health and would have considerable value in shortening epidemiologic tests and trials of chemotherapeutic agents. The Minnesota study involves 750 children, six to eight years old. Data on their dental history, dietary habits, and oral hygiene are being collected. Plaque samples are taken at two-week intervals from noncarious surfaces of permanent first or primary second molars and assayed for the presence and relative proportions of cariogenic streptococci and lactobacilli. In addition plaque is assayed for ability to synthesize polysaccharide. Based upon correlations between plaque microbiology and metabolic characteristics and developing caries, the University of Minnesota scientists will attempt to design a test to predict caries in children. This test also should have value in predicting certain types of caries in adults.

Caries Grant Programs Branch
National Caries Program
National Institute of Dental Research
Summary Statement

The Caries Grant Programs Branch (CGPB) administers the support of that research carried out by means of grants in the NCP. These grants span the spectrum from caries-relevant basic research to the clearly applied. A major responsibility of the CGPB is to develop a rigorously tested base of information concerning caries etiology and to facilitate the maturation of basic research findings to the stage where they are suitable for highly applied research and development.

Administrative Activities

The staff of the CGPB reports to the Associate Director, NCP. The activities of the CGPB are closely coordinated with contract activities and direct operations of the NCP to achieve program goals, and like these other entities it seeks guidance and program review from the Dental Caries Program Advisory Committee. In its management of grants, however, the CGPB functions according to the policies and procedures of the EP. Thus, after the Study Section review of new grant applications, staff of the CGPB presents those that are assigned to it because of caries relevance to the National Advisory Dental Research Council for final recommendations. CGPB staff also periodically reviews for Council the activities and program plans of the Branch and analyzes for it the current grant support of caries research.

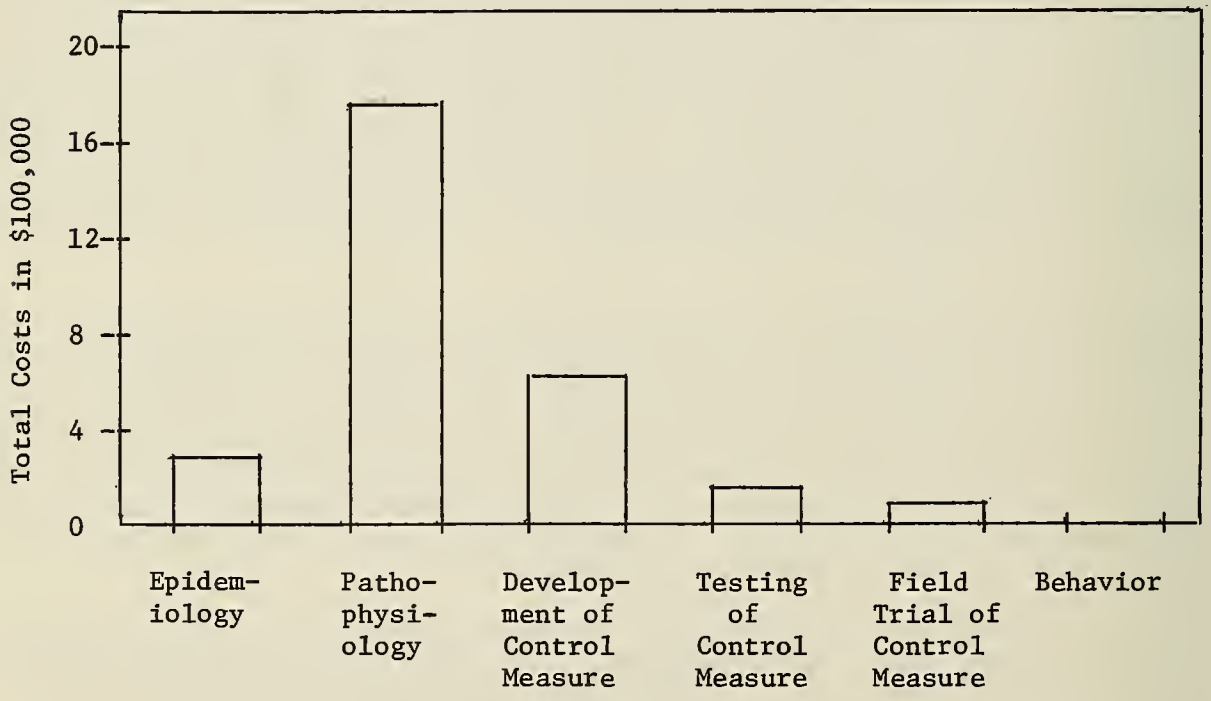
In FY 1972 the CGPB provided financial support for 46 research grants at a total cost of $2.7 million. Early in FY 1973 we faced a level budget but had urgent program needs for research described in approved grant proposals. To rectify this problem the NCP reprogrammed $0.275 million of contract funds for use in the grants program. Later in the year a major administrative step was achieved through establishing an independent yearly budget for the CGPB. This budget, fixed at $2.993 million for FY 1973, allowed 8 research grants to be initiated and 2 to be renewed. In total during the year the Branch has had administrative responsibility for 59 grants.

In NCP's "Blueprint" and other plans we have described many questions which must be answered and could be addressed now. Not only would this information materially shorten the time to achieve NCP objectives, but scientists are eager to carry out required research, and our staff has the capacity to handle additional projects. The reality that we face in FY 1974, however, is a reduced budget leading to probable cutbacks in individual grants and little new research. CGPB staff will attempt to compensate for this cutback by re-balancing its grants program to make it even more effective. In each area of the program the urgency of NCP requirements, the contribution of ongoing research and our investment will be scrutinized, and grants budgets will be adjusted

accordingly. In doing this it is quite possible to "cut too close to the quick" of research. With advice from Council and advisory groups, however, we will do what is possible and prudent to prevent FY 1974's cutbacks from slowing NCP progress.

As shown below, much more research is supported through grants on the pathophysiology and etiology of caries than on the development, testing and field trial of caries control measures. This distribution provides a strong base of information on fundamental aspects of caries to serve as a resource out of which can come better caries control measures in the future. One may expect, however, as the program matures that relatively increased support will be given to studies dealing more directly with caries control. Significantly, research supported through contracts compliments that supported through grants in the areas of epidemiology and development of control measures.

Grants by Relation to Control Measure FY 1973 Funds



Evaluation and Advisory Meetings

To obtain information upon which to base programming decisions the National Caries Program has exposed its staff in FY 1973 on almost a daily basis to visitors representing the top talent in caries research. In addition it has sponsored four "State-of-the-Art" workshops (Immunology, Trace Elements, Anti-Microbial Agents, and Topical Fluoride Treatment) and has held a retreat to coordinate planning among staff. Recommendations from these meetings and subsequent staff interactions have led to the development of a 14-page NCP Blueprint that establishes priorities in NCP requirements for information in

FY 1974. This document was presented March 19-20 to the newly organized Dental Caries Program Advisory Committee. It is used as a major guide by the CGPB in program planning and in coordinating its activities with other elements of the NCP.

Combatting the Microbial Agent

Research on the microbial etiology of caries is progressing through the following phases: first, isolation and identification of microorganisms from carious lesions and plaque; second, development of techniques for rapid enumeration of these organisms; third, animal and epidemiologic studies to confirm the pathogenicity of suspected organisms; and fourth, research on metabolic and antigenic characteristics of implicated organisms to develop control measures.

Greatest attention currently is being given to a species, Streptococcus mutans, believed to be the chief, but not only, etiologic agent of smooth surface caries. Certain strains of this organism are much more pathogenic than others. This pathogenicity is thought to be highly dependent on the mixture of organisms that live symbiotically in plaque. These concepts can be tested through epidemiologic studies comparing caries prevalence with the particular species and strains of microorganisms found at carious sites in the mouth. Unfortunately the classification of strains of S. mutans remains unsatisfactory with, among other problems, major differences existing among the strains of organisms grouped collectively in the species. Furthermore, no easy method exists for the identification and enumeration of microorganisms of plaque samples collected in epidemiological studies. To resolve these problems the CGPB currently is providing considerable support to research having the objective of developing a classification scheme for S. mutans similar to the Lancefield serologic scheme for B-hemolytic streptocci and to studies leading to rapid procedures for identifying and enumerating microorganisms in plaque samples.

For serologic identification of S. mutans the Branch is supporting research in two general areas: (1) to isolate and identify antigens that truly represent each S. mutans group and (2) to improve the specificity of available antisera. In both of these areas we believe significant advances have been made by CGPB grantees. At the University of Florida one of these scientists has reported the isolation and main structural characteristics of glycerol teichoic acids from the cell walls of representative organisms of S. mutans, Jablon-Zinner groups I and II. He reports that each of these teichoic acids appears to be specific for its group. At Villanova University a scientist reports isolation of a rhamnose-containing glycoprotein antigen from cell wall of S. mutans group II. There is evidence that the sugar groups of the glycoprotein antigen and the teichoic acid antigen of group II are similar. The latter work appears to be corroborated by reports from Northwestern University concerning a very similar group II cell wall antigen that occurs in both polysaccharide and glycoprotein forms. These results will help tremendously in the effort to develop serologic methods for accurate identification of organisms of these groups.

In the second of the areas mentioned above the CGPB is supporting research at the Florida Institute of Technology, at the University of Michigan and at the University of Miami to work out the preparation of group specific, high

potency fluorescent antibodies. At the latter institution a research team has been attacking the particularly difficult problem of preparing fluorescent antibody that is specific for Bratthall strain b. Certain unidentified oral streptococci that are non-cariogenic and not members of S. mutans have been found to react strongly with group b antiserum and to have caused erroneous interpretation of results in some epidemiologic studies. The University of Miami team now reports that certain strains of S. Sanguis will adsorb the cross-reacting antibody from the antiserum. It will be interesting to see if antiserum prepared against the group b antigens described above also obviates the problem of interference.

Alternatives to the fluorescent antibody technique are being examined also. At the University of Alabama and the University of Maryland, CGPB grantees are employing the phenomenon that microorganisms are strain specific in their susceptibility to infection by bacteriophages, and in the production of and sensitivity to bacteriocins, as aids in classification and identification. These investigators are designing batteries of bacteriophage and bacteriocin indicators which exactly identify an unknown microorganism by the specific pattern of inhibition that is obtained. The design and preparation of these arrays of indicators require many months of technical effort but once accomplished will significantly facilitate the identification of caries isolates.

The Branch is supporting smaller amounts of research on other microorganisms contributing to smooth surface caries and to microorganisms associated with pit and fissure caries and caries of tooth roots. Generally research on the microbial etiology of the latter types of caries is following a path similar to that of research on smooth surface caries. Thus in investigating deep dentinal caries a CGPB grantee at the New Jersey Dental School is isolating lactobacilli found in these sites, separating their cell wall components, and testing these as possible group specific antigens. This investigator reports that with fluorescent antisera prepared against L. casei (Sharpe's group C) he finds homologous organisms in the dentinal lesions of the majority of teeth that he examines.

With the methods of identification now available, caries researchers are making some headway with epidemiologic studies. Using fluorescent antibody techniques, the research group at the University of Miami has found in a study on caries-free and caries-active school children that S. mutans is found on normal tooth surfaces of caries-active and caries-free students, as well as on carious surfaces. On the other hand, this group has found that the total numbers of certain streptococci and that certain types of S. sanguis and S. mutans are related to caries status and that diets high in sucrose and poor oral hygiene are positively correlated with the disease.

These correlations between organisms and caries are not as marked as one might expect, and other groups who have examined the relationship have not been convinced that differences in either the number or type of organism in plaque were significant. It is obvious that if differences can be ascertained--for instance, that a specific microorganism is present wherever plaque is pathogenic--the result will greatly help dental researchers to focus their attention on these organisms or phenomena actually responsible for the disease. At Eastman Dental Center a grantee has been convinced for some time that such

differences do exist but are masked by the large diet and hygiene effects on plaque metabolism. Through extreme care in standardizing diet, oral hygiene and plaque sampling in her subjects, this investigator now has demonstrated clear differences in plaque associated with pathogenicity.

At Forsyth Dental Center a CGPB grantee has been examining specific tooth surfaces in individual children to establish the rate at which changes develop in the resident microflora. This investigator reports that some children harbor S. mutans consistently on certain surfaces but not on others, suggesting that the spread of this organism is very slow. To test this possibility some of the surfaces that had consistently shown the presence of S. mutans were sterilized with iodine solution. Half of these surfaces remained free of S. mutans for several months afterward, demonstrating that periodic removal of S. mutans might prove to be an effective method for caries control.

In addition to epidemiologic studies aimed at establishing the identity of microorganisms involved in caries and determining their transmissibility, the Branch is providing considerable support to research to explain how these organisms adhere to the tooth surface and initiate plaque formation. At Forsyth Dental Center, Northwestern University and other institutions, scientists supported by the CGPB theorize that special structures in or on the microbial surface provide each microorganism with the ability to adhere to the particular oral surfaces that it colonizes. In the case of S. salivarius and S. pyogenes the attachment to epithelial surfaces is through an outer fibrillar "fuzzy coat." In the case of S. mutans, attachment to tooth surfaces, pellicle and plaque is through strands of high molecular weight dextran bound to the cell membrane. Other organisms such as S. sanguis, S. miteor, A. viscosus and A. naeslundii react with salivary glycoproteins to form insoluble aggregates that bind to and become part of the matrix of plaque. It is believed that blocking the initial adhesion of the pathogen presents some of the best possibilities for control of caries and other oral diseases.

Because of sugar's involvement in formation of plaque, and because sugar metabolism leads to acid production on the tooth surface, the Branch is providing major support to studies on carbohydrate utilization by cariogenic streptococci. Many plaque organisms produce characteristic proportions of dextran and levan polysaccharides from sucrose. These are produced on the surface of the microorganism or are synthesized extracellularly. In addition, large amounts of monosaccharides are produced in this process or are formed by hydrolysis of sucrose or other carbohydrates. Each of these monosaccharides is phosphorylated, carried through the cell wall by specific enzymes and either polymerized into a storage form of carbohydrate or utilized for energy with eventual liberation of organic acids.

It would appear that an outstanding prospect for control of smooth surface caries is provided by the seeming dependence of plaque on these polysaccharides. Since selection and design of antimetabolites to block enzyme synthesis of plaque polysaccharides would be greatly simplified by information on the active sites on the enzyme and the mechanism of enzyme action, CGPB grantees at Villanova University, the University of Miami and University of San Francisco are isolating dextran and levan sucrases (the polymer forming enzymes), both cell-associated and free, of several plaque microorganisms and studying their

properties. It was reported last year that one of these scientists had determined the bush-like structure of dextran produced by S. mutans. This year he has isolated and purified the extracellular enzyme that makes this dextran and proceeded to isolate and study the cell wall enzyme of S. salivarius that is responsible for levan synthesis. He finds that the latter enzyme forms levans ten times more rapidly than the S. mutans enzyme forms dextran. He also describes techniques for extracting and purifying levan sucrase from S. salivarius and presents data on major characteristics of this enzyme. This is important because levans are known to be easily hydrolyzed to fructose by some plaque microorganisms and could provide reserves of carbohydrate to be utilized during intervals when foodstuffs have been washed out of the mouth. Another scientist has been isolating and characterizing the cell-wall dextran sucrase of a strain of S. mutans. He finds that this enzyme is continuously produced by sucrose-grown cells but becomes progressively entrapped in the insoluble dextran being synthesized and is eventually inactivated.

Previously it has been observed on purifying cell-free dextran sucrase from one strain of S. mutans that several enzyme fractions are obtained during chromatography. At a national scientific meeting this year it was reported that the number of observed fractions is from one to six, depending on the strain of S. mutans from which enzyme is isolated. This observation is important because dextran formation involves several processes (chain elongation, branching and formation of high or low molecular weight dextrans), and we do not know whether more than one enzyme is required to carry them out. The observed multiplicity of enzymes could represent these special functions, or they might represent simply a family of isozymes having a common function but various molecular configurations. In any case there is convincing evidence that different strains of cariogenic streptococci produce polysaccharides with different structures and different susceptibility to enzymatic attack. The clinical situation is even more complex since human plaque contains polysaccharides from a variety of microorganisms. Therefore it appears that it will be necessary to employ a mixture of hydrolytic enzymes if successful dispersion of plaque is to be achieved through this approach. To obtain initial evidence on this possibility one investigator has isolated the mixture of extracellular dextran-synthesizing enzymes from each of 6 strains of S. mutans and 1 strain of S. sanguis and incubated each mixture with sucrose to obtain typical samples of mixed dextrans produced by each organism. The polysaccharides were found to be almost exclusively polymers of glucose but otherwise differed considerably. By direct observation in an electron microscope some were found to be spheres, some ellipsoidal, and some were aggregated in chains and clumps. Furthemore, when incubated with dextranases (hydrolytic enzymes) from several commercial sources, hydrolysis varied widely; and with several commercial preparations of amyloglucosidase (another hydrolytic enzyme) there was almost no breakdown of dextran. It is believed that these variations are due both to the position, number and type of branch points in the dextran molecule and to the different ability of dextranases to hydrolyze the particular glucose-to-glucose bonds at these points.

An alternative to dispersion of plaque is caries control based on prevention of plaque formation. Conceivably, the involved enzymes could be blocked by molecules similar to sucrose to prevent synthesis of dextrans and levans. To investigate this possibility the CGPB grantee at the University of California

is proceeding empirically by testing inhibition of these enzymes by a number of natural and synthetic sugars and sugar derivatives that are structurally related to sucrose. An alternative way to identify likely compounds is to predict the structure that would block the enzyme on the basis of information gained from studies on the structure of the active site and on the mechanism of reaction. This kind of information is being generated as one phase of the research carried out by the University of Miami investigator mentioned above.

It has been learned in the last two years that several important cariogenic streptococci have the ability to hydrolyze (or invert) sucrose as well as use it for polysaccharide synthesis. The enzyme, invertase, now has been established as being constitutive in S. mutans but inducible by sucrose in S. sanguis. In both organisms there appear to be mechanisms induced by sucrose which control the transport of monosaccharides, such as glucose and fructose into the cell. One of the most interesting findings this year comes from the University of Oregon, where a CGPB grantee reports that glucose and fructose intake in most oral streptococci appears to involve phosphorylation of the monosaccharide by phosphoenolpyruvate. Since the synthesis of phosphoenolpyruvate is quite sensitive to fluoride, this investigator wonders whether the caries protective effect of fluoride is strictly related to alterations in the solubility of enamel. The Oregon scientist also has obtained interesting data that suggests that when S. mutans is grown on sucrose, fructose derived from it is the source of cellular energy and plaque acid. Presumably fructose could derive equally well from other sources, such as levan in plaque or fruit in the diet.

Possibilities for caries control through inhibition of enzymes is not limited to the area of carbohydrate metabolism, since a number of enzymes that attack non-carbohydrate substrates are liberated by cariogenic streptococci and evidently provide for necessary features in the development of plaque or the caries lesion. Some of these extracellular enzymes are neuraminidase, hexosaminidase, B-galactosidase, fucosidase and protease. Since their role in caries etiology is unclear, a CGPB grantee at Rush-Presbyterian-St. Lukes Medical Center is isolating and purifying these enzymes and characterizing them with regard to substrate and products. Other CGPB grantees at Ohio State University, Temple University and the University of Miami have been developing defined media that will support growth of S. mutans, sanguis, and salivarius. Utilizing the new media, these investigators have determined amino acid requirements of these organisms, point out that some amino acids must come from hydrolysis of saliva and food proteins, and are investigating changed requirements and proteolytic capability under anaerobic conditions of plaque.

In last year's report it was mentioned that a CGPB grantee at the University of Miami had found that all cariogenic strains of streptococci carried a bacteriophage. This is a highly interesting observation that suggests that bacteriophage in S. mutans, as in certain other microorganisms, caries genetic information that causes virulence. To test this hypothesis it is necessary to decontaminate the streptococcus of all genetic information introduced by the bacteriophage and demonstrate that the cured bacterium is no longer cariogenic. Since it is difficult to demonstrate a complete cure, several methods are usually tried with the expectation that one is successful. During the

current year this scientist has succeeded, at least in part, in curing strains of cariogenic streptococci of their phage in two different ways. When tested in a rat model, the first of the strains was found to retain much of its virulence. Strains cured by the second technique now are undergoing the test for cariogenicity.

To develop measures for caries control based on chemotherapeutics the CGPB currently supports a small but active program in five laboratories. The goal is an agent that could be used at infrequent intervals specifically to eliminate cariogenic microorganisms. Since there is a continuous flow of saliva in the mouth, techniques must be devised to keep the agent in contact with the microorganisms long enough for effect. Furthermore, in addition to being non-toxic and having other desirable features, the agent should possess the ability to penetrate and disperse plaque. At the University of Tennessee one research team is studying compounds that carry three or four "punches." These compounds, organic amine halides, are surface active and potentially capable of dispersing plaque and clinging to surfaces. In addition, the compounds have aliphatic amine and fluoride functions that are active in several ways against cariogenic microorganisms. Another research team at the same University is studying cogeners of chlorhexidine and derivatives of urea for capability to lyse plaque. Rather than plaque itself, this team is using monomolecular films which are dispersed or form micelles in the presence of lytic agents. Both of these groups are employing advanced computer techniques to predict configurations of molecules with desirable features.

At Tufts University a CGPB grantee is attempting to bind agents to the tooth surface to prolong and concentrate their anti-bacterial effect. He reports having achieved a 12-fold prolongation of the effect of chlorhexidine-gluconate (one of the more interesting anti-plaque chemicals) by pre-treating the tooth with dilute solutions of ferric ion. Also he reports encouraging results in in vitro tests using new analogs of 8-hydroxyquinoline (another compound with pronounced ability to bind metals). At the University of Michigan and at Forsyth Dental Center other groups supported by the Branch are clinically testing the feasibility of using periodic topical applications of antibiotics for plaque control. In this technique antibiotic in gel form is held for several minutes against the teeth with a "mouth guard" appliance. This approach, used up to now with vancomycin and kanamycin, appears to cause a marked, prolonged reduction in gram positive organisms and has evident promise for use by individuals who cannot manage brushing or similar forms of oral hygiene. Possible overgrowth by monilia and development of resistant strains of bacteria are concerns in using antibiotics for extended periods. To assess these possibilities the University of Michigan group has now completed a 45-week trial without brushing in which a maintenance level of kanamycin was applied to the teeth every five weeks. It is reported that during this long trial supragingival plaque dropped to about 50 percent of normal, gingival health improved somewhat, and that yeast and kanamycin-resistant organisms did not appear to increase. This group now plans to extend these studies for several years to see if actual reductions in caries incidence can be achieved in caries-rampant individuals.

Improving the Caries Resistance of Teeth

Research on the tooth as a factor in the etiology of caries is progressing through the following phases: first, a detailed description of the development of teeth and their structure and properties with emphasis on the parts of teeth involved in early caries; second, epidemiologic studies to identify caries-associated differences in teeth; third, an analysis of the mechanism through which these differences modify the resistance of the tooth; and fourth, research taking advantage of this information to increase the capacity of the tooth surface to withstand the metabolism of cariogenic microorganisms. Research is under way in all of the above phases at the present time. In general, however, the approach is to: (a) decrease the solubility of enamel in acids or other microbial products, or (b) seal the surface so that these products do not penetrate to the enamel.

It should be noted that little is known about the mechanisms governing the initial development of teeth or the long process of extracellular maturation of enamel; thus, the CGPB is allocating some of its resources to this area. At the U.C.L.A. School of Dentistry an investigator supported by the Branch is studying the Golgi apparatus within ameloblasts and odontoblasts (enamel and dentine-forming cells) that synthesizes and "packages" materials for transport to the extracellular region where mineralization occurs. During fever and nutritional deficiencies the metabolism of these cells is abnormal. This leads to a relatively permanent hypoplastic defect in the forming enamel and dentin. At the University of Illinois College of Dentistry a CGPB grantee has found that, at least in dentine, hypomineralized areas are easily mineralized in vitro and has carried out most interesting electron microscopic studies on the initial stage of calcification in dentine. Scientists have speculated that collagen provides the nucleus for calcification. Now the University of Illnois scientist reports that mineralization occurs first in discrete vesicles apparently derived from dentine and then spreads to and follows the collagen bundles.

At the State University of New York at Rochester a CGPB grantee is studying the nucleating potential of human dentin collagen, synthetic hydroxyapatite (the mineral phase of bone and teeth) and finely ground enamel. He reports that the material which initially grows from the collagen surface is not hydroxyapatite and must go through at least two crystalline precursor phases before becoming hydroxyapatite. A series of experiments now is under way to establish the identity of these phases and the effects of trace ions in accelerating these transitions. In this regard he reports that at levels as low as 10^{-6} molar, polyfunctional phosphonates, which have been suggested as agents for coating teeth, completely inhibit the growth of hydroxyapatite crystals. Additional information on the effect of trace ions on hydroxyapatite crystallization is reported by a CGPB grantee at New York University. This scientist finds marked effects on the crystal morphology and rate of crystal growth. For example, carbonate ion, known to occur in enamel hydroxyapatite in significant concentrations and observed to be variably present in human enamel, caused the crystallites to assume rod-like or even spheroidal shapes, depending on concentration. Fluoride also caused the crystallites to assume rod-like conformations.

Epidemiologic evidence suggests that fluoride is not the only trace element that modifies hydroxyapatite properties and caries susceptibility. This evidence is being tested by careful studies in experimental animals of those elements already implicated in in vitro experiments and in epidemiologic studies. At the University of Alabama in Birmingham a nutritional biochemist supported by the Branch is examining the effects of some of these trace elements in rats, when administered pre-eruptively, i.e., by stomach tube during the first 17 days of life. He finds that doses of strontium, molybdenum, lithium and boron at levels which do not affect growth induce changes in the teeth, causing them to be more susceptible to caries.

These effects in the rat were initiated when the enamel was forming and undergoing initial mineralization; however, secondary effects of the trace elements probably occur during remineralization that accompanies caries. Similarly one can categorize effects of trace elements applied to the surface of the tooth or from saliva as affecting resistance of enamel due to slow diffusion into it or as affecting remineralization when caries occurs. Staff of the NCP considers it urgent to clarify these phenomena occuring in the outer few microns of enamel. Just to mention a few areas, we must obtain information on the rate and mechanism of diffusion of ions through enamel, on the size and abundance of pores and cracks in enamel and the characteristics of capillary phenomena, on the properties of the solvent layer close to the charged surface of hydroxyapatite crystals, and on the mechanism of re-crystallization and the properties of new mineral phases formed on enamel by topical agents.

Though transformation of hydroxyapatite into minerals that are more resistant to acid remains the most probable explanation for the anti-caries activity of fluoride, several dental scientists believe that a fluoride effect on cariogenic microorganisms may contribute to the cariostatic action. The CGPB is supporting two very interesting studies to evaluate these possibilities. In one of these studies a scientist at Ohio State University is attempting to establish whether systemic fluoride treatment still prevents caries in rats if the cariogenic microorganism is rendered fluoride resistant. Thus he hopes to distinguish a tooth protective effect from a bacteriostatic effect. In his first year's work this investigator has succeeded in making certain S. mutans strains resistant to fluoride concentrations as high as 700 ppm and now is testing them for cariogenicity in rats. In another study a scientist at Emory University is investigating possibilities that: (1) an enzyme, phosphoprotein phosphatase, liberated by cariogenic bacteria, destroys the mineral-binding capacity of enamel protein; (2) fluoride given systemically has an effect on cariogenic microorganisms; and (3) these effects are related. He reports that the expected 50 percent reduction in caries of rats exposed to fluoride during the period of early tooth formation is overcome by reinoculating them with feces from rats not exposed to fluoride, but is not overcome by reinoculating them with feces from fluoride-exposed rats. This data suggests that fluoride is reducing the cariogenicity of oral microorganisms.

While research continues to determine how fluoride works in reducing caries and to increase its effectiveness, the CGPB also is supporting research on new control measures for enamel protection. In the last several years polymer sealants have been demonstrated to be promising for preventing the impaction of bacteria and food debris into pits and fissures of molar teeth. Major draw-

backs to increased use of sealants is that their durability appears highly dependent on the skill with which the teeth are prepared and the sealant applied. To overcome these problems the Branch is supporting a materials scientist at the University of Connecticut in research to improve the adherence and abrasion resistance of sealants and to decrease the skill and time required for preparing the teeth for treatment. The possibility that bacteria might remain alive under sealants appears to be resolved with a report this year that numbers of recoverable bacteria decrease rapidly with the period that the sealant has been in place.

During the last year or two dental scientists also have discussed the possibility of sealing pits and fissures permanently through use of laser energy. One would either fuse the enamel itself or sinter a ceramic-like material to it. If this approach is to be useful, it is necessary first to demonstrate that teeth can withstand the heat input of laser treatment. To answer this initial question the Branch is supporting a scientist at the University of Utah who is measuring the temperature rise in the tooth pulp during simulated laser treatment. He reports temperatures are well within the tolerable range and now is proceeding to prepare and evaluate materials for sintering to teeth in animals.

Increasing the Effectiveness of Salivary Protective Systems

It is known that a small number of individuals is largely resistant to dental caries. It is also known that in individuals lacking a normal flow of saliva the disease tends to be rampant. These and other observations indicate that salivary factors including bactericidal, pellicle-forming and immunity systems may be highly important in establishing individual caries rates. An immediate NCP goal is to establish the nature of these systems and the degree to which they keep caries under control. If these salivary systems are found to have a significant capacity to limit caries and found to be of a type that can be reinforced by therapeutic measures, it is likely that the NCP will increase its research investment in this area.

At Forsyth Dental Center a research team supported by the CGPB is studying salivary proteins that adsorb to hydroxyapatite. Seven of these proteins now have been partially characterized with respect to molecular weight, amino acid composition, and strength of adsorption to hydroxyapatite. At the American Dental Association research laboratories in Chicago a CGPB grantee is studying the cationic proteins (a largely unstudied group of proteins that travels to the cathode during electrophoresis) with regard to possible correlations to caries status. She reports that these proteins are typical glycoproteins containing large amounts of glutamic acid, proline and glycine and currently is studying their content of carbohydrate and association with salivary lysozyme. Another part of this project involves an epidemiologic correlation of the electrophoretic pattern of salivary proteins and caries status of recruits at the Great Lakes Naval Training Station. Research concentrating on the lysozyme system (a natural bactericidal system in saliva) and again seeking correlation to caries status is being supported at the University of Nebraska. As natural defense systems these salivary proteins may be quite important to individuals, but means of reinforcing the systems as public health measures aren't obvious. Therefore our current policy is to provide

For a small but high quality program in this area, limited to evaluating the actual defense capabilities of these salivary proteins that function through other than immune mechanisms.

In the area of salivary immunoglobulins the CGPB is supporting a considerably larger program of research. Epidemiologic studies, basic research on the nature of the antibody and more applied research to establish procedures for increasing antibody titers are being carried out through this support. At N.Y.U. one of these grantees reports that in a survey of persons in the 10-20 and 20-30 year age ranges, those with elevated salivary IgA (immunoglobulin A found in external secretion) tended to have lower caries rates. In persons over 30 the relationship was not apparent; and it is suggested by the investigator that new antigens, perhaps from periodontal disease, are masking the reaction due to caries. At the University of Alabama a grantee is studying the molecular structure of secretory IgA and evaluating the level to which this system provides protection to caries. Currently he reports being able to elicit through gingival injection of protein antigen local inflammatory response and production of antibodies of the IgG class in regional lymph nodes of rabbits. He also reports being able to detect IgA in addition to mixed salivary proteins in supragingival plaque.

At Forsyth Dental Center a research career development award from the Branch is being employed with an NCP contract to establish whether vaccination with S. mutans antigen modifies subsequent caries experience. For this experiment the scientist had to develop extremely sensitive techniques to identify immunoglobulin classes in saliva of pathogen-free rats. Using these techniques the awardee found in repeated experiments that salivary IgA is increased, caries decreased and level of S. mutans slightly decreased subsequent to injection of formalin-killed S. mutans in the vicinity of the salivary glands. Current experiments at Forsyth are directed at establishing the mechanism--perhaps, in some cases, interference with adhesion--through which the cariogenicity of S. mutans is decreased by immune reactions. Similar information comes from Northwestern University and the University of Southern Illinois where two grantees report that vaccination of rats with an S. mutans extracellular enzyme also causes reduced levels of plaque, S. mutans and caries. These reports provide rather concrete evidence that antibodies can be generated by antigens injected locally to salivary glands, that the antibodies are carried by saliva to the lesions on the tooth, and that under ideal conditions reduction in plaque and caries scores result. Thus we are at the stage where at least we know that caries vaccination is not an impossibility; whether it can provide the basis for a practical control measure, however, is a question that will take much hard work to answer.

Report of the Caries Prevention and Research Branch
National Institute of Dental Research
Summary Statement

The Caries Prevention and Research Branch functions as the intramural scientific effort of the National Caries Program. The caries-related research conducted by the fifteen independent investigators is of both a fundamental and applied nature and involves laboratory animal and human studies. The Branch was reorganized during Fiscal Year 1973 into four sections designed to facilitate research into the discovery, development, clinical trial and public health application of caries prevention agents and technics. This new organization with an Etiology Section, Preventive Methods Development Section, Community Programs Section and a Biometry Section, provides attention to all aspects of the caries research spectrum.

The Etiology Section seeks basic scientific information on the carious process with the current emphasis on research into the causitive role of certain oral bacteria. The Preventive Methods Development Section seeks new agents and technics capable of reducing caries with the current emphasis on fluorides to increase host resistance and anti-microbiological agents to reduce or eliminate causative bacteria. The Community Programs Section evaluates various preventive procedures in terms of patient benefit and researches delivery systems for effective and economical public health programs. The Biometry Section collaborates with the balance of the Branch in the compiling, processing and analysis of their data and conducts independent studies of problems unique to the handling of the caries data.

In addition to their intramural studies, members of the Branch are vital participants in the development of the overall program of the National Caries Program. Input by Branch scientists is instrumental in identifying research questions for approach through the extramural activities of the Program. This input serves as the basis for programming of caries research grants and the initiation of collaborative studies. Branch scientists also participate in the administration of extramural activities as Project Officers on collaborative studies which are either extensions of their intramural activities or are in areas of their expertise and serve overall Program interests. Currently each Branch scientist has Project Officer responsibility for one or more contracts.

Report of the Biometry Section
Caries Prevention and Research Branch
National Caries Program
National Institute of Dental Research
Summary Statement

The Biometry Section has two major roles. The first is one of collaboration with and consultation and service to the rest of the Branch and the Caries Program. Members of the section consult with branch staff, other intramural scientists and investigators from other agencies on design of studies. They perform systems analyses, train individuals in data collection, process data, and perform statistical analyses for intramural and collaborative research projects. To provide these services most efficiently, general systems to handle data sets and study designs of a similar nature are utilized whenever possible.

The second role of the Section is one of independent investigation. Research is conducted to determine methods best suited for analysis of data peculiar to the caries field. Results of these endeavors are then applied in the design and analysis of trials, surveys and experiments.

In the systems area, a prototype was developed for the processing and analysis of data from clinical trials of sealants. In addition preliminary plans were made for the analysis of sequential cross-sectional caries data and for developing a system for the growing volume of microbiologic data.

All of the existing systems have been modified to allow updating and editing to be carried out through the remote, on-line terminal. The process of preparing raw data files, editing, updating and correcting and merging new data into master files, is now handled entirely by the statistical assistants.

Also in this area, a data bank and information retrieval system for caries contracts, grants, and intramural projects was developed. The form for collecting DMFS information was modified to facilitate mechanization of forms preparation and a new machine readable form for the recording of cost benefit data was developed.

In their consultation role, members of the staff were active in the planning stages for a number of studies and the analysis of results from several others. A computer program for numerical taxonomy, developed at Georgetown University, was modified and rewritten for microbiologic data and the BMD general linear model program was tested and used for the analysis of data from one clinical trial.

The use of the general linear model is of particular note because of its direct bearing on the investigation of methods for reducing residual variation in caries incremental data. It is hoped that this effort will yield methods by which differences among groups may be detected at an earlier time. In addition to the BMD computer program, routines developed under contract with the Research Triangle Institute and Columbia University for multivariate

analysis and univariate analysis utilizing a blocking design have been evaluated and tested.

During the coming year, the Section will continue in its collaborative and service function. It will maintain, operate and update the general systems; provide consultation and systems analysis for new projects; develop new systems when appropriate; modify existing computer programs or write new ones when necessary for unique projects; and perform statistical analysis on study data. In particular the feasibility of systems for processing data from cost-benefit studies and root caries surveys will be determined.

Research currently under way to identify methods by which residual variation in caries increment data may be reduced will be continued. These methods, as they are identified and tested, will be implemented.

Community Programs Section
Caries Prevention and Research Branch
National Caries Program
National Institute of Dental Research
Summary Statement

Since the Community Programs Section was established in the fall of FY 72, the principal role of the Section has been broadened. The evaluation of agents, methods and procedures that have both proven, and potential, efficacy for the prevention of dental caries now is encompassed in the revised charge of the Section. The foremost mechanism employed to accomplish program objectives is direct operational research. Emphasis, however, is also given to research contracts in cooperation with the Office of Collaborative Research, NIDR. Staff has been strengthened by the addition of a dental hygienist and a dentist to assume the duties of a project scientist. Both persons have permitted the planning and initiation of a few new direct operational studies, although the basic responsibility of the project scientist is to provide general guidance to contractors and to monitor the technical aspects of the assigned contracts.

Direct Operational Research

Self-administration of topical fluorides offers decay preventive benefits to large numbers of persons with minimal demands on professional manpower and cost. In Santa Clara, California, the potential of annual, supervised tooth-Ebrushing with a stannous fluoride-zirconium silicate prophylaxis paste is under evaluation. Examinations which were conducted in September 1972 marked the end of the clinical phase of the three-year collaborative study. A joint report with Indiana University briefly summarizing two-year findings has been sent to the Santa Clara Research Foundation, the study's sponsoring agency. As soon as the processing of the three-year data is completed, a final report will be prepared.

Initial work with an adhesive resin (Bis-GMA) in preventing occlusal caries has produced encouraging results. To more fully explore the effectiveness and retentive properties of the new material, a study was initiated in Kalispell, Montana in May 1970. First- and second-year follow-up examinations have been carried out. A joint report with the staff of the Dental Health Center containing one-year findings has been submitted to the Journal of the American Dental Association for publication. A brief summary of two-year findings has been sent to the manufacturer of the sealant. Arrangements have been made for Dr. Sven Poulsen, Visiting Scientist with the Etiology Section, CP&RB, to participate as an examiner on the forthcoming three-year examinations in May 1973. Dr. Poulsen will evaluate the retentive properties of the sealant.

Fluoridation of school water supplies is a feasible method of bringing the benefits of fluorides to children residing in areas which lack central water supplies. Currently, a level of 4.5 times the optimum for community fluoridation in the geographic area is recommended. To help develop the procedure to its maximum potential, the effect of school fluoridation at 7 times the optimum is being tested in Seagrove, North Carolina. Four-year examinations

were conducted in Seagrove in May 1972, to determine the interim benefits to teeth of children exposed to fluoridated water at school. Initially, the study school contained children in grades 1-12. Recently, students in grades 9-12 were transferred to a new high school and it became necessary to fluoridate the water supply of the new school at the same level of 6.3 ppm. A report containing the four-year findings will be presented at the 51st General Session of the International Association for Dental Research (IADR) and the manuscript submitted to an appropriate scientific journal for publication.

Because self-administered procedures are well adapted for use in public health programs, there is a need to determine the total effectiveness of a combination of some of the most feasible and promising methods. In Nelson County, Virginia, a combination of self-administered procedures is being studied. Base line dental examinations of children enrolled in all public schools in the County were carried out in September 1972. Immediately following, an eight-year preventive program was implemented consisting of daily administration of chewable fluoride tablets in school, weekly rinsing with an 0.2% solution of sodium fluoride in school, use at home of an "accepted" therapeutic dentifrice and general instruction in dental health education. Children in grades 1-6 are participating actively in the program, but evaluation will be conducted approximately every two years.

Several studies in the United States and abroad have demonstrated a caries-inhibitory effect when fluoride tablets were administered in a school program. The effect of a "swish and swallow" acidulated phosphate-fluoride chewable tablet is being determined in Wayne County, North Carolina. First follow-up dental examinations (after 30 months of study) were carried out in April 1972. In addition to assessing caries experience, enamel biopsies of a random sample of children were made to determine fluoride content. Children will be ending their fourth year of participation in the study in June 1973. A brief paper on 30-month findings will be presented at the 51st General Session of IADR and subsequently a full report will be submitted to a national dental journal for publication.

A combination of two procedures which independently have been shown to be successful in the control of dental caries and gives promise of complementing each other in the type of benefits conferred is the subject of investigation in Robeson County, North Carolina. Base line dental examinations were conducted in September 1972 in three study schools in the County. Participants consume fluoridated water at school at 4.5 times the optimum recommended for community fluoridation in the geographic area and engage in weekly mouth-rinsing with an 0.2% sodium fluoride solution. To evaluate the long-term benefits of the combined treatments, follow-up examinations will be conducted every two years until the completion of the program in 1984.

Ongoing Research Contracts

To assess whether the topical application of amonium fluoride (NH_4F) has a greater anti-caries effect than other standard topical fluoride compounds, two clinical trials are being conducted by Forsyth Dental Center. One study

is designed to compare the effectiveness of professionally administered, semi-annual, topical applications of acidified NH_4F with that of acidified phosphate-fluoride solutions. A group of children that receive a placebo solution serves as control. Six hundred 9 to 12 year old school children in Cambridge, Massachusetts were examined at baseline and one year after initiation of treatments, DMFS and fluoride biopsy data are being evaluated.

The other study involves a supervised, daily, one-minute rinse in school with either an acidified NH_4F, neutral NaF or a placebo mouthrinse. In all other respects, both study designs are similar. DMFS and fluoride biopsy data for baseline and one-year, post-treatment exams are being analyzed.

European investigators have reported that enhanced benefits are derived when fluoride is complexed with an organic molecule, both in terms of fluoride uptake by enamel and in caries reduction. At the University of Miami, a study of 730 school children, 6-13 years of age, is being conducted in Puerto Rico. The levels of fluoride uptake and the anti-caries effect of two short regimens of a professionally applied amine fluoride gel are being compared with those obtained with an acidulated phosphate-fluoride gel. Clinical examinations performed at base line and 12 months after initiation of treatments include dfs, DMFS, PI, and enamel fluoride biopsy information. The data are presently being analyzed. Follow-up exams after 24 months will be conducted in January 1974.

There is a need to determine the total benefit that can be derived by using combinations of proven cariostatic measures. A 3-year contract was established with the University of Michigan in July 1972. The contract is to evaluate the combined use of an acidulated phosphate-fluoride gel, an adhesive sealant and oral health education in Ypsilanti, an optimally fluoridated community. A test group of 600 children receive the preventive procedures and in addition receive comprehensive restorative care. A comparable group of controls receive only oral health education. Baseline DMFS, OHI, PI examinations were conducted in February 1973. Initial preventive and restorative treatments have been given. Follow-up evaluations will be made annually for 3 years.

Another 3-year study was initiated with SUNY, at Buffalo, whereby the effectiveness of a combination of several caries preventive measures, topical application of an acidulated NaF gel, an occlusal sealant, and a special behavioral motivational program, is being evaluated. The study was implemented in a community with water fluoridation and where the study population has access to routine restorative care and routine oral hygiene instruction. Base line DMFS and plaque data are being processed.

The presence of trace elements other than fluoride could explain the marked differences in caries prevalence observed in communities which, otherwise, are very similar in all other aspects. This hypothesis is being tested by Forsyth Dental Center, with samples of water, soil, saliva, food, and teeth obtained from two such qualifying communities in Colombia, South America. Final results are being evaluated statistically.

Samples of teeth from areas of high and low caries prevalence in the United States, but with similar fluoride levels, are being analyzed for content of trace minerals by the Eastman Dental Center in a three year study. The third year of effort will be initiated in July 1973. Results might provide a basis for new measures of controlling dental caries.

Other Activities

Staff of the Section provided consultation during FY 73 to the Council on Dental Therapeutics, American Dental Association; the Food and Drug Administration; the Division of Dental Health; the Commission on Classification and Statistics for Oral Conditions (COCSTOC), F.D.I.; the World Health Organization; and State Health Departments. Several manuscripts for publication in scientific journals were referred to Staff for review of technical merit. Dr. Horowitz has stepped down from the position of Chairman of COCSTOC's sub-group on dentofacial anomalies but has assumed the Chairmanship of COCSTOC's Working Group I on Caries Incidence Studies. Additionally, he had the prime responsibility for updating the FDI document "Principal requirements for controlled clinical trials of caries preventive agents and procedures." Dr. Heifetz served as Chairman, Committee on Research, American Association of Public Health Dentists, for the year 1972.

Publications

During FY 73, staff of the Community Programs Section published or submitted for publication the following papers:

Partial defluoridation of a community water supply and dental fluorosis. Health Services Reports 87:451-455, May 1972. (H.S. Horowitz, S.B. Heifetz, W.S. Driscoll).

Effect of partial defluoridation of a water supply on dental fluorosis - final results in Bartlett, Texas after 17 years. Amer. J. of Pub. Health 62:767-769, June 1972. (H.S. Horowitz, S.B. Heifetz).

Test of a method for estimating prevalence of DMFT. J. of Pub. Health Dent. 32:165-168, Summer 1972. (B.J. McClendon, A.M. Abrams, H.S. Horowitz).

Clinical trials of preventives for dental caries. J. Pub. Health Dent. 32: 229-233, Fall 1972. (H.S. Horowitz).

Application of Dunnett's "t" test to dental studies with multiple treatment groups and a control. Pharmac. and Therap. in Dent. 1:157-167, April 1971. (J. Doyle, J. Barbano, H.S. Horowitz, A.M. Abrams).

A clinical trial of a calculus-inhibitory dentifrice. J. Periodont. (J.D. Suomi, H.S. Horowitz, J.P. Barbano, V.W. Spoksky, S.B. Heifetz).

The current status of topical fluorides in preventive dentistry. p.34-59 (In Newbrun, E. ed. Fluoride and dental caries. Springfield, Thomas, 1972). (H.S. Horowitz, S.B. Heifetz).

Fluoride dentifrices. p.22-33 (In Newbrun, E. ed. Fluoride and dental caries. Springfield, Thomas, 1972). (S.B. Heifetz, H.S. Horowitz).

School fluoridation for the prevention of dental caries. Internat. Dent. J., June 1973. (H.S. Horowitz).

Evaluation of self-administered prophylaxes and supervised toothbrushing with acidulated phosphate-fluoride. Caries Res. (H.S. Horowitz, S.B. Heifetz, B.J. McClendon, A.R. Viegas, L.O.C. Guimaraes, E.S. Lopes).

The effect on dental caries of weekly rinsing with a neutral sodium fluoride or an acidulated phosphate-fluoride mouthwash. J. of Amer. D. Assn. (S.B. Heifetz, W.S. Driscoll, W.E. Creighton).

The control of dental caries and periodontal disease: a fundamental approach. J. Pub. Health Dent. 33:2-6, Winter 1973 (S.B. Heifetz, J.D. Suomi).

An appraisal of mass plaque control programs. J. Pub. Health Dent. (S.B. Heifetz, F.A. Bagramian, J.D. Suomi, V.A. Segreto).

Utilization of fluorides in areas lacking central water supplies. J. of Canad. D. Assn. (S.B. Heifetz, H.S. Horowitz, W.S. Driscoll).

Retained anti-caries protection from topically applied acidulated phosphate-fluoride: 30- and 36-month post treatment effects. Internat. J. of Preventive Dent. (H.S. Horowitz and M.C.W. Kau).

Factors affecting the prevalence of dental fluorosis in Lucknow, India. Arch. Oral Biol. (R.S. Nanda, I. Zipkin, J. Doyle, H.S. Horowitz).

Report of the Etiology Section
Caries Prevention and Research Branch
National Caries Program
National Institute of Dental Research
Summary Statement

The bulk of the Etiology Section's activity during the past year has continued to center in the relationships between oral bacteriology and clinical caries. Its goals have been pursued both by Direct Operations and the Contract mechanism.

In the former, the major effort has been to perfect procedures for culturing and differentiating oral streptococci and other organisms obtained from different types of human source material. An improved transport medium has been developed which will facilitate laboratory studies of bacterial inocula collected during geographically separated field studies. Methods for differentiating streptococci by colony form, biochemical reaction and fluorescent antibody technics have been brought to an advanced level of refinement. The last phase of the work has been considerably aided by means of Contract and Inter-Agency Agreements.

Other bacteriological activities have been directed toward finding out the extent to which human familial characteristics determine the presence of mutans or other streptococcal types in the mouth. Also in the same area of inquiry, a study has been initiated to determine at what stage of an infant's life Streptococcus mutans establishes itself in the mouth and, as far as is possible, what factors determine that event.

The results in another clinically-based investigation are of special interest. Following the finding that Streptococcus mutans was present in the mouths of an isolated caries-free population of Yanomamo Indians in Venezuela, one of the isolated Strep. mutans strains from that source was tested in gnotobiotic rats to determine whether it was capable of causing caries. Caries was produced, indicating that, at least in the Yanomamo Indians, the presence of a cariogenic organism was not of itself capable of producing caries. It is hoped that this finding can be expanded and correlated with other observations on the dietary, bacterial, and cultural practices of that or other caries-free populations.

Several studies have been initiated under contract to determine more fully the relationships that exist between foodstuffs and bacterial activities in the mouth as they might relate to polysaccharide formation or enamel destruction. Preliminary findings indicate that factors other than the sucrose content of foods influence enamel destruction. This suggests the need of expanded study of dietary factors in caries causation.

The effect of deprivation of saliva on the bacterial population of the mouth is also under study in patients with xerostomia.

On the clinical side, the pattern of caries attack in deciduous teeth is being studied by a guest scientist, Dr. Sven Poulsen. In addition, a study

of combined preventive procedures is being made in cooperation with dentists working with the Bureau of Indian Affairs.

Preventive Methods Development Section
Caries Prevention and Research Branch
National Caries Program
National Institute of Dental Research
Summary Statement

The Preventive Methods Development Section was established early in FY 1973, with three investigators, Dr. R. Shern, Dr. H. Englander, and Dr. R. Larson, Chief. The purpose of this Section is to identify and develop agents and technic to prevent dental caries to a point where they are ready for full-scale testing in human field trials.

The work done during this first year has been largely a continuation of projects which had been started in other Sections the previous year. Fortunately, that work has fit into the mission of the Section.

The major emphasis of all three investigators has been directed toward an increased understanding of the mode of action of fluoride and determining optimum methods of delivery. The major need is to develop methods of delivery for those individuals who do not have access to fluoridated drinking water. The second need is to determine if there is a different or additional exposure which could provide greater protection against caries than fluoridated water as presently recommended.

An earlier finding has been confirmed showing that in the rat continuous exposure to 10 ppm fluoride in the drinking water which is associated with no increase in enamel uptake of fluoride, offers more caries protection than short exposure to high levels of fluoride, which results in high levels of uptake in the enamel. The relative merits of these methods of exposure have been adequately demonstrated in the rat and are now ready to be tested in clinical trials.

If it should be shown that continuous exposure is more important in humans than an increase in fluoride content of the enamel it would be wise to replace widely spaced topical treatments with methods involving more frequent exposure. Several concentrations and frequencies of treatment are being studied in the rat in an effort to select the more promising means of frequent exposure which would then need to be evaluated in clinical trials.

Adherent characteristics of amine fluoride make it particularly attractive as a means of keeping fluoride in the mouth continually with low levels of total intake. The result of two short field trials run in the last year have shown that this agent is safe and acceptable for human use and restricts plaque without disturbing the oral ecosystem.

Priorities for testing agents in field studies are established as a result of information resulting from in vitro and animals' screening. This method allows a profile of predictive attributes to be constructed so that optimal dosage can be anticipated for restriction of plaque, caries and gingivitis prior to testing these agents in humans.

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Publications by the Section during the year include:

1. Englander, H. and Jordan, H. V. Relation between S. mutans and smooth surface caries in the deciduous dentition. J.D.R. 51: 1505 (1972).

2. Larson, R. H., Clemmer, B., and Scherp, H. W. Reduction of rat caries by trimetaphosphate on different tooth surfaces: variations produced by diet and oral flora. Arch. Oral Biol. 17: 883-887, 1972.

3. Larson, R. H., Spalter, C. D., Clemmer, B. A., and Scherp, H. W. Continuous vs. intermittent feedings of different levels of trimetaphosphate in relation to caries development in the rat. Arch. Oral Biol. 17: 1537-1541, 1972.

4. Larson, R. H., and Zickus, C. S. Patterns of dental caries in Osborne-Mendel and NIH Black rats in relation to length of the caries-test period. J. Dental Res. 51: 1375-1381, 1972.

5. Theilade, R., Larson, R. H., and Karring, T. Microbial studies of plaque in artificial fissures implanted in human teeth. Caries Res. 7: 130-138 (1973).

REPORT OF THE EXTRAMURAL PROGRAMS

NATIONAL INSTITUTE OF DENTAL RESEARCH

July 1, 1972 - June 30, 1973

by

Dr. Clair L. Gardner
Associate Director for Extramural Programs

For the Extramural Programs of NIDR, Fiscal Year 1973 was a year marked by successful adaptation to changing conditions. The year was characterized by changes in leadership, in staffing, in program reorganization and in Government policy as well as by new approaches to administrative and scientific problems. In response to the situation there has been a ferment of staff activity which has led to the gradual development of a smoothly functioning, extramural staff intimately involved in the direction of programs which are advancing toward a state of maturation. Early in the year emphasis was placed on strengthening the professional staff so that all of the scientific programs could be manned as effectively as possible. Through these efforts, a highly competent group with an abundance of scientific versatility has now been assembled. A reorganization of programs and staff at midyear brought forth a more propitious alignment of personnel and scientific management activity. Since that rearrangement, great strides have been made by staff in program analysis, program planning and program monitoring. The area on training has been pursued with vigorous dedication in spite of the phaseout of training announced in January, 1973. At the present time phaseout plans have been developed in fine detail and are proceeding on course.

The newly reorganized Extramural Programs is described below:

Special Assistants to the Associate Director:

Dr. Anthony A. Rizzo, Program Coordination;
Dr. Robert J. Schuellein, Research Manpower;
Dr. Emil Rigg, Dental Research Institutes and Centers.

Categorical Programs:

Craniofacial Anomalies Program

Chief, Dr. Richard L. Christiansen
Dr. H. George Hausch

Mineralization, Salivary Secretions and Nutrition Program

Chief, Dr. Paul D. Frazier
Dr. Matthew Kinnard

Pain Control and Behavioral Studies Program

Chief, Dr. Aaron Ganz
Dr. Edward J. Driscoll

Periodontal and Soft Tissue Diseases Program

Chief, Dr. John F. Goggins
Dr. Richard L. Hayes
Dr. Anthony A. Rizzo
Dr. Robert J. Schuellein
Dr. Thomas M. Valega

Restorative Materials Program

Chief, Dr. Louis W. Wachtel
Dr. Louis J. Pecora

This new administrative format appears to have distinct advantages over previous arrangements, the chief one being a much higher degree of communication between staff members, leading to more frequent application of collective judgments. The creation of two Special Assistants to the Associate Director, that dealing with research coordination and that dealing with manpower, appeared to fulfill long existing needs of the Extramural Programs to heighten the awareness of all staff members about the activities of every program, and to bring together in a more concerted way actions which affect all programs. The decision to include the Dental Research Institutes and Centers (DRIC) in Extramural Programs and to designate a Special Assistant for this program again facilitates the involvement of all Program staff in this heretofore somewhat separate DRIC Program. It is rather obvious that these changes were made in an attempt to respond to pressing demands that we streamline both our research and our training programs. Since the phaseout of training programs has been enacted, the new staff position related to manpower has become an even greater challenge.

The rationale underlying the programmatic content modifications derived from multiple considerations. The new Craniofacial Anomalies Program is basically similar in outlook and purpose to a previous program of that nature. This Program, however, is presently somewhat more unilaterally directed toward the solution of disease problems. A new Program, Mineralization, Salivary Secretions and Nutrition, was established. While the admixture of the three elements of this latter Program may seem somewhat inappropriate, there is, nevertheless, a unifying reason for combining these different subject areas. The organization of this unit reflects an attempt to single out the subject areas and highlight them for special attention because each of them seems to require special scrutiny at this time. For example, in regard to our large and long-continuing programs on mineralization, great

emphasis should be placed upon evaluating the significance of these programs. On the other hand, insofar as nutrition is concerned, there appears to be good reason to expand these programs and develop them along new avenues that somehow have not been followed as a logical outgrowth of traditional scientific inquiry. In recognition of the fact that research on pain control has been grossly neglected, it was felt appropriate and timely, and perhaps urgent, to give specific attention to this important concern. Accordingly, a new Program, designated as the Pain Control and Behavioral Studies Program, was created to satisfy the Institute's responsibilities for research on pain. The new Program is also expected to begin to explore the potential contributions that behavioral studies can make to the solution of oral health problems.

The remaining alteration in program content involved the consolidation of two former programs to make up the new Periodontal and Soft Tissue Diseases Program. Since the two former programs clearly contained vast research areas of common interest, it was indeed fitting to assimilate them into one scientific administrative unit.

Committees

During the course of the year, special committees, four internal and two external, have been formed to bring the Extramural operation to its most efficient and effective stage. These varied committees have already proved to be of immense value to staff in meeting its wide spectrum of obligations. One of the external committees formally established this year was the Periodontal Diseases Advisory Committee, a standing Committee of eight members, formed to give broad advice on the future direction of periodontal research. This committee is intended to offer recommendations to the Institute as a whole, and to specifically aid the Periodontal and Soft Tissue Diseases Program in program planning. An advisory group has also been developed to lend counsel to the Restorative Materials Program. This Materials Advisory Panel will be convened on an ad hoc basis, but is expected to have a core of continuing participants, so that it will be able to provide recommendations for future goals and priorities for a number of years.

One of the four internal committees formed for specific purposes was given an assignment to develop guidelines for the various types of verbal and written reports that must be prepared by staff. A second committee is expected to develop guidelines for the various kinds of meetings (such as State-of-the-art workshops) that are initiated by or are participated in by staff, and a third committee has the purpose of setting down specific, up-to-date instructions for staff to carry out the various administrative procedures for which staff is held responsible. Finally, a fourth committee was assembled to explore the possibilities of storing and retrieving Extramural Programs' data by employment of computer facilities. The first committe, under the Chairpersonship of Dr. Halegua has already gone far in reaching its goals. A document designed to be of help in preparing Annual Reports was made available and has already been put to use. In another effort, this same committee has assembled a comprehensive set of guidelines which should be extremely helpful in a large number of specific situations in which staff must make reports or presentations. Meanwhile, the second committee is at work

developing the conceptual framework and practical guidelines to plan and conduct effective meetings. This committee is exerting efforts to deal effectively not only with the problem of scientific progress assessments, as would be achieved by state-of-the-art meetings, but also with the evaluation of the overall effectiveness of NIDR's programs. The Computerization Committee, under the Chairmanship of Dr. Kenneth C. Lynn, (1) reviewed the various record-keeping activities of the Extramural Program, (2) examined the data needs for reporting purposes, and (3) identified the specific data elements for inclusion in the system. With this information in hand, Dr. Lynn and his staff began to work on a computerized data processing system for grants and awards. By utilizing the data base captured by DRG, they were able to, with a minimum of input personnel, develop a working model of an NIDR system capable of responding to the most frequent information needs more accurately and in less time than ever before. The model system was described to the committee and there was general agreement that such a system was not only compatible with the needs of the Institute but highly desirable. Cost estimates were presented in terms of dollars and man years for various combinations of data elements in the base. Only two major factors prevented the system from becoming operational--(1) procedural details and (2) additional personnel. Of the two, only the latter was considered to be serious. Current organizational alignments promise to alleviate these obstacles, and the committee is expected to continue as an advisory body to John Wilson, who now has the responsibility for all data processing activities at NIDR. One Program has already put the retrieval system to good use. It is expected that employment of these facilities will be rapidly expanded. Another group has essentially completed an Extramural Procedures Manual which should prove to be extremely valuable to staff and, in some instances, to consultants as well.

Training

As a part of its overall fostering of research, NIDR, along with other Institutes of the NIH, has had a long history of supporting the training of research personnel, and the Institute has successfully implemented this policy through the award of training grants, fellowships, and career development funds. Beginning in 1957 with the award of 20 training grants at a total level of $500,000, the Institute's training programs expanded tremendously to a level of more than 100 training grants a few years ago with a commensurate increase in funding. In FY 1973, NIDR continued to maintain a strong program of research training. During this past year support was awarded for 61 training grants at a level of $3 million; for 64 fellowships, 23 career development awards and 3 career awards, at a level of $1,000,000. Earlier in the year the NIDR Extramural Programs had set up a number of conferences with training program directors to make up-to-date assessments of the current and projected impact of the training programs. It was the intention of these conferences to explore the effectiveness of the training programs in fulfilling the NIDR's mission of fostering research to control and prevent oral diseases. Six of these conferences were held in Calendar Year 1972, and all of them turned out to be fruitful exchanges of viewpoints on the part of both the staff and the training program directors.

When the decision was made in January of 1973 to terminate training grants and fellowships, it was deemed inadvisable to hold the three remaining scheduled conferences. Thereafter, staff concerns have been preoccupied with implementation of the phaseout plan developed by NIH as a whole, and by the need to maintain some kind of positive activity insofar as training interests are concerned. One approach adopted by staff is to closely monitor programs being phased out over a period of years. Staff has taken the position to encourage training program directors to maintain their programs with funds from other sources, such as State funds or university funds. Moreover, in exploring ways to keep the training efforts viable, staff will be alert to the possibilities of incorporating research associates into research grants that are funded. It is also planned that staff make visits to training programs being phased out to ensure continued excellence in research training.

In accord with the NIH general phase-out plan, projected training support for predoctoral, postdoctoral, and other forms of training activity has been completed. The Table to follow shows the current and projected distribution of training funds through FY 76, which is expected to complete the execution of the phaseout plan. Subsequent to that year only the 3 career research awards and a minimal number of trainees in the special clinical training grants supported by NIDR are expected to continue. NIDR maintains a strong sense of commitment to continued responsibility for assessing the research and academic manpower needs of dentistry. Therefore, the Institute plans to continue evaluation studies of past and present training efforts. Meanwhile, efforts will be made to take advantage of training opportunities of the present, however meager, and also to plan appropriate types of training mechanisms for the future.

CURRENT AND PROJECTED DISTRIBUTION OF FUNDS $(000s)

	FY 72	FY 73	FY 74	FY 75	FY 76
No. Training Grants	90	61	53	33	12
No. Trainees	435	284	161	83	26
Predoctoral	144	124	90	50	15
Postdoctoral	291	160	71	30	11
Funds	$5,698*	$2,989	$2,529	$1,212	$431
No. Postdoctoral Fellows	30	16	5	-0-	-0-
Funds	$285	$158	$49	-0-	-0-
No. Special Fellowships	31	22	6	-0-	-0-
Funds	$487	$349	$100	-0-	-0-
No. Career Development Awards	26**	22	17	15	11
Funds	$582	$506	$380	$317	$288
Total No. Individuals	522	344	189	98	37
Total Funds	$7,052	$4,002	$3,058	$1,583	$719

*Total level of support was reduced by unobligated funds from previous year.
**Includes 3 career awards.

Research Funding

During FY 73, Extramural Programs of NIDR funded 276 individual research grants. Table I shows the distribution of funds for these awards according to the six Programs included. The Dental Research Institutes and Centers are also not included in the foregoing items. Among the research projects funded this year were 43 new research grants, 30 competing renewal grants and 199 non-competing continuation grants. Forty-eight of the grants funded this year were small (R-23) grants for young investigators. These research grant funds were sufficient to maintain activity encompassing all of the

areas in which the Institute should have activity but not sufficient to allow ideal development of certain needed areas of research.

Program Area	Research Grants No.	$(000s)	Training Grants No.	$(000s)	RCA and RCDA Grants No.	$(000s)	Fellowship Grants No.	$(000s)
(1)*	49	3,074	1	105	3	63	2	19
(2)*	76	3,873	15	667	6	132	14	171
(3)	64	3,756	14	595	4	93	10	150
(4)*	36	1,435	9	494	2	45	2	33
(5)*	39	2,305	6	322	4	107	7	89
(6)*	12	215	9	331	3	66	3	45
	276	14,658	54	2,514	22	506	38	507

(1)* Caries Grant Programs Branch

(2)* Periodontal and Soft Tissue Diseases Program

(3)* Craniofacial Anomalies Program

(4)* Restorative Materials Program

(5)* Mineralization, Salivary Secretions and Nutrition Program

(6)* Pain Control and Behavioral Studies Program

Staff Activities

As usual, Extramural Program staff paid visits to a large number of academic institutions both in this country and abroad for programming, monitoring, and evaluative purposes, and also to participate in meetings. The magnitude of this activity varied according to the nature, size, and state of development of the Program. For example, in the Program entitled "Mineralization, Salivary Secretions and Nutrition Program" little programming activity was carried on because the needs of this Program at this time require much analysis and evaluation before effort is invested in programming. On the other hand, the Program entitled, "Pain Control and Behavioral Studies" is so underdeveloped that there is a tremendous need to stimulate interest and encourage investigators to become involved in research of this type. Accordingly, in this area, programming has consumed a major portion of staff energies. Through the specific dental school visits conducted by Dr. Kreshover in which Extramural staff participated, and through Extramural-initiated

programming visits, there has been a great deal of interaction with investigators this year. A special effort was made to set the stage for as much communication as possible with applicants or potential applicants at the American Association of Dental schools and the International Association for Dental Research meetings in Washington, D.C. This year for the first time letters were sent out to all grantees and potential grantees to inform them that they would have opportunities to speak with the appropriate NIDR staff during the course of both of these meetings. The response to this invitation was gratifying.

This year, also, saw the initiation of two new types of evaluation models for the assessment of broad scientific programs that have been operative over periods of years. In one of these, a study was made to assess the significance of research dealing with the mechanisms of mineralization. NIDR has supported research in this area for many years in the belief that studies of the basic physical properties of calcium phosphate minerals would provide key insights into the various disease processes. Since the ultimate value of this extensive research effort is in question at present, plans were made to carefully consider the significance of the mineralization studies and to establish specific goals for the future. Accordingly, a special ad hoc advisory panel was selected from investigators who do not have grant support from the NIDR. The committee was instructed to consider program productivity, duplication of effort and research areas where emphasis should be increased, decreased, redirected or eliminated. Since it is believed that a committee composed of non-grantee consultants would be less likely to show unconscious bias, the results of the special advisory panel dealing with mineralization was carefully observed. This evaluative model proved to be so successful that other NIDR Programs will also use advisory committees selected in this manner.

In addition to their participation in a large number of meetings on a wide variety of scientific subject matter, extramural staff specifically supported two large conferences, a series of workshops, two advisory committee meetings, and in addition made available some assistance in the development of two international symposia. One of the larger meetings was the International Conference on "The Comparative Molecular Biology of Extracellular Matrices" held in California for a 5-day period. This impressive conference, and its published proceedings, supported via grant mechanism, stimulated great interest in many important questions related to the nature of developing connective tissues. The Conference on Craniofacial Malformations brought out discussion of the recent breakthrough in surgical procedure which now allows successful treatment of disfiguring craniofacial syndromes, which were previously untreatable. The several workshops dealt with a variety of clinical cleft palate research areas. Included were discussions on etiology, otolaryngology, speech, surgery, psychosocial considerations, dentistry, anatomy, and physiology. Of the two symposia, one brought together most of the leading investigators of the pain phenomena from all over the world to share their research findings and to develop plans for the future. Of immense interest were discussions of plans drawn up to scientifically evaluate the true efficacy of acupuncture in pain relief. The other symposium dealt with the relationship between prosthetic appliances and the tissues of the body. This symposium, held at Clemson University, provided discussion on

two subjects of specific interest: maxillofacial prostheses and dental implants. Finally, two meetings of the Periodontal Diseases Advisory Committee were held. The principal recommendations offered by this committee were that NIDR should immediately develop plans for short-range projects on the control of dental plaque, and also should initiate coordinated projects, involving both clinical and laboratory components, to elucidate the many biological mechanisms involved in the disease process.

Research Highlights

The research accomplishments reported throughout the year by grantees and by investigators at the university-based dental research institutes and centers are described in the individual program reports to follow. However, among the array of significant new research material, several instances of scientific progress are sufficiently outstanding to be mentioned here. For example, research in the field of periodontology has brought forth new information on the microbiology of periodontal disease which modifies etiologic concepts, and has also introduced new evidence linking the chemical and cellular events of inflammation with tissue destruction. The latter findings give substance to theories on disease mechanisms. In the area of craniofacial anomalies attention should be drawn to the new and dramatically successful methodologies for the treatment of severe facial disfigurement, and to experimentation which suggests that orthodontic treatment might be carried out at an earlier age. Studies which appear to give a firm support to the concept of fluoride deficiency as an authentic state of malnutrition serve as yet another example of the kind of scientific progress that has come forth this past year.

The studies on the microbial etiology of periodontal disease are significant because they indicate that different bacteria are associated with different forms or stages of human periodontal disease. Now that investigators have available a comprehensive battery of laboratory tests by which to characterize oral microorganisms, it has been possible to systematically study and identify them. It has been shown, for example, that in early periodontal disease Gram-positive organisms are present at the gum line and are also present below the gum line. Surprisingly, Gram-negative organisms are not associated with this level of periodontal disease. In contrast, Gram-negative organisms are present in high numbers in cases of severe periodontal disease with pocket formation and a substantial amount of bone loss. Moreover, in cases of periodontosis, in which there is an extreme degree of bone loss, the bacteria associated with this condition are still different from that so far outlined. All of these findings underscore the possibility that the distinctly different forms of periodontal disease recognized clinically may be caused by different microorganisms. Thus the likelihood of this disease having a single, principle, etiologic agent becomes less and less likely.

Exciting developments in regard to mechanisms of tissue destruction and disease have shown that the process of inflammation may account for a state of depressed protein synthesis, as well as a state of enhanced destruction of the structural elements of the periodontal tissues. Emerging evidence indicates that the inflammatory cells including macrophages, lymphocytes,

and mast cells have distinct roles in the destruction of cells, fibers and bone and also in inhibiting protein synthesis in general. In some instances a specific destructive enzyme has been identified and its source localized. For example, collagenase was found this year to be produced by the macrophages. Moreover, it has been shown that the destructive chemical processes of these cells can be activated by dental plaque extracts or by individual components of the plaque either directly or indirectly through the activities of other cells. In a related study it was shown that gingival inflammation raises the levels of prostaglandin, a chemical substance capable of causing bone resorption. The exciting possiblities of this finding is that known prostaglandin inhibitors are already on hand and conceivably could be used to stop the production of this potentially destructive body chemical.

Another highlight of research considered to be an actual breakthrough is the new surgical treatment of disfiguring craniofacial syndromes. In this technique an intracranial approach is followed which allows a more effective alignment of the displaced portions of the tissue components. Studies on the modification of muscle forces has led to a new appreciation of the value of applying judiciously contolled forces early in life in the treatment of orthodontic disorders. Finally, the new evidence supporting the thesis that lack of fluoride is a true nutritional deficiency has important implications. According to the results reported, a low fluoride diet caused progressive infertility in mice, which was correctible by adding the element to the diet of the mothers. Continued study of other important aspects of this problem may indeed lead to findings of great impact in human nutrition.

Future Plans

In addition to plans of the future already mentioned in the foregoing portions of this summarizing report, Extramural Programs of NIDR intend to place heavy emphasis on evaluation, on the monitoring of research and training grants, and on explorations of the research potential of the contract mechanisms and the small research grants program. Consistent with HEW concern for evaluation and the provision of 1% set-aside funds for this purpose, the Extramural Programs plan to make full use of such funds so that comprehensive analysis, and evaluation of both research progress and administrative programs can be properly made. In this way optimally effective program planning can be achieved. In accord with the position taken by NIDR in developing the initiatives described in the Blue Print document submitted to Congress and to the Administration, Extramural Program staff intends to closely monitor all research and training grants to make certain that projects continue to parallel the aims and responsibilities of NIDR. Staff will make project site visits to consult with investigators and training program directors. Whenever there seems to be a need for reorienting and restructuring programs, the appropriate discussions will be held with the investigators to implement the course of action that evolves. A schedule of visits to training grant directors is presently under preparation, and a similar schedule of project site visits to research grantees will soon be developed. More expanded use of the contract mechanisms for research activities will be employed so that badly needed research efforts will not be neglected. It is believed that a certain portion of funds in the budgets of all of the categorical

areas would provide the flexibility of project orientation necessary to comprehensively fulfill the important needs of the day. Staff also intends to explore the possibilities of expanding the Special Awards programs for young investigators. It has been suggested that expansion of this well-received program might be a useful device to accelerate research accomplishment in certain particular fields and to recruit basic scientists into dental research.

In spite of stringencies in research funds and the definitive phaseout of all NIH training programs, NIDR extramural programs have made considerable progress in program analysis, evaluation and program planning. This past year each categorical program developed a formal presentation embracing these three aspects of science administration. These accountability presentations were prepared with great depth and understanding and were favorably received by the leaders of the Institute. This activity and the others described above will be continued by the NIDR Extramural Programs.

PERIODONTAL AND SOFT TISSUE DISEASES PROGRAM

INTRODUCTION

The Periodontal and Soft Tissue Diseases Program was established this fiscal year as a component of the Extramural Program of NIDR by combining the Soft Tissue Stomatology Program and the Periodontal Diseases Program into a single entity. This newly consolidated program has the responsibility for extramural research in periodontal diseases, oral ulcerative diseases, oral cancer, and other oral soft tissue disorders including those of the dental pulp. The ultimate mission of the program is to develop new knowledge which may lead to the prevention and eradication of these diseases throughout the world. Since the diseases named are multifactorial in etiology and extremely complex in manifestation, the program supports research and training efforts in a wide variety of subject matter. Included are studies on bacteria and viruses, their biology, chemistry, effects on the host, and behavior in the oral environment, as well as studies on the molecular biology of the normal and abnormal metabolism of the mucosa and periodontium. Within the broad spectrum of biological studies supported, special attention is given to the specific biochemical and immunologic mechanisms involved in the pathologic processes of inflammation and tissue destruction. It is hoped that comprehensive studies of the chemical steps involved in periodontal and soft tissue disease progression will reveal optimum points at which inhibitory, preventive agents can arrest these diseases.

ADMINISTRATIVE

During the past year the program awarded research funds of approximately $3.3 million to support 6 program projects, 55 regular research grants, and 18 small grants for young investigators. The program also awarded approximately $1.1 million for 22 training grants which provided stipends for 64 trainees. In addition, $145,000 was made available for 7 research career development awards, and $224,000 was expended to support 21 fellowships.

Table I shows the distribution of research grant funds according to subject area. It should be pointed out that the periodontal diseases component of the program consumed approximately 85% of the grant funds expended. As the table indicates, there was a small amount of activity in the areas of oral tumors and dental pulp studies, but extremely minimal activity in the highly important areas of Herpesvirus infections and recurrent aphthous ulcers. Since there has been little oral cancer research at NIH in the past, NIDR and the National Cancer Institute now have an agreement to cooperate in stimulating research in this important area. Under this agreement NIDR staff will identify needed research in oral cancer and will develop programs of research to meet the need, and NCI will supply funds to support the projects. This beneficial arrrangement provides the means whereby NIDR and NCI staff can work together in many ways to foster oral cancer research.

TABLE 1

I.	Periodontal Disease	
	A. Etiology	
	Plaque (ecology, chemistry and metabolism, adhesion, toxic activity, salivary and dietary factors)	$ 744,744
	Miscellaneous factors	125,683
	B. Pathogenesis and Pathology	
	Inflammatory response (Chemotactic factors, vascular effects, immune response)	485,284
	Tissue destruction and healing (epithelial integrity, connective tissue and bone metabolism, effect of inflammation)	1,342,041
	Miscellaneous	29,579
	C. Diagnosis and Treatment	62,534
	D. Prevention	47,837
	Subtotal:	2,837,702
II.	Herpes Simplex Virus Infection	19,883
III.	Recurrent Aphthous Ulcers and Similar Disorders	60,274
IV.	Tumors of Oral Mucosa and Connective Tissue	174,493
V.	Tumors of Salivary Glands	82,680
VI.	Dental Pulp Studies	158,274
	Total:	$ 3,333,306

STAFF ACTIVITIES

During the year program staff made visits to a large number of institutions both in this country and Europe to program, monitor, and evaluate proposals, and also to participate in meetings. Earlier in the year programming visits were made to five institutions in Europe considered to be potential strongholds of periodontal research. This effort has resulted in at least two approved applications of high relevance and merit. Visits were made also to approximately 25 universities in the U.S. to discuss a variety of potential studies in the fields of periodontal and oral soft tissue diseases. In addition, staff attended project site visit reviews of approximately ten research grant proposals. Staff also participated in a number of meetings:

1. Second International Conference on Periodontal Research, Aarhus, Denmark;

2. 58th Annual Meeting of the American Academy of Periodontology, San Diego;

3. 13th National Meeting of the American Academy of Pharmaceutical Sciences, Chicago (Invited speaker);

4. Workshop Conference of the Chemotheraphy of Herpesvirus Diseases, Ann Arbor, Michigan;

5. Conference on Clinical Indices in Periodontal Disease, Philadelphia;

6. American Association of Dental Schools, Washington, D.C.;

7. International Association for Dental Research, Washington, D.C.;

8. Symposium on Controlled Release of Drugs, Birmingham, Alabama;

9. NIH Collaborative Extramural Programs Retreat, Warrenton, Virginia;

RESEARCH HIGHLIGHTS

The research findings described in this section follow the general outline in Table 1. The major research efforts in the periodontal diseases have involved studies on pathogenesis and pathology, in which emphasis has been placed on the nature of the inflammatory response and the mechanisms of tissue destruction. Nevertheless, a significant amount of attention has also been devoted to etiologic factors, with the main emphasis being in the area of microbiology and dental plaque formation. It is noted that research efforts dealing with diagnosis, prevention, and treatment of periodontal disease have been negligible.

Etiology of Periodontal Disease

The dento-bacterial plaque is thought to be the initiator of the chain of events which leads to inflammation of the soft tissues, bone destruction and the eventual loss of teeth. Thus, studies on the biology of the plaque have assumed great importance. Paramount among these investigations have been projects designed to discover which organisms in the plaque set the disease process in motion. Investigators have sought to identify pathogenic bacteria by two general approaches: by isolating bacteria in pure culture and demonstrating pathogenic potential in experimental systems, and by testing to see which oral bacteria caused the production of antibodies. It is assumed that organisms causing an antibody or hypersensitivity response in blood or tissue are also causing local disease in the periodontal tissues.

The most comprehensive attempt to identify organisms in the periodontal plaque is that being carried out at Forsyth Dental Center. In this study, emphasis is being placed upon individual organisms. Studies are being conducted of the morphologic, biochemical and nutritional characteristics of individual strains of bacteria and tests for their pathogenicity are being carried out with single cultures or with combinations of different cultures or strains. These studies are beginning to bring forth information on the types and

distribution of bacteria in the different forms of periodontal disease. In these systematic studies, attempts are being made to identify and quantify all of the organisms involved in the development of plaque from its earliest inception until it reaches a degree of maturity. After cultures are isolated and identified, individual strains or groups of organisms are tested by monoinfecting gnotobiotic rats to see if the inoculum causes periodontal disease. Another pathogenic characteristic being evaluated is the ability of organisms to form plaque on surfaces in vitro. Studies have also been made to identify organisms associated with the different forms or degrees of severity of human periodontal disease. In these recent studies the cultivable micro-organisms in early and in severe periodontitis were determined using culture techniques which have been carefully developed over a period of years. In the early form of disease in which bone loss is just beginning, it was found that dental plaque at the gum line included many Streptococcus sanguis organisms and Actinomyces viscosus-like bacteria. Anaerobic micro-organisms including Gram-positive filamentous rods and Peptostreptococci were present in higher proportions below the gums. Surprisingly Gram-negative organisms were not detected in these subgingival samples. In ordinary cases of severe periodontal disease, deep pockets had been shown by earlier workers to contain a variety of bacteria including Gram-positive anaerobic filamentous organisms and Gram-negative anaerobic rods and cocci. In the periodontosis patients bacteria were cultured from areas of extreme bone loss and from areas which did not show bone loss. Differences were found between the bacteria taken from these two sites. The samples taken from the normal sites were similar to those just described for the early disease categories. The microbiota of the pathologic sites, however, were dominated by the presence of Gram-negative anaerobic rods. Two clearly distinguishable types of these were present: a motile curved rod and a non-motile small rod. Both of these organisms turned out to be extremely difficult to culture and unusually slow in growing. Two weeks were required for them to attain maximum growth. Since these organisms were quite different from those seen in the early cases of disease and it seems possible that there may be distinctly different forms of periodontal disease caused by distinctly different groups of micro-organisms. The studies cited seem highly significant because they bring at least some degree of order out of the confusion in the cultural approach to the etiology of periodontal disease. While it is still not possible to implicate a single organism as a causative agent in human periodontal disease, it seems likely now that groups of organisms may be responsible for the initiation and progression of this disease process.

In another investigation a University of Iowa scientist has studied the bacteriology of the advanced periodontal pocket. In these studies he first isolated pure cultures of organisms under special anaerobic conditions and identified them. In more recent studies he has taken 25 representative strains isolated from the deep surfaces on the roots of teeth in human periodontal pockets and tested them in vitro to determine whether they form dental plaque in the laboratory. He found that 16 of the 25 formed plaque when grown on a sucrose medium, and many formed plaque when grown on a glucose medium as well. Notable among the plaque-formers were the anaerobic species Lactobacillus, Actinomyces, Bifodobacteria,

Arachnia, Proprionobacteria, and facultative Gram-positive cocci. In an effort at the University of Pennsylvania Center for Oral Health Research, measures are being taken to separate and identify the different streptococcal bacteria associated with periodontal disease by using a combination of cultural and immunologic procedures including fluorescent antibody techniques. In the immunologic approach to the identification of pathogenic organisms, workers at the State University of New York (SUNY) at Buffalo have studied the blood of patients with localized gingivitis and patients with periodontitis to find out which bacteria were causing an antibody response. Using the technique of indirect immunofluorescence, they found that antibodies to four or more of the bacteria were present in all of the blood samples tested, but the amount varied considerably in different individuals. The levels of antibody were significantly greater in the periodontitis patients than in the less severe gingivitis patients.

At the Forsyth Dental Center, important investigations on the adherence of bacteria to soft tissue cells lining the mouth were continued. The same investigators had shown in earlier investigations the highly significant finding that bacterial adhesion to soft tissue cells was an important factor in local infections. Recent studies showed that this adhesion could be inhibited by enzymes which break down proteins and fats and by fatty substances themselves. Detergents and certain forms of antibody were also shown to reduce adhesion. In studies to determine why some bacteria reside in the mouth while others reside in the gut, organisms from each of these areas were inoculated into germ-free rats and their eventual distribution was examined. The results suggest that adherence to tissue cells may be a very important factor in determining which bacteria localize in specific sites in the mouth.

In another project at the Forsyth Dental Center, an investigator is working out the lipid composition of organisms known to be pathogenic for periodontal disease in germ-free rats. In collaboration with a colleague, extracts from these organisms have been tested in animals and have been shown to possess bone resorption activity. In other studies on the toxic activity of plaque conponents conducted at the University of Minnesota, the hypothesis that hydrogen sulfide may be involved in the etiology of periodontal disease has been examined further in humans. In these studies, patients with periodontal pockets exceeding 4 mm in depth practiced oral hygiene on one side of the mouth only. Even though each subject demonstrated good brushing techniques, there was no reduction in the hydrogen sulfide generation in the deepened pockets on the brushed side of the mouth. Apparently brushing fails to remove gas-producing micro-organisms from the deep inaccessible areas. Thus it would appear that the noxious effects of this toxic gas may continue in spite of local preventive measures.

In recent efforts to understand the role of the salivary glands in the formation of plaque, studies have been made on the earliest acquired pellicle which precedes the participation of bacteria. This early pellicle apparently deposits on a clean tooth surface within a few hours. At the Center for

Research in Oral Biology at the University of Washington in Seattle, and at the Institute of Dental Research at the University of Alabama, it has been found that certain salivary proteins are selectively adsorbed onto teeth, thereby forming the pellicle. Recent evidence indicated that the enzyme lysozyme in an active form is present in a pellicle two hours old.

Pathogenesis and Pathology of Periodontal Disease

Since there is a net loss of collagen fibers in the gingival tissues in periodontal disease, it has been believed that the disease is caused by agents which directly or indirectly cause fiber destruction. However, since connective tissues are continually undergoing buildup and degradation, it is necessary to know the normal turnover rate in health before it is possible to understand the disease process. Recently investigators at the Center for Research in Oral Biology at the University of Washington in Seattle proposed that the net loss of collagen may be a consequence of depressed production rather than enhanced destruction. They suggested that the collagen of the gingiva may be subjected to an inordinately high turnover rate. They then attempted to gain definitive data to support the elevated turnover rate hypothesis by following the incorporation and conversion of proline-UC 14 into collagen in the small primate Saguinus oedipus. The results indicated that the turnover rate may be six times higher in the gingiva than in other mature connective tissues studied. Furthermore, they found that most of the radioactive counts incorporated into the insoluble collagen of gingiva were lost during the next 17 weeks, while those present in other mature connective tissues persisted. The results obtained do support the hypothesis that the net loss of collagen observed in periodontal disease could be the result of depressed formation.

There is, of course, no reason to expect that net collagen loss should be due only to depressed formation. Indeed, it seems more reasonable to expect that depressed synthesis and enhanced degradation may both be at work. This concept of dual mechanisms is buttressed by more and more emerging evidence that the inflammatory process alone provides mechanisms which bring about not only the destruction of cells, fibers, and bone, but also the inhibition of protein synthesis. As a result of several collaborative studies between investigators in Europe and the U.S. on the mechanisms by which the host may destroy its own periodontal tissues, the activities of inflammatory cells are being elucidated.

Not too many years ago, the functions carried out by different inflammatory, or white cells, of the body were exceedingly mysterious; now it is possible to define a good many of the biological activities of all of the participating white cells. This past year findings have come forth concerning three important inflammatory cells: macrophages, lymphocytes and mast cells. Macrophages normally serve to protect the host by engulfing foreign material such as bacteria or particulate debris. To do this these cells contain powerful chemicals which digest such substances. However, in local inflammatory diseases such as periodontal disease, it is thought that these cells simultaneously turn their weapons on their own tissues. Since macrophages frequently

make up the predominant cell population in tissues affected by delayed hypersensitivity and other inflammatory reactions, it seems imperative that these cells be extensively studied. Investigations at the University of Washington at Seattle and joint collaboration with workers in England and studies at NIDR have shown that macrophages can become activated directly by minute amounts of plaque material associated with gross periodontal disease. This activation process releases from the macrophages a battery of chemicals which could directly cause widespread and long-term tissue damage. Related studies at these same institutions have shown that the macrophages can be activated by specific bacterial endotoxins and antigens from the plaque.

Other morphological studies carried out by the same Seattle workers in collaboration with investigators in Zurich have shown an indirect mechanism by which these macrophages may become activated and play a role in periodontal disease. In this indirect process the plaque substances apparently first react with lymphocytes, and the lymphocytes in turn activate the macrophages. The findings are based both on morphological ultramicroscopic examination correlated with biochemical study of diseased human tissue itself. These studies show that lymphocytes in contact with macrophages cause the macrophages to release chemicals which are thought to destroy structural elements of the periodontal tissues.

One of the specific tissue-destructive chemicals produced by these macrophages is an enzyme which breaks down the collagen fibers. The discovery of this enzyme activity was made by workers at the University of Alabama Institute of Dental Research, who showed that the newly discovered enzyme is similar to other animal collagenases. Related studies at NIDR have shown that the macrophages can be activated to produce more collagenase by specific bacterial endotoxins or by products released by lymphocytes.

Another cell important in the breakdown of collagen fibers is the mast cell. Studies at the University of Texas Dental Science Institute point to a significant role for the mast cell in the in vivo destruction of collagen fibers. In earlier studies rat mesenteric connective tissue was used to test the activity of a gingival collagenase enzyme because the mesentery represents connective tissue in its natural state. In these studies it was found that mast cells enhanced the dissolution of collagen fibers through the activity of its granules. In more recent studies it was determined that mast cells were essential to the collagen breakdown reaction in mesenteric connective tissue, while, on the other hand, mast cells were not required for enzymatic dissolution of artificially reconstituted collagen fibers. Subsequent studies indicated that the mast cell factor necessary for attack on native fibers is not an enzyme, nor is it heparin or histamine. Neither is it proteinaceous in nature. The studies suggest that the enhancing role of the mast cell may be due to its ability to block serum inhibition of collagenase.

Besides serving to activate the macrophages, lymphocytes seem to be involved in the inflammatory process in other ways as well. It was shown in earlier studies by NIDR investigators that lymphocytes activated by substances from dentobacterial plaque produced factors which cause destruction of the fibroblast cells that make collagen and which also inhibited protein synthesis. Recent experiments by a scientist working under Special Fellowship support in collaboration with NIDR investigators have shown that these cells produce an osteoclast-activating factor which stimulates bone resorption in a manner similar to that of parathyroid hormone. The evidence cited above for the mechanisms of tissue destruction rest upon results obtained from biochemical, morphological, electron microscopic, and tissue culture studies.

Continuing studies by Harvard scientists to isolate and identify a bone resorption stimulating factor from human gingiva have shown that this factor is a high molecular weight compound which is produced by fibroblast-like cells and by epithelial cells as well. Earlier studies at the University of California on the bone resorption in periodontal disease had shown evidence that prostaglandin E2 (PGE_2) could be involved. In a recent study the actual levels of prostaglandin $E2^2$ in diseased human periodontal tissues were measured and compared with normal healthy tissues. It was found that inflamed gingival tissue contained twice as much PGE_2 as that obtained in healthy tissues. Moreover, the highest levels measured were in the inflammatory exudate, where the values were greater then 400ng/ml. This concentration corresponds to that which maximally stimulates bone resorption in tissue culture. These findings provide direct support for the hypothesis that local prostaglandin increase causes bone resorption in periodontal disease.

Diagnosis and Treatment and Prevention of Periodontal Diseases

Investigators at Forsyth Dental Center have developed a method which may prove useful in objectively assessing periodontal disease activity at an early stage. These investigators measured albumin in the saliva on the assumption that most of the albumin comes from the gingival crevice tissues. They found that the concentrations of albumin in the saliva of subjects with experimentally induced gingivitis was related to the degree of inflammation. For example, the average albumin at the onset of the experiment in healthy subjects was 1 mg% when the average disease scores were only 0.1. When the gingival index of disease had increased to an average score of 0.95 at day 20 of the experiment, the average albumin had risen to 5 mg%. The finding cited earlier that gingival prostaglandin levels are elevated in periodontal disease suggested to the investigators (University of California, S.F.) that treatment of patients with drugs which inhibit prostaglandin synthesis might inhibit gingival inflammation and periodontal bone resorption. One of these inhibitors is α-tocopherol (Vitamin E). Because of its low toxicity, this substance was selected for initial clinical testing. In a study supported by the University, subjects were given Vitamin E daily for 21 days with no other form of periodontal therapy, while control subjects were given a placebo lactose capsule. Sulcus fluid flow from the gingival crevice was taken as a measure of gingival inflammation. The results indicated that the Vitamin E treatment caused a significant decrease in the sulcus fluid flow, whereas that of the placebo-treated subjects remained unchanged. The reduction in inflammation is thought to be due to Vitamin E inhibition of prostaglandin,

which normally causes an increase in vascular permeability and fluid leakage during inflammation. Whether such topical treatment could inhibit the bone resorption seen in disease must await studies of longer duration. This experiment is of great interest because it represents a successful attempt to prevent a cardinal feature of inflammation by the local application of a specific blocking agent.

While the results obtained with prostaglandin inhibitors seem to offer cause for optimism in developing preventive measures, other experimental prevention methods have not turned out well. For example, efforts to show that fluoride applied topically to the alveolar bone may prevent chronic destructive periodontal disease have continued to fail, and tests of the broad spectrum antibiotic gantrisin against periodontal disease in beagle dogs have shown that after one year the gantrisin therapy seems to have no effect on plaque scores, calculus scores or gingival inflammation.

In an immunologic approach to the prevention of dental plaque and periodontal disease, the oral organism Streptococcus mutans in complete Freund's adjuvant was inoculated into oral tissues of germ-free rats to immunize them. Subsequently the rats were infected with the same organism and placed on a diet which induces plaque and periodontal disease. Bone loss and antibody levels were followed. It was found that the immune animals demonstrated elevated salivary antibody levels which appeared to consist almost entirely of IgA. Although bone loss was observed in all animals to some extent, the mean bone loss scores were lower in the immune group. Furthermore, a reduction in the numbers of plaque bacteria was noted in many, but not all, of the samples taken. The results suggest that specific antibody in the saliva directed to S. mutans may bring about a reduction in bone loss and may affect plaque formation.

Animal Models for Periodontal Disease

One of the impediments to progress in the field of periodontal disease research has been the lack of suitable animal models. It is therefore encouraging to note several specific advances that have developed in recent years. Since the beagle dog is considered to be one of the best characterized animal models for periodontal disease studies, it is of great interest that this animal is now being studied in the germ-free state. In a joint project developed between Louisiana State University and the Center for Oral Health Research at the University of Pennsylvania, investigators have examined the periodontal status of germ-free beagle dogs ranging in age from 20 months to 3 ½ years. Although some chronic inflammatory cells were found to be present in the gingival connective tissue, it was clear that no tissue destruction had taken place even in the older animals. The healthy status of these germ-free dogs also underscores the high probability that periodontal disease in conventional animals is caused by micro-organisms.

While rodents and other animals as well as dogs have served as useful periodontal models for certain purposes, there is a continuing need for an animal model in which the disease can be produced with essentially all of the

characteristics seen in man. Recently, investigators at the State University of New York at Buffalo have conducted experiments in Macaca speciosa monkeys which have shown that it is possible to produce in these animals all of the clinical, histologic and microbiologic features of human periodontal disease. Their data indicate that this primate is a suitable model for the study of early periodontal disease in man.

In the peculiar condition called Chediak-Higashi syndrome (CHS), a disease which occurs in mink, mice, and man, the polymorphonuclear leucocytes which normally protect against disease, do not function normally. It has been shown that those affected with this disease do show increased susceptibility to periodontal disease, but this increased susceptibility has not been quantified and detailed. At the University of Connecticut and at the Center in Seattle, recent studies of mink and mice with this condition have been conducted. Assays were made of oral debris, gingival inflammation bone resorption, epithelial proliferation, and the number of polymorphonuclear leucocytes in the epithelium and connective tissues. While similar amounts of oral debris were observed in both the normal and disease groups, those with Chediak-Higashi syndrome showed more gingival inflammation and more bone resorption than normal animals. The histological changes in the affected animals included epithelial proliferation and disintegration, vascular proliferation and large numbers of polymorphonuclear leucocytes within the epithelium adjacent to calculus deposits. They also showed significantly more bone resorption. Although the exact mechanism of this susceptibility remains to be determined, it is presently believed that the abnormal lysosomal function of these polymorphonuclear leucocytes either represents a failure of protective mechanisms against bacteria, or that the lysosomes contribute to the destructive process themselves by releasing enzymes.

Oral Ulcerations

In a study at the Royal Dental College in Copenhagen, the effect on oral leukoplakia of the cessation or reduction of cigarette smoking was assayed in a total of 123 patients. Thirty-one stopped smoking permanently, 60 stopped smoking for three months, and 32 decreased their tobacco consumption to less than half for a period of three months. In the first group the percentage with disappearance of the leukoplakia was 67.7; in the second group, 31.7, and in the third group, 6.3. Thus, a decrease in daily tobacco consumption does not seem to have much effect whereas cessation has a marked effect after only 3 months. Permanent cessation leaves only 16.1% unchanged. Therefore, cessation of smoking is an important measure to be used in the treatment of recurrent leukoplakia. As a follow-up to the preliminary report on the immunologic aspects of oral leukoplakia conducted by the same group of scientists and recorded in the 1972 Annual Report, it appeared that the loss of blood group antigens from tissue may be used in the early diagnosis of malignant changes in oral leukoplakia. Further quantitative studies have substantiated this. However, the presence of group Antigen A in normal buccal tissue showed that it is not possible to use a general standard if the loss or decrease in amount of blood group substances is to be used in the early diagnosis of malignant changes. The amount of blood

group substances in each patient's normal mucosa must in every case serve as baseline against which malignant changes are evaluated.

MEETINGS SPONSORED

The Periodontal Diseases Advisory Committee was established this fiscal year as a standing committee to provide the Institute with broad advice in developing appropriate programs for the pursuit of periodontal disease research. Eight outstanding individuals were appointed to the committee, with Dr. Maynard K. Hine of Indiana-Purdue University serving as chairman. The remaining members are Dr. Henry M. Goldman, Boston University; Dr. Particia J. Keller, University of Washington; Dr. Barnet M. Levy, University of Texas; Mrs. Patricia Ann McLean, Columbia University; Dr. Ralph Snyderman, Duke University Medical Center; Dr. William D. McHugh, Eastman Dental Center; Dr. Kirk Hoerman, American Dental Association, Chicago. Dr. Anthony A. Rizzo of NIDR staff was appointed Executive Secretary of this Committee. The Committee met twice during the course of this fiscal year.

At the first meeting, December 6-7, 1972, Dr. Kreshover discussed the role and function of the committee. Drs. Gardner, Greulich, and Carlos then described the programs under their direction, and Dr. Rizzo discussed the extramural periodontal diseases program. Specific discussion was held on the following topics: status and implementation of preventive measures; the need to study other medical conditions which could lead to a better understanding of periodontal diseases; the need for good model systems, both in vitro and in vivo; and the need for close communication and collaboration between the National Caries Program and the Periodontal Diseases Program. Dr. McHugh prepared a draft of a research strategy and Dr. Hoerman presented a written plan of an implementation mechanism. There was discussion on the order of priority of research areas in the periodontal diseases program, but no agreement could be reached at that time.

At the second meeting of the Committee, one session was devoted to presentations by members of NIDR Intramural scientific staff in order to further acquaint the Committee members with the Institute's programs on periodontal disease. In the second session of that meeting, the Committee discussed their potential role in serving the Institute and made specific recommendations. After discussing the question of whether the control of dental plaque would prevent periodontal disease, the Committee recommended that NIDR develop contracts to determine the effect of plaque control measures on the periodontal tissues. Specific plans to develop plaque control systems was presented in a draft by Dr. McHugh and his written suggestions were discussed by the Committee. The Committee again recommended that close collaboration with the National Caries Program be maintained. They specifically suggested that a workshop on dental plaque be arranged so that participation by both the National Caries Program Advisory Committee and this Committee could participate. In order to accomplish this, it was suggested that the next meeting of this Committee be held immediately before or after that of the National Caries Advisory Committee.

Seminars

Program staff sponsored four seminars during the year, three dealing with periodontal disease, and one in which the subject of recurrent aphthous ulcers was discussed. One seminar featured two speakers from Sweden, Dr. Jan Egelberg and Dr. Rolf Attstrom, who presented data on the role of polymorphonuclear leucocytes in gingival inflammation and described experiments on the role of delayed hypersensitivity in beagle dog periodontal disease. In another seminar, Dr. Jan Lindhe, also from Sweden, described several studies in which the beagle dog was successfully used as a model for important periodontal disease studies. A third seminar on periodontal disease was given by Dr. J. M. Goodson from the University of California at San Francisco who spoke on prostaglandin activity in relationship to periodontal disease in humans. The seminar on recurrent aphthous ulcers was presented by Dr. Irwin Ship of the University of Pennsylvania. Dr. Ship described the epidemiologic and immunologic aspects of this condition.

FUTURE PLANS

In periodontal disease research, emphasis will be placed on defining the numerous steps in the disease process itself in order to develop rational means for the prevention and control of the disease. Increased programming efforts will also be made to stimulate research dealing with aphthous ulcers, Herpesvirus infections and oral cancer.

The periodontal projects to be developed will include specific studies aimed at plaque prevention. Toward this end a variety of antibacterial compounds will be tested for efficacy against dental plaque formation. Increased efforts to study the metabolism of the bacteria of the plaque and of overall plaque biology will be made so that it will be possible to develop chemotherapeutic agents on a rational basis. Among these studies will be investigations on the mechanisms of adhesion. If the binding sites or other mechanisms of bacterial adhesion to teeth were known, then specific measures to block adhesion itself could be employed. Ongoing studies designed to develop fundamental knowledge necessary to control the tissue destructive aspects of the immune and the inflammatory response will continue to receive strong support. It is hoped that such efforts will clarify the role of immune mechanisms in periodontal disease. Since the loss of the connective tissues in periodontal disease is thought to result from an inbalance between synthesis and breakdown, continuing emphasis will be placed upon the basic metabolism of these tissues. Studies on tissue repair will also be included with a view towards developing better methods to restore lost periodontal tissues. In the area of soft tissue diseases efforts will be brought to bear upon the development of better diagnostic methods for Herpes simplex virus infections so that earlier treatment will eventually be possible. Attempts will be made to determine the factors involved in the latency, tissue localization, and replication of the virus. Greater efforts will be made to stimulate research on recurrent aphthous ulcerations, since this widespread condition has been essentially neglected. It is also recognized that the problem of oral cancer must become more visible to the scientific community. Therefore, efforts will be made to work with the medical community, with the National Cancer

Institute and particularly with the regional cancer centers to develop a more coordinated effort in the study of this important neoplastic disease. In accord with the foregoing description of future plans, certain specific workshops and contract projects are already in the planning stages. These projects are outlined in the following paragraphs.

Three workshops are planned for the coming year. The first, planned for autumn of 1973 is entitled, "Mechanisms of Connective Tissue Degradation." A second workshop is also planned for the autumn of 1973 entitled "Problems and Perspectives of Recurrent Aphthous Stomatitis." The third, entitled "Assessment of Delayed Hypersensitivity Mechanisms in Periodontal Diseases," will be held in the spring of 1974. At this stage of planning, chairmen have been selected, programs drawn up and tentative participants selected. The primary purpose of these workshops will be to determine the state-of-the-art of research in the respective areas and to identify promising areas for future research.

Contract proposals to be initiated during FY '74 include three important projects on periodontal disease and three of equal or greater urgency on other oral diseases. The titles of these proposals are:

1. "Association of Recurrent Herpes Simplex Infection and Oral Cancer"

2. "Evaluation of Treatment Methods for Herpes Labialis"

3. "Electron Microscopic Search for Viruses in Aphthous Lesions Using Fractionation Techniques for Viral Isolation"

4. "Study of the Immune Status of Patients with Severe Periodontal Disease"

5. "The Study of Periodontal Disease on Patients with Abnormalities of the Immune System"

6. "In Vitro Screening of Chemical Agents with Potential to Prevent Human Periodontal Plaque Formation"

SUMMARY

The Periodontal and Soft Tissue Diseases Program was formed this year to consolidate extramural research in periodontal diseases, oral ulcerations, oral cancer and other disorders. Since these diseases are complex, the program supports a variety of studies on oral microorganisms, their biology, chemistry and ecology, as well as multi-faceted biological studies on the metabolism of oral tissues. Special encouragement is given to studies on disease mechanisms, because they offer promise of improved health.

During the past year, nearly $5 million was awarded for research grants, training grants and fellowships. Approximately 85% of the research funds were expended for periodontal disease projects. The majority of these projects dealt with pathogenesis and pathology, but significant attention was also devoted to studies on etiology.

Since the dentobacterial plaque is thought to initiate the chain of events leading to inflammation and bone destruction, etiologic studies have emphasized its role in disease. In the last few years, efforts to identify pathogenic bacteria have become systematic and comprehensive. It is now becoming clear that different forms of periodontal disease may be caused by different bacteria. Indeed, recent studies of the microbiology of the classical severe syndrome of periodontosis, in which there is great bone loss and pocket formation but little inflammation and plaque formation, indicate that extremely peculiar organisms are associated with this disease. Moreover, other organisms usually present in pockets seem to be absent in this conditon. These findings suggest that these patients with periodontosis may have an unusual pattern of susceptibility to certain organisms. These studies cited have also shown that certain organisms will cause disease in otherwise germ-free rats and that certain oral bacteria have the capacity to produce plaque in pure culture by utilizing certain substrates.

New information on mechanisms of tissue destruction that may operate in periodontal disease have provided evidence that the net loss of connective tissues observed is probably the result of depressed formation as well as enhanced degradation. Emerging evidence indicates that the inflammatory process offers mechanisms which bring about both of these effects. For example, white blood cells found in inflamed tissues have been shown to produce chemicals which destroy cells, fibers, and bone. Efforts to develop suitable animal models for periodontal disease studies have been encouraging on three fronts. Recent studies have highlighted the potential value of beagle dogs, monkeys, and animals with Chediak-Higashi syndrome.

During the year, staff carried an extensive array of professional activities which included the sponsorship of four seminars, and programming visits to at least thirty universities in this country and abroad. Staff also participated in nine important scientific meetings. In addition, the NIDR established a standing Periodontal Diseases Advisory Committee to advise the Institute in developing new research. This Committee has held two fruitful meetings which resulted in a number of specific recommendations.

Projects planned for the future in the periodontal area include not only studies aimed at plaque prevention but also comprehensive studies on the biology of the plaque including its mechanism of adhesion. It is also clear that emphasis will continue on the tissue destructive aspects of the immune inflammatory response. Meanwhile, attempts to devise better diagnostic methods for Herpes simplex infections and to solve the challenging problem of the etiology of aphthous ulcerations will be given special attention. Specific efforts to stimulate research in these areas will include the development of three contract proposals on periodontal disease and three contracted projects involving Herpes virus, oral cancer, and aphthous ulcers.

ANNUAL REPORT FY 73

CRANIOFACIAL ANOMALIES PROGRAM

INTRODUCTION

The Craniofacial Anomalies Program supports research in the etiology, prevention, and treatment of craniofacial malformations, including congenital, acquired and postnatal disfigurements. The program also supports developmental biology research on the normal and abnormal development of the cranium, face, and oral structures from the prenatal period through adulthood.

ADMINISTRATION

The program name has been changed from Developmental Biology and Oral-Facial Anomalies to Craniofacial Anomalies. This new name better describes the programmatic research support which extends beyond the oral cavity and face to include the cranial structures as well. Research support is balanced between (a) congenital malformations such as cleft lip/palate and other syndromes which may involve numerous craniofacial structures; (b) acquired malformations from surgery or accidents; and (c) malocclusion of teeth and jaws. These efforts are supported by fundamental research in prenatal and postnatal developmental biology.

During FY 73 the program supported 68 research grants and 11 program projects, which were funded at a total of $3,930,204. Six special dental awards (small grants) were included. In addition, the program supported 19 training grants covering the subject area or disciplines of developmental biology, genetics, basic sciences, and clinical sciences. These grants furnished stipends for 72 trainees. Postdoctoral fellows supported numbered 24, and research career development awards were made to 6 investigators. As a result of the phasing out of training programs, FY 74 will see a reduction of predoctoral trainees to 26 and of postdoctoral trainees to 42.

STAFF ACTIVITIES

(a) Visits to 12 universities in response to an invitation from the craniofacial investigators to discuss types of extramural programmatic research support, or to assist in the assessment of the State of the Art of research in craniofacial anomalies at the field level. Grantees and other relevant researchers were consulted during these visits.

(b) Participated in professional scientific meetings; consultation service was made available to researchers in attendance at the meetings.

(c) Participated in 12 workshops or conferences, training program directors' meetings and served as observer at numerous study section site visits during grant review.

(d) A programming visit was made to Poland. Medical (health) Academies were visited in Warsaw, Poznan, Lodz, and Krakow to identify promising and mutually beneficial areas of research interest, and to inform the Polish investigators of the mechanisms of PL 480 research support. Over 70 investigators were contacted.

RESEARCH HIGHLIGHTS

A. Basic Studies of Mechanisms of Normal and Abnormal Growth and Development -

The pathogenesis of congenital malformations may be tied to defect(s) of early cellular or tissue differentiation. In the development of epidermal organ systems, there is thought to be transfer of developmental information between epithelium and mesenchymal tissues. At the University of Southern California, indirect evidence for this hypothesis has been obtained from in vitro heterotypic cell recombination experiments, and from diffusion of various isotopic precursors from one tissue to another. In recent experiments on tooth formation, it was found that matrix vesicles in the intercellular organic matrix between the epithelium and mesenchyme contained ATPase enzyme activity. This finding suggests that these vesicles may be carriers of developmental information.

In studies of communication between cells in the matrix of connective tissues, it has been possible to isolate RNA in a relatively pure form from the outer surface of fibroblasts. Tests are now underway to determine whether the base composition of the intercellular RNA is similar to that obtained from intracellular locations. Such evidence may add weight to the hypothesis that RNA serves as an intercellular mediator of information. Another study at the University of Southern California has concentrated on cyclic AMP as an intracellular second messenger mediating the action of hormones. At Brandeis University, investigators are studying the role of chemotaxis in cellular migration. Specifically, they are studying the mechanism of action of cyclic AMP in inducing aggregation of cells in normal growth. The information they obtain will add to our knowledge of the biochemistry of embryogenesis.

Laboratory studies are in progress at the University of Southern California to study the effects of parathyroid extracts and heparin on the activity of degradative enzymes in bone resorption. Also under way is a study of the effects of polyamine on fibroblast growth in skeletal matrix formation and a project in which the effects of serum calcium altering drugs on the cartilage-bone calcified junction in growing animals is being observed. In another approach to experimental skeletal control the effect of various drugs on the disappearance of osteoclasts or osteoblasts is being examined.

A critical process in embryogenesis and tissue development is vascular supply. Normal development in vertebrate organisms depends upon transport of nutrients to cells and exchange of waste products. It was noted in studies at Brandeis University that RNA and specific proteins are apparently

required for endothelial growth while factors which inhibit endothelial cell division are still unknown.

Recent studies reveal that the degree of collagen cross-linkage is related to the structure and function of this protein in various tissues. New data also indicate that vitamin D affects the quantitative relationship between crosslinks and aldehydic precursors in the collagen molecules of bone. Additional studies will determine if this vitamin influences the crosslink patterns in tissues other than bone.

Studies at the University of Michigan on pulpal change following resection of the inferior alveolar nerve in mice reveal an increase in mitochondrial activity in the odontoblasts and rapid increase in formation of dentin. These observations suggest that after parasympathetic denervation the sympathetic system stimulates an over-production of dentin. This model system may be useful in parasympathetic-sympathetic control of skeletal tissues.

B. Cleft Lip/Palate -

(1) Embryology

Laboratories, including those at the University of Michigan and the University of North Carolina, are investigating the development of palatal shelves in vitro. The structures are dissected from the developing embryo at varying stages and with varying amounts of additional surrounding embryonic tissue, such as tongue and mandibular structures. In these organ cultures the nutrient media and other environmental factors can be controlled, which permits more precise observation of teratogenic agents on the palatal tissues.

The fusion capabilities of embryonic oral tissues are studied through epithelial-mesenchymal interaction. At the University of Minnesota the adhesion of palatal shelf epithelium, local areas of adhesion, and force of adhesion between shelves are under study. It is hypothesized that the adhesion between shelves may be greater than that between cells. Qualitative cytochemistry studies of enzyme systems such as malate dehydrogenase are being done to compare the enzyme levels in the critical epithelial-mesenchymal tissues of the palatal shelves with other structures as the tongue.

A study at the University of Michigan of human facial development during the 5-11 intrauterine week interval revealed that significant asymmetry of facial development exists in 28% of the cases.

Several studies have involved searching for the mechanisms of cleft palate formation. In one study chemical analysis of the palatine shelves of mice during days 14 and 15 of gestation revealed that there was an apparent deficiency in one specific isozyme in those animals receiving teratogenic doses of cortisone acetate.

The teratogenic effect of disruption of fluid balance between the fetal-maternal complex continues to receive attention. Studies on fluid imbalance indicates that many different stimuli may effect cellular activity unfavorably and result in clefting or other congenital anomaly.

An investigation at the American Dental Association has involved crossing different mice strains to attempt evaluation of the different rates of spontaneous clefting. It has been determined that the strains with different clefting rates also differ in the biochemistry of the mitochondria and in other systems such as the content of nicotinamidases. In back-crossing the two differing strains A/Jax and C57 mice, differences were noted in the maternal cytoplasm. It is speculated that these strain differences are due to genetic differences in the way the mice metabolize the 6-aminonicotinamide teratogen. Cross-breeding studies of this type may lead to a better understanding of the genetic components of cleft palate etiology.

A critical event in the embryonic development of the normal palate is the transposition of the right and left palatine shelves from a vertical to a horizontal plane just before they begin to fuse at the midline. It has been proposed that spontaneous muscular movements of the whole embryo offer the primary force for transposition to occur. In testing this hypothesis in mice at Michigan State University, tranquilizers and barbiturates were given to reduce general muscular activity. It was found that mice receiving the drugs sustained an increased frequency of cleft palate. This finding suggests that the spontaneous movements are important in closure of the secondary palate. Another hypothesis to explain the vertical-to-horizontal-plane transposition of the palatal shelves states that active contractile components are contained within the palatine tissues, and that these specialized tissues pull the palatal shelves into a horizontal position. Evidence in favor of this hypothesis has been obtained from studies at the Children's Hospital in Cincinnati in which biochemical identification of actin-like and myosin-like proteins have been made in 14.5 day old mice embryo palatal shelves.

(2) Epidemiology

Genetic-epidemiological studies are in progress to develop a better scientific basis for cleft lip/palate. An Indiana University study in Denmark involves 3600 cleft lip and palate patients and 900 isolated cleft palate cases. Information will establish recurrence risks by (a) type of cleft, (b) severity of cleft, and (c) sex of the affected. In studies by the University of Minnesota of the Haliwa Indians of North Carolina it was found that all cleft lip/palate patients trace ancestory to one woman. Five family lines account for over 50% of all known cleft cases in the geographic area.

In Hawaii standardized information on prenatal and postnatal conditions of mothers and infants is being collected and analyzed to determine whether cleft lip/palate occurrence is related to 32 sociological, genetic, biological and medical factors. So far, only diabetes in the mother seems related to risk of cleft lip/palate. When frank diabetes was present, the frequency was doubled.

(3) Psychosocial

The psychosocial complexities involved in families in which one or more children has a cleft lip/palate have been studied at two cleft palate centers. Three years ago the University of Pittsburgh Cleft Palate Center held group therapy sessions for mothers of cleft children. The mothers were able to help each other work out feelings that surrounded the birth of a child with a defect and to make progress in coping with child management problems. The sessions were so successful that a six-month program was developed to train the mothers as para-professional group leaders. The mothers now lead five 8-week group sessions each year under careful supervision. Investigators at Duke University are studying 100 cleft lip/palate patients who were operated on 22 to 27 years ago. The studies indicate that appearance and accomplishments were generally satisfying. The patients perceived their habilitated malformation as having little influence on their lives and they possessed a high level of satisfaction. The typical adult with a cleft appeared to be happily married, gainfully employed, and a contributing member of society. Twenty-seven percent had gone to college and eight percent had received a college degree.

(4) Growth and Surgery

Surgical treatment to correct cleft lip/palate malformations has frequently been followed by retarded growth of the palate. To develop ways to avoid this unfavorable sequela, experiments on normal dogs have been done at the University of Iowa in which surgical procedures have been produced and the pattern of healing monitored. The results show that when the surgical procedures include separation of the periosteum from the palatal bone, early wound contraction occurs and retardation of upper jaw growth follows. If the periosteal manipulation is too close to the maxillary central incisors, these teeth are retarded in their anterior growth. Because of the importance of the mucoperiosteum, transplantation of this tissue from other areas of the oral cavity to the maxilla is also under study.

The child with a cleft palate generally manifests a constellation of craniofacial features which are characteristically abnormal. It is important to know whether these features are genetically determined or whether this type of pathology represents the normal response of adjacent tissues to the cleft lesion. To study this problem, 21 normal rhesus monkeys were treated surgically at the University of

California, San Francisco, to create unilateral palatal clefts and medial movement of parts. The subsequent craniofacial growth pattern developed a characteristic form: (a) the angle of the mandible increased significantly, (b) the lower incisors became upright and extruded, (c) the lower arch lengthened and intermolar width increased, and (d) overall mandibular length and facial height remained similar to the control group. The study clearly illustrates that compensatory mechanisms are operant when a single lesion is created in the craniofacial complex.

Establishing the optimum age at which to perform surgical correction of the cleft is an important question still under consideration. In a recent retrospective study at Duke University, young cleft patients were examined and categorized according to age at the time of initial surgery. In patients treated before 17 months of age, it was found that the velopharyngeal closure was successful in 86% of the cases, whereas when surgery was done after the patient was more than 30 months of age, the success rate was only 52%.

New, encouraging technics to align the maxillary arch are also under way. At Duke University, craniofacial maxillary alignment and growth guidance of the structures into a normal configuration is being attempted. Meanwhile, at the University of North Carolina, the hypothesis that palatal skeletal voids can be eliminated by controlled biomechanical stimulation of the existing small palatal shelves is being proved in dog studies. These studies may harness the natural potentials of the deformed structures and improve habilitative procedures.

(5) Dentistry

Functional evaluation of habilitated cleft patients at Duke University (mentioned previously) revealed that all cases presented a cross-bite malocclusion of maxilla and mandible and 95% were in need of immediate dental care. Seventy-one percent of those with cleft lip and palate exhibited adequate velopharyngeal closure, while only 26% of the patients with a cleft-palate-only had adequate velopharyngeal closure, and 32% exhibited marked nasality in voice quality.

(6) Otolaryngology

In studies in otolaryngology at the University of Pittsburgh, it was found that the eustachian tube of cleft palate patients was more flaccid and unable to clear fluid normally. Functional failure of the tube was found to be due not only to lack of tension in the tube itself but also to muscular dysfunction in the soft palate. The effect of ear surgery to alleviate the otitis media problem was also investigated. A study of 34 children who received ear surgery during the first months of life indicated that the children had an I.Q. above 100 and did not show the mild depression in mental function

previously reported in cleft children without corrective ear surgery.

C. Other Craniofacial Anomalies

In the area of congenital craniofacial anomalies exclusive of cleft lip/palate, studies are underway on premature craniofacial synostoses. Examination of 53 patients at the University of Illinois revealed: (a) it is usually possible to distinguish radiographically between Crouzon and Apert syndromes; (b) the craniofacial growth pattern in these syndromes appears different at different times, that is, the face at one age is not necessarily representative of the condition when examined at a later age, even though the face is abnormal at all ages; (c) coronal craniectomy of the craniostenosis group permitted cranial remodeling, including displacement of bone, and transformed an abnormal cranial morphology into a more normal form; (d) distinctive patterns of nystagmus (eye motion) was observed in Apert and Crouzon syndromes, while size of the eyeball was related to size of the bony orbit, and proptosis was found to be a function of underdevelopment of the bony orbit and forward displacement of the greater wing of the sphenoid; (e) the unusual palate seen in the Apert syndrome has revealed large quantitites of mucopolysaccharide containing soft tissue.

At the University of Minnesota investigators have prepared a computer program to diagnose pediatric disorders and plan to extend the program to adult malformations. The descriptions of seven craniofacial syndromes have been expanded and redefined, while that of nine new syndromes have been added. This comprehensive compilation includes all known syndromes of facial clefting and syndromes of retinal detachment. Data on congenital oral anomalies among the Eskimo population of the Canadian archipelago has also been entered and analyzed. These studies will clarify our understanding of these syndromes and will minimize nomenclature problems.

D. Malocclusion

(1) Epidemiology

The effects of hereditary factors on the occlusion of various racial groups have been studied in the Hawaiian population. The studies indicated that Orientals, when compared to Caucasians, have a greater tendency to mesioclusion, crowded teeth, malalignment, and often missing teeth. Filipinos demonstrated buccal segments much like Caucasians, while children of Hawaiian parentage were intermediate between Caucasians and Orientals. There was a suggestion of maternal transmission involved in malalignment, incisor width, and lingual crossbite. The overall racial effects were largely additive and there was no significant

effect on malocclusion of hybridity of child or recombination. Human racial crosses apparently present no added risks for malocclusion.

(2) Growth Studies

Studies at the University of Michigan show that as longitudinal data on growth become more complete and analysis more automated, it will be possible to predict at an early age the patterns of development and anticipate future needs for treatment. Because growth and ultimate adult dimensions have extremely wide variation, a new approach is being used to simplify the classification of craniofacial morphology. In this approach, individuals who have a certain malocclusion form are clustered together. Within each cluster there is a limited, but defined range of variability. The proposed classification would allow a basic diagnosis and treatment plan to be developed for each malocclusion form.

In a longitudinal series of 21 subjects at the Royal Dental College of Copenhagen, Denmark, the eruption paths of teeth and growth changes were studied by means of metallic implants. A general feature of the facial development was a more or less marked forward rotation of the face, especially in the mandible which was strongly associated with condylar growth. At the lower and posterior borders of the mandible most of the mandibular rotation was masked by compensatory remodeling. Rotation of the maxilla was masked by remodeling of the nasal floor which remained almost unchanged in inclination. The marked facial rotation also required adaptation of eruption of the teeth. What has previously been described as an eruption of the upper molars appeared to be a combination of active eruption of the teeth and rotation of the maxilla. It would appear that malocclusions are to a greater extent due to incomplete compensatory guidance of eruption than to dysplastic deformation of the dental arches. Interceptive measures should therefore focus on the factors potentially responsible for impairing the compensatory mechanism.

(3) Craniofacial Orthopaedics

The relationship between genetic and environmental factors no longer appears to be as independent of each other as was previously believed. Animal experiments reveal clearly that variations in craniofacial form can take place through environmental changes. At the University of California, San Francisco, it was hypothesized that by lowering the postural position of the mandible, tooth extrusion will increase as will facial height. When an oral appliance was placed in 18 of 36 rhesus monkeys to stimulate the tongue and cause them to hold their mandibles in a lower position, the animals did develop an increase in facial height through increased tooth eruption. Changes were also observed at

the mandibular angle and the chin of the experimental group. It was shown in another experiment that mouth breathing also causes tooth extrusion and increased facial height. In animals with experimentally-induced mouth breathing both dental arches became smaller, the maxillary dental arch length was reduced, and the tongue became thinner in back, bulkier in the middle, and more pointed at the tip.

At the University of Michigan, 6 rhesus monkeys were guided into an anterior occlusion of the mandible. This treatment was followed by alterations throughout the craniofacial complex with a significant increase in rate and amount of growth at the head of the condyle. At the end of 3 months, all experimental animals exhibited mandibular prognathism and after 5 months all demonstrated a skeletal prognathism.

The principle of selective oral-facial sensory stimulation to produce changes in muscle activity and influence skeletal morphology has been applied at the University of California, San Francisco, in a new treatment for correction of congenital underdevelopment of the mandible. Procedures used up to now have relied on bone grafting alone to enlarge the mandibular ramus and were not successful. In the new treatment, a unique intraoral appliance protects the graft from excess pressures and allows muscular adaption to accept the new skeletal morphology. This treatment has been successful on 9 patients.

(4) Surgical Orthodontics

The treatment of severe malocclusion has historically been by orthodontics or surgery. The combined surgical-orthodontic management of these cases has produced improved clinical results but questions regarding the preservation of blood supply are still unanswered.

At the University of Texas vertical interdental and subapical osteotomies of the cortical plate of rhesus monkeys were performed to test if blood supply via the bone marrow was sufficient to maintain vitality of the fragments. The results indicate that such a procedure will imperil the intraosseous and intrapulpal circulation. However, revascularization was achieved in monkeys which had undergone total maxillotomies. The results indicate that the entire maxilla can be moved with minimal alteration of circulation to the mobilized bone and teeth. In other experiments the periosteal blood supply was removed from the growing condyles in young monkeys; by the end of six weeks the condylar cap had completely regenerated.

In human studies at the University of Kentucky, tongue pressures have been measured in pre- and post-mandibular surgery cases.

Evidence of lingual muscular tissue compensation was still observed for more than a year after surgery.

(5) Speech and Oral Function

The complex relationship between neural parameters and motor activity in speech and oral perception are under study in several laboratories. At Purdue University, it was noted that in oral-sensory deprived conditions produced by anesthesia, the tongue shifted posteriorly, moved more slowly and produced increased pressure during speech. The University of Washington's experiments showed that anesthetization of a specific part of the mandibular nerve caused jaw opening to be reduced. In faster speech exercises there was a more significant reduction in jaw movements. Thus, when greater demands were placed upon the mechanism, greater disruption occurred.

MEETINGS SPONSORED

1. An International Conference titled "The Comparative Molecular Biology of Extracellular Matrices" was held at the University of Southern California Marine Biological Laboratory on June 4-8, 1972. The conference included discussions of research of the biology of extracellular matrices. Attention was also given to the question of how individual cells influence and control their micro-environment and, in turn, how the microenvironment affects cell structure and function. Great interest was also expressed in cellular-intercellular interactions as they relate to embryonic induction and the expression of potential development during cell differentiation and the ultimate formation of cranial facial structures. The proceedings, edited by H. C. Slavkin, have been published in book form.

2. State of the Art Workshop -- "Cleft Palate: Clinical Research," (NIH-NIDR-71-643) contracted to the American Speech and Hearing Association. A series of workshops were held in a variety of disciplines relevant to the clinical aspects of cleft palate research: etiology, otolaryngology, speech, surgery, psychosocial, dentistry, anatomy, and physiology. Three publications have resulted from this conference this past year:

A. Clinical Research in Cleft Lip and Cleft Palate: The State of the Art, Cleft Palate Journal, 10: 113, 1973, (D. Spriestersbach, Chairman).

Recommendations:

(a) Identify variables in cleft populations and the nature of their interactions.

(b) Support research to establish uniformity of cleft palate centers.

(c) Objective documentation of the lesion and progress in development and treatment.

(d) Develop reliable standards to permit prediction of prognosis.

(e) Prospective longitudinal studies.

(f) Develop a centralized data bank for objective information.

(g) Develop programs of research in concert with other craniofacial anomalies.

B. Etiology and Pathogenesis of Congenital Cleft Lip and Cleft Palate an NIDR State of the Art Report, Teratology, 6: 255, 1972, (F. C. Fraser, Chairman).

Recommendations:

(a) Application of molecular biology to mammalian teratology; understand environmental predisposing factors.

(b) Investigate feasibility of national system to make available human fetus material for study.

(c) Epidemiological studies should be done only to test specific hypotheses.

(d) Twins, especially discordant monozygotic twins, shall be explored to identify predisposing factors.

(e) High risk mothers (mothers of a cleft child) should be studied in detail during subsequent pregnancies.

C. Cleft Lip and Cleft Palate: Research relevant to clinical management in dentistry, an NIDR State of the Art Report. American Journal of Orthodontics, 63: 398, 1973, (S. Horowitz, Chairman).

Recommendations:

(a) Overall strategy to correlate morphologic and physiologic maturation through long-term longitudinal studies.

(b) Continuum of coordinated treatment and research documentation from birth to adulthood.

(c) Development of interdisciplinary craniofacial anomalies centers.

(d) Standardization of research methodology to allow pooling of data.

3. Conference on Craniofacial Malformations (S. Pruzansky, Chairman).

This workshop was designed to discuss the state of the art of research in congenital craniofacial anomalies exclusive of cleft lip/palate and malocclusion. Although infrequent in occurrence, these anomalies are important because

they are severely disfiguring. Highlights of the conference included discussion of the recent breakthrough in surgical procedures for the treatment of several disfiguring craniofacial syndromes which were previously untreatable. In the new technic an intracranial surgical approach is followed. It was brought out, however, that more information is needed on the effect of these surgical procedures on subsequent growth processes. It is also essential that complete functional evaluations be made before and after surgery. Proceedings of the conference will be published in an appropriate journal.

FUTURE PLANS

The concept of Craniofacial Anomalies centers to achieve a cohesive effort in investigations of all congenital craniofacial anomalies will be under exploration. This is timely because of the recent breakthrough in surgical procedures of severe craniofacial deformities and the achievements of the interdisciplinary research within the cleft palate centers. In addition to the more radical surgery, surgical-orthodontic experiments on subhuman primates will quantitatively investigate the reinervation and revascularization of tissue following maxillotomy procedures. The control and guidance of craniofacial growth through orthopaedic measures will be explored in research and assessed in a State of the Art workshop. Teratogenic studies on cleft lip and palate will focus on the activity of enzymes, drugs and environmental factors on palatal closure. These studies will be done on experimental animals and it is planned that studies of high risk mothers and discordant monozygotic twins will be started to provide definitive information about the multifactorial etiological factors in humans.

SUMMARY

The Craniofacial Anomalies Program supports research on structures of the oral cavity and face and on cranial structures as well. Attempts are made to maintain program balance so that funds are provided not only for projects on congenital and acquired malformations and malocclusion of teeth and jaws, but also for fundamental research in prenatal and postnatal developmental biology. In FY 73 nearly four million dollars was awarded for 79 research grants or programs.

Laboratory research on clefting has brought the technic of organ culture of embryonic palates of experimental animals to a high level of sophistication. It is now possible to examine in detail the epithelial-connective tissue interactions involved in the fusion of the palatal shelves and to test for enzymatic control of the developmental processes. This model system is also highly suitable for studying mechanisms of teratogenesis.

Objective studies on experimental surgical procedures and wound healing have provided sound data upon which to base treatment technics which will lead to better control of bone growth in the habilitation of congenital anomalies. Studies in subhuman primates are providing valuable information on revascularization and reinervation of component skeletal fragments which have been surgically repositioned.

The most common form of craniofacial malformation is seen as a discrepancy between the size of the jaws and that of the teeth. Through research on this problem, more precise understanding of the roles of environment and genetics in developing the final form and function of oral facial structures has evolved. It is now believed that the potential for modifying the growth and alignment of teeth and jaws is greater than was thought to be true earlier. Thus, the stage is set to attempt to intercept or prevent certain malocclusions by applying local forces to guide growth in a favorable manner.

In future research, prospective studies will be carried out to secure data on prenatal events associated with cleft lip and palate, especially in mothers of affected children. Subsequent offsprings of such mothers could be expected to have a high incidence of clefting. These timely studies will be directed at folate level, vitamin A metabolism and an anti-insulin factor. The mothers will also be followed through subsequent pregnancies to identify yet unrecognized predisposing environmental factors which may be involved. In addition, detailed study of twins, particularly discordant monozygotic twins, will be conducted to identify predisposing features and to evaluate the effect of the malformation on the total phenotype.

Methods for research on the extensive new surgical procedures for the severe craniofacial anomalies will be explored. It is vital to determine the proper time for surgical intervention along with the functional, esthetic, and psychosocial disabilities and adjustments. Consideration will also be given to the present confusion in syndrome nomenclature and the need for an international classification system. In addition, attention will be given to the lack of information on incidence and prevalence of the numerous but less common noncleft congenital craniofacial anomalies. It is recognized that many of these efforts will require the efforts of both clinical and basic science investigators.

ANNUAL REPORT FY 73

RESTORATIVE MATERIALS PROGRAM

INTRODUCTION

Since the highly specialized tissues of the mouth cannot replace themselves when they are lost, treating the effects of dental disease is still largely based upon the effective use of available materials. Therefore, the principal objectives of the Restorative Materials Program are to provide better dental care by fostering the development of improved materials and methods to restore lost oral tissues to normal form and function. To achieve these goals, support is furnished to academic and industrial research groups for the conduct of basic and applied studies. Both grant and contract mechanisms are employed in this support.

The Restorative Materials Program has five broad categories of interest: (1.) restorative materials for repairing the damage from dental diseases, (2.) plastic adhesive sealants for protecting the surfaces of teeth against decay, and for sealing the margins of fillings, (3.) maxillo-facial prostheses for replacing tissue defects and mutilations resulting from birth defects, accidents, or surgery, (4.) artificial tooth implants to provide either serviceable replacements for missing teeth, or posts to anchor bridges or complete dentures in place, (5.) development of devices, equipment, and standards for materials.

This year research emphasis was placed on developing projects for solving some of the many problems which exist in determining the best materials, designs, and procedures for use with implants. Several Requests for Proposals have been issued which present in detail questions for which the NIDR seeks answers in this field. The projects which will be developed in response to these Requests should bring us closer to the time when implants can be recommended for general use in dentistry.

It is anticipated that a state-of-the-art conference will be held early in FY 74 to discuss the four specific areas of Program interest, and to obtain advice on National research goals and priorities in dental biomaterials for the next several years.

ADMINISTRATIVE

Table I shows the distribution of funds for research grants, contracts and interagency agreements in FY 72, in which expenditures were made of $1.3 million for 35 grant awards and $0.5 million for contracts and interagency agreements. It may be noted that approximately one-third of the total funds were expended for research to improve or develop new filling materials and about one-third was spent to continue projects dealing with adhesives. Only 14% of the funds was allotted to implant investigations, while no funds at all were provided for studies on maxillo-facial prostheses. Six of the grants were special dental awards made to young investigators.

FY 72...Distribution of Research Funds (in $1,000)

Interest	Grants(35)	Contracts(5)	Interagency Agreements(3)	Total	%
Filling Materials	$556 (42%)	-	-	$556,000	30
Adhesives	245 (19%)	280	*120	645,000	35
Dental Prostheses					
Intra Oral	138 (10%)	-	-	138,000	8
Maxillofacial	-	-	-	-	
Implants	151 (12%)	102	-	253,000	14
General	217 (17%)	11	5	233,000	13
Overall Total:	$1,307	$393	$125	$1,825,000	

Table I

* Includes part of the National Bureau of Standards program.

Table 2 shows the distribution of funds for training grants, fellowships, and career development awards in FY 72. The training grants included programs to train scientists in metallurgy and materials science (50%); chemical and mechanical engineering (25%); and in chemistry and physics (25%). Stipends were furnished for 47 trainees in FY 73. Because of the phase out of training only 39 trainees will be supported in these programs during FY 74.

Distribution of Training Funds - FY 73

	No.	Amount ($1,000)
Grants	13	620
Fellowships	2	31
Career Developments	1	20
Total:		$671

Table 2

Table 3 shows the distribution of research funds according to category of effort: clinical, applied, basic. Thirty-one projects funded at just more than $1.0 million were designated as applied or clinical studies, and 12 projects funded for nearly $0.8 million were considered to be basic in nature.

Science Interest	CATEGORY OF EFFORT FY 72 CLINICAL	APPLIED	BASIC	TOTAL
Filling Materials	$184,000*(5)	$103,000(5)	$269,000(3)	$556,000(13)
Adhesives	43,000 (2)	218,000(5)	384,000(5)	645,000(12)
Dental Prostheses				
Intra Oral	61,000 (1)	20,000(2)	57,000(2)	138,000(5)
Maxillofacial	-	-	-	-
Implants	-	253,000(5)	-	253,000(5)
General	-	154,000(6)	79,000(2)	233,000(8)
Total:	$288,000(8)	$748,000(23)	$789,000(12)	$1,825,000(43)

Table 3

*( ) = No. of projects including contracts.

An estimate of expenditures in FY 73 is given in Table 4. This table shows an increase in anticipated research expenditures of about $400,000, which reflects primarily the expected funding of two new contracts on dental implants. The Request for Proposals for these contracts have already been advertised, and the proposals submitted in response to the RFP have been reviewed and are now awaiting funding.

FY 73...Distribution of Research Funds (in $1,000)

Interest	Grants(36)	Contracts(9)	Interagency Agreements(3)	Total
Filling Materials	$667	$ -	$ -	$667,000(29%)
Adhesives	225	280	120	625,000(28%)
Dental Prostheses				
Intra Oral	138	-	-	138,000(6%)
Maxillofacial	-	-	-	-
Implants	170	502	-	672,000(30%)
General	143	11	5	159,000(7%)
Overall Total:	$1,343	$793	$125	$2,261,000

Table 4

STAFF ACTIVITIES

In an effort to stimulate the development of new research programs, staff made specific visits with Dr. Kreshover to five dental schools and held planning consultations with potential applicants at the International Association for Dental Research and American Dental Schools meetings. To keep abreast of developments and to help define future directions, staff participated in two ADA sponsored meetings dealing with materials. Staff also made four monitoring visits to contractors with ongoing projects in order to assess progress and offer suggestions. In addition, staff made visits to two potential contractors and to five academic institutions for purposes of scientific evaluation. A list of these visits is presented below.

Dental School Visits (programming):

Loma Linda University	Aug. 1972
University of Pittsburgh	Oct. 1972
St. Louis University	Oct. 1972
Medical College of Georgia	Dec. 1972
University of the Pacific	Mar. 1973

ADA Meetings and Conferences:

Materials Council	Nov. 1972
Research Conference	Feb. 1973

Site Visits (Monitoring) - Contractor:

NIH-NIDR-71-2383	Midwest Research Inst.	Sept. 1972
NIH-NIDR-71-2022	University of Utah	Oct. 1972
NIH-NIDR-71-2386	Battelle, Northwest	Nov. 1972
NIH-NIDR-70-2238	Franklin Research Inst.	Jan. 1973

Site Visits (Evaluative) - Contract Proposals (implants):

(1) Battelle-Columbus, Ohio
Clemson University, South Carolina — Jan. 1973
University of Florida

(2) Harvard University, Massachusetts
Loma Linda University, California — Jan. 1973
University of Southern California

Site Visits (Evaluative) - Grant Applications:

(1) Training:

T01 99	Polytechnic Inst. of Brooklyn	Aug. 1972
T01 127	Georgia Institute of Technology	Dec. 1972
T01 104	University of Pennsylvania	Jan. 1973

(2) Research:

R01 3504	Emory University	Dec. 1972
R01 3754	University of Southern California	Feb. 1973

RESEARCH HIGHLIGHTS

Continuing studies of artificial dental root implants in three different experimental animals have shown that implants made of titanium, vitallium, or ceramic can remain successfully anchored in the jawbone for periods up to one year. These findings have been obtained in studies carried out in dogs at the Medical University of South Carolina (titanium), in baboons at the University of Illinois (vitallium), and in minipigs at the University of California (titanium) and at Battelle Memorial Laboratories at Richland, Washington (titanium and ceramic). In these studies, the implants have been fabricated in various root designs including conical, cylindrical and blade shapes with or without porous surfaces. Proper surgical technique has been found to be an important factor in achieving success, particularly the necessity for absolute stabilization of the implant during the healing period of 2-3 months. Tight fits achieved by driving implants into slightly undersized drill holes, wedging of blades snugly into grooves, or the use of Cr-Co castings as splints have all been found to be effective in achieving proper stabilization. These findings indicate the need for determining the relative importance of surgical technique when evaluating various designs and materials used in implants.

Continuing contracted research at Battelle Memorial Laboratories in Columbus, Ohio, and Franklin Institute in Philadelphia have brought forth further advances in the study of marine animal adhesives. Recently a method for solubilizing the proteinaceous cement secretions of barnacles and mussels has been accomplished, and chemical characterization of the material has begun. Nevertheless, it is still not clear at this point whether or not these efforts will lead to a useful product applicable to oral problems. In a contract project at Midwest Research Institute in Kansas City, several methacrylate base polymers with adhesive properties have been formulated and have been shown to exhibit a tensile strength of approximately 1000 pounds per square inch. The efficacy of these materials as elastomeric liners for fillings in teeth is now being tested in animals.

MEETINGS SPONSORED

A grant was made to Dr S. F. Hulbert, Clemson University for the purpose of planning and executing an international Symposium on "Prostheses and Tissue: The Interface Problem." The Symposium held at Clemson University on April 15-18, 1973, provided a forum for the discussion of many problems in Biomaterials, including two of specific interest to the NIDR Restorative Materials Program: Maxillofacial Prostheses and Dental Implants. It is expected that as a result of this Symposium, problems associated with prostheses tissue interfacing will be better understood, and that new approaches to solving these problems will be developed.

FUTURE PLANS

In the immediate future the Restorative Materials Program will give special emphasis to research on artificial dental implants and will attempt to initiate projects to develop better maxillofacial prostheses. Through contract mechanisms the program is presently following a more systematic approach to the research and development of implants than has been attempted previously. It is expected that three significant contracts, soon to be let, will provide solid progress towards achieving an effective implant. In one contract the influence of tooth design on implant acceptability will be studied in animals. In this study the effects of tooth shape, as well as the effects of grooves, slots, and porosity will be evaluated. In the other two studies, the influence of the chemical composition of the tooth implant will be assayed. Artificial teeth made of ceramic, metal, and plastic will be tested under these contracts.

In an effort to sharpen program planning for all components of the Restorative Materials Program, arrangements have been made to obtain the comprehensive expert advice of a number of consultants early in FY 74. To do this, plans were initiated and developed for holding a Symposium/Conference on "Dental Biomaterials - Research Priorities" at the O'Hare Inn (Chicago O'Hare Airport) August 7-9, 1973.

The symposium will consist of four half-day sessions covering each of the following subjects: Restorative Dentistry; Protective Coatings; Maxillo-facial Prostheses; Implants. The session on Implants will be co-sponsored by the American Dental Association. Papers by experts will be presented to analyze the current status of the fields, to identify important gaps in knowledge, and to recommend avenues of research. A general discussion period after each half-day session will allow members of the audience to offer suggestions and comments.

Following the Symposium an ad-hoc Materials Advisory Panel will confer to discuss the information gathered from the Symposium, to evaluate its significance, and to recommend future goals and priorities for the next several years. This special advisory panel is composed of ten outstanding research leaders drawn from the fields of materials science and prosthetic dentistry.

Proceedings of the Symposium will be published to acquaint the dental materials researchers and other interested professionals with current needs.

ANNUAL REPORT FY 73

MINERALIZATION, SALIVARY SECRETIONS AND NUTRITION PROGRAM

INTRODUCTION

The Mineralization, Salivary Secretions and Nutrition Program is a new program which supports basic and applied research efforts designed to expand knowledge of (a) the role of nutrition in the growth, function and health of the craniofacial complex; (b) the normal structure and function of salivary glands and their secretions; and (c) the mechanism of the mineralization process. It was formed in January 1973 from research and training grants which were formerly administered by other categorical programs of NIDR. The rationale for the formation of the new program area was to bring together under one administrative unit those activities which provide fundamental underpinning to the disease oriented program areas, thereby facilitating programmatic objectives. Under the present guidelines, only undifferentiated research is assigned to this program area, with targeted research on mineralization and salivary secretions being administered in the more disease-oriented extramural programs.

ADMINISTRATION

The program presently administers 49 research grants and 15 training grants. Currently, only four individual research grants have been identified as dealing with nutrition. The total support for these grants amounts to less than $80,000 per year. Twenty-three active grants deal with basic studies of salivary glands and their secretions. Support for these grants was just over .5 million dollars per year. During the Fiscal Year, four new applications dealing with salivary gland secretions were approved but were not funded, because of the unavailability of funds. For mineralization research the funding level for FY 73 was slightly over 1.5 million for 22 grants. During the current fiscal year twelve training grants, funded at $750,000 were administered by this program. Of these, ten emphasized hard tissue research, one program dealt with metabolism and nutrition, and one offered training in oral pathology. Of the 54 trainees supported by these grants, 28 were postdoctoral and 26 were predoctoral. For FY 74 this number will be reduced to 44 trainees, and the cost of the grants will approximate $466,500. In addition to the training grants, 9 postdoctoral and special fellows were supported at a cost of $119,000; and 4 career development awardees were supported at a cost of $108,000.

RESEARCH HIGHLIGHTS

Mineralization - Interesting studies of the effects of impurities in apatite structures have been carried out by investigators at the Georgia Institute of Technology. They have shown that the interaction between the chlorine and hydroxyl ions in apatite seem to be much stronger than the interaction between fluorine and hydroxyl ions. This suggests that chlorine, which is nearly always present in enamel, may have an important role in the

solubility of enamel and, perhaps, in susceptibility to caries. Future studies will attempt to elucidate the mechanism of this chlorine-hydroxyl interaction. Investigators from the same laboratory have provided sound crystal structural evidence of differences between tooth enamel and pure hydroxyapatite synthesized in the laboratory. They have examined a number of specimens of human enamel and synthetic hydroxyapatite with several techniques under identical conditions. Their results revealed differences between enamel and hydroxyapatite which have been ignored in the past.

Investigators at Case Western Reserve University have obtained additional information on the relative solubility of enamel through the development of a method whereby etched surfaces of enamel blocks could be simply infiltrated with embedding medium and serial thin sections for transmission electron microscopy could be cut. The microscopic findings reinforce the concept that orientation of the enamel crystals is a major factor in the rate of dissolution. Areas in which crystals were oriented perpendicular to the plane of the facet were definitely the most vulnerable. Inasmuch as the crystals in enamel show a high central solubility, in contrast to their outer shells, attempts are also being made to determine if the central region has a different chemical composition from the outer shell.

Recent evidence indicates that therapeutic agents, such as fluoride, can be manipulated to enhance their penetration into enamel. A study by grantees at the National Bureau of Standards has shown that the application of both dicalcium phosphate dihydrate (pH 2-3) and phosphoric acid (pH 4) bring about the formation of dicalcium phosphate dihydrate (DCPD) at various depths in enamel. This treatment does not produce undesirable surface etching. Since DCPD reacts with fluoride to form fluorapatite, it appears that production of DCPD in the depths of enamel prior to F treatment will increase both the penetration of F and the yield of fluorapatite formation, thus enhancing protection against caries.

An important advance recently made by scientists at the Institute of Materials Science, University of Connecticut, was the development of a hydrothermal method for growing large hydroxyapatite crystals. Such crystals measure as much as 3 X 3 X 7 mm. The advantage of large crystals is that they enable scientists to study the surface properties thoroughly without interference from contaminating impurities. Since biological apatite mineral undergoes critical reactions with organic substances and other ions through its surface properties, it is important that the nature of these crystal surfaces be thoroughly clarified. Such studies may allow scientists to discover the exact relationship between the organic and mineral components of teeth and bones, so that the processes of growth and development, as well as those involved in normal maintenance metabolism will be better understood.

Since it first became known that increased levels of the enzyme carbonic anhydrase were associated with parathyroid-induced bone resorption, it has been hypothesized that parathyroid hormone operates through mechanisms

involving carbonic anhydrase. Further evidence to support this hypothesis was recently obtained by a grantee at the University of Louisville, who demonstrated that the specific carbonic anhydrase inhibitors acetazolamide and ethoxzolamide inhibit parathyroid-induced bone resorption also. Their data show that the ability of these drugs to inhibit parathyroid varied directly with their ability to inhibit carbonic anhydrase. For example, ethoxzolamide is 60 times as potent as methazolamide in inhibiting parathyroid hormone. It was also demonstrated that an analogue of acetazolamide which is unable to inhibit carbonic anhydrase is also unable to inhibit parathyroid. This new data supports the hypothesis that carbonic anhydrase is involved in parathyroid-induced bone resorption in mammalian species.

Additional evidence supporting this hypothesis was recently obtained from experiments on disuse osteoporosis, the common phenomenon that bones undergo resorption when not in use. This form of resorption was produced in rat humerus bones by sectioning the brachial nerve plexus, but resorption could only partially be inhibited by parathyroidectomy. Now it has been conclusively shown that this manifestation of bone resorption can be inhibited by simply adding the carbonic anhydrase inhibitor acetazolamide to the rat diet.

In other studies on bone resorption, grantees at Boston University have explored the ability of 5-methyl-2-thiophenecarboxylic acid (5-MTC) to lower serum calcium and phosphorus and to inhibit pathological loss of bone. The results indicate that this compound is relatively non-toxic and that it has the most prolonged duration of action of all the known hypocalcemic agents. It does not enhance urinary calcium excretion nor does it appear to interfere with calcium absorption from the gastro-intestinal system of rats. The study of 5-MTC has now been extended to hamsters since regulation of extracellular calcium in this species is more strongly influenced by the actions of parathyroid hormone on the kidney than is the case for rats. The importance of this lies in the fact that hamster regulation of calcium metabolism is similar to that of humans. The results show that 5-MTC also has marked and prolonged hypocalcemic and hypophosphatemic actions in the hamster. Moreover, the drug retains its actions after removal of the kidneys, thus indicating that the hypocalcemic and hypophosphatemic actions of the drug are the result of its ability to inhibit bone resorption. Future studies will include the addition of 5-MTC to a diet which causes periodontal disease in hamsters to determine if the drug will affect the development, or severity of the disease.

Bone rebuilding has also been studied by a collaborative effort between investigators at the Boston University and the University of Illinois. In these studies, decalcified cortical bone matrix allographs (DABM) have been used to replace alveolar ridges in edentulous dogs, to fill surgically created bone defects around roots of teeth, and to replace defects in long bones. This easily handled graft material can be trimmed to fit the defect site exactly. It apparently is a strong inducer of new bone growth and is itself resorbed in time, being entirely

replaced by new bone. To determine factors affecting the ability of DABM to stimulate new bone formation in rats, the investigators have examined a situation in which the overall rate of bone growth is diminished. When DABM was implanted into a limb bone undergoing atrophy because of denervation, rapid new bone formation occurred in spite of the overall regressive, atrophic changes taking place. While it has previously been shown that DABM can stimulate new bone growth in dogs, rats and monkeys it is possible that other factors which can be controlled, may affect its ability to serve as an inducer of new bone formation. For example, low intake of asparagine and glutamine (generally considered not essential) reduces the amount of new bone formed in response to DABM. Results, however, suggest that administration of exogenous growth hormone seems to enhance the activity of DABM in mature female rats. The effect of fluoride will also be considered in future investigations.

Investigators at the Dental Research Institute at the University of North Carolina have shown that there are differences between hard tissue collagen and soft tissue collagen. Their findings may help answer the question as to why certain collagens calcify in the body and others do not. The answer to this fundamental question would be a significant step toward identifying the control mechanism(s) in mineralizing systems. Investigations into the stabilization of collagen macromolecules through cross-linking indicate that following reduction with a tritiated borohydrate (sodium borotritide NaB_3H_4), both bone and dentine collagen contain only a few labeled substances compared to soft tissue collagen. On the other hand, reduced aldehydes, hydroxynorleucine and dehydroxynorleucine are abundant in mineralized collagens and are thought to represent the major crosslink (60%). Other results suggest that the marked insolubility of bone and dentine collagen is due to the fact that 25-50% of the crosslinks are in a chemically reduced state *in vivo*. In a companion study which relates more directly to mineralization it was shown that vitamin D is necessary for a normal crosslink pattern in chick bone collagen. This new evidence suggests that the role of vitamin D in bone metabolism is greater than was previously believed. It now appears that this vitamin not only serves as a regulator of mineral metabolism, but also influences the normal maturation of the bone matrix.

SALIVARY GLAND AND SECRETION STUDIES

Salivary glands are important regulators of the ecological status of the oral cavity, and as such are fundamental determinants of oral health. The glands also serve as indicators of certain local or systemic pathological conditions.

In studies on salivary glands at the University of Minnesota, investigators have obtained data which appears to contradict a universally held biological principle. Normally the more specialized a type of cell is, the less it divides and proliferates. However, in adult rat parotid glands, the investigators have found that the terminal cluster of cells

serves as the source of most of the gland's functional or parenchymal cells. Terminal cluster cells are not the most specialized cells in the gland, however, it had been thought that the intercalated duct cells which appear less differentiated than the terminal cluster cells were the source of functional parotid cells. These conclusions are based upon counts of mitotic figures in the various cell populations.

Investigators at Temple University are successfully using an experimental model in which they stimulate cellular growth in salivary glands by giving a drug called isoproterenol to mice and other rodents. Using this system they are making important contributions to our understanding of the control of protein synthesis, cell proliferation and a cyto-differentiation in biological systems in general. They have, for example, shown that isoproterenol stimulation causes activation of the genetic portion of the cell by first bringing about ribosomal RNA synthesis. They have also shown that cellular glycolipid synthesis is specifically stimulated by isoproterenol. While the exact role of these glycolipids is not yet clear, these substances are of great concern because they become altered during cell transformation due either to viral infection or malignancy.

Salivary gland secretions contain nearly 30 different proteins. At the present time two interesting groups of these proteins are receiving concentrated attention by NIDR supported scientists, because of their potential importance in health and disease. At Boston University, biochemists have identified a group of proteins in fresh saliva which are rich in proline, glycine, glutamic and aspartic acid. Partial characterization indicates that there are 4 proteins in this group and together they constitute about 4% of the total parotid saliva proteins. They have similarities to known structural proteins and are cleaved by clostridial collagenase A suggesting a peptide sequence similar to collagen. The unusual aspects of these proteins is that they are not found in freshly collected whole saliva and they disappear when mixed with relatively small amounts of whole saliva in vitro. Since these proteins have a high affinity for hydroxyapatite, they may contribute to pellicle formation, to plaque formation, or they may be involved in the re-mineralization of tooth structure.

Another exciting study of salivary proteins is being conducted at the University of Wisconsin. There investigators have identified a group of basic proteins which demonstrate genetic polymorphism. Three phenotypes have been observed. The results of studying several families support the hypothesis that these basic proteins reflect an autosomal codominant inheritance pattern. Because of the similarities of these proteins to lysozyme and cationic proteins of granulocytes, it is believed that they may have antibacterial activity.

It has been reported in annual reports for the last several years that studies of secretory immunoglobulins hold great promise for the ultimate prevention of oral diseases. Studies have now progressed to the stage where specific antibodies have been stimulated and the mechanism of interaction between antibody and certain microorganisms have been demonstrated. NIDR supported scientists at SUNY at

Buffalo and the Forsyth Dental Center in Boston have shown that local immunization in and around the salivary glands with killed microorganisms (Streptococcus mutans) produces partial protection from tooth decay in rats. Local immunization in and around the salivary glands routinely seemed to stimulate antibody in saliva and blood, while injections made in other parts of the body produce antibody primarily in the blood and only inconsistently in saliva. Other investigators have shown that the immunoglobulin A(S-IgA) coats specific bacteria, causing them to clump together, thus inhibiting their ability to colonize on mucous membranes and tooth surfaces. The mechanism of the S-IgA reactions with bacteria differ from other antibody reactions in that the organisms are not killed nor are they removed by phagocytosis.

Investigators at the Center for Oral Health Research, University of Pennsylvania, and the Institute of Dental Research, University of Alabama, have been developing highly sensitive analytical methods to study both colostral IgA and parotid saliva IgA and their associated components. In related studies attempts are being made to understand the relationship of local immunity to the systemic immune response so that the development of more effective local immunization procedures will be possible.

Studies at SUNY in Buffalo have investigated the changes that occur when salivary glands are infected by mumps virus. In these studies, Rhesus monkeys were inoculated with mumps virus by retrograde instillation into the parotid gland. For up to one week after this inoculation it was possible to isolate virus from buccal swabbings and parotid biopsies. Within three weeks a vigorous serum neutralizing antibody response occurred and the parotid gland itself showed a marked monocytic infiltration of the stroma. The predominant inflammatory cells were non-immunoglobulin-containing mononuclear cells resembling lymphocytes. However, plasma cells containing IgG, IgA, IgM and IgE increased in numbers in the gland after infection. The greatest increase was in IgG-containing plasma cells. Extracts prepared from the infected glands contained neutralizing antibodies as early as 36 hours after infection. The highest level of neutralizing activity was observed in tissue samples taken 3 weeks after infection. Infected tissue extracts also contained interferon. When previously infected animals were challenged locally with new mumps virus, gland tissue showed an increase in monocytic infiltration as well as an increase in immunoglobulin-containing plasma cells. However, the re-infection was apparently short-lived, since no virus was cultivable from the tissues. This experimental infection model system is proving very useful in elucidating local antibody production and local cell-mediated immune mechanisms.

NUTRITION

Kwashiorkor is an extreme form of protein malnutrition which is well recognized in some parts of the world. Studies of humans, however, have provided no satisfactory clues as to the essential biochemical or structural lesions attributable to the protein deficiency itself. Thus investigators have turned to experimental animal models which can be made

to simulate the syndrome of human kwashiorkor in order to increase our understanding of the precise role of proteins in growth and development and in basic metabolic processes. Investigators at the Institute of Dental Research at the University of Alabama and the Center for Research in Oral Biology at the University of Washington are conducting interesting studies in animals on the effects of reduced protein intake in the hope of clarifying the immediate and long-term effect of such treatment. Another effect of protein-calorie malnutrition is enlargement of the salivary glands, a condition that has been observed in both humans and animals. Gland enlargement has also been observed in patients suffering from malnutrition who have been rapidly restored to normal nutrition. Salivary gland enlargement has been observed in diabetes mellitus, in liver disease and alcoholism with or without liver disease. In other studies, undernourished rats showed an increased rate of amylase synthesis which paralleled parotid gland enlargement for the first 12 days of food restriction. This was followed by a marked decrease in synthesis. This unexpected result indicates that there are control mechanisms regulating salivary secretions beyond those already known. Since salivary secretions are of considerable importance in oral health, further studies will be undertaken to determine the mechanism for triggering the increased synthesis and subsequent decrease.

For many years fluoride research has continued to have fascinating ramifications quite apart from its capacity to prevent tooth decay. Although the ingestion of insufficient fluoride to prevent tooth decay has been described by some authors as a fluoride deficiency this view has not been taken too seriously by many investigators, and could hardly be supported scientifically as a valid hypothesis. Recently, however, scientists at the University of Minnesota have adduced evidence that may support the validity of the concept that a true deficiency of fluoride may indeed exist. These investigators have demonstrated that progressive infertility was observed in mice on a low fluoride diet containing 0.1 to 0.3 parts per million of the element. In a more recent study, they repeated the earlier infertility experiments and then proceeded to show in a more clear cut fashion that low fertility was associated with low fluoride in the diet. In these latest experiments, mice with impaired breeding performance were divided into two groups: one maintained on the original low fluoride diet, and the other given drinking water containing 50 parts per million fluoride. After a twenty week period, 80 to 90% of those on the high fluoride diet produced four litters, whereas only 50% of those on the low fluoride intake produced four litters. The scientists concluded that fluoride deficiency had been the cause of the relative infertility of the experimental animals. Since the addition of fluoride alone prevented the infertility, fluoride can be considered essential in the nutrition of the mouse. Related studies are now in progress to evaluate the possible effects of fluoride deficiency on the virility of the male mouse, and to determine the effects of low fluoride on the fertility of second generation female animals. In other studies in the same laboratory it has been found that severe anemia occurs in pregnant mice on low fluoride diets and in their pups during their rapid-growing stage. Strangely enough, however, anemia is not observed in the new-born pups. At the

present time there is little information to explain the mechanism of the anemia, because the effect of the fluoride on the hematopoietic system has not been thoroughly investigated, nor has its effects on iron secretion in milk been studied. It is believed however that fluoride may be essential for intestinal absorption of factors involved in hematopoiesis.

PROGRAM PLANS

NIDR has traditionally assumed a major role in supporting research dealing with the mechanisms of mineralization because caries, periodontal disease, and craniofacial malformations involve mineralized tissues. For many years it was strongly believed that studies on basic physical properties of the calcium phosphate minerals, including crystalline hydroxyapatite, and on mineral deposition would provide key insights into the various disease processes. This approach, however, has not borne fruit and appears even less promising today than before. Today many believe that the problems of health and disease may be solved more readily by attention to molecular biology than by continued emphasis on the physical aspects of hard tissue metabolism. Thus, plans have been made to carefully consider the significance of mineralization studies and to establish specific goals for future research. Accordingly, a special Ad Hoc Advisory Panel was convened to evaluate accomplishments in the mineralization field and to outline a future course of action. The Committee was asked to examine the program and make recommendations concerning the scope and balance of NIDR support in mineralization research. The Committee was instructed to consider program productivity, duplication of effort, and areas where emphasis should be increased or redirected. It is the intention of staff to closely supervise progress in this field, and to relate this area of research to the new information being generated in other disciplines.

Plans are being prepared to expand research in nutrition in two areas. Laboratory studies will be developed to associate the deficiency state with impairment of general oral health; and studies involving special populations will be implemented. The clinical studies will include projects on the specific condition of linear enamel hypoplasia which appears in areas where malnutrition also exists. This condition is known to exist in locations in this country and in Central and South America. The proposed studies will be designed to show clearly whether or not nutrition is involved in the prevalence of this enamel defect. A small workshop will soon be held to identify other specific problems of nutrition that would be of special interest to NIDR. Two laboratory projects to be initiated are already planned for funding by contract means. One of these is a study of the specific relationship of protein malnutrition to oral epithelial cell turnover rates, and the other is concerned with the effects of protein deficiency on other aspects of oral tissue metabolism.

Although there are no specific program plans to expand research in salivary secretions, it is considered that this area offers bright promise. Young investigators have shown great interest in salivary glands because these

tissues can be useful as model systems for studying a number of intriguing biological processes including protein synthesis, morphogenesis, calcium metabolism, and cell transformation. Such a system can also be used to study detailed events associated with transformation, such as glycolipid synthesis in relation to cell membranes and electrical potential changes.

ANNUAL REPORT FY 73

PAIN CONTROL AND BEHAVIORAL STUDIES PROGRAM

INTRODUCTION AND BACKGROUND

Pain and its relief are major health concerns of the American people, as indicated by the intense public interest in acupuncture. Two specific aspects of the overall pain problems are of particular interest to NIDR. The first is the pain and associated fear and anxiety identified with almost all types of dental treatment. Such fears are undoubtedly responsible for the fact that millions of Americans avoid dental treatment, and thereby settle for poor oral health. The second type of pain of interest to NIDR is that associated with a variety of orofacial pain syndromes such as tic douloureaux (trigeminal neuralgia), temporomandibular joint syndrome, glossodynia and others. These conditions are widely distributed among the population and the pain is often excruciating. Its victims suffer economic loss and severe psychosocial debilitation as well. Yet these conditions remain poorly understood and inadequately treated because research on these two aspects of pain has been grossly neglected. In recognition of this situation, pain control has been identified in the title of a new program which now assumes the responsibility for stimulating investigations into the nature, etiology, pathophysiology and treatment of these major pain problems associated with the oral-facial complex.

The Pain Control and Behavioral Studies Program was established as a new and discrete program when NIDR reorganized its extramural programs in January 1973. Until that time, studies on pain had been part of the now discontinued General Oral Sciences Program, as had behavioral research. The link between behavioral influences and pain has already been mentioned, but it is also clear that psychosocial factors are important in a variety of oral disease states. Therefore, the title of the new program was also intended to give visibility to the potential contributions that behavioral studies can make to the solution of oral health problems in general.

Since the research areas covered by the new program have been so severely neglected, it has been necessary for staff to exert tremendous efforts to initiate the needed research wherever opportunities could be identified. While these extensive programming efforts seem to be proceeding successfully, nevertheless, it must be recognized that actual research accomplishments will only be realized after an appropriate, critical interval of time has elapsed. Therefore, the primary content of this report will deal with programming activities rather than research findings.

This report will summarize the results achieved during the previous year in implementing the objectives of the NIDR pain control program. It will also describe the current status of the program and will outline plans for future activities. In the behavioral studies area, this report will

attempt to develop a framework for action which will focus on the identification of research needs and opportunities in a wide variety of behavioral science areas relevant to all of NIDR's programs.

ADMINISTRATIVE

Apart from the major organizational change mentioned above which provides a new focus and visibility for the effort in pain control and for the new thrust in behavioral studies, other important administrative changes have taken place during the year. Dr. Matthew Kinnard, who served as scientist administrator in the General Oral Sciences Program in 1972 was transferred early in 1973 to assume similar responsibilities in the Mineralization, Salivary Secretions and Nutrition Program. Until recently, Dr. Edward J. Driscoll had devoted a major portion of his time to the pain control program. Since he is now serving as Clinical Director of NIDR, he will be able to devote only a small proportion of his energies to the program. However, his interest and enthusiasm in the NIDR pain initiative remains undiminished and he will continue to make important contributions to its planning and program development. As of this writing, however, the Program Chief, is the only professional devoting full time to this important program. Hopefully, additional staff assistance will soon be forthcoming.

The current level of NIDR extramural support for research in the area of pain control amounts to approximately $626,000. Of this amount, approximately $472,000 supports research in these areas in the Dental Research Institutes, chiefly at the University of North Carolina and the University of Washington. The balance of approximately $154,000 supports seven research grants scattered throughout the country. This low level of overall support reflects the underdeveloped state of these fields in comparison with other programs. Therefore, increasing the level of research support for worthy projects in pain control and the behavioral sciences will be a major objective during the coming year.

The distribution by specific area of interest of the currently-supported research program in pain control and behavioral studies is shown in the following table:

PAIN CONTROL & BEHAVIORAL STUDIES PROGRAM - FY 72

Overall Current Distribution of Extramural Research Support by Area of Interest

Interest	Grants or Projects	Total (1,000's)	% of Total
Local Anesthesia	2	67.2	10.73
General Anesthesia	1	28.1	4.49
Oral-Facial Pain	3	100.3	16.02
Basic Neurophysiology	9	381.4	60.90
Behavioral Science	2	49.1	7.84
Total	17	626.1	

This table demonstrates the level of NIDR-supported research in the important areas of local anesthesia, general anesthesia, oral-facial pain, and in the behavioral sciences generally. These programs will will receive particular attention during FY 73.

Since the General Oral Sciences Program staff were occupied for most of the past year with aspects of all NIDR extramural programs, particularly the training programs, less than optimal attention was devoted to pain control manpower needs specifically. Therefore, an in-depth evaluation of the relatively modest NIDR research effort in pain control will be deferred until the next annual report.

STAFF ACTIVITIES

Because of the newness of the program, much of the past year's staff effort has been devoted to programming activities. At least 15 specific visits to stimulate program-oriented research were conducted, and several visits were made to dental schools with Dr. Kreshover. In addition, staff participated in five meetings in which important questions related to program development were discussed.

Specific Programming Visits:

1. July, 1972, New York University. Discussed a pain research center proposal with Dr. Frederic Leibman and staff.

2. August, 1972, Ohio State University. Discussed progress of NIDR's first training grant in pain control and dental anesthesiology. This

grant was intended to train individuals to assume key research and leadership roles in the development of pain control units in dental schools. Progress was satisfactory, but the decision to eliminate training has prevented the achievement of this program's objectives.

3. September, 1972, University of Iowa Dental School. Discussed a possible training grant in pain control and dental anesthesiology. A formal application for such a program was to be submitted when the decision to phase out training grants was announced.

4. September, 1972, New York University College of Dentistry. Discussed possible training programs with particular emphasis on a combined D.D.S.-Ph.D. program.

5. October, 1972, American Dental Association, Chicago. Discussed planning with ADA staff for NIDR participation in a meeting of the Council on Dental Research of the ADA dealing with pain control and acupuncture.

6. October, 1972, Zoller Institute for Dental Research, Chicago. Discussed mutual research interests and opportunities.

7. November, 1972, U.S.P.H.S. Dental Health Center, San Francisco. Discussed behavioral science research and planning for a symposium on pain control to be held at the Spring, 1973 meeting of the International Association for Dental Research with Dr. L. Luccachini.

8. November, 1972, University of North Carolina Dental Research Institute. Discussed the major pain research activities of the UNC Research Institute and the possibility for developing a major pain research center there.

9. November, 1972, University of Pennsylvania. Discussed anesthesiology research and training with Dr. Harry Wohlman, Chairman of the Department of Anesthesiology, College of Medicine. Encouraging progress was made toward involving the excellent resources of this highly research-oriented department in pain and anesthesiology research of interest to NIDR.

10. December, 1972, Battelle Institute, Columbus, Ohio. Discussed the possibility of developing a basic research program on the nature and effectiveness of electrical anesthesia. Their interest is strong and their resources appear to be unique to carry out such a study.

11. April, 1973, Massachusetts General Hospital. Met with Dr. Richard Kitz, Chairman of the Department of Anesthesiology, to discuss a research program designed to evaluate the effectiveness of acupuncture in dental pain situation. The prospects are encouraging, as this environment appears to be strong for this type of study.

12. April, 1973, Harvard School of Dentistry. Discussed behavioral science research with staff at this institution.

13. April, 1973, College of the Pacific School of Dentistry, San Francisco. Discussed basic biochemical and biophysical research proposal with Drs. S. Eleter and J. Cohen. They propose to study membrane effects of local and general anesthetic agents using nuclear magnetic resonance techniques.

14. April, 1973, San Francisco General Hospital. Met with Dr. Robert Smith, a leading authority on electrical anesthesia, to obtain his views on the current status and future prospects of this area. Problems blocking its use in humans were discussed as were means to resolve these problems.

15. April, 1973, Regional Primate Research Center, Seattle. Visited with Dr. E. Luschei to discuss behavioral research.

Dental School Visits with Dr. S. J. Kreshover and other NIDR Staff:

1. August, 1972, Loma Linda University, Loma Linda, Calif.

2. October, 1972, University of Pittsburgh Dental School, Pittsburgh, Pa.

3. February, 1973, University of Kentucky Dental School, Lexington, Ky.

Meetings and Conferences

1. August, 1972, American Dental Association, Chicago. Participated in the Evaluation Conference of the Dental Research for College Students program. This program is designed to introduce carefully selected, highly qualified college students into dental research and motivate them towards careers in dental research and education.

2. October, 1972, San Francisco, Calif. Annual meeting of the American Dental Association.

3. November, 1972, American Dental Association, Chicago. Participated in the discussions of the ADA Council on Dental Research dealing with pain and acupuncture research. Assisted the ADA in the development of official policy statements dealing with each of these areas.

4. April, 1973, Washington, D. C. Annual meeting of the Association of American Dental Schools and the International Association for Dental Research. A plenary session on pain control was a key part of the program. Dr. Driscoll was a featured speaker at this session.

5. May, 1973, International Pain Symposium, Seattle, Washington. The Pain Control and Behavioral Studies Program was instrumental in developing a major International Pain Symposium held under the auspices of the University of Washington School of Medicine in May 1973. This symposium was particularly timely; pain research is now at a critical stage. New

theories of pain perception and its transmission are in need of evaluation, and a more adequate basis for the evaluation of acupuncture in pain relief needs to be developed. In addition, there is a need for more knowledge of recently-introduced psychosedative and amnesic agents. To discuss these problems, the symposium brought together most of the leading pain investigators from all over the world to share their research findings and to develop new leads for future endeavors in basic and applied areas.

RESEARCH HIGHLIGHTS

Because there has been little time yet for program development, no extensive research findings are available at this time. Nevertheless, reports from two research efforts can be cited as examples. The findings of these interesting studies are briefly outlined in the following paragraphs.

During the past year, progress in improving treatment of one type of facial pain syndrome, called the myo-facial pain dysfunction syndrome (MPD) was reported by NIDR supported investigators at the University of Illinois. This condition is characterized by severe facial pain often accompanied by jaw clicking and limited jaw movement, when no demonstrable pathology exists. The investigators have tested their theory that the pain is caused by habitual tooth grinding and clenching in response to stress. This clenching can produce painful muscle spasms. Laboratory tests have shown that MPD patients respond to physical and emotional stress by clenching their teeth whereas non MPD patients respond in other ways, with increased heartbeat, for example. In treating MPD patients the investigators found that many are helped by muscle-relaxing and tranquilizing drugs as well as by placebos. All treatments are more effective when the doctor provided reassurance and genuine interest in the patient. Even with the use of splints, some of which were designed to be non-functional (no alteration of occlusal patterns) the benefits were far greater when reassurance and psychological support were provided. These findings suggest that the psychological factors, particularly the quality of the doctor-patient relationship, are of far greater importance in treating MPD patients than was previously believed.

The physiological and pharmacologic responses of hospitalized patients receiving general anesthesia have been comprehensibly documented, but the response of ambulatory subjects on general anesthesia in the dental office have only begun to be examined. In a recent study investigators at the University of Washington, Seattle, examined the effect of different postural positions of cardiovascular, respiratory, and electroencephalographic responses in control individuals and in those receiving an intravenous anesthetic agent. The findings indicated that sitting upright is the least desirable position, while semi-reclining at a 45 angle is the most desirable position for this type of anesthesia.

FUTURE PLANS

Plans for increasing the level of research support for the pain control and behavioral science areas have been developed and are already being implemented. These plans are consistent with the recommendations of the NIDR Ad Hoc Advisory Committee on Pain Control which were convened in 1971 and 1972.

Pain Control - in the pain control area, the following initiatives are now being undertaken or will be developed during FY 73:

1. The research leads and opportunities identified during the International Symposium on Pain discussed above will be carefully evaluated for potential significance to NIDR's program and will be followed up by programming visits for possible future research.

2. A state-of-the-art workshop focusing specifically on oral-facial pain problems will be convened in the fall of 1973 to obtain additional information and opinion on key research needs and opportunities.

3. Because of the highly complex multidisciplinary and interdisciplinary nature of the overall pain problem, including both its objective and subjective components, efforts will be made to foster the development of oral-facial pain research centers in which pain problems can be studied at both the basic and clinical levels.

4. Efforts will be made to achieve a healthy balance in support between basic and applied pain research. Although there are great difficulties in developing a strong clinical pain research program, it must nevertheless be recognized that solutions to human pain problems are far more likely to emerge from human pain research than from animal pain research. Accordingly, emphasis will be given to the development of clinical pain research progams wherever possible.

5. Contacts will be maintained with all of the Dental Research Institutes to further their understanding of NIDR's pain control initiative and to encourage them to make maximum use of their resources.

6. At the University of Washington in Seattle, where a strong pain research center now exists, programming efforts will continue to explore the possibility of developing a program project focusing on oral-facial pain. A pain research program has already been developed as part of the existing Dental Research Institute.

7. At Massachusetts General Hospital, Boston, discussions are underway with the Department of Anesthesiology to stimulate an application to evaluate acupuncture in the treatment of dental pain. This institution appears to have unique strengths for such a study. At present only one acupuncture research project is being supported by all of the NIH.

8. At the University of Pennsylvania and at Philadelphia General Hospital, possibilities for a project to study the underlying causes of a variety of oral-facial pain syndromes are being explored. Such a project should lead to improved treatment. In addition, as a result of NIDR initative, plans are being developed to establish a pain center at the University of Pennsylvania. Finally, the Department of Anesthesiology of the University of Pennsylvania, one of the strong research anesthesiology departments in the U.S., is developing plans for studying dental and ambulatory anesthesia.

9. At the Battelle Research Institute in Columbus, Ohio, preliminary discussions are underway which may lead to a uniquely important study in some of the basic aspects of electrical analgesia and anesthesia. This physical modality has great promise but requires additional technical information not yet available, as well as more basic physiological and neurophysiological data, before clinical application can be attempted. Because of the nature of the needed information, the contract approach will be explored.

10. Close cooperation has been established with the leaders of NIDR's intramural program in the neurophysiological aspects of pain and other sensory mechanisms. It is anticipated that this on-going program may be extended to include a clinical component so that the framework for an overall NIDR intramural pain research center may eventually emerge.

11. Taste is another sensory response which falls within the general mission of NIDR and the Pain Control and Behavioral Studies Program. A small number of research projects in the taste area are now being supported by NIDR and it is anticipated that this support will be continued. Evidence exists for a relationship between clinical taste and pain problems, and efforts will be made to clarify the nature of this relationship.

Behavioral Studies - In the newly initiated Behavioral Studies Program area the following approaches will be utilized to begin a serious effort to involve NIDR more effectively:

1. Expert advice will be obtained from knowledgeable behavioral scientists familiar with both the dental significance of behavioral approaches as well as with opportunities for improvement in the current state-of-the-art relating to psychosocial information on the solution of dental problems.

2. It is anticipated that, following assessment of the information obtained from consultants of the type described above, planning will proceed, leading to the convening of a workshop of key behavioral scientists who will identify specific research needs and opportunities. It is expected that they will recommend studies aimed at improving our understanding of behaviorally related dental problems, including pain, craniofacial anomalies and the preventive aspects of caries and periodontal disease.

3. Along these lines the Pain Control and Behavioral Studies program will work with and coordinate the behavioral aspects of all of NIDR's extramural program areas to provide assistance in project development and evaluation.

DENTAL RESEARCH INSTITUTES AND CENTERS PROGRAM

The Dental Research Institutes and Centers Program consists of the comprehensive and multidisciplinary research activities of the five institutes and centers at the Universities of Alabama, Michigan, North Carolina, Pennsylvania, and Washington. The present program, which was initiated with a budget of $3 million 6 years ago, is now funded at $6.5 million with each of the five centers funded at above $1 million for the current year. This year the institutes and centers program was administratively transferred from the NIDR Director's Office to the NIDR Extramural Programs to facilitate and stimulate more involvement of the Extramural staff with the research programs of the institutes. In view of this increasing coordination of research by NIDR staff, it was decided to include the research highlights from the institutes and centers in the individual reports of the appropriate categorical program areas. The remaining aspects of the institutes and centers program are treated in this report.

Important personnel considerations include the recent appointment of a new director at the University of Michigan, the departure of the director from the institute at North Carolina and the impending retirement of the director at Pennsylvania. Searches are now being made to fill the directorships at the latter two universities.

The Dental Research Institutes and Centers Program was initiated as an experimental approach to the problem of how to accelerate the development of the knowledge needed to solve the problems of dental and oral diseases. The underlying idea of the program was to plan and develop institutes or centers which would bring together all of the appropriate resources of the parent universities and focus these resources upon the problems of oral health in an ideal research and training environment. It was expected that the institutes and centers would create a sufficiently stimulating atmosphere to attract investigators and students from disciplines not generally associated with dental research.

The five institute models have been under development for the past six years. During this period a considerable amount of effort on the part of NIDR staff and the Dental Research Institutes and Special Programs Advisory Committee (DRISPAC) has been devoted to developing and refining guidelines for these new programs. Attempting to promote understanding of the institute concept at all levels within the universities has been a major challenge, and the most difficult part of this challenge has been to communicate the idea that these grants should be university based rather than dental school based. The designation of goals, selection of personnel, development of quality control procedures, the evaluation of policy, and the establishment of decision-making apparatus has inevitably brought out problems unique to each university. In many

instances, however, great progress has been made in solving these problems through the annual staff project site visits, the thorough comprehensive annual evaluation by DRISPAC with their feedback reports to the universities themselves, and through the frequent and extensive discussions between NIDR staff, DRISPAC members and representatives of the universities. These continuing evaluations have led to desirable modifications in the programs of all of the institutes. Thus, the emphasis so far has been placed on strengthening the five institutes already in existence with relatively little attention being paid to the planning of additional institutes. It is hoped, however, that in the future, increased budget allowances will enable NIDR to increase the number of institutes to ten.

Based upon annual peer reviews of the centers and NIDR staff impressions through close interaction with the staff of the programs, the centers appear to be successful in meeting their goals to varying degrees. They have indeed attracted scientists who were not previously involved in dental research. Among these are investigators with expertise in disciplines such as biochemistry, X-ray crystallography, microbiology, immunology, virology, immunogenetics, polymer chemistry, engineering and others. As a result of these new infusions of scientific talent, the quality of research seems to be improving steadily. For example, a very strong program in connective tissue biochemistry is evolving in the institute at the University of Alabama. The institute's utilization of research associates is proving to be an important source of future scientific manpower.

As their programs undergo maturation, the institutes are becoming more mission oriented. The University of Washington is now primarily concerned with problems related to periodontal disease; the University of North Carolina is increasing emphasis on pain control, in addition to growth and development; the University of Pennsylvania is focused mainly on periodontal and soft tissue diseases; the University of Alabama is concentrating on caries and periodontal disease; and the University of Michigan is in the process of reorganizing its research programs to provide mission orientation in several directions.

In November 1972 a one-day meeting was held at NIH to discuss ways to promote more interaction between the institutes and dental schools at universities not having institutes. Participants included the directors of the five institutes and the dental deans from their universities, a cross-section of other dental deans, representatives of the American Association of Dental Schools and NIDR staff. The group felt that collaborative efforts should be encouraged wherever resources and interest would permit, and that a first step towards this goal would be to communicate more information about the institutes to all dental institutions. It was agreed, however, that the anticipated collaboration might be limited unless funds for this purpose were found.

Associated with these large and complex programs are many intangibles which need to be examined in special ways in order to develop an understanding of their total impact. Planning programs of this nature in

the future demands that evaluation processes more comprehensive than the regular peer review be established and conducted. In two meetings held with key NIDR staff and one consultant intimately familiar with the objectives and the history of this program, the rudiments of an encompassing evaluation method have begun to emerge. Through a series of such meetings, we expect to specify important questions for which answers are needed and thereby provide a basis for making sound judgments about the program. We will then seek whatever help is necessary to answer these questions once they have been formulated.

SPECIAL TRAINING GRANTS

Four special training programs all designed to train clinical investigators, are assigned to the Dental Research Institutes and Centers Program for administration. Three are "dual degree" programs, and the fourth is a combination of "dual degree" and post-D.D.S. training. During the phase-out of these grants, trainees will receive support for varying periods, in order to complete their training. NIDR staff will maintain close contact with the program directors and with the trainees to assure that high quality training continues to be provided throughout the phase-out.

Report of the Computer Processing and Analysis Section*
National Institute of Dental Research
Summary Statement

Background

This section provides a centralized focus for the design, development and implementation of an on-line computer system which serves a broad spectrum of research needs throughout the Institute's direct operating programs. Functionally, the section seeks to improve various intramural research efforts through the use of computer technology. Moreover, it provides the setting, both technical and intellectual, in which the investigator can expand the horizons of his research, ultimately permitting him to attempt experiments or pose questions which heretofore had not been amenable to exploration. The ability of the machine to perform simple repetitive operations at microsecond speed can serve substantially to complement the intuition, knowledge, and judgement of each researcher. Together, this man-machine synergism offers the prospect of significantly enhancing both the breadth and the efficiency of research at NIDR.

With the assistance of hardware and software specialists from the Division of Computer Research and Technology at NIH, the NIDR Computer system has been expanded to include the functions described below.

Progress During the Past Year

Applications

During the past year an X-ray diffractometer was interfaced to the system and regular processing of acquired diffractometer data is being performed. The bacteria monitoring and control experiment was expanded to include a fluoride electrode. Computer control experiments were conducted which monitored pH and fluoride ion levels. Programs were written or improved to further support on going, on-line applications in neurophysiology, amino acid analysis, scintillation counting, spectrophotometry and gas chromatography.

*This report is based upon progress achieved during the year in project numbers NIDR-DIR001a68 and NIDR-DIR002a72.

System

The dual processor (H516 - H316) system was further improved by several software adjustments. The design goals established for this approach--increased core storage, reliability and flexibility--were fully realized. In February 73, a fixed head disc was added to the system. Software to support this peripheral, which will significantly improve the response time of the system, is being implmented. An interactive graphics package was implemented.

Plans for the Coming Year

Further expansion of the closed-loop bacteria control experiments is projected. The system will also be interactively involved with neurophysiological-behavioral studies of primates.

Report of the Environmental Mechanisms Section*
National Institute of Dental Research
Summary Statement

Background

The Environmental Mechanisms Section has the mission of investigating the interactions among cells and the interactions of cells with their environment. Interest has grown in characterizing physiological and biochemical adaptations of specific cells to their particular ecological situation. Considering the basic mission of the NIDR, the area appropriate for study by this section is the relationship between bacteria and the oral cavity. Specific studies have taken three main directions, namely: 1) the investigation of the metabolism and growth of oral streptococci 2) the development of a computer controlled system to simulate the oral environment, and 3) research on cellular slime molds in an attempt to understand the biochemical events accompanying aggregation and differentiation as an environmental response.

The first area of concentration has been that of studying the environmental role of various simple sugars and complex carbohydrates in the growth processes of cariogenic streptococci, especially Streptococcus mutans. Such information should contribute directly to the development of a system for physically simulating the natural environment of such organisms namely, the mouth.

The second area of research is an extension of the study of cariogenic streptococci, but incorporates modern computer technology. It is clear that many of the microbially mediated phenomena which take place in the mouth are the result of highly complex, multiparametric interactions. Accordingly, an on-line computer system is being interfaced to control a fermentor in the laboratory in order to simulate oral conditions; in a sense comprising an "artificial mouth". By this technique, simultaneous multiparameter changes may be affected and multi-variate responses noted. In a related effort, computer techniques are being developed to permit a uniform coding system for describing bacteria. This system will become the foundation of an vitally important international microbial data bank.

*This report is based upon progress achieved during the year in projects number NIDR-DIR101b68, NIDR-DIR102b72, NIDR-DIR103a70, NIDR-DIR104a71, NIDR-DIR105b71, NIDR-DIR106b71, NIDR-DIR107b71, NIDR-DIR108a71, NIDR-DIR109a72.

The third area of research has been that of studying the biochemical and environmental control of differentiation in the cellular slime mold, or social amoeba, Dictyostelium discoideum. Of particular relevance is that a number of cellular properties of D. discoideum are strikingly similar to those noted during certain stages of differentiation in mammalian species.

Progress This Year

Growth and Metabolism of Oral Bacteria

When grown in the presence of sucrose, S. mutans forms extracellular glucans and fructans. One of the characteristics of Streptococcus mutans is the formation of elevated colonies on Mitis-Salivarius agar. It is generally believed that polysaccharide production from sucrose contributes to this special colonial morphology. Certain S. mutans strains produce, in addition, a clear exudate which can be seen either as a bubble on top of the colony or as a puddle around the colony. The combination of colony appearance and exiadate is used diagnostically in epidemiological studies linking S. mutans with the formation of carious lesions. Our present aim is to analyze this exudate. Preliminary results indicate that the major constituent of the exudate is a polyglucan. Minor amounts of sucrose, glucose, fructose and lactic acid have also been detected.

An important consideration in the impact of the micro-environment on these cocci is the study of the various enzymatic systems responsible for the initial attack on the sucrose molecule. Presumably, all the mechanisms have the cleavage of the glycosidic bond of the sucrose in common, irrespective of the nature of the products resulting from that cleavage. Thus, we are beginning the purification of polysaccharide-producing enzymes. The invertase activities which are responsible for about 90-95% of the cleavage of sucrose in growing or non-growing cultures are also being isolated for further characterization.

The Use of Computer Technology

The second area of investigation, carried out with the close cooperation of the Computer Processing and Analysis Section, is related to the first in that it involves the methods of using computers to study the oral bacteria. Many innovative continuous monitoring transducers are necessary to fully implement a control system. For example, in order to measure turbidity in microbial cultures with an on-line computer, an autoclavable turbidity probe has been built to measure bacterial growth in situ. It has been tested for long- and short-term stability. The probe has also been used to monitor the growth of Streptococcus mutans. It performed well over a wide range of bacterial concentrations (comparable range of absorbancy measurements at 600 nm is 0.01-10). Mathematical functions to convert the output, as recorded by the computer, in absorbancy units have been evaluated and a cubic polynomial choosen for preliminary calibration purpose.

As a result of the encouraging results obtained with the prototype, four new light probes with different design parameters are being built.

The fermentor-computer system has been expanded and tested. Turbidity and fluoride ion activity data are now also collected in real-time, in addition to pH, acid addition and base addition. Consequently, the data acquisition and control program was updated to account for these changes. The program

was modified to allow the user to shift the pH up or down at a specified rate. Both hardware and software were extensively tested and performed to specification. The actual pH control observed was within ± 0.02 of the setpoint during the entire course of a fermentation or during pH shift experiments.

The kinetics of growth and acid production by Streptococcus mutans in glucose, sucrose, or fructose-containing media have been measured. Data were acquired in real-time by the digital computer system described above. Afterwards, a PDP-10 computer and MLAB (a powerful modeling program with visual display capabilities) were used to evaluate various mathematical models to describe the results. A simple differential equation was found to accurately represent the acid production data during logarithmic growth. The developed model also allows the calculation of the specific growth rate from the acid production data. The results obtained agreed with these obtained by conventional methods.

Presently transient states, such as those occurring after a downward pH shift or a sodium fluoride addition, are being studied. The effects of a fluoride addition on the growth and acid production rate of the cariogenic streptococci are more pronounced at lower pH. For instance, at pH 6.0 a significant decrease occurs with 40 ppm F, while at pH 5.5 a similar decrease is observed with 20 ppm. These pH values and shifts are quite analogous to those reports in plaque on the surface of the teeth. However, the fluoride ion concentration is much higher than normal found in plaque on teeth even in fluoride treated populations. These very preliminary results cast doubt on the hypothesis that fluoride leaches out of the tooth, inhibits the plague bacteria and thus inhibit caries formation.

Under the operating system of the NIDR computer, procedures were developed to acquire data from the gas chromatograph and calculate areas under the curves. This system now operates in conjunction with the central PDP-10 using an interactive graphics package.

The Gilford spectrophotometer was interfaced with the same computer. A complex set of modular programs is in various stages of development for the multiplicity of uses possible with this instrument, including enzyme kinetics, end-point assays, scanning densitometry, spectral analysis, etc.

In depth studies of the ecological distribution and epidemiology of oral microorganisms require the handling of microbial strain data by large scale computers. Definition and standardization of the questions that are asked about microbial strains in a computer-compatible form is the first goal. Programs have been developed to enter data and retrieve it in a variety of ways 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. Already agreeing to cooperate are the University of Queensland, Australia; the American Type Culture Collection, the Food and Drug Administration, United States; the National Collection of Type Cultures, England; and the World Federation of Culture Collections, International Association of Microbiological Societies.

A data file of primary data on a large number of bacteria found in the oral cavity and related types is being established. This file will provide a resource for asking both ecological and epidemiological questions of interest in dental research.

Aggregation and Differentiation in Dictyostelium discoideum

The transition from single amoebae to a multicellular organism such as occurs in the differentiation of the cellular slime mold, D. discoideum, provides an elegantly discrete model for studying the control of chemotaxis, the process by which individual cells aggregate toward a common center. Cyclic AMP has been implicated as the agent responsible for chemotaxis in D. discoideum.

Recently, we have been studying adenyl cyclase which forms cyclic AMP. It was found to be stimulated by thiamin pyrophosphate. Purification and further characterization of this controlling enzyme will be continued.

A new metabolite arising from cyclic AMP has been detected. Its identity and physiological function are being studied.

We have been able to grow five strains Dictyostelium discoideum in axenic liquid culture. Also, these strains can be grown on agar surfaces, but slowly. Thus, stock cultures of axenically grown strains can be maintained without the short term liquid cultures previously required.

The axenic strain (AX-1) responds chemotactically to cyclic AMP, almost like the wild type NC4 strain. However, the concentration required is both lower and critical. Too much cyclic AMP does not elicit chemotaxis even though the cells differentiate. We found that folic acid causes chemotactic responses at the same concentrations in both strains.

Folic acid, at the proper concentration, inhibited differentiation in the axenic strain without losing its chemotactic properties. The cells retained their motility and normal appearance although they do not differentiate at any time on agar.

Time lapse cinematography of strain NC4 has shown that folic acid and cyclic AMP act at different stages of development. A cell suspension, placed on agar containing both chemotactic agents shows two concentric rings of cells. The outer ring has the same diameter change with time as that with folic acid alone. The inner ring corresponds to that obtained with cyclic AMP alone.

Summary Report of Neural Mechanisms Section*
National Institute of Dental Research

The Neural Mechanisms Section was established to study neural mechanisms of sensation and movement in the oral-facial region. In the last year, the major efforts of the Section have focused on mechanisms of pain and temperature sensation.

Our ultimate aim is to study neuronal events associated with behavioral responses in awake monkeys trained primarily to avoid potentially noxious thermal stimuli. The trigeminal area is particularly well-suited for such studies because the central projection pathways are accessible for chronic electrophysiological recording techniques with minimal surgical intervention. In addition, earlier behavioral studies indicate that a lesion in man or monkey which interrupts connections between the rostral and caudal poles of the trigeminal brain stem nuclear complex, results in decreased facial sensibility to noxious and thermal stimuli. This procedure allows one to study neuronal events in the brain stem in trained animals under two distinct conditions: before and after sensory loss almost completely restricted to the detection and recognition of pain and temperature sensation.

Our approach in the last year has been multidisciplinary and includes the following research activities: (1) neurophysiological studies of the responses of trigeminal nerve fibers and brain stem neurons to noxious and innocuous thermal stimuli applied to the monkey's oral-facial area; (2) neurocytological studies of the organization of the trigeminal brain stem nuclear complex, with particular emphasis on those peripheral and central structures in the "pain" pathway; (3) behavioral studies in monkeys trained to escape noxious thermal stimuli, and (4) psychophysical studies of pain and temperature "thresholds" in man.

Progress During the Last Year

Anatomical Studies

The observation that surgical transection of the spinal trigeminal tract results in a marked loss in the appreciation of thermal and painful sensation has led us to study the morphology of the trigeminal brain stem nuclear complex. The above observation suggests that the integrity of the pathway to the lower end of the spinal trigeminal nucleus (nucleus caudalis) is essential for the transmission of sensory information about pain. During the past year we have considered the following questions. First, do the neurons and synaptic circuitry in nucleus caudalis differ morphologically from that found at more rostral levels of the trigeminal nuclei? Second, does some part of nucleus caudalis receive trigeminal nerve axons which differ morphologically from those entering more rostral parts?

The neurons of the trigeminal nuclei have been divided into two types based on dendritic morphology visualized with the Golgi technique. Class I neurons include those found rostral to nucleus caudalis and in the magnocellular layer of nucleus caudalis. Their dendrites extend for

long distances in the medial and lateral directions, span the entire width of the nucleus, and are oriented in the transverse plane of the brain stem. Their dendrites have relatively few spines. Class II neurons include those found in the marginal and substantia gelatinosa layers of nucleus caudalis. Their dendrites are oriented primarily in the long axis of the brain stem. The dendrites of the substantia gelatinosa layer are confined to that layer and emit numerous fine dendritic spines. The dendrites of marginal and magnocellular neurons also enter the substantia gelatinosa layer. On entering this layer they emit numerous spines, become oriented in the long axis of the brain stem and become intertwined with the dendrite of the substantia gelatinosa neurons. It is this tangle of spiny dendrites derived from the neurons of the three layers of neucleus caudalis which forms the post synaptic component of a second major trigemal circuit.

Electron microscopic studies indicate that, with the exception of the marginal and substantia gelatinosal layers of nucleus caudalis, the axonal endings of the trigeminal nerve sunapse mainly on dendritic shafts of Class I neurons in relatively simple glomeruli. In this Type I glomerulus, a centrally located trigeminal nerve ending synapses on several dendritic shafts and in turn receives an axonaxonic synapse from small axons which originate from intrinsic neurons. On the other hand, in the substantia gelatinosa layer, trigeminal nerve endings synapse on the spine-laden dendrites of Class I and II neurons in more complex glomeruli. This type II glomerulus contains five kinds of neuronal processes which are linked by axodendritic, axoaxonic, and two kinds of dendrodendritic synapses. The trigeminal nerve axon, which is centrally located in the Type II glomerulus differs from the trigeminal nerve ending of the Type I glomerulus. It has an irregular, scalloped outline, is darker than the dendritic elements, and forms axodendritic synapses mainly on dendritic spines of Class I and II neurons.

These studies suggest that the spinal trigeminal tractotomy procedures interrupt a specific component of the sensory root of the trigeminal nerve which innervates the glomeruli of the substantia gelatinosa layer of nucleus caudalis. The integrity of this trigeminal nerve-substantia gelatinosa pathway may be critical for the appreciation of oral-facial pain and temperature sensation.

Physiological Studies

Our approach to the study of pain mechanisms involves the use of noxious heat stimulation to determine the trigeminal peripheral and central neuronal population which is capable of transmitting information about noxious stimuli. For this purpose, a thermal probe was developed to deliver precisely-controlled temperature stimuli. Step temperature changes of $10^{o}C$ per second are possible, and the probe temperature range is 20 to $60^{o}C$, permitting the investigator to deliver rapid, controlled noxious heat stimuli.

The first question we asked was: how do the trigeminal nerve fibers innervating the face and lips of the monkey respond to innocuous and

noxious thermal stimuli? Detailed quantitative analysis of the response properties of these fibers was enhanced by utilization of the NIDR computer system. Neuronal impulse data is stored on magnetic tape in the laboratory and also can be transferred on-line to the disc storage area of the computer. Summary response data is displayed graphically in the laboratory.

Our present data indicates the following types of fibers responsive to noxious thermal stimuli (greater than 45°C) applied to the monkey's face. Cold fibers, whose discharge rate increased to cooling and was suppressed by warming, often responded initially to thermal stimuli above 55°C. Repeated rapid temperature shifts into the noxious range "sensitized" these fibers and lowered their thresholds to below 50°C; the response to rapid cooling steps ultimately was depressed even though consistent, repeatable discharge rates occurred in response to noxious heat. Cold fibers had mostly single-spot receptive fields of less than 300μ in diameter and conduction velocities in the A delta range. Warm fibers whose discharge rate increased to warming, and was suppressed by cooling, sometimes responded to temperatures above 45°C. Repeated noxious stimuli depressed their responses to temperature shifts in the 30 to 40°C range, although spike discharges persisted to stimuli above 40°C. Warm fibers had single spot receptive fields (300μ in diameter) and conduction velocities in the A delta and C fiber range. A few fibers similar in most respects to warm fibers responded to thermal stimuli only above 45°C. High-threshold mechanoreceptors, responsive to pinprick stimulation, had conduction velocities in the A delta range, and sometimes responded to thermal stimuli above 50°C. Many larger A beta fibers exhibited low threshold, slowly-adapting responses to single hair movement which were suppressed by temperatures above 45°C. In summary, noxious heat stimulation increased the activity of most small myelinated (A delta fibers) and unmyelinated (C fibers) afferent fibers innervating the oral-facial area of the monkey. The activity of many larger myelinated (A beta fibers) fibers was suppressed. These reciprocal events may play an important role in the recognition of pain sensation produced by noxious heat stimulation.

The second question concerns the response properties of brain stem neurons to similar stimuli. To what part of the trigeminal brain-stem nuclear complex do the above small fiber populations responsive to noxious heat stimulation project? How do these responses compare with those of the incoming fibers? Our preliminary data in anesthetized monkeys suggests that noxious heat activates neurons located primarily in the caudal part of the trigeminal brain-stem nuclear complex and that low-threshold mechanical stimuli, innocuous thermal stimuli, and noxious input converge on the same neurons.

Behavioral Studies

Facial pain and temperature "thresholds" are being investigated in monkeys by operant conditioning and psychophysical procedures. In recognition and detection paradigms, the temperature step must be detected, as present, or recognized as warm or cold, to receive reinforcement. Each paradigm is combined with large temperature shifts into the noxious heat range which the monkey can abort, but which lack the opportunity for reinforcement.

These experiments should provide information about temperature and pain "thresholds". Since different paradigms are used, the independence of the threshold measurements and the testing methods can be evaluated. Similar threshold measurements are performed on humans to determine whether the discriminatory capabilities of monkey to noxious and innocuous thermal stimuli are comparable to humans.

Future plans

Our ultimate aim is to study those central neuronal events associated with pain sensations and reactions in awake monkeys performing the sensory discriminations discussed above. Neuronal activity, before and after trigeminal tractotomy operations, will be compared.

Future anatomical studies will be directed towards correlating the dendritic elements of the substantia gelatinosal layer with the neuronal cell types of the three layers of nucleus caudalis to more precisely define this neural circuit.

*This report is based upon progress achieved during the year in project numbers NIDR-DIR201b66, NIDR-DIR202b70, NIDR-DIR203b65, NIDR-DIR204a69, NIDRDIR205b72, and NIDR-DIR206a72.

Summary Report of the Experimental Pathology Branch
National Institute of Dental Research

The Experimental Pathology Branch was established in 1969, under the leadership of Dr. Harold M. Fullmer, then Chief of the Histochemistry Section in the Institute's Laboratory of Histology and Pathology. The functional charge to the new Branch was that of generating and correlating basic and clinical research information relating to oral pathoses, particularly periodontal disease and diseases of the oral mucosa. As initially constituted, the Branch was comprised of 23 professional and supporting staff whose competence lay for the most part in the areas of experimental and clinical pathology.

Working individually and collaboratively during the subsequent several years, the members of the Branch succeeded to a considerable extent in meeting the difficult challenge placed before them, and by so doing also established a number of new collaborative links between the clinical and laboratory endeavors of the Institute.

More recently there has seen a serious diminution of the Branch's capacity to fulfill its functional role, a situation reflecting not on the ability or quality of individuals within the Branch, but rather on the continued loss of key personnel, including Dr. Fullmer, occurring during a prolonged period of government austerity wherein staff recruitment was not possible.

That the Branch continued to be contributive, however, is clearly indicated by the sectional summary reports which follow these paragraphs. On the other hand, the viability of the Branch very clearly became a matter of concern as its manpower resources became more and more limited, for a Branch or a Laboratory, just as a total Institute requires a minimal critical mass to perform its role effectively.

Accordingly, midway through the current reporting year, it was concluded that the interests of the Institute would best be served by abolishing the Branch and consolidating the efforts of its remaining staff into the activities of a new laboratory unit, the Laboratory of Oral Medicine. This Laboratory, with a research mission in the general area of oral soft tissue diseases, and encompassing a variety of professional competence drawn from other segments of the intramural program, including virology, clinical medicine and clinical dentistry, seemed a logical setting for the research input of the experimental pathology staff.

This programmatic decision was implemented sufficiently late in the reporting year to warrant issuing the sectional summary reports for the last time as though coming from the Experimental Pathology Branch. At the time of this writing, however, the new Laboratory of Oral Medicine has already achieved notable progress in assimilating the professional and supporting staff of the Branch.

Summary Report of Diagnostic Pathology Section
Experimental Pathology Branch
National Institute of Dental Research

The primary function of this Section is to perform service and research activities relative to the diagnosis and study of oral pathologic processes in human beings. Created in June, 1969, the Section is concerned with recording, processing, and appropriate documentation of human oral tissues submitted for pathologic examination from intramural and extramural Public Health Service sources, and the use of this material for research, clinicopathologic correlations, longitudinal study of oral diseases and the application of established techniques, such as histochemistry, electron microscopy, immunofluorescent procedures, etc., to these tissues.

The Diagnostic Pathology Section provides histopathologic service and clinical consultation in support of clinical activities in the Dental Services Branch of the NIDR. A total of 987 accessions were processed in calendar year 1972. Of these, 763 represented human source material while 224 constituted animal research accessions. Almost 14,000 sections were prepared on this material, and a variety of histochemical and immunofluorescent procedures performed on many of the sections. Tissue examination reports (S.F. 515) were prepared on the human material and accessioned as well in the Laboratory of Pathology, NCI, which acts as a central repository for pathology specimens at the Clinical Center.

The primary emphasis of the Section is the development of data correlating clinically-observed changes with the microscopic observations in various oral tissues. Those areas of current clinicopathologic interest include studies of oral mucosal diseases, the dentition in acquired and inherited metabolic diseases, and disorders involving minor salivary glands. In collaboration with the Institute's Histochemistry Section, clinicopathologic studies of human oral mucosal diseases have employed histochemical approaches to further characterize some of the changes observed in certain clinically unique oral disorders.

Correlative clinicopathologic and histochemical studies have provided valuable data on mucosal changes in environmentally induced disorders. Changes in the superficial epithelium of chronically inflamed oral mucosa commonly associated with chronic denture irritation have been identified histochemically as being rich in sulfhydryl groups and representing a degenerative epithelial alteration. These observations warrant further investigations of the local oral environmental factors as well as possible allergic effects by proprietary agents. Histochemical studies of subepithelial deposits associated with "snuff-dipper's keratosis" has revealed that these represent an unusual glycoprotein, and no amyloid as previously reported. Their occurrence in association with a particular type of snuff

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This report is based upon progress achieved during the year in Project Nos. NIDR-EPB003a63, NIDR-EPB004a66 and NIDR-EPB005a70.

("Copenhagen" brand of U.S. Tobacco Co.) further emphasized the need for clinicopathologic investigations, since "snuff-dipper's keratosis" is known to predispose to "snuff-dipper's carcinoma."

Clinical studies of deciduous and permanent teeth showing alterations associated with metabolic diseases have been pursued; however, access to histologic materials has proved to be a problem because of the limited availability of teeth for study. Permanent teeth generally are not available unless removed for periodontal disease, caries, impaction, etc., while deciduous teeth constitute a more readily accessible reservoir. Teeth from patients with various metabolic disorders, especially those patients under study at the Clinical Center, are considered most desirable. Of particular interest are teeth from patients with hereditary hypophosphatemia, hypophosphatasia, pseudohypoparathyroidism, and infantile hypercalcemia.

The clinical and morphologic studies of disorders of minor salivary glands have centered chiefly on histologic changes seen in Sjögren's syndrome and reflect a particularly successful collaborative research effort between investigators from NIDR and those affiliated with the Arthritis and Rheumatism Branch of the National Institute of Arthritis and Metabolic Diseases. The minor salivary gland biopsy has been clearly shown to be an essential parameter in the diagnostic evaluation and therapeutic follow-up of these patients, and is being pursued further for its ability in such disorders as lupus erythematosus, sarcoidosis, and other connective tissue diseases. Application of immunofluorescent procedures to salivary gland tissue using sera from patients with Sjögren's syndrome has indicated the presence of antigenic sites in the ductal-periductal region. This technique was correlated with autoradiographic procedures performed by Dr. Norman Cummings of the Oral Medicine and Surgery Branch of NIDR, demonstrating the specificity and stability of the isolated and radiolabeled gamma globulin fraction from the serum of a patient with Sjögren's syndrome.

Future plans center on the continued development of data correlating clinical and morphologic changes and the utilization of research techniques on these tissues. The purpose is to further our understanding of pathogenetic mechanisms of human oral mucosal diseases, dental alterations in metabolic disorders, and morphofunctional changes in salivary gland diseases. Several areas that need to be further developed include genetic and epidemiologic data as it relates particularly to the oral mucosal and dentitional studies. Continuation of the salivary gland studies and the collaborative efforts with the Arthritis and Rheumatism Branch and intramural (NIDR) groups are necessary for further elucidation of the anti-salivary duct antigen.

Although there are many opportunities to collaborate with clinical as well as experimental investigations both intramurally and extramurally, the service activities of the Section necessitate only selective support for these studies. The Sectional activities will continue to provide consultative and professional liaison with the Laboratory of Pathology of the National Cancer Institute, as well as formal association and liaison with the Armed Forces Institute of Pathology, the U.S. Naval Dental School, The Army Institute of Dental Research, Georgetown and Howard University Dental Schools and the Smithsonian Institution.

Summary Report of the Histochemistry Section
Experimental Pathology Branch
National Institute of Dental Research

Histochemistry evolved from the biologists', anatomists', histologists', and pathologists' attempt to relate substance to structure and function, and while it is classically envisioned as a morphological tool, the working definition of histochemistry employed in this Section includes a wide range of methodology in the study of the qualitative and quantitative chemistry of tissues. Histochemical, biochemical and cell and tissue culture studies have evolved from several lines of inquiry with the intent of further understanding the physiologic and pathologic processes occurring in oral tissues. While the emphasis has been placed on periodontal disease, the results of these studies have had a much broader range of applicability. It has been demonstrated that the histochemical approach, when employed in proper relation to other disciplines, provides a unique view of biological processes.

In keeping with the multidisciplinary approach of this section, progress during the year and its significance will be considered under four separate headings.

1. Biosynthesis and degradation of acid mucopolysaccharides

Continuing studies of the biosynthesis of acid mucopolysaccharides have demonstrated the existence of a biosynthetic precursor of hyaluronic acid. Studies carried out in tissue culture have shown intracellular hyaluronic acid to have a considerably higher molecular weight than that found external to the cell in the medium.

2. Histochemical study of dihydroorotic dehydrogenase in oral tissues

Previous work in this laboratory and others indicated a correlation between increased staining for dihydroorotic dehydrogenase and increased requirement for nucleic acid synthesis in certain cells. This correlation has been strengthened by the finding that increased staining for dehydroorotic dehydrogenase occurs in isoproterenol stimulated salivary glands.

3. Folic acid deficiency studies

A collaborative pilot study (Dr. H. Spencer, Metabolic Section, Hines V.A. Hospital, Hines, Illinois) on the induction of a folic acid deficiency in man has been completed. Following a starvation phase of about 50 days several obese patients were refed with a folic acid deficient diet. Serum folate levels tapered off during the starvation phase, but no deficiency state was induced. On refeeding, however, a precipitous drop in serum folate was noted. Before and during starvation and during refeeding oral cells were collected and are now undergoing cytoanalysis.

This report is based upon progress achieved during the year in Project Nos. NIDR-EPB001b64 and NIDR-EPB002b66.

4. Human Dental Plaque Studies

Continuing studies of the enzyme histochemistry of human dental plaque made possible by the development of a technique for cutting fresh frozen sections of plaque collected on mylar strips have revealed the existence of considerable activities of succinic, lactic, malic, isocitric, glutamic, and glycerophosphate, β-hydroxybutyric, 6-phosphoglucomic and glucose-6-phosphate dehydrogenases. The results emphasized the importance of both glycolysis-citric acid cycle and the hexose monophosphate pathways of carbohydrate metabolism and that clycolysis, the citric acid cycle, the hexose monophosphate short and fatty acid metabolism are active components in the metabolic activity of dental plaque as a whole. The considerable variation in enzyme activities observed indicate large differences in metabolic activity on different teeth, on different surfaces of the same tooth and even within the same plaque.

During the coming year, histochemical, biochemical, and cell and tissue culture investigations will continue. The areas of specific interest will be the metabolic analysis of normal and disease tissues as revealed by qualitative and quantitative enzyme and end-product histochemistry.

An interagency agreement is being negotiated to provide support for a collaborative project with Dr. H. Spencer, Hines V.A. Hospital, Hines, Illinois, using human volunteers on a closely controlled folic acid study, and a contract has been made with Litton Bionetics for the electron microscopic localization of certain hydrolytic enzymes in the periodontal ligament of the rat.

Summary Report of the Oncology Section
Experimental Pathology Branch
National Institute of Dental Research

Oral Cancer - Oral Premalignant Conditions

The Section of Oncology has approached its main research interest - oral cancer - from several avenues. First, the most productive of these in the clinical sense has been the seven year P.L. 480 funded epidemiological oral cancer-oral premalignant lesion study in rural Indian villages in 5 states. The extensive scientific findings from the first six years work have been reported in book form, co-authored by Drs. Mehta, Pindborg, and Hamner (*note reference). Work continues in India on the 10-year follow-up of cases.

The second chief endeavor has been in the field of histopathology. The Section of Oncology was designated as a Collecting Center for the W.H.O.'s International Reference Center in Copenhagen to participate in a collaborative, international effort to study oral premalignant epithelial lesions and arrive at a common terminology. The Oncology Section has contributed cases and continues to participate in this mutual endeavor. Dr. Hamner participated in a conference of all the Chiefs of W.H.O. Collecting Centers held in Copenhagen, Denmark in May 1973. Similarly from the histopathological viewpoint, the Section continues to collect data on fibro-osseous jaw tumors, which will also be tabulated in book form.

The third effort lies in basic research on betel quid chemical carcinogenesis supported under N.I.D.R. contract funding at the Southwest Foundation for Research & Education in San Antonio, Texas. Gross lesions, demonstrating marked epithelial atypia, have been produced in the baboon cheek pouch mucosa after 48 months of exposure to betel leaf, areca nut, lime and tobacco. This basic research study coincides with the clinical findings in our Indian project patients. This study is described in greater detail in the contract's annual renewal report (PH-43-67-1475).

The Indian P.L. 480 project (#01-022-1) has been continued for an additional three years April, 1972-1975. It is hoped to complete the book on fibro-osseous lesions during 1973-74. The betel quid carcinogenesis study in baboons will be continued for another year, half of the animals with gross lesions being removed from treatment to note if their lesions regress, progress, or remain the same.

Implant Biomaterials Research

These investigations have been conducted as direct contracted research with the Southwest Foundation for Research and Education. The detailed reports for this contract have been channeled through the Science Information

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This report is based upon progress achieved during the year in Project No. NIDR-EPB006c66.

Exchange. In brief Summation, the artificial tooth implantation studies have encompassed ceramic, plastic, carbon, and metal bladevent replica implantation in the jaws of baboons. Clinical, radiographic, and histopathologic evaluations have been made in regard to each of these replacement biomaterials. Details are described in greater context in the annual contract report (PH-43-67-1476).

In the forthcoming year research under this contract will continue to focus on metal and carbon bladevent insertion into sub-human primate jaws and the use of calcium aluminate ceramic for periodontal osseous defect obliteration.

References

1. MEHTA, F. S., PINDBORG, J. J., and HAMNER, J. E., III: Oral Cancer and Pre-Cancerous Conditions in India, (Munksgaard: Copenhagen, Denmark, 1971).

2. HAMNER, J. E., III, MEHTA, F. S., PINDBORG, J. J., and DAFTARY, D. K.: "An Epidemiologic and Histopathologic Study of Leukoedema Among 50,915 Rural Indian Villagers", O. Surg. 31:58-65, 1971.

3. HAMNER, J. E., III, MEHTA, F. S., PINDBORG, J. J., and Daftary, D. K.: "The Altered Staining Reaction of Connective Tissues in 53 Submucous Fibrosis Patients", J. Dent. Res. 50:388-392, 1971.

4. HAMNER, J. E., III and REED, O. M.: "Betel Quid Carcinogenesis in the Baboon", J. Med. Prim. 1:75-85, 1972.

5. HAMNER, J. E., III: "Betel Quid Inducement of Epithelial Atypia in the Buccal Mucosa of Baboons", Cancer 30:1001-1005, 1972.

6. HAMNER, J. E., III: "Oral Implantology and Oral Carcinogenesis in Primates", Amer. J. Phy. Anthropol. 38:301-308, 1973.

7. HAMNER, J. E., III, and REED, O. M.: "The Current Status of Artificial Tooth Implantation", Acad. Gen. Dent. J., 19:35-38, 1971.

8. HAMNER, J. E., III: "Natural and Artificial Tooth Implantation in Baboons", in Proceedings of the 3rd International Congress of Primatology, S. Karger: Basel, Switzerland, 1971.

9. HAMNER, J. E., III and REED, O. M.: "The Complex Factors in Tooth Implantation" in Proceedings of the Conference on Engineering in Medicine - Bioceramics, Vol. 2:297-310 (New York, N.Y., Wiley Interscience, 1972.

10. HAMNER, J. E., III, REED, O. M., and GREULICH, R. C.: "Ceramic Root Implantation in Baboons", J. Biomed. Mat. Res. Symposium: Biomaterials for Skeletal and Cardiovascular Applications, Vol. 3:1-13 (New York, N.Y.: Wiley Interscience, 1972).

11. AUTIAN, J. and HAMNER, J. E., III: A Toxicity Profile for Calcium Aluminate", J. Dent. Res., 51:880, 1972.

12. HAMNER, J. E., III, and REED, O. M.: "Implantable Ceramic Teeth" in J. Biomed. Mater. Res. Symposium: Materials and Design Considerations for the Attachment of Prostheses to the Musculo-Skeletal System, p. 217-234, (New York, N.Y.: Wiley-Interscience, 1973).

13. HAMNER, J. E. III, and REED, O. M.: "Pyrolite Carbon Dental Implants in Baboons: A Preliminary Report" in Medical Primatology, Part II, p. 177-181 (Basel, Switzerland: S. Karger, 1973).

Summary Report of the Human Genetics Branch
National Institute of Dental Research

Due in a large part to continuing restrictions on personnel, a decision was made some three years ago to narrow the focus of the activities of the Human Genetics Branch. Thus, congenital malformation in general, and craniofacial anomalies, in particular, were emphasized as major program areas. During the past year the activities of the Branch have been devoted almost exclusively to these areas.

The Developmental Genetics Section is concerned with etiology and pathogenesis of craniofacial malformations in experimental animals. Emphasis is in two areas: basic studies in facial embryology utilizing interspecies grafting techniques and studies of environmental factors contributing to the production of congenital anomalies in genetically susceptible strains of animals. Using strains of animals which develop facial defects spontaneously either because of single gene mutation or polygenic predisposition, studies are directed at defining the developmental basis of these defects and at modifying environmental factors in order to alter the expression of the genes. The aim of these studies is to develop models of genome-environment interaction which may ultimately lead to practical preventive measures in humans.

The Population Genetics Section conducts studies on human families and populations with the aim of better elucidating genetic mechanisms in craniofacial anomalies and identifying agents in the maternal and/or fetal environment which also contribute to the formation of defects. Human studies are designed to produce leads which can be further elaborated and refined through animal studies. As more definitive models are developed through laboratory experimentation the results are examined and tested, in the field, for relevance to the human situation.

Two particularly noteworthy results were obtained during the past year. One is the confirmation of a suspected increase in risk for cleft lip and palate among children of epileptic women. There is considerable evidence that this association is due to the anticonvulsant medication taken by these women. One of the known effects of these medications is the reduction of serum folate levels. This suggests an important role of folic acid metabolism in the genesis of oral clefts. Further studies are being developed both in animals and humans to further test this hypothesis. Cleft lip and palate occurs spontaneously in A/Jax mice as the result of a multigenic predisposition. Low, single doses of x-radiation have been found to markedly increase the frequency of the defect. Furthermore, the results suggest that at a very precise developmental period radiation will selectively kill embryos with cleft lip. These findings are of significance for two reasons: 1) they demonstrate responsiveness of the expression of a genetic defect to relatively minor environmental insults and 2) the possibility of selective abortion of defective embryos is compatible with human data collected within the Branch suggesting that the risk of early fetal loss in humans is positively related to genetic predisposition to oral clefts.

Staff of the Branch includes six investigators, three of these are permanent, one is a research associate, and two are part of the Visiting Program. Although the small number of permanent senior investigators has severely limited the ability of the Branch to move forward, evidence

of continued productivity is attested to the publication record. Seven papers and two books authored or co-authored by staff members have been published during the last year and seven additional papers are in press.

Summary Report of the Developmental Genetics Section
Human Genetics Branch
National Institute of Dental Research

The major focus of the Developmental Genetics Section is on the control of normal and abnormal development of the face. Embryological studies are concerned with the processes by which the head and face develop. Mutant and highly inbred strains of mice with CLP or CP or predisposition to these traits are examined for differences from normal animals during morphogenesis and for their responses to teratogenic stimuli.

Embryology

The research program in this area was concerned with the completion of descriptive studies utilizing cell marking procedures and the initiation of new studies concerned more with the mechanism of neural crest migration.

Further details concerning the contributions of cranial neural crest to head mesenchyme have been elucidated, particularly the role this mesenchyme plays in the development of the eye, skeletal muscle and vascular tissues.

Only partially analyzed have been the contributions of crest cells to the inner ear and other sensory receptors. The contributions of "vagal" crest cells to the ultimobranchial body and carotid body were confirmed and we also observed extensive contributions to the wall of the internal carotid artery and other vessels derived from the embryonic aortic arches where they presumably function as baroreceptors. Interaction between crest and placodal cells in this region appears to be limited largely to the nodose and petrosal ganglia.

The derivatives of the paraxial mesoderm have been studied with R. D. Hazelton. In confirmation of his earlier studies, the occipital somite mesoderm was found to give use to the hypoglossal musculature. This mesoderm was also found to form the cervical strap musculature as well as other muscles in the cervical area. More anterior paraxial mesoderm was found to form the extrinsic ocular muscles and the visceral arch musculature. In all these cases very extensive cell migrations occur. The mesoderm transplant cases also provide a valuable check for the crest mesenchyme experiments since the mesenchyme not formed by crest is formed by these mesoderm cells.

Biochemical and radioautographic studies of matrices synthesized in relation to crest cell migration are being studied in collaboration with R. M. Pratt. It appears that hyaluronate is the primary intercellular mucopolysaccharide and its rate of synthesis (per mg protein) doubles at the onset of crest cell migration. Much of the material appears in the "cell-free spaces" within which the crest cells migrate.

This report is based upon progress achieved during the year in Project Nos. NIDR-HGB001a54, NIDR-HGB002a62, NIDR-HGB003b67, NIDR-HGB004b70 and NIDR-HGB005a72.

Time lapse studies of avian embryos using conventional transmission and interference contrast microscopy are being conducted with J. Hassel and L. Love. They are still in preliminary stages and will be correlated with collaborative scanning EM studies being conducted by A. J. Steffek.

Cleft Lip and Cleft Palate (CLP)

The A/J mouse has about 10% CLP at birth. The cleft is morphologically similar to that found most often in humans. The genetic difference between the susceptibility of A/J mice compared to resistant C57Bl/6 mice is due to several genes. This is similar to the polygenic model of CLP suggested by data on man.

One of the properties of a polygenic multifactorial hypothesis for CLP is that a variety of teratogenic stimuli should shift animals with high genetic predisposition from the unaffected to the cleft phenotype. Therefore the animals of a strain predisposed to spontaneous CLP like A/J might be expected to be more susceptible to teratogens than a genetically resistant strain like C57Bl/6.

To test the hypothesis that A/J is more susceptible to induced CLP than C57Bl/6, pregnant C57Bl/6 mice were treated with x-ray at different periods of gestation. The most sensitive period was found for embryos on day 7 when a dose of 100R gave 3.5% facial clefts. When 100R was given to pregnant A/J mice on day 7 about 24% of the embryos had CLP and over 45% had CLP on day 8. The morphology of the CLP produced in A/J generally similar to that occurring spontaneous CLP while those produced in C57Bl/6 were dissimilar. This result demonstrates increased susceptibility of the predisposed A/J strain towards CLP induced by x-ray as compared to C57Bl/6. They also illustrate the close interaction between genetic predisposition and response to teratogens in the polygenic multifactorial pattern of inheritance.

When A/J mice were radiated on day 10 the surviving fetuses showed no CLP in 16 litters compared to 20% in sham irradiated controls. Litter size was reduced by the amount equal to the expected CLP in the radiated litters. There is no evidence for increased lethality in normal fetuses suggesting that, on day 10, CLP fetuses are being killed while the normals are unaffected.

Thyroxine has been proposed as a preventive for CLP in A strain mice when given during early pregnancy. A pilot experiment to evaluate the reproducability of this phenomenon gave equivocal results but suggested that maternal age may be a major factor in thyroxine response. An experiment designed to include this factor is in progress.

To measure the serum levels of thyroxine in treated animals a sensitive radioimmune assay has been set up in the laboratory. It is able to detect 0.4 nannograms per 100 ml (n%) using samples of 0.025 ml of serum. It has been compared to competitive binding assays and gives comparable results. We have found that dwarf (dw/dw) mice which are known to be panhypopituitary have serum levels of about 1 n% while full size litter mates are separated into two groups. Those with values about 3.5 n%, some of which have had dwarf offspring and are therefore obligate Dw/dw, and others, with values about 5 n%, which have had no dwarf offspring and are therefore probably Dw/Dw. Those results suggest that Dw acts additively as regard to thyroxine levels although it is dominant in

regard to body size. A/J mice have thyroxine levels between 1.5 and 2.5 n% which is lower than Dw/dw but above the dwarf animals.

Isolated Cleft Palate (CP)

Cleft of the secondary palate (CP) results from a variety of teratogens. Among the most effective are the glucocorticoids. Different strains show different susceptibility to these adrenal steroids. We have concentrated on the effects of the natural glucocorticoid of mice, corticosterone, on A/J the most sensitive strain known. During the past year we have utilized the sensitive radioimmune assay for corticosterone developed in this laboratory to determine the pattern of serum glucocorticoid levels in normal A/J pregnancy and in animals treated with corticosterone.

During the first 10 days of gestation in A/J the pattern of diurnal corticosterone change is between 5 and 20 n% and response to injected corticosterone is like that of a nonpregnant animal. By day 12 of pregnancy the absolute levels in both diurnal variaton and response to injection increase between 5 and 10 times. They rise to between 10 and 20 times the nonpregnant levels on day 15. These changes are occurring in the period during which steroid treatment has been found to be most teratogenic.

Stress to the A/J mother, as produced by removal of drinking water and treatment in a dry moving air environment results in serum corticosterone levels during day 12 to 15 similar to those resulting from an injection of 2.5 mg of corticosterone and also in similar CP rates (about 40%). Detailed examination of correlation between individual corticosterone levels, lethality and malformation rate in fetuses are in progress.

A strain of mice has been discovered which appears to be as sensitive to the stress system used as A/J. This strain is "Star" a line used in Europe in crosses with Dwarf to produce F_1 mothers that respond to a variety of environmental stimuli by producing up to 30% CP offspring. We have repeated the Star x Dwarf cross experiment with our stress system and find only 6% CP in the offspring of F_1. Dwarf strain mice do not show CP after stress but the Star mice have about 50% CP among surviving offspring along with about 50% lethality. These mice are unrelated to A/J and have no CLP. The strain A/Heston which is closely related to A/J also has CP in response to stress but somewhat more fetal death than A/J.

CP associated with open eyes at birth occurs in the strain oel as a recessive trait. We have found this strain to contain another recessive trait, anencephalus. Anencephalus and CP have never occurred together in over 1,600 abnormal animals examined.

Histological sections show that both the normal and cleft palate animals have reduced facial bone and increased sinus and nasal air space. In cleared alizeran stained specimens there is a reduced number of caudal vertebrae. Examination of embryos on day 10 shows that a heterogeneity exists with some showing open anterior neural tubes while others with the same number of somites have closed tubes.

Analysis of the segregation ratio of litters in which both traits occur shows that the results are not compatable with the hypothesis of two independent loci but are compatable with the hypothesis that the two traits are results of allelic genes with a normal heterozygote and a normal wild type allele. If this should be born out by further experiments

it makes it probable that there is some common factor in the formation of these two defects since alleles usually affect a single enzyme or other molecule or instruction in development.

The occurrence of two genes, possibly allelic, affecting the midline structures is suggestive of the T (tailess) complex of genes, a region on chromosome 9 in the mouse at which there are many "alleles" showing frequent "mutation". The oel strain was originally part of a T locus study of the allele phocomelia. We are therefore testing these two genes for allelism to T.

Future Plans

Embryological examination of the events of early facial development will be combined with examination of CLP and CP development in mutants and inbred lines using genetic techniques to control the type of defect under study in various test systems.

Appropriate cell markers will be used to study the contributions of crest cells to the inner ear and to sensory receptors in the facial region. EM, fluorescence microscopy and other methods will be utilized to determine the functions of cells derived from quail grafts transplanted to chick embryos. Considerable progress has already been made in some of these projects--e.g., EM examination (by R. Peach) of the neurons in the chick trigeminal ganglion whose origins from crest or placodal quail grafts are identified from adjacent Feulgen-stained "thick" sections.

The time lapse and scanning EM studies on migrating crest cells will be extended from avian embryos to mammals, both normal and abnormal. Study of other migrating embryonic cell populations is also proposed.

Demonstration of increased sensitivity to radiation in a strain with genetic predisposition to malformation will be the basis for further studies. During the sensitive period a dose-response curve will be needed as well as studies of the relation between lethality and malformation. The apparent increase in lethality of x-ray for CLP embryos on day 10 will be followed up with examination of the events in development in these embryos on days 11 and 12. Particular attention will be given heart development because of the active morphogenesis of the heart on day 10 and the lethality of major cardiac malformations. The strain CL/FR which has higher frequency of CLP than A/J will also be examined for similar responses.

Large differences in corticoid biochemistry that are under major gene control have recently been observed among mouse strains. These appear to be related to CP susceptibility after "stress". The specific enzymes involved are now subject to assay. This opens the possibility of evaluating the ability of the fetus to handle the high corticoid levels in stress or corticosterone treated mother. Since steroids and thyroid hormone are both known to be powerful inducers of new enzyme synthesis in embryogenesis these hormones will be studied for their actions in modifying CLP and CP or correlations between fetal levels of hormone and phenotype. The radioimmune assays also permit the observation of placental transport of hormones at levels not previously practical. Possible differences in human response of the two CP prone strains A/J and "star" will be examined. Studies of the biochemistry of palate shelves in these mice will be undertaken in collaboration with Dr. R. M. Pratt.

Further genetic and developmental studies will be carried out on the oel strain to determine the process by which clefts and anencephalus are formed and the relationship between the genes controling these processes.

Summary Report of the Population Genetics Section
Human Genetics Branch
National Institute of Dental Research

The Population Genetics Section was established to study both normal and abnormal genetic variation in human populations. During the past year the focus of the section has been primarily on the study of congenital malformation, particularly cleft lip and palate utilizing both family and population data. Major areas of study of the section include (1) characteristics of families with oral clefts, (2) maternal factors in congenital malformation, (3) congenital malformation in birth characteristics in American Indians, (4) genetic studies of oral disease anomalies and development, and (5) genetic analyses of human populations.

Progress

Studies on facial morphology in relatives of oral cleft patients are being conducted in collaboration with the Lancaster Cleft Palate Clinic and the Department of Orthodontics at the University of Michigan. The objectives of these studies are to utilize parameters of facial morphology in an attempt to define the different subgroups of cleft families and to determine if facial measurements can be useful for genetic counseling. In addition, if certain aspects of facial morphology can be shown to be predisposing to the production of oral clefts this information may be useful in better understanding the pathogenesis of these defects. Data from frontal and lateral cephalograms have been utilized. The sample consists of 223 parents of children with cleft lip with or without cleft palate, 124 parents of children with isolated cleft palate and two control groups of parents, one group consisting of 115 individuals from Lancaster, Pennsylvania, and the other of 132 parents of normal children from Ann Arbor, Michigan. All parents of cleft children were obtained from Lancaster. The most striking findings are observed in the cleft lip with or without palate group. The parents of these children show small but highly significant increases in interorbital distance as well as significantly decreased upper face height. These changes result secondarily in increased mandibular prognathism and a somewhat concave facial profile. Although these changes are consistant in both sexes they are most striking among the fathers. These findings are consistent with the hypothesis that a relative deficiency of embryonic facial mesenchyme may play a role in the production of cleft lip with or without cleft palate.[1] It is further suggested that this relative deficiency may have a heritable component.

Significant differences are also found in facial measurements of parents of children with isolated cleft palate. Although these changes are not as striking or as consistent between sexes, they are generally similar to those observed for the CLP parents.

This report is based upon progress achieved during the year in Project Nos. NIDR-HGB006b67, NIDR-HGB007a58, NIDR-HGB008a63, NIDR-HGB009a58 and NIDR-HGB010a73.

Multivariate analysis of these data by use of discriminate functions show highly significant differences between the parents of both cleft groups and the controls. The parents of the two types of clefts do not however differ from each other. The most striking result from this aspect of the study is that the difference between the two control groups is greater than the distance between the cleft groups and the Lancaster control. Therefore although there are real differences in facial morphology of parents who have produced children with oral clefts these differences are less than the combined effects of ethnic variation of the two control samples plus the differences which may have been introduced by slight technical variations introduced in the two control series. It seems highly unlikely then, that using the present approach measures of facial morphology will have practical use in genetic counseling.

In order to determine the maximum changes that may occur in so called "normal" relatives of oral cleft patients, similar analyses of 30 unaffected monozygous co-twins of CL/P probands are being conducted. Each of these 30 individuals has a genetic constitution identical to an affected individual and has shared a very similar pre- as well as post-natal environment. This group therefore is expected to show the most extreme deviations in facial morphology to be found in relatives of oral cleft patients. Age and sex matched control group of 8-10 individuals has been obtained for each of the 30 twins.

Another study in this area has involved the analysis of sex-ratio in 1,700 CL(P) pedigrees. Differences in sex-ratio were found among sibships with 1, 2 and 3 children affected with cleft lip with or without cleft palate. The data have resulted in the formulation of a hypothesis of a two threshold model of causation for CLP.[2] Three classes of individuals are hypothesized to be associated with CLP liability, 1) normal, 2) live born with clefts and 3) early abortuses. It is further hypothesized that as liability increases an increasing proportion of males are aborted thus accounting for the changes in sex-ratio observed. Findings suggestive of similar interaction between early fetal death and cleft production have been observed in studies involving radiation of A/Jax mice (see summary report of Developmental Genetics Section).

Studies of maternal factors in congenital malformations have centered around analysis of computerized data from U.S. Air Force Hospitals for the period, 1965 through 1971. Summary data are available for the hospital records of 347,097 live born infants and their mothers. Of particular interest was the outcome of pregnancies to epileptic women. Considerable evidence has accumulated in recent months suggesting an increasd frequency of cleft lip with or without cleft palate and perhaps heart defects among infants born to epileptic women--particularly those taking anticonvulsant drugs during the first trimester. Uncertainty remains regarding the magnitude of the effect and whether other specific malformations are involved. Epilepsy was diagnosed on 410 maternal obsteric records. 4.2% of their offspring were malformed compared to 2.7% among all births. This increase in malformation rate is significant at the 5% level.

Three cases of CL/P were found among the births to epileptic mothers giving a rate of 7.32 per thousand compared to 1.52 per thousand for all births. This difference is also significant with probability .025. Congenital heart defects also occurred with about twice the expected frequency among the epileptic group. Again this increase is statistically

significant. No other specific malformation category was significantly increased.[3] These data are consistant with other population reports and collectively suggest a 5 to 6 fold increase in the incidence of CLP among epileptic women.

From these data it can be estimated that approximately 3% of the CLP cases born each year are associated with maternal epilepsy and likely to be the direct result of ingestion of dilantin or other anticonvulsant drugs.

Studies of birth characteristics of American Indians have resulted in the finding of a highly significant association between prenatal care and death of the newborn. Thus, women who receive no prenatal care showed approximately a 40% increase in death among their newborn infants and about a 4% decrease in birth weight. These effects remained after correcting the analysis for several genetic and environmental factors which might be associated. The possibility remains however that other uncontrolled factors are responsible for these effects rather than prenatal care per say.[4]

Two human populations can be assumed to differ genetically both in the frequency and type of alleles present. For qualitative traits the differences can be demonstrated simply by comparison of the frequencies of alleles at one or more loci. However for heritable quantitative traits, especially those with environmental components which result from cultural factors, there appears to be no direct method for interpopulation comparison. Genes controlling variation in these traits cannot be identified and the effects of differing physical and cultural environment, the complications of social-heredity and genotype-environment interactions, all make the prospect for comparisons poor. Thus, there appears no direct answer to the question of whether there are genetic differences between races either in genes or gene frequencies with respect to continuously varying traits related to behavior. In certain cases however migrants from two populations have intermixed to form distinct hybrid populations. If such intermixture occurs over a long time the contribution of either ancestral population to the genetic background of an individual in the hybrid population can vary between 0 and 100%. Our studies in this area have been directed toward development of mathematical techniques for estimating ancestral admixture within specific individuals utilizing qualitative genetic traits. These estimates can then be used in regression analysis to relate each individual's ancestry to his quantitative scores for continuously varying genetic traits.[5] This method is now being applied to studies of hypertension in the Negro population. The Black population of the U.S. has a significantly greater incidence of hypertension than the white population but this difference has not been observed in studies outside of the U.S. For this reason it has been suggested that this increased incidence may be, at least in part, attributable to the effects of adverse environmental factors. Our studies are based on 433 Black Americans age 30 years or older examined at the Strong Memorial Hospital, Rochester, N.Y. Blood samples were obtained and typing done for ten different genetic systems. Using these data individual estimates of ancestry were calculated using the methods previously discussed. Blood pressure values were then regressed on these admixture estimates and other concominant environmental variables. The results indicate that slightly less than 10% of the variation in hypertension in this

population can be attributed to genetic factors specific for race. The remainder is due to environmental influences or genetic factors common to the races.

Future Plans

The major portion of the activities of this section will involve continued family and population studies of cleft lip and palate. Particular emphasis will be placed on the identification and description of possible maternal factors which may play a role in the etiology of oral clefts. These studies are directed toward identifying etiologically different subgroups of oral clefts and the relative importance of each. They are also concerned with identifying aspects of fetal environment which might provide possibilities for the application of preventive measures.

There is now considerable evidence that anticonvulsant drugs are associated with the production of cleft lip and palate. Studies[6] have shown that anticonvulsant drugs lower serum folic acid levels. In addition, folic acid antagonists, for example, methotrexate are known to produce malformations and abortion.[7, 8] There are also suggestions of reductions in the reoccurrence rates of clefts among the mothers on vitamin supplementation, including folic acid, after the birth of one affected child.[9, 10] Studies are being undertaken in collaboration with Dr. Wertelecki at the University of South Carolina and Dr. Victor Herbert at Columbia University in an attempt to determine if certain women who have produced cleft lip and palate children may have detectable defects in folic acid metabolism. Blood samples from approximately 100 mothers of children with clefts and 100 normal women are being obtained for an evaluation of their serum and tissue folate levels. Assay of thyroxine levels will be conducted concurrently.

The other studies covered by this report are being continued but since Dr. MacLean has left the section during this year little new work is anticipated in relation to genetic analyses of human populations.

References

1. ROSS, R. B. and JOHNSTON, M. C.: Cleft Lip and Palate. Williams and Wilkins Co., Baltimore, 1972.

2. NISWANDER, J. D., MACLEAN, C. J., CHUNG, C. S. and DRONAMRAJU, K.: Sex ratio and cleft lip with or without cleft palate. Lancet, pp. 858-860, Oct. 21, 1973.

3. NISWANDER, J. D. and Wertelecki, W.: Congenital malformation among offspring of epileptic women. Lancet, p. 1062, May 12, 1973.

4. IBA, B. Y., NISWANDER, J. D. and WOODVILLE, L.: Prenatal care, birth weight, major malformation and newborn death in American Indians. HSMHA Health Reports, in press, 1973.

5. MACLEAN C. J. and WORKMAN, P. L.: Genetic studies on hybrid populations. Ann. Hum. Genet. 36: 341-351, 1973.

6. MALPAS, J. S., SPRAY, G. H., and WITTS, D. J.: Serum folic-acid and vitamin-B levels in anticonvulsant therapy. Brit. Med. J. 1: 955-957, 1966.

7. MILUNSKY, A., GRAEFF, J. W. and GAYNOR, M. F.: Methotrexate induced congenital malformations. J. Pediat. 72: 790, 1968.

8. THIERSCH, J.B.: Therapeutic abortions with a folic acid antagonist, (4 amino PGA) administered by the oral route. Amer. J. Obstet. Gynecol. 62: 1298, 1952.

9. PEER, L. A., GORDON, H. W. and BERNHARD, W. G.: Effect of vitamins on human teratology. Plast. and Reconstr. Surg. 34: 358-362, 1964.

10. CONWAY, H.: Effect of supplemental vitamin therapy on the limitation of incidence of cleft lip and cleft palate in humans. Plast. and Reconstr. Surg. 22: 450-453, 1958.

Summary Report of the Laboratory of Biochemistry
National Institute of Dental Research

Karl A. Piez, Chief of the Laboratory of Biochemistry, has been on assignment since August 1972 in the Laboratory of Molecular Biophysics at Oxford University, Oxford, England. He is scheduled to return to the NIDR in August 1973. During this period he is collaborating with Oxford University scientists on computer analyses of the amino acid sequence in collagen looking for the sites of molecular interactions and also applying x-ray diffraction to the study of fiber formation. During this period, George R. Martin, Chief of the Connective Tissue Section, Laboratory of Biochemistry, has been serving as the Acting Chief of the Laboratory.

The research efforts of the Laboratory are continuing in the areas described in last year's report, namely connective tissue chemistry and metabolism, the transglutaminase enzymes and the regulation of cell division and specialized functions. Studies on connective tissue are directed toward an understanding of normal functions and alterations that occur in disease states. The transglutaminases are a widely distributed class of enzymes with diverse and as yet incompletely understood functions, including those in blood clotting and in wound healing. Studies on the regulation of cell division and function have uncovered new control mechanisms that may be exploitable for therapeutic intervention in congenital or acquired disorders.

We have initiated a governmental interagency agreement with scientists at the National Bureau of Standards to study the structure of connective tissue proteins by nuclear magnetic resonance rather than acquire the expensive equipment and experienced personnel. Even now this project has attained important new insight into the structure of these proteins and the forces that maintain their structures. In a related study, chemical mapping of a transglutamine has brought new understanding of the manner in which enzymes function and the physical basis of enzyme specificity.

Close collaboration has been established with the Departments of Medical Genetics and Orthopedics at the Johns Hopkins University. Three guest workers have reached the Laboratory from this collaboration. Joint efforts involve research on inherited defects of connective tissue in humans and have revealed the nature of the enzymatic defect in one of these disorders.

Summary Report of Cell Biology Section
Laboratory of Biochemistry
National Institute of Dental Research

Background

The genetic information stored in DNA is ultimately translated into the physiological activity of differentiated animal cells and tissues. Studies in progress in the Cell Biology Section are aimed at elucidating some of the biochemical processes by which this translation is achieved and controlled.

The current work of the section is divided between two major aspects of this problem:

1. How are alterations in the differentiated state of the cell correlated with changes in the synthesis and processing of various classes of RNA, the primary intermediate in the translation of genetic information? What biochemical processes regulate such changes?

2. How is the differentiated state of the cell determined by the activity and organization of the protein synthesizing mechanism of the cell and how are these factors controlled?

Progress

For several years, the RNA metabolism of human peripheral lymphocytes has been under investigation. This cell offers certain notable opportunities for research: the physiological activity of this cell *in vivo* includes prolonged periods of non-growth, followed by abrupt onset of growth in response to an immunologic stimulus. This sequence may be studied *in vitro* (1). The capacity to vary its growth rate in response to external stimuli may be viewed as an aspect of the differentiated function of the lymphocyte. An understanding of the biochemical mechanisms which control the growth of lymphocytes *in vitro* will then bear on two major problems in modern biology and medicine: the control of cell growth *per se*, and the regulation of cell differentiation. Moreover, knowledge in this area will extend our understanding of problems in immunology.

Improved understanding in these fields is currently of great importance to modern Dental Research. Disordered growth and development of oral-facial structures are major causes of pathology in this area. Since proper development depends critically on precise differential control of cell growth and non-growth, improved understanding of such control is vital to a reasoned approach to prevention and treatment. In addition, immune phenomena at the cellular level, involving lymphocyte reactivity to foreign and host materials and tissues, are now thought to play an important role in oral ulcerative disease and in periodontal disease, two widespread diseases of the American population. Since lymphocyte participation in these conditions is mediated through the growth response to specific stimuli, it is exceedingly important that we understand the means by which the lymphocyte recognizes, interacts with, and responds to such stimuli. Only by knowing the steps in this

*This report is based upon progress achieved during the year in Project Nos. NIDR-LBC401b62, NIDR-LBC402b67, NIDR-LBC404b72 and NIDR LBC405b72

sequence in great detail can we hope to intercede at the proper points to control lymphocyte responses for the benefit of the patient.

Perhaps of greatest significance is the relevance of these studies to the problem of oral cancer. A thorough understanding of the biochemical basis for the control of normal cell growth must underlie any rational attempt at prevention and non-surgical treatment of cancer. This is especially vital for oral cancer, since surgical treatment is often tragically disfiguring, so that the "cured" patient is hardly able to make use in any normal way of the years added to his life.

Previous studies in this section have established the patterns of RNA metabolism characteristic of normal resting and growing lymphocytes [reviewed recently (1,2)]. In the course of these studies, alterations in RNA metabolism characteristic of the shift from the resting to the growing state have been identified (3,4). In particular, a previously undescribed mechanism of cell growth regulation, based upon the control of degradation of newly formed ribosomal RNA, was reported (5). The growth of resting lymphocytes is restricted by a process which limits the rate at which new ribosomal RNA can be accumulated. RNA molecules synthesized in excess of this limited amount are degraded. Such "wastage" may affect more than half of the newly synthesized ribosomal RNA molecules in resting lymphocytes. That control of such wastage may be important in cell growth regulation is shown by the rapid reversal of such wastage following the onset of lymphocyte growth (6,8).

In a recent study (9), the process of rRNA degradation in resting lymphocytes was examined in detail. It was found that the two components of rRNA - 28S and 18S rRNA's - are degraded independently in the nucleus at different times following their synthesis, despite their having been synthesized together as parts of a common precursor molecule. Thus, 18S molecules are degraded immediately after their formation, while 28S molecules synthesized at the same time are degraded only after a prolonged period of processing. It is apparent that a very precise control exists over the nuclear degradation of ribosomal RNA, which is consistent with the hypothesis that this degradation and its reversal constitute an important growth regulating mechanism.

This study provided additional evidence that turnover of cytoplasmic ribosomes, presumably during their activity in protein synthesis, also occurs in both resting and growing lymphocytes. Ribosomal turnover was distinguished from the "wastage" of ribosomal RNA described above because both 18S and 28S RNA's were degraded in parallel during turnover. It was found that a marked increase in half life of new ribosomes occurs after growth stimulation. However, careful analysis revealed that this was due to the accumulating ribosome content of growing cells, resulting from increased synthesis and decreased intranuclear wastage of rRNA. The absolute number of ribosomes degraded per unit time changed very little after growth stimulation. Thus, the degradative portion of the ribosome turnover process does not seem to be a major point of regulation in the control of lymphocyte growth.

Evidence was obtained which showed that the survival of newly synthesized ribosomal RNA molecules is dependent on the availability of proteins ("ribosomal protective proteins") which appear to protect the RNA from de-

gradation (7,8). The number of such protein molecules available at any moment will determine the number of ribosomal RNA molecules which are protected; the remainder are degraded. Since normal cell growth is dependent on the accumulation of new ribosomes, control of the rate of synthesis of ribosomal protective proteins may be the means by which rRNA survival and degradation is determined, and hence may be the critical regulatory point in this process of cell growth control.

We propose that ribosomal protective protein is one of the large complement of proteins which are components of the ribosome. A major effort is in progress to isolate, characterize and purify this protein. This is being done coordinately in lymphocytes and in HeLa cells, the latter being used as a source of large quantitites of material for study. To date, ribosomes from normal growing lymphocytes and from HeLa cells have been isolated, their proteins separated and analyzed by acrylamide gel electrophoresis. Examination of the relative rates of synthesis of the various ribosomal proteins in rapidly growing lymphocytes is currently in progress in an effort to localize components of this complex system which may be produced at limiting rates. Results to date indicate that particular proteins show the rapid labeling expected of a ribosomal protein whose production is rate limiting for ribosome assembly.

In continuing this line of investigation, we have successfully developed the methodology for preparation from HeLa cells and from lymphocytes of large numbers of ribosomes and ribosomal subunits labeled with a variety of radioactive precursors. Degradation of these particles into smaller ribonucleoprotein components by controlled ribonuclease digestion has also been achieved together with the procedures for selectively detaching proteins from these fragments. At present, progress is being made toward the goal of separating ribonucleoprotein fragments according to certain of their biochemical properties. This will permit the eventual separation and preparation of ribosomal proteins which are associated with specific regions of ribosomal RNA. It will then be possible to study the activity of such proteins in protection against ribonuclease attack and in relation to other ribosome functions. Following the identification of the specific ribosomal protective protein(s) in HeLa cells, it will then be feasible to study the synthesis of these proteins in relation to lymphocyte growth regulation.

Another major class of cellular RNA is the messenger RNA which transmits genetic information stored in nuclear DNA to the cytoplasmic ribosomes for translation into proteins. The means by which animal cells control the transcription of various messenger RNAs in the nucleus and their translation in the cytoplasm is a subject of intense study in many laboratories. In this aspect of regulation must lie the basis of cellular differentiation, wherein different cells of the body, all containing the same genetic information, produce different assortments of proteins and of enzymologically determined products. The lymphocyte offers a unique opportunity for studying this question with great precision, since this cell undergoes physiological differentiation steps in cell culture. Few laboratories in the world have taken advantage of this fact.

Using a newly-discovered biochemical property of messenger RNA in animal cells, the possession of an extensive polyadenylate segment, we have begun a study of

the metabolism of messenger RNA in resting and growing lymphocytes (11). The kinetics of its nuclear synthesis, processing, and transport to the cytoplasm are currently being examined, together with growth-induced alterations. The distribution of mRNA among various compartments of the cytoplasm has been studied. One significant preliminary finding is an indication that the resting lymphocyte may transcribe and transport to the cytoplasm messenger RNA's which are not used to make proteins in the resting cell, but which are rapidly utilized when growth stimulation occurs. This may provide a clue to the mechanism by which the lymphocyte is able to respond quickly to external stimuli with the elaboration of an array of physiological products.

To aid in these studies, a computerized approach to the analysis of the kinetics of RNA precursor pool labeling by radioactive materials was developed and applied to the study of RNA labeling in lymphocytes (10). This method permits the investigator to correct RNA labeling data for changes in precursor pool specific activity without the necessity for measuring the pool specific activity directly. For work with lymphocytes, this is essential, both because it was shown that slow labeling of RNA precursor pools markedly influences the labeling of RNA, and because the amounts of lymphocyte material available are too limited to permit direct precursor pool quantitation.

In a related study, a unique ribonuclease, widely present in animal tissues and fluids, was discovered by Dr. Stern in this laboratory (12). It has been further studied, with the following results:

A. Molecular weight: 40-45,000 Daltons
B. Slightly basic, co-electrophoresing with γ-globulin.
C. Specific for pyrimidine sequences of double stranded RNA only.
D. The RE cells of the lung are the cells which are most active in enzyme synthesis.
E. Associated with the rough endoplasmic reticulum fraction of the cell.

In the same area, it was learned that all cells examined synthesize small amounts of double-stranded RNA. The site of such synthesis was localized to the nucleus (14), with subsequent transport to the cytoplasm. Double-stranded RNA's are becoming very important in molecular biology since it is now thought that they may play a role in the control of initiation of protein synthesis. Complete understanding of this process is of basic importance to a rational approach to the treatment of viral infection, of both infectious and oncogenic types.

Differentiated animal cell systems are often characterized by the production of large amounts of specific proteins. Such production varies not only among different tissues, but also during various stages of development of particular tissues. The means by which such differentiated states of protein synthesis are determined and maintained is being sought at the biochemical level by an analysis of the protein synthesizing elements in a cell-free system.

This effort involves the cell-free synthesis of collagen. Since the orderly laying down of collagen as part of connective tissue is essential to normal

cranio-facial development, this work has clear relevance to a major NIDR goal. Much evidence indicates that the production of proteins is controlled, in part, by factors which act during the synthesis of the protein molecule. These factors, and their modes of action, have only begun to be understood, and that understanding has come only through the development of cell-free protein synthesizing systems. Application of this methodology to collagen biosynthesis will provide information as to the regulation of production of this protein at the most basic level. The possibility exists that such knowledge will permit us to intercede locally with appropriate biochemical treatment and correct problems of improper bone development in the jaw and other facial structures. Thus, malocclusion may eventually become a biochemically treatable disease, providing a less expensive and more physiological approach to this problem than that currently in use.

In a more general sense, fuller understanding of the basic controls exerted over collagen synthesis will play a role in unraveling the causes of congenital cranio-facial anomalies. There can be little doubt that both environmental and genetically-determined developmental disorders result, in many cases, from deranged control of the production of specific proteins. Disordered regulation of collagen synthesis may be involved in the production of cranio-facial developmental anomalies and it is important to identify the particular biochemical steps which are susceptible to derangement, either through genetic defect, through toxic effects of the environment, or because of unusual stresses at critical times in uterine development.

Major progress has been made in this undertaking.

Cell-free protein synthesizing systems have been successfully set up using rabbit reticulocytes and Krebs ascites tumor cells as sources of ribosomes and required factors. These systems have been used to translate various added messenger RNA's into protein: hemoglobin mRNA; polyuridylic acid; certain viral RNA's.

Successful synthesis of complete collagen chains in the cell-free system has been achieved. Products of synthesis have been evaluated by molecular sieve and CM cellulose column chromatography and act as suitable substrates for collagenase digestion. Cyanogen bromide peptide chromatography and proline hydroxylase assays are additional procedures which are being developed currently in a continued identification of the products of synthesis. Work is in progress to isolate and purify, using poly T cellulose column chromatography, the messenger RNA of collagen, for translation in this system.

The current source for collagen mRNA is the 14 day old chick embryo calvarium. This tissue devotes up to 60% of its protein synthetic activity to collagen production. A major question is whether this emphasis is based upon the proportion of mRNA devoted to collagen, or upon the extensive utilization of a small amount of mRNA.

Evidence for the former possibility is being sought through attempts to label and isolate the collagen mRNA and mRNA-bearing particles from developing chick calvaria. The second possibility is being investigated in terms of the enzymes and transfer RNA molecules which are essential to the translation of

the collagen mRNA. The unique amino acid composition of collagen (high glycine and proline content) suggests that tissues active in the production of collagen may be enriched in the amino acylating enzymes and/or isoacceptor tRNAs required for those amino acids. tRNA preparations have been isolated from chick calvaria and from liver as a control. A procedure has been developed for the separation of isoacceptor RNA;s for proline, glycine and arginine in order to determine whether enrichment for collagen-related amino acid isoacceptors is present. Initial studies in this determination suggest that such enrichment may occur.

Significance

The particular relevance to Dental research and oral disease of the studies of the Cell Biology Section have been noted in the previous section.

In summary, our work relates directly to high-priority Institute programs concerned with:

1) Cranio-facial development (cell growth control; cell differentiation; control of collagen biosynthesis).
2) Oral soft tissue lesions:
   a) Oral cancer (cell growth regulation)
   b) Peridonal disease) Control of lymphocyte
   c) Oral ulcerative disease) growth response to antigens and other substances
   d) Host-response to viral infection (double-stranded RNA synthesis and specific enzyme production)

In addition to these specific applications to dental problems, our work has broad implications for medicine in general. The major killers of our citizens cardiovascular disease and cancer, are diseases which stem from derangements at the level of cell growth and metabolism. In the area of cancer, much emphasis is currently placed on finding and proving the viral etiology of the disease. There is growing awareness among those involved in that effort that, if it is successful, the next essential step must be to understand how the putative virus interferes with cell metabolism at the biochemical level and where it may be vulnerable to medical intervention. Our work aims at providing basic biochemical information about normal regulation of cell growth, so that viral-induced derangements may be recognized, understood and hopefully corrected. If the search for a cancer virus should prove abortive or of limited application, sound advances in the basic biochemistry of cell growth regulation will be absolutely essential to provide new avenues of investigation.

Relevant Bibliography

1. Cooper, H. L.: Effect of mitogens on the cell cycle: a biochemical evaluation of lymphocyte activation. In Effects of Drugs on the Cell Cycle. Eds: Zimmerman, Padilla and Cameron, Academic Press, N. Y. In press.

2. Cooper, H. L.: Studies on RNA metabolism during lymphocyte activation. Transplantation Reviews 11:3, 1972.

3. Cooper, H. L.: Alterations in RNA metabolism in lymphocytes during the shift from resting state to active growth. In Biochemistry of Cell Division. Ed. R. Baserga; C. C. Thomas, Springfield, 1969, p. 91.

4. Cooper, H. L.: Early biochemical events in lymphocyte transformation and their possible relationship to growth regulation. In Proc. 5th Annual Leukocyte Culture Conference, Ed. J. Harris, Acad. Press, N. Y., 1970.

5. Cooper, H. L.: Ribosomal ribonucleic acid production and growth regulation in human lymphocytes. J. Biol. Chem. 244:1946, 1969.

6. Cooper, H. L.: Ribosomal ribonucleic acid wastage in resting and growing lymphocytes. J. Biol. Chem. 244:5590, 1969.

7. Cooper, H. L.: Control of synthesis and wastage of ribosomal RNA in lymphocytes. Nature 227:1105, 1970.

8. Cooper, H. L. and Gibson, E. M.: Control of synthesis and wastage of ribosomal RNA in lymphocytes. II. The role of protein synthesis. J. Biol. Chem. 246:5059, 1971.

9. Cooper, H. L.: Degradation of 28S RNA late in rRNA maturation in non-growing lymphocytes and its reversal after growth stimulation. Submitted for publication.

10. Cooper, H. L.: Analysis of precursor pool labeling kinetics without pool size measurements: a computer-assisted approach. Analyt. Biochem. in press. 1973.

11. Cooper, H. L.: Studies of poly A-bearing RNA in resting and growing human lymphocytes. To be presented at Cold Spring Harbor Symposium, May, 1973 (by invitation), with publication late in 1973.

12. Stern, R.: A nuclease from animal serum which hydrolyses double-stranded RNA. Biochem. Biophys. Res. Commun. 41:608, 1970.

13. Friedman, R., Barth, R. and Stern, R.: Anomalous effects of heterologous normal serum on interferon production in mice. Nature, New Biol. 230:17, 1971.

14. Stern, R., Twanmoh, A. and Cooper, H. L.: Site of actinomycin-resistant RNA synthesis in animal cells. Exp. Cell Res., in press.

SUMMARY REPORT OF THE CONNECTIVE TISSUE SECTION*

Background

Numerous human disorders involve connective tissue. These disorders include developmental anomalies, inflammatory conditions, and tissue destruction due to aging, infection or other disorders. Major programs by the NIDR on dental diseases involve research in these areas.

It is the object of the Connective Tissue Section to apply new information on the chemistry and biology of connective tissue when possible to disease states. In close collaboration with investigators in the Protein Chemistry Section, we apply new methodology and chemical procedures to biological systems. In addition, we study acquired and inherited disorders of connective tissue trying to identify the underlying defect.

Progress

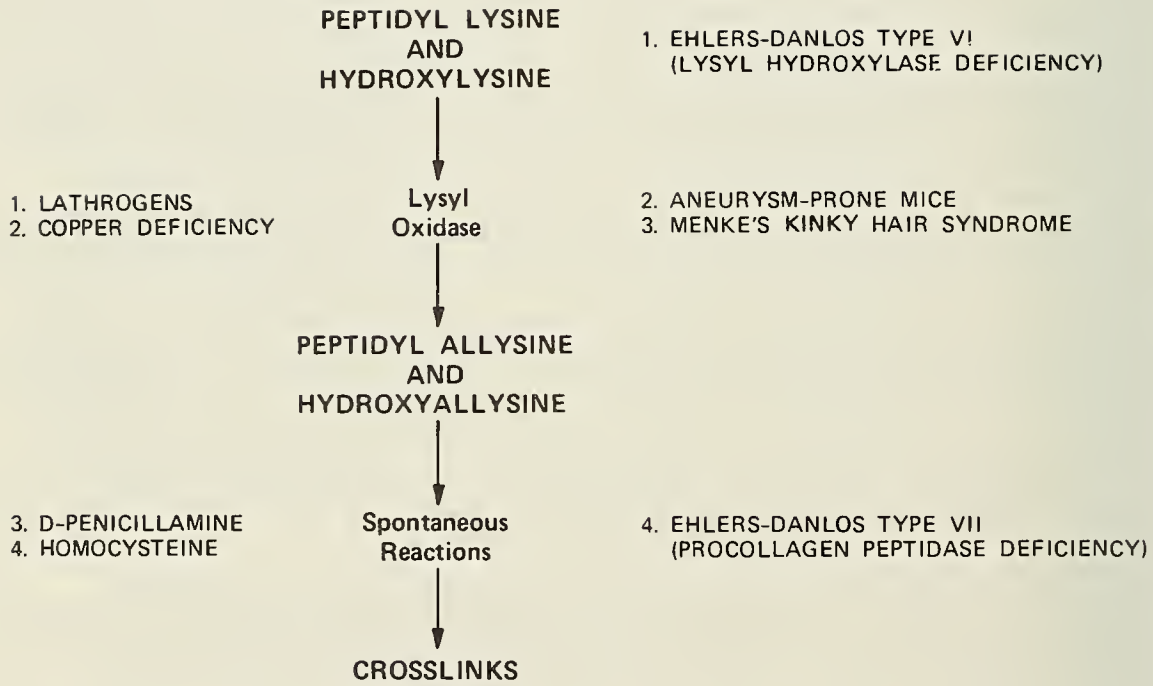
In recent years particular attention has been directed by the Laboratory to the synthesis and maturation of collagen. These studies have led to a general understanding of the steps in collagen formation and maturation and some application to disease states. The acquisition and application of this information is illustrated in Fig. 1. From studies on the structure of collagen by the Protein Chemistry Section, it was learned that collagen undergoes cross-linking following synthesis, as an essential step in the development of normal connective tissue (1). Subsequently, it was discovered in this Laboratory that certain lysines and hydroxylysines in collagen were converted to aldehydes and these in turn were incorporated into cross-links (2). The enzyme involved in cross-linking, lysyl oxidase, was first identified here (3). The site of action of a number of drugs and a deficiency (copper) that interfere with the crosslinking of collagen have been established and are shown in Fig. 1. In addition, defects in crosslinking account for some congenital diseases in humans and animals. Some patients with the Ehlers-Danlos syndrome (Type VI) have collagen lacking hydroxylysine (4). Bone deformities, impaired wound healing and numerous other defects occur in these patients as hydroxylysine is important in crosslinking.

Aneurysm-prone mice and patients with Menke's Kinky Hair syndrome have many clinical similarities. We have found that the aneurysm-prone mice have a generalized defect in the crosslinking of collagen and elastin which we have localized to the enzymatic step (5) involving lysyl oxidase.

Recently we have found patients with still another form of the Ehlers-Danlos Syndrome (Type VII) to have a defect in the conversion of procollagen to collagen (6). Procollagen is the biosynthetic precursor of collagen first reported from this Laboratory (7,8). These patients have short stature, multiple joint dislocations and hyperextensible ligaments. Using cultures of fibroblasts from the skin of these patients the defect was localized to the enzyme that converts procollagen to collagen. Cultures of skin

*This report is based upon progress achieved during the year in Project Nos. NIDR-LBC201b62 and NIDR-LBC202b61.

Figure 1


PEPTIDYL LYSINE AND HYDROXYLYSINE
1. EHLERS-DANLOS TYPE VI (LYSYL HYDROXYLASE DEFICIENCY)
1. LATHROGENS
2. COPPER DEFICIENCY
Lysyl Oxidase
2. ANEURYSM-PRONE MICE
3. MENKE'S KINKY HAIR SYNDROME
PEPTIDYL ALLYSINE AND HYDROXYALLYSINE
3. D-PENICILLAMINE
4. HOMOCYSTEINE
Spontaneous Reactions
4. EHLERS-DANLOS TYPE VII (PROCOLLAGEN PEPTIDASE DEFICIENCY)
CROSSLINKS

fibroblasts from these patients have an increased rate of synthesis of collagenous protein, possibly related to the inability of these cells to convert procollagen to collagen. These studies suggest that one control of collagen synthesis may involve the peptides released from procollagen.

We are also studying the reactions relevant to the removal of collagen from bone and other tissues. Previous studies (9) have indicated that in chronic inflammation, collagen destruction was associated with cell mediated effector systems. The source of collagenase was not identified. Now our studies suggest that the macrophage plays an important role in the degradation of collagen. Little or no collagenase was produced by peritoneal macrophages in culture. However, when endotoxin was added to the culture media to activate the macrophages, high levels of collagenase were found. Thus it appears that the activation of macrophages is an important event in the degradation of collagen in wound healing and inflammatory reactions.

Recently it has been discovered that the collagen in certain tissues is specific: that is, the collagen in cartilage, basement membrane, blood vessels, etc. is unique to that tissue (10). We have investigated the type of collagen produced by cartilage cells in the presence of certain drugs. Normally cartilage cells in culture synthesize a cartilage specific collagen. However, if these cells are exposed to BUDR, the synthesis of fibroblast specific collagen and mucopolysaccharide begins. These studies indicate a way to induce and study the control of gene expression.

The manner in which certain inflammatory cells are attracted to infected or inflamed areas is under study. Substances exuded by E. coli and derived from C5A are chemotactic for polymorphonucleocytes (PMN). The bacterial factor can compete with C5A factor in attracting PMN's. The E. coli chemotactat is a small peptide as indicated by its behavior on molecular sieves and by its inactivation by certain proteases (pronase and subtilisin but not trypsin). Chemical modifications indicate that a free carboxyl group is required for activity. Preliminary analyses are consistent with a hexapeptide containing 3 glycine, 2 serine and 1 alanine residues.

Significance

While connective tissues were once considered to be metabolically inactive, it is now known that synthetic and degradative processes in these tissues undergo marked alterations in response to physiological stimuli and disease processes. We have attempted first to understand the steps in the formation and development of the major structural components of connective tissue and second, to apply this information to diseases affecting these tissues. In the past particular attention was directed to experimental disorders. Using techniques developed during the study of these conditions, several significant advances have been made in the understanding of inherited and acquired disorders of connective tissues in humans.

The directed migration of cells is a general biological response which is important in development, host resistance and repair. Methods used to study the chemotaxis of PMN leucocytes may be applicable to other cell types and be essential to the understanding of a variety of cellular reactions.

Future Plans

We expect to continue to investigate the biochemical steps involved in the formation and destruction of connective tissues. Mutants both animals and human will be sought which have defects in connective tissue. Specifically additional patients with inherited defects in connective tissue will be examined particularly those having bone involvement. More mouse models of defective connective tissue will be sought and the collagen in tumors will be investigated. Finally, the factors important in the degradation of connective tissue during inflammation will be studied.

Relevant Bibliography

1. Piez, K. A., Eigner, E. A. and Lewis, M. S.: The Chromatographic Separation and Amino Acid Composition of the Subunits of Several Collagens. Biochem. 2:58 1963.

2. Bornstein, P. and Piez, K. A.: The Nature of the Intramolecular Crosslinks in Collagen. Biochem. 5:3460 1966).

3. Pinnell, S. R. and Martin, G. R.: Crosslinking of Collagen and Elastin: the Enzymatic Conversion of Lysine in Peptide Linkage to α-Aminoadipic-δ-semialdehyde by an Extract from Bone. Proc. Natl. Acad. Sci. 61:708 1968.

4. Pinnell, S. R., Krane, S. M., Kenzora, J. and Glimcher, M. J.: A New Heritable Disorder of Connective Tissue with Hydroxylysine-deficient Collagen. New Eng. J. Med. 286:1013 1972.

5. Rowe, D. W., McGoodwin, E. B., Martin, G. R. and Grahn, D.: An Inherited Defect of Collagen and Elastin Cross-linking in Mice. Clin. Res. 21:534 1973.

6. Lichtenstein, J. R., Kohn, L., Sussman, M. D., McKusick, V. A. and Martin, G. R.: Human Deficiency of Procollagen Peptidase: the Defect in a Form of the Ehlers-Danlos Syndrome. Clin. Res. 21:724 1973.

7. Layman, D. L., McGoodwin, E. and Martin, G. R.: The Nature of the Collagen Synthesized by Cultured Human Fibroblasts. Proc. Natl. Acad. Sci. 68:454 1971.

8. Martin, G. R., Byers, P. H. and Smith, B. D.: On the Nature of the Polypeptide Precursors of Collagen. Proc. Conf. on the Fibroblast. in press. 1973.

9. Nigra, T., Friedland, M. and Martin, G. R.: Controls of Connective Tissue Synthesis: Collagen Metabolism. J. Invest. Derm. 59:44 1972.

10. Miller, E. J. and Matukas, V.: Chick Cartilage Collagen: a New Type of α1 Chain not Present in Bone or Skin. Proc. Natl. Acad. Sci. 64: 1264 1969.

Summary Report of the Enzyme Chemistry Section
Laboratory of Biochemistry
National Institute of Dental Research

Background

Studies carried out during the last three years have been directed toward characterization of enzymes, termed transglutaminases, that are responsible for the formation of γ-glutamyl amide bonds in proteins and polypeptides. These enzymes catalyze transfer reactions at the carboxamide group of peptide-bound L-glutamine with a high degree of stereospecificity (for review see (1)). In the presence of acceptor amines, the transfer reaction results in the formation of substituted amides ($-CONH_2 + RNH_2 - CONHR + NH_3$).

One of the well-characterized covalent cross-links between and within protein molecules is the ε-(γ-glutamyl)lysine bond. There has been increasing evidence of occurrence of this bond in various proteins, e.g., insoluble fibrin clots, cell membrane, glycerinated myofibril of muscle, proteins of seminal plasma, native wool keratin, and citrullin-containing protein fraction of hair (for review see (1)). The wide occurrence and obvious importance of γ-glutamyl amide bonds has led us to focus attention on the enzymes responsible for their formation.

Progress

In addition to the three major classes of transglutaminases defined by earlier studies in this laboratory (1,2,3) a further distinct class of these enzymes has been found in mammalian seminal plasma. These enzymes, which appear to be involved in the fertilization process, are responsible for coagulation of seminal plasma. The origin of the enzymes is the prostate gland from which they have been isolated in the guinea pig and human. One has a molecular weight of 180,000 to 200,000, exists in the active form, and displays a specificity similar to that of the tissue transglutaminase. The other, of molecular weight 160,000, exists as a zymogen that is activated by thrombin. Each enzyme requires Ca^{2+} for activity and coverts soluble blood fibrin to insoluble or stabilized fibrin by ε-(γ-glutamyl)lysine cross-link formation.

The transglutaminases isolated from red blood cells have been studied kinetically. The enzymes from human and guinea pig erythrocytes display identical kinetic properties which are also identical to those of the guinea pig tissue enzyme. These red blood cell enzymes also cross-link fibrin by means of ε-(γ-glutamyl)lysine bonds.

A number of molecular properties of human plasma protransglutaminase (blood coagulation factor XIII) and of the active enzyme (factor XIIIa) formed by thrombin activation have been determined. Several properties of the protransglutaminase from blood platelets have also been investigated. These include: a) The disulfide - sulfhydryl distribution in the subunits of the

*This report is based upon progress achieved during the year in Project Nos. NIDR-LBC301b72 and NIDR-LBC302b52.

plasma zymogen. The catalytic subunits (a chains) contain 10 -SH groups per molecule and no disulfide bonds. The noncatalytic subunits (b chains) have no -SH groups, but contain 35 to 40 disulfide bonds per molecule (4); b) The subunits of the plasma zymogen have been separated. The noncatalytic subunits may be combined with platelet zymogen to form a zymogen indistinguishable from that of the plasma; c) An active site group of the plasma and platelet enzymes has been identified as an -SH. The stoichometry of inactivation of the enzymes by [^{14}C]iodoacetamide and by 3-nitro-4-hydroxy-α-bromoacetophenone shows that total loss in enzymatic activity is accompanied by incorporation of one mole of inactivator per molecule of enzyme. These findings suggest that both enzymes catalyze their reactions by a so-called "half of the site" reaction requiring participation of a subunit structure for catalytic activity (4); d) The plasma zymogen is not dissociated by Ca^{2+} and remains as a tetramer structure containing 2 a chains and 2 b chains. Upon activation by thrombin in the absence of Ca^{2+} the enzyme is found also to be a tetramer containing 2 b chains and 2 modified a chains. However, when Ca^{2+} is added to the active enzyme it dissociates into a chain dimers and b chain dimers (4). The dissociation constant for Ca^{2+} estimated from the requirements for this dissociation (1 to 2mM) is close to that determined from Ca^{2+} in its function as an activator for the amine incorporation reaction (5). This suggests that it is the a chain dimer, dissociated from b chains, that is catalytically active: e) The subunits of thrombin-activated plasma transglutaminase have been separated by gel filtration in the presence of Ca^{2+}. Removal of Ca^{2+} has been shown to result in reassociation of the subunits to form a tetramer indistinguishable from that found in the absence of Ca^{2+}; f) The activation peptides, the materials formed by thrombin hydrolysis of a chains of plasma protransglutaminase, have been isolated, in direct evidence for a proteolytic activation process.

The Ca^{2+} requirements for catalytic hydrolysis and transfer of active esters by plasma and platelet transglutaminases has been examined. It was surprising to find that this requirement (K_a, Ca 50mM) is significantly higher than that for amine incorporation at glutamine residues. The opposite has been found to be true with the tissue enzyme (6).

Active site mapping studies of transglutaminases, commenced last year, have been extended, with particular emphasis on the tissue and blood enzymes. Earlier findings using straight- and branched-chain aliphatic amides indicated that glutamine substrate attaches to the enzymes with its β and γ methylene groups situated in a hydrophobic binding region and with its carboxamide group directed toward the active site -SH group of the enzyme (7). The dimensions of this binding cleft on the enzymes were estimated from atomic model studies to be about 5A X 5 A. Evidence has been obtained from inhibitor and kinetic studies that the enzymes undergo a conformational alteration at some step during catalysis, probably proceeding or together with enzyme acylation. These findings with aliphatic amides have been confirmed by using peptide derivatives of methylglutamine isomer (8). That the derivatives of L-α-methylglutamine only are substrates for transglutaminases is strong evidence for the postulated active sites. The observations with the methylglutamine derivatives have also formed the basis for a theory as to how the enzymes exert their stereospecificity, i.e., their strong preference for L-glutamine residues. This follows from the suggestion that peptide-bound L-glutamine residues are oriented on the enzyme surface with their α-hydrogen

projecting away from the surface. In the case of D-glutamine residues the α-hydrogen, abutting the enzyme surface, perturbs the normal mechanism.

A corollary to inhibition studies carried out in conjunction with active site mapping work has been the development of a general theory for inhibition in the case where inhibitor is present in constant proportion to variable substrate (9). The presentation of this theory should be of special value to investigators designing inhibition experiments. Further, this theory will enable workers to recognize inhibiting impurities in substrate preparations.

A series of heptapeptides of the general structure, X-X-X-L-gln-X-X-X, where X is glycine or L-leucine and L-gln is L-glutamine, have been prepared. The formylated derivatives of these peptides have been tested in a preliminary way in an effort to gain evidence that transglutaminases have an extended active site, and that the variations in specificity of the various classes of enzymes are a function of the amino acid residues surrounding the glutamine residue; the findings to date are very encouraging, and indicate pronounced effects of hydrophobic residues as far as three amino acid residues away from the glutamine.

Significance

A comprehensive study of various transglutaminases has shown that the enzymes are widely distributed in various organs and tissues, and that they may all be involved in the formation of ε-(γ-glutamyl)lysine crosslinks within and between protein molecules. These linkages are essential in maintaining the permanent rigid structure of many protein molecules.

The physiological importance of the protransglutaminases of plasma and platelets is well known. The finding of protransglutaminases in placenta and uterus indicates another regulatory control site of the blood coagulation process. Estrogen and progestrone regulation of transglutaminase levels in rabbit uterus has been observed (10).

The localization of specific transglutaminases in seminal plasma and prostate gland suggests a role of these enzymes in the fertilization process. Indications that inhibitors of thrombin, the activator for protransglutaminases, lower the incidence of fertilization in test animals (11) suggests that coagulation of seminal fluid is important in this process.

There is recent substantial evidence for ε(γ-glutamyl)lysine cross-links in polymeric collagen (12). It is suggested that the function of this cross-link is to reduce the hydrophilic nature of the component collagen molecules polymerized within the fibrils. It seems almost certain that a transglutaminase catalyzes the formation of these bonds in collagen.

Understanding of the molecular characteristics of the transglutaminases is vital to determination of the function of these enzymes in normal and diseased tissues, pharmacological control of activity and hormonal regulation.

The minimal substrate structural requirements for liver transglutaminase have been defined over the past year through the studies described above. We now

have a better understanding of the mechanism of enzyme-substrate interactions in transglutaminase-catalyzed reactions. The technique of active site mapping, using straight and branched chain substrate analogs, methylglutamine isomers, and large glutamine peptide derivatives is one which may be applicable to other enzymes and most certainly will be applied to studies with other transglutaminases.

Future Plans

Preliminary studies suggest a primary role for transglutaminases in wound healing. Attention will be focused on the possible interaction between fibrinogen and collagen as catalyzed by transglutaminases and the possible relationship of this interaction to wound healing.

The possible use of liver transglutaminase in localizing and determining the orientation of membrane proteins and other structural protein is under investigation.

Studies of the effect of various amino blocking groups on the reactivity of model peptide substrates will be continued. The effect of various metal ions on these activities of transglutaminases will be investigated.

There are plans to expand the substrate specificity studies described to some of the other transglutaminases currently under investigation in this laboratory.

Relevant Bibliography

1. Folk, J. E. and Chung, S. I.: Molecular and Catalytic Properties of Transglutaminases, Advances in Enzymology, in press, 1973.

2. Chung, S. I.: Comparative Studies on Tissue Transglutaminase and Factor XIII, Ann. New York Acad. Sci. 202; 240, 1972.

3. Chung, S. I. and Folk, J. E.: Transglutaminase from Hair Follicle of Guinea Pig., Proc. Natl. Acad. Sci. 69: 307, 1972.

4. Chung, S. I. and Folk, J. E.: Transglutaminases of Plasma and Platelets (Activated Coagulation Factor XIII): Effects of Calcium Ion, Abstracts, 9th International Congress of Biochemistry, Stockholm, Sweden, July 1-7, 1973.

5. Chung, S. I. and Folk, J. E.: Kinetic Studies with Transglutaminases: The Human Blood Enzymes (Blood Coagulation Factor XIII) and the Guinea Pig Hair Follicle Enzyme., J. Biol. Chem. 247: 2798, 1972.

6. Folk, J. E., Cole, P. W. and Mullooly, J. P.: Mechanism of Action of Guinea Pig Liver Transglutaminase III. The Metal-dependent Hydrolysis of p-nitrophenyl acetate: Further Observations on the Role of Metal in Enzyme Activation, J. Biol. Chem. 242: 2615, 1967.

7. Gross, M. and Folk, J. E.: Mapping of the Active Sites of Transglutaminases I. Activity of the Guinea Pig Liver Enzyme toward Aliphatic Amides. J. Biol. Chem. 248: 1301, 1973.

8. Gross, M. and Folk, J. E.: Mapping of the Active Site of Transglutaminases II. Activity of the Guinea Pig Liver Enzyme toward Methylglutamine Peptide Derivatives. J. Biol. Chem., in press 1973.

9. Cleland, W. W., Gross, M. and Folk, J. E.: Inhibition Patterns Obtained Where an Inhibitor is Present in Constant Proportion to Variable Substrate, J. Biol. Chem., in press, 1973.

10. Laki, K., personal communication.

11. Zaneveld, L. J. D., Polakoski, K. L., Robertson, R. T. and Williams, W. L.: Trypsin Inhibitors and Fertilization, in Protease Inhibitors in Mammalian Fertilization, Academic Press, Inc., N. Y., 1971.

12. Stevens, F. S., Coy, G. R. and Jackson, D. S.: Evidence for Intra-Chain Masked Carboxyl Groups in Polymeric Collagen, Biochim. Biophys. Acta 271: 114, 1972.

Summary Report of the Protein Chemistry Section*
Laboratory of Biochemistry
National Institute of Dental Research

Background

Collagen is the major structural protein of all vertebrate and many invertebrate species. It is found not only in the major connective tissues, skin, tendon, bone and cartilage, but is an important component of tissues such as basement membranes, cornea and major blood vessels. The basic structure common to all or most collagens is a rod-like molecule about 3000 x 15A composed of three polypeptide chains of about 1000 amino acids each (95,000) molecular weight). Each chain is helical throughout most of its lengths and the three chains are coiled together in a major helix. The major interstitial collagens contain two kinds of chains (two $\alpha 1$ and one $\alpha 2$) but other collagens have identical chains.

Superimposed on this basic structure are variations inherent in the structural genes or added during the biosynthetic steps following translation. These include differences in amino acid sequence, degree of hydroxylation of proline and lysine, amount of attached carbohydrate, proteolytic alterations at the ends of the chains, amount and location of lysine- and hydroxylysine-derived aldehyde, and (in the fibril) the type and degree of covalent cross-linking. These and other aspects of collagen chemistry and structure have been recently reviewed (1).

Progress

Certain aspects of the broad problem are presently being emphasized in the program of the section.

These include: 1. The isolation of peptides with cross-links and determination of the positions of the cross-links in the sequence. 2. Species and tissue comparative studies on collagen chemistry. 3. The role of the amino-terminal, non helical regions of the α chains in the specific alignment of molecules in the fibril. 4. Computer analysis of the amino acid sequence of collagen to elucidate the origins of molecular packing. 5. Cross-linking of collagen in implants. 6. A nuclear magnetic resonance study of collagen structure. 7. A similar NMR study on elastin.

An amino acid sequencing laboratory has been established. One purpose of the facility is to locate the amino acid residues involved in intermolecular cross-linking. It is known from earlier studies that lysyl and hydroxylysyl residues near the amino- and carboxyl-termini of the $\alpha 1$ and $\alpha 2$ chains of collagen are involved in cross-linking after conversion to aldehydes. To find the other side of the cross-links, double-chain peptides containing cross-links are being isolated. By comparison with the peptides from soluble collagen not containing cross-links, the exact residues involved can be determined. Studies have so far shown that cross-links usually involve three or more chains and several sites along each chain.

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*This report is based upon progress achieved during the year in Project Nos. NIDR-LBC10152 and NIDR LBC10262

The fact that the major collagens of higher animals contain two different kinds of chains suggests that the difference is important to molecular and/or fibril structure. Computer analysis of a part of the α2 chain sequence has shown that it is very similar to the α1 chain and that both evolved from a common precursor (2). Further studies of the sequence should elucidate the presumptive advantage conferred on a collagen containing two types of chain over one having identical chains.

Chemical studies have so far been confined largely to collagens from higher animals. To understand function, it is important to have data from lower animals. Presently, two such collagens are being studied. The one from codfish skin shows most of the characteristics of mammalian collagens. The collagen from ascaris cuticle, on the other hand, is very different.

Although collagen is largely helical in conformation, it contains regions at the amino- and carboxyl-termini that have a different type of chemistry. These regions have special properties and perform special functions, the best established of which is that of serving as a site of cross-link formation. There is also indirect evidence that these regions may play an important role in the specific interactions that are involved in fibrillogenesis. The details of which are now being studied in an in vitro system where rates of aggregation can be followed. Peptides containing the N-terminal region when added to collagen under aggregating conditions inhibit aggregation. However, the large concentrations necessary suggest that the effect is non-specific. Many other substances inhibit or accelerate aggregation by poorly understood mechanisms.

X-ray diffraction studies of rat tail tendon fibers have shown that collagen in this tissue is packed in a highly ordered fashion. The concept that the primary structure of a polypeptide chain carries the information that determines its three-dimensional structure suggests that it should be possible to understand and observe aspects of the origins of molecular packing of collagen by analysis of the amino acid sequence of the α chains. The sequence of the α1 chain of rat and calf skin collagen is now available and its analysis is in progress. Since there are 1052 residues in the α1 chain, computer analysis is required to make the many comparisons necessary. Preliminary results assuming a one-dimensional molecule charge interactions and hydrophobic interactions are maximal when molecules are staggered by multiples of 234 $\pm$ residues (3). This result is consistent with the stagger of 670 A determined by electron microscopy and x-ray diffraction. It should be possible to extend the analysis to three-dimensions and hopefully determine the helical parameters that describe the exact structure.

Cross-linking of collagen in vivo is being studied at the Hadassah School of Dental Medicine under a PL 480 agreement (No. 06-041-1, Amendment 2) as an extension of the laboratory program. Purified collagen of various types when implanted in special chambers in experimental animals will continue to cross-link. The process can be followed easily since the implants can be readily removed and analyzed. Support for this study under the PL 480 program ends this fiscal year.

Although the helical structure of collagen is quite well understood in outline, details of the factors that enter into molecular stability have long been argued. Nuclear magnetic resonance provides a new approach to this problem since it is sensitive to restrictions on the movement of atoms (protons or carbon atoms) and can therefore determine the state of certain functional groups in collagen in the native and denatured state. Proton magnetic resonance studies on the native and denatured states of α1-CB2, a well-characterized peptide of 36 residues from collagen, and α chains suggest that side chain interactions may be involved in stabilizing the molecular structure. Specifically, NMR measurements of single-chain helical structures such as polyhydroxyproline show much greater mobility of the pyrrolidine rings than is seen in collagen. The three-chain hydrogen bonded structure as well as the stereochemical restrictions imposed by the pyrrolidine rings and side chain interactions is therefore critical to stability.

Similar studies on elastin structure utilizing carbon magnetic resonance have been initiated. It has been shown (4) that the polypeptide chains of elastin in the solid state have mobilities approaching those of random chains in solution. This indicates that kinetically free chains are the basis for the elasticity of elastin as in the case of rubber and similar elastomers. This study as well as the study on collagen is a collaborative study with the Polymer Division, National Bureau of Standards and is supported by Interagency Agreement No. NIDR-13.

Significance

Since the biological role of collagen is largely structural, a knowledge of its structure is necessary to an understanding of its function. As basic knowledge is obtained it can be directly applied to biological and medical problems. This is already occurring in the connective tissue field. The number of programs in orthopedics, dermatology, rheumatology, genetic disorders, calcification, wound healing, inflammation, ageing and dental medicine that are studying collagen and elastin at the molecular level and the enzymes involved in the biosynthesis and degradation of collagen is rapidly increasing. The program of the Protein Chemistry Section has not only contributed information valuable to these efforts but has been an important source of training for investigators entering these health oriented areas.

Future Plans

No fundamental changes in direction are planned. Major emphasis will be placed on amino acid sequencing, particularly with regard to the location of cross-links in collagen, on characterization of collagens from lower animals on NMR studies of collagen and elastin structure, and on computer analysis of the primary structure of collagen.

Relevant Bibliography

1. Traub, W. and Piez, K. A.: The chemistry and structure of collagen. Adv. Prot. Chem. 25:243-352, 1971.

2. Piez, K. A., Balian, G., Glick, E. M. and Bornstein, P.: Homology between the α1 and α2 chains of collagen. Biochem. Biophys. Res. Communs. 48:990-995, 1972.

3. Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A. and Woodhead-Galloway, J.: Analysis of the primary structure of collagen for the origins of molecular packing. J. Mol. Biol., in press.

4. Torchia, D. and Piez, K. A.: Mobility of elastin chains as determined by ^{13}C nuclear magnetic resonance. J. Mol. Biol., 76: 419-424, 1973 .

Summary Report of the Laboratory of Biological Structure
National Institute of Dental Research

Despite continuing constraints on personnel and facilities the Laboratory has maintained a notable level of research accomplishment during the past year. In concert with the Laboratory's functional mission, its staff has persevered in a wide-ranging series of basic and applied research investigations, all having in common the goal of characterizing structural-functional relationships, knowledge of which should clearly facilitate understanding of a variety of normal and abnormal biological processes affecting oral health. As in the past, progress for the year is reported in the sectional summaries which follow below.

In view of the existing diminution of resources, implementation of research activities has necessarily involved an ever-broadening system of collaboration with other units both within and outside of the Laboratory. The mutual benefits accruing from this type of doing business are evident in the summary reports from this Laboratory as well as in those from other NIDR research elements. Further, the Laboratory has for the first time undertaken collaborative programs outside of NIH through the contract mechanism. Assuming continued stringencies locally, it is anticipated that even more use will be made of the contract instrument in the future. For it seems clear that the strengths of the Laboratory have now reached a state of attenuation such that our capacities for making research progress will depend almost completely upon yet farther broadened collaborative arrangements.

During the reporting year, the professional staff of 7 Senior Investigators, 2 Staff Fellows and 3 Guest Workers submitted 13 manuscripts and 8 abstracts for publication. As in the past, members of the staff were identified for particular scientific recognition, as reflected by invited participation in national and international symposia and workshops; in editorial tasks; and in scientific review functions relating to NIH and other granting agencies. Finally, the Laboratory Chief, Dr. Marie U. Nylen was accorded a singular and richly deserved honor during the year, namely the degree of Doctor of Odontology (hon.caus.) awarded by the University of Copenhagen, of which she is a graduate. (RCG)

Summary Report of the Experimental Morphology Section*
Laboratory of Biological Structure
National Institute of Dental Research

The basic objectives of the Experimental Morphology Section are to advance knowledge concerning the structure and function of normal and experimentally altered tissues and cells. Light and electron microscopy, electron diffraction, microradiography, enzyme cytochemistry, extra-and intra-cellular tracers as well as specific chemical detection methods are the principal technique which are used.

Studies presently underway include: (1) the effect of tetracycline on molars and incisors in rats of various ages; (2) ultrastructure and cytochemistry of secretory and post-secretory ameloblasts in incisors of young rats; (3) ultrastructure and cytochemistry of salivary glands and (4) biochemical and ultrastructural studies on tissue fixation by protein crosslinking reagents. Progress in three of these areas will be described in more detail in this summary report.

Previous investigations in this laboratory have shown that single intraperitoneal injections with 130 mg tetracycline hydrochloride (TC) per kg bw give rise to 100% gross enamel lesions in the incisors of 75-day-old rats. Australian investigators, on the other hand, have described gross enamel lesions in rat molars following much smaller dosages. These investigators suggested not only that molars are more sensitive to TC than incisors but also that the sensitivity of the incisors varies with age. Differences in experimental condition, however, made it difficult to compare their data with ours. Since they also suggested that the rat molar would be a more suitable object than the incisor for routine screening of drugs suspected of affecting the human dentition it was considered important to repeat and expand their experiments.

To date our experiments have shown that a single dose of 200 mg per kg bw or three injections, 24 hours apart, of 75 mg per kg bw consistently cause gross lesions in the incisors of 4-day-old rats. In contrast the molars in rats of the same age develop 100% lesions following a single administration of 130 mg per kg bw or three injections, 24 hours apart, of 50 mg per kg bw. Giving dosages of 130 mg per kg bw to animals ranging in age from 4 to 75 days shows that it is not until the animals reach age 75 days that they develop 100% gross incisal lesions. Measurements of serum concentrations in 4 and 75-day-old animals which received 130 mg per kg bw reveal that the TC concentration is higher in the 75 than in the 4-day-old animals during the first two hours after the single injection although the difference is not significant. After 6, 24 and 48 hours, however, serum levels of TC in the 4-day-old rats are significantly higher than those in the older animals. After six days no TC can be measured in the serum of the adult rats while the young ones have a concentration of 3 µg/ml serum. The sustained higher levels of the drug in the

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*This report is based upon progress achieved during the year in project Nos. NIDR-LBS101b63, NIDR-LBS111b68, NIDR-LBS112b71, NIDR-LBS121b73.

blood of the younger animals are also apparent morphologically in that the dentin of these animals is much more extensively labeled than the dentin of the 75-day-old rats. Administration of 200 mg per kg bw to 4-day-old animals, a dose which consistently gives rise to gross lesions in these animals, results after one hour in serum levels similar to those measured in 75-day-old animals one hour after they received 130 mg per kg bw.

Our data thus confirm that, in the rat, molars are more sensitive to TC than incisors and that a distinct variation exists with age in the effect of TC on incisor development. The variations in response between molars and incisors may be caused by a difference in tissue distribution of the antibiotic although considering the early and rapid development of the molars in these animals, the rat molar enamel organ may be more sensitive than the incisor enamel organ. That no tooth in the human dentition forms under similar circumstances contradicts the contention that the rat molar is a suitable object for routine screening of drugs suspected of affecting the human dentition.

Future studies will be aimed at identifying cellular events associated with the development of gross enamel lesions in response to injections of TC. In addition the extent and surface morphology of these defects will be explored using our recently acquired facilities for scanning electron microscopy.

The basic objective of the study of salivary glands is to provide a better correlation between structure and function in secretory cells. Cell fractionation, autoradiography, and in vitro systems have provided a general concept of protein synthesis, transport and secretion in animal cells, but specific details of the process are lacking in many areas. Efforts this past year have been concerned with cytochemical demonstration of peroxidase activity since detection of this enzyme cytochemically presents an opportunity for careful study of the cellular compartments concerned with protein synthesis and transport. Thus far, results have mainly been on a technical level, i.e., determining proper fixation and incubation procedures to obtain optimum reaction conditions. An interesting finding is that the acinar and duct cells of all the salivary glands, the lacrimal gland and the exocrine pancreas contain organelles called peroxisomes. These organelles were previously thought to exist only in liver and kidney cells. The function of this organelle is largely speculative, but it has been suggested that it plays a role in carbohydrate and cholesterol metabolism, and may protect the cell against high levels of hydrogen peroxide.

In addition to the above studies, the ultrastructural cytology of the different cell types in the three major salivary glands of the adult hamster has been investigated. Parotid acinar cells, intercalated duct cells of all three glands, convoluted granule tubule cells of the submandibular gland (CGT), and demilune cells of the sublingual gland all contain electron dense, zymogen-type granules. With the exception of the CGT cell, all of these cells have an extensive rough surface endoplasmic reticulum surrounding an irregularly shaped, basally located nucleus, numerous polysomes and vesicles, mitochondria, and Golgi complexes in relatively sparse numbers. Numerous fibrous bundles are also seen which probably are involved in maintaining the shape of the cells. The sublingual acinar cells have the usual complement of cellular

organelles and are packed with discrete or fused electron lucent granules. Since there is no polarity to the type of formations found in each acinar cell it is believed that the fusion of granules may be an artifact of fixation or preparation. Evidence was obtained suggesting that mucin granules are extruded together with part of the plasma membrane after fusion of the outer leaflets of the granule limiting membrane and the plasma membrane. Submandibular acinar cells are similar to sublingual acinar cells although their granules sometimes are more electron dense and rarely appear to fuse. Both types of mucous acinar cells contain very few fibrous bundles suggesting the latter are not required for maintaining cell shape in this type of cell or -- if the presence of the fibrils is related to cell secretion -- that mucous extrusion is not accompanied to any degree by any contractive force. Although mucous cells are said to contain only glycoprotein and to lack peroxidase, our studies indicate that the adult hamster sublingual gland does synthesize peroxidase and that this peroxidase appears to be finally localized in the mucin secretion granule.

The studies on hamster salivary glands will be discontinued at this laboratory due to the departure of the principal investigator. Our other efforts in this area, however, will continue. Future plans include the utilization of appropriate cytochemical methods to elucidate the role of various organelles in the functions of the cell and the development of new histochemical methods for the detection of lipase activity.

The studies concerning the development of new fixation procedures are directly associated with our interest in cytochemistry. Although glutaraldehyde and formaldehyde are wisely and successfully used in morphological and cytochemical studies, other protein crosslinking reagents have been largely ignored as possible tissue fixatives. One particular class of bifunctional crosslinking reagents, the diimidoesters, appear particularly promising, since they are water soluble, can be prepared with various internal chain lengths, do not alter protein charge, and have been shown to cause little or no alteration of the enzymatic and immunological properties of certain purified proteins. In order to determine optimum conditions for fixation with these compounds, a simple assay for "crosslinking" was developed based on the assumption that the aggregates formed by crosslinking would be water insoluble and could be easily separated from the remaining soluble proteins by homogenization and centrifugation. Rat liver was chosen as our model system, and a correlated biochemical ultrastructural, and cytochemical study was undertaken.

Using this crosslinking assay, optimum conditions for fixation with the diimidoester dimethyl suberimidate (DMS) were determined. Best results produced an insoluble fraction of 92.1% of the protein, while only 3.3% of the protein diffuse out of the block during fixation. Ultrastructural studies indicate that the preservation of the tissue is comparable to that obtained with various aldehyde fixatives although some swelling of smooth endoplasmic reticulum and Golgi cisternae usually occurs. Cytochemical studies indicate good localization and retention of the activity of glucose-6-phosphatase, thiamine pyrophosphatase and peroxisomal catalase. Preliminary quantitative determinations of enzymatic activity indicate that DMS retains about three times more activity of glucose-6-phosphatase than does glutaraldehyde. Light microscope cytochemical studies suggest that DMS may be the fixative of choice

when the detection of aldehyde groups is desired, as with the PAS or Feulgen procedures, since excellent cytological detail is obtained without the introduction of new aldehyde groups into the tissue.

It is planned to complete the quantitative enzymatic and cytochemical studies on DMS-fixed tissue and to initiate new work on the biochemical aspects of tissue fixation.

Summary Report of the Experimental Pharmacology Section*
Laboratory of Biological Structure
National Institute of Dental Research

The incidence of oral-facial congenital malformations in the human population is second only to those of the heart. In addition, these malformations are a great drain on the budget, not only because of the expenses involved in surgical repair, but much more so in rehabilitation of the patient so he, or she, can become a useful member of society.

Congenital malformations occur not only in the human species but in all animal species. It is true that the majority of them are probably of genetic origin but it has also been amply demonstrated that a great number are induced by (1) environmental contaminants, such as dioxan in 2, 4, 5-T insecticide sprays; (2) toxic plants eaten by grazing animals, such as Veratrum Californicum and the loco weed that has resulted in a great economical loss to agriculture; (3) viruses, such as rubella and finally; (5) medications, or drugs, taken at critical periods of gestation. A good example of this is the thalidomide induced phocomelia.

Investigations carried out in the section started about 12 years ago when the great majority of scientists in the field were convinced that the human embryo developed in an impervious environment. Then in 1962 the thalidomide tragedy made it all quite evident that this supposition was incorrect and that indeed the placenta was not an impermeable barrier.

The aim of the Experimental Pharmacology Section is not only to induce specific oral facial malformations but to do so in a reproducible manner in order to be able to study the metabolism, placental transfer and binding of the drug or contaminant used. In this way then one can approach investigations on the etiology of the malformation induced, not only at gross morphological levels but also the biochemical and molecular pathways by which they may act.

In the past we have been able to induce the malformation of cleft palate in animal strains that normally do not have a spontaneous incidence of the defect. This has been accomplished by the administration of (1) Vitamin A, (2) Lathyrogens or (3) Benzhydrilpiperazines. In all of these instances the defect was associated with other skeletal aberrations. During the past year we have been able to induce the malformation of cleft palate alone for the first time. This was done by the administration of specific phenothiazine derivatives at critical stages of organogenesis. These agents are active in the rat, mouse and rabbit.

Preliminary results indicate that in order for a phenothiazine derivative to be teratogenic it has to contain a piperazine ring in its molecular structure

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*This report is based upon progress achieved during the year in project Nos. NIDR-LBS401b63, NIDR-LBS411b70, NIDR-LBS21b72, NIDR-LBS441b73, NIDR-LBS431b63.

and it has to be administered only at critical stages of palatal growth (days 12 to 15). Prolonged administration stimulates the enzymatic breakdown of the compound and negates its teratogenic action.

Administration of these compounds during early gestation (days 0 to 5) inhibits nidation and if they are administered for prolonged periods gestation can be prolonged from the normal 21 days to up to 36 days in the rat and from 19 to 25 days in the mouse. The young that are born from the prolonged gestation appear grossly normal and are able to reproduce once they attain sexual maturity.

Studies are underway to determine the various mechanisms of action of these compounds. Of particular interest is the development of the fertilized ovum that is prevented from implanting in the endometrium for such a prolonged period of time and yet is able to develop to an apparently normal fetus.

The role that growth plays in the formation and development of the palatine shelves has been a relatively unexplored area. Last year a study was initiated which involved quantitatively measuring the growth of the palatine shelves and the effect that teratogens may have on this growth. Techniques were designed to follow the growth of the palatine shelves in terms of DNA, and RNA accumulation from early day 14 to early day 17. Preliminary studies indicate that Vitamin A and Diazo-oxo-norleucine (DON) significantly reduced the accumulation of DNA, RNA and proteins while the Lathyrogen β-amino propionitrile (BAPN) did not affect any of these growth parameters. These findings indicate that certain cleft palate teratogens may act by inhibiting the proliferation of cells which comprise the palate.

The Lathyrogen BAPN administered as a single dose on day 15 of gestation induces cleft palate in 100% of the offspring and it was reported last year that BAPN temporarily inhibits the crosslinking of collagen in the palatal shelves.

Investigations are currently underway to determine the localization of the collagen fibrils before and after shelf rotation. This is being done by specifically staining the fibrils with silver methenamine and examining their distribution under the electron microscope. Preliminary results indicate that late on day 14 the fibrils are most commonly located between mesenchymal cells adjacent to the oral epithelium or against the nasal epithelium. However, by early day 16 just prior to rotation, one also notes the appearance of fibrils closely associated with the basement membrane of the oral epithelium suggesting that these late appearing fibrils might be involved in the rotation of the shelf. Parallel studies using the light microscope demonstrate that a PAS positive material is present in the intercellular spaces adjacent to the nasal epithelium on day 16 but not on day 15, furthermore the distribution of this PAS positive material is disrupted in animals treated with BAPN. Presently work is being carried out to identify the nature of the PAS positive material.

Palatine shelf epithelial cell breakdown and fusion. The breakdown of fused epithelial cells represents one of the few instances during embryo genesis

when the death of epithelial cells contributes to the formation of an adult structure. The Epidermal Growth Factor (EGF), extracted from male salivary glands by Dr. Stanley Cohen, stimulates epidermal cell proliferation and keratinization. Studies are now in progress to determine the effect of EGF on the breakdown of fused epithelial cells. Preliminary results indicate that EGF can prevent the breakdown of fused epithelial cells in tissue culture suggesting that cleft palate may also be produced by the inhibition of epithelial death cell. Work in progress will determine if the same situation exists in vivo.

The surface properties of the palatal epithelium are being investigated by using Concanavalin A (Con A) which is a specific carbohydrate binding protein. A procedure has been developed in this laboratory, which allows the visualization of Con A binding sites in frozen tissue sections and experiments are underway to determine the effect of different teratogens on the palatal shelf and the nature of the proposed "sticky substance" on the prefusion shelves. This material appears to be a glycoportein(s) synthesized on the surface on the fusion epithelium between days 15 and 16. At present EM studies are being carried out to determine the binding of Con A to the palate at specific times, furthermore other studies are planned on other embryonic tissues, to ascertain whether these glycoproteins on the fusion surface are specific for this area, and whether they are important in the initial fusion of the palatal shelves since a failure to achieve initial contact between the shelves would lead to a cleft palate due to the rapid lateral growth of the head.

It has been demonstrated in this laboratory that hyaluronic acid (HA) is the predominant acid mucopolysaccharide (AMPS) present in the rat and mouse palatine shelves just prior to and during rotation. Experiments are now in progress to determine what role, if any, HA plays during palatogenesis.

Benzhydrilpiperazine teratogenesis. Previous reports from this laboratory have established that norchlorcyclizine (NorCC) is the metabolic moeity that is responsible for the teratogenic action of the benzhydrilpiperazine drugs. In addition it was also reported in 1972 that NorCC may act by competitively inhibiting the binding of calcium in the developing embryo. Further studies using equilibrium dialysis techniques have shown that Ca^{+2} and positively charged NorCC bind to hyaluronic acid and chondroitin sulfate and that the presence of either ion inhibits the binding of the other. For these reasons, the synthesis and distribution of acid mucopolysaccharides (AMPS) were determined in embryonic tissues during development. AMPS were radioactively labeled in vivo by intrauterine application of ^{3}H glucosamine to day 13, 14, or 15 control embryos or embryos from rats treated with chlorcyclizine. The animals were killed 24 hrs later and the embryonic areas affected by the chlorcyclizine treatment (i.e., palatal shelves, mandibles and limbs) were dissected out and the individual AMPS isolated by DEAE-cellulose chromatography. The results indicate that the synthesis of AMPS in the palate was greatly altered after chlorcyclizine treatment as compared to control specimens. Chromatographic peaks normally containing hyaluronic acid and chondroitin sulfate were reduced from the control by approximately 80 and 60% respectively, while 70% of the radioactive macromolecular material present was contained in a peak that preceded the known AMPS peaks. In contrast to the

different AMPS "profiles" of control and experimental palates, the quality of AMPS synthesized in the mandible and limb appeared to be the same in the control and experimental situation, however, the synthesis of AMPS was reduced in these tissues after chlorcyclizine treatment.

Studies are in progress to identify the macromolecular material synthesized after chlorcyclizine treatment and to investigate its role in the development of the palate.

In conclusion, the work of the Experimental Pharmacology Section has centered on studies of the etiology of various types of drug induced cleft palate, particularly in investigating the presence and function that certain extracellular macromolecules play in palatogenesis. The three major classes of macromolecules discussed were the acid mucopolysaccharides, collagen and glycoproteins.

Lately, it has been repeatedly stated by a number of outstanding scientists in the field, that the effect of drugs or environmental contaminants in the unborn child will have to be determined in man. We contend that, like in many other situations, animal models can be used, and the data obtained intelligently translated to human experience.

Summary Report of the Molecular Structure Section*
National Institute of Dental Research

The Molecular Structure Section was established to investigate the physico-chemical and ultrastructural properties of calcified tissue and its collagenous and mineral components. Major attention is also focused on naturally occurring and synthetic calcium phosphate compounds related to the mineral components of hard tissue. During the past year research efforts were nearly equally divided between studies on calcified tissues per se and studies on synthetic analogues to the mineral phases investing these tissues.

Amorphous calcium phosphate (ACP) is the initial solid to form in the in vitro precipitation of calcium phosphates from physiological-like solutions. This compound is also a major component in hard tissues and is believed to be the precursor to biological apatite. Electron microscopic studies conducted during the past year demonstrated that the formation and transformation of ACP from aqueous solutions is a complex series of events involving the unstructured coalescence of calcium and phosphate ions from supersaturated solutions followed by a continuing dehydration of the resulting highly solvated calcium phosphate complex, with conversion to anhydrous apatite as the final step. This picture of progressive disolvations casts doubt on the applicability of classical nucleation and growth theory to describing the mineralization events leading to apatite formation in vivo.

The associated chemical events leading to apatite formation are also quite complex. It is well known that the Ca/PO_4 molar ratio of apatite is higher than for its amorphous progenitor. Carbonate levels in the precipitated solid, on the other hand, were found to either rise or fall upon conversion of ACP to apatite, depending on the concentration of carbonate in solution. In carbonate-free preparations, no significant change in HPO_4^{2-} levels were found upon conversion. Unexpected difficulties were encountered, however, when the standard assay method for HPO_4^{2-} was applied to carbonate-apatites. It was found that carbonate interferes with $P_2O_7^{4-}$ production upon sample pyrolysis, an essential step in the assay. Since the assay in question is the only available method for quantitative analysis of HPO_4^{2-} in hard tissues, the presence of appreciable carbonate in these tissues raises the possibility that previously reported chemical measurements for HPO_4^{2-} are largely associated with the ACP component.

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*This report is based upon progress achieved during the year in Project Nos. NIDR-LBS200b70, NIDR-LBS201b73, NIDR-LBS202b70, NIDR-LBS203b73, NIDR-LBS204b73, NIDR-LBS205b73, NIDR-LBS206b63.

The structural nature of dried ACP continues to be a moot question. The dispute is centered about whether this material is truly non-crystalline or possesses residual crystalline order not detectable by current methods used to establish the crystalline nature of solids. Infrared, far infrared and laser Raman spectroscopy studies conducted during this past year indicate that the local atomic environments in ACP are only minimally affected by nearest neighbor groups, a perturbation completely explainable from considerations of coordination chemistry principles. No evidence of macrocrystalline order was observed in these studies.

Infrared spectroscopy studies completed this past year have demonstrated that biological apatites and synthetic apatites prepared at pH 7.4 are severely deficient in hydroxide ions, contain considerable internal distortion, and have a significant portion of their carbonate on the crystal surface. When recrystallized by boiling or heating in air, these apatites picked up OH^- ions, altered the carbonate environment, and selectively removed internal distortions, i.e., crystals were no longer structurally representative of the original apatite. Since the spectroscopic properties of these altered apatites were now typical of well-crystallized apatites in general, it was concluded that rigorous structural extrapolations to biological apatites should not be made from idealized, well-crystallized compounds such as those obtained from geological sources or prepared by high temperature syntheses.

In addition to the above described investigations into the structuro-chemical nature of isolated calcium phosphate systems, studies were conducted during the past year on calcium phosphate-macromolecular complexes. It was found from infrared, Raman, and electron spin resonance spectroscopic examination that basic amino acid side chains bind to phosphate groups located in the mineral phase and acidic side chains bind to the mineral through calcium bridges. With neutral polymers, mineral phosphate groups bind to the carbonyl oxygen within the peptide bond itself by calcium bridges. Electron microscopy revealed that the strong affinity between the mineral and the polymeric molecules resulted in more compacted clusters of ACP spherules than normal, and, similarly, in more closely packed arrays of apatite crystals. Frequently, the ACP-polymer co-precipitates exhibited very little, if any, electron beam damage at exposure levels which normally resulted in the formation of electron-lucent centers in pure ACP controls. These data indicate that the mineral in calcified tissue may possibly be attached to any number of functional sites on the surrounding matrix protein. The electron spin resonance spectroscopy was done in collaboration with Dr. I. Pullman and associates at New York Medical College.

While it appears that multiple sites exist in the matrix of calcified tissue for the binding of mineral particles, the effect of this bound mineral in altering the properties of matrix collagen may not be as severe as some investigators have suggested. A recently completed study on the mineral-collagen relationships in turkey leg tendon demonstrated that the presence of apatite crystals did not affect the ability of the collagen molecules to adjust their lateral intermolecular distances to changing water levels in this tissue. The apatite crystals were neither wedged in between the adjacent molecules comprising the microfibrillar unit of collagen nor were they so

tightly ringed about the microfibril as to squeeze the molecules within the latter into a tight anhydrous bundle. Therefore, despite the possibilities for chemical union and the fact that the crystals appear to be peripheral to the microfibrils, the apatite is not imposing a restraint on the ability of the microfibrils to swell in water. Another interesting result from this study was the finding that there is no free water or water loosely bound to the mineral fraction in calcified tendon. The majority of the water in this tissue goes to hydrate the collagen component only. These data would suggest that the vast majority of hard tissue mineral is not in aqueous communication with the body fluids.

As part of a study on the X-ray diffraction properties of bone mineral in disease, the hydrazine procedure described in FY 1972 Annual Report was used to deproteinate a series of bones from rats with chronic uremia. Results revealed smaller apatite crystals and an increased ACP fraction in the bone mineral of the uremic rats as compared with age-matched, pair-fed control animals. This retardation in bone mineral maturation became progressively more severe the longer the uremic state was maintained. To facilitate data collection and processing, the x-ray diffraction equipment used in this study was automated and interfaced with the NIDR computer during the past year. This study is being conducted in collaboration with Dr. Louis V. Avioli, Washington University Medical School, St. Louis, Mo.

In collaboration with Dr. M. H. Gottlieb, NIAMDD, lamellar lecithin-water mesophases are being studied by x-ray diffraction procedures. During the past year it was found that the crystalline-liquid crystalline transitions of dipalmitoyl lecithin-water mixtures were not thermally sharp but took place over a temperature range of several degrees. This transition behavior is empirically similar to that observed by others with bacterial membranes.

Two contractural agreements were finalized during this past year. One, with Dr. A. Franklin of the National Bureau of Standards, is concerned with the preparation and physical properties of large single crystals of hydroxy-apatite and related calcium phosphates. These well characterized apatites will be used as spectroscopic reference materials. The other contract is with Dr. I. Pullman, New York Medical College, and involves electron para-magnetic resonance measurements on mineral-protein complexes.

The future plans of the section are varied and involve the initiation of new projects as well as the continuation of current research efforts. Among the present projects to be continued and, in many cases, extended are:

(1) Chemical and electron microscopic studies on synthetic calcium phosphates with emphasis on recrystallization phenomena. Planned are investigations into the recrystallization events which occur upon exposing a crystalline calcium phosphate such as apatite to an aqueous environment designed to produce instabilities at the crystal-solution interface. The results from such studies may yield useful information on how enamel crystal-lites are altered by exposure to oral fluids.

(2) Infrared and Raman spectroscopy studies on calcium phosphates. Included here are plans for investigating fluor-hydroxyapatite, carbonate-apatite and dental enamel.

(3) Spectroscopic and electron microscopic studies of mineral-polymer complexes. Future work will focus on collagen-mineral and dentin phosphoprotein-mineral interactions.

(4) X-ray diffraction studies on hard tissue mineral. The plan is to extend current studies to include human diseases such as osteopetrosis and osteogenesis imperfecta, and toxicity effects of tetracycline and fluoride on bone and dentin mineral maturation.

(5) All collaborative efforts.

An entirely new project currently under development is a chemical investigation of the interactions of monofluorophosphate (MFP) with amorphous calcium phosphate and crystalline apatite. To be examined are the extent of MFP adsorption on these salts, the stability of adsorbed MFP, and the inhibition of MFP adsorption by other anionic species such as fluoride, sulfate, and condensed phosphates. The reactivity of MFP will be correlated with the composition, texture, and prior chemical history of the calcium phosphate salts used. From the results obtained in this study it is hoped that a better understanding of the effectiveness of MFP as an anticaries agent will emerge.

Summary Report of the Structural Interactions Section*
National Institute of Dental Research

The basic objectives of the Structural Interactions Section continue to revolve around interest on plaque deposits and may be grouped into three general areas (1) predominant organisms, (2) their polysaccharide by-products, and (3) development of methods for control of plaque deposition on both hard and soft tissues. This interest is particularly well documented by our continuing efforts to further evaluate the usefulness of fluorescent antibody techniques and radioimmune assays for rapid identification and quantitation of specific microorganisms in plaque. Polysaccharides from oral bacteria have long been of concern to our group due to their importance in the adherence of the microorganisms to the tooth. New methods of studying these products have been developed recently opening up other avenues of approach toward fruitful investigations. Studies on control of microorganisms important in the caries process have focused on investigations into the mode of action of fluoride. A project which has just been completed examined the problem of possible immunization against caries active streptococci.

The development of methods for the rapid detection of oral Actinomyces and actinomycete-like bacteria remains a prime undertaking of this section. To date fluorescent antibody reagents have been prepared for A. viscosus serotype 2, A. naeslundii serotype 1, and serotypes 1 and 2 of A. israelii. Fluorescent antibody conjugates are currently being developed and evaluated for A. naeslundii serotype 2, Actinomyces sp. strain N_{16} which may be a third serotype of A. naeslundii, and Bacterionema matruchotti. In addition an interagency agreement with the Mycology section of CDC has been initiated for the development and evaluation of FA conjugates for A. odontolyticus, Arachina propionica and Rothia dentocariosa.

Investigations utilizing the plant lectin Concanavalin A (Con A) have demonstrated that this compound is a useful tool in studies concerning the molecular structure of various bacterial polysaccharides since it binds to glucose, fructose or mannose moieties. These saccharides constitute a liberal portion of many of the polysaccharides produced by oral organisms and appear to be significant in the etiology of caries and periodontal disease. We have initiated studies using Con A to determine if some common denominator exists between polysaccharides which can be related to the disease process. To date Con A has been reacted with numerous strains of oral streptococci and filamentous diphtheroids grown in either sucrose, glucose, fructose or maltose. Results indicate that significantly more Con A is bound by cells grown in sucrose than in either of the other sugars. The amount of Con A bound by cells grown in glucose falls within a narrow range only slightly different from that bound by cells grown in fructose and maltose. The difference may be related to the organisms ability to adhere and cause disease in animals fed sucrose. An example of this possibility which is being actively pursued

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*This report is based upon progress achieved during the year in Project Nos. NIDR-LBS301b73, NIDR-LBS302b73, NIDR-LBS321b73, NIDR-LBS320b69.

in this laboratory is that, at least in one instance, a caries active, adhering strain of S. mutans binds approximately twice as much Con A as does a caries inactive non-adhering mutant after growth in sucrose media. The use of compounds such as Con A may afford an opportunity to rapidly survey organisms for similarities or differences related to the etiology of oral disease.

The anti-caries activity of various fluoride compounds has been under investigation for many years with few concrete results concerning the mode of action of this widely used agent. Several different alternatives as to its action have been proposed. One hypothesis of considerable merit is that fluoride present in the tooth interferes with the metabolism of the organisms in the plaque resulting in cell death with subsequent diminished acid production. For this to be true, the bound fluoride must be made available and be concentrated within the plaque until a concentration is reached which is bacteriocidal. Until now, no procedures or techniques have been sufficiently sensitive to either prove or disprove this hypothesis.

The Structural Interactions Section, in close collaboration with the Molecular Structure Section of this laboratory, has developed an in vitro model which closely mimics the in vivo tooth surface plaque relationship and has also developed methods to monitor possible effects of fluoride on plaque. In essence, microbial deposits of various caries associated microorganisms are grown on pressed discs of hydroxyapatite containing known concentrations of fluoride. Since these discs can be prepared using hydroxyapatite of different crystalline textures, parameters other than fluoride concentration may be studied. Several indices of growth such as total protein, DNA, dry weight and total viable organisms have been examined. Of these actual determinations of total viable organisms present appear to be the most reliable index of the total numbers of organisms present.

Initial studies demonstrated that fluoride incorporated at 10-100 PPM into growth media inhibits growth and subsequent acid production by many of the organisms associated with caries and periodontal disease. When a specific caries-associated streptococcus was grown on pressed discs of hydroxyapatite with bound fluoride (HF) and the total number of organisms at various times compared to the numbers on discs of hydroxyapatite (HA) only, certain interesting observations emerged. The first was that plaque deposition is clearly a cyclical event. In the particular time sequence of these experiments, maximum numbers of organisms were present on both types of discs after 32 and 64 hours incubation while the minimum number of organisms were present at 48 hours. Approximately seven times as many organisms, however, were retained on the HA disc as were present on the HF disc at the peak of the growth curves while similar numbers were present at the lowest points. One interpretation of this effect, which is consistent with the hypothesis of fluoride elution from hydroxyapatite, is that a certain minimum number of organisms is required before sufficient acid is produced to dissolve the hydroxyapatite on a microscale and to "elute" the bound fluoride. When this point is reached the fluoride begins to exert its cidal or static effects on the flora resulting in reduced numbers of viable organisms with the concurrent reduction in acid production. This allows more organisms to attach and/or those surviving to multiply and the cycle is started again. The rapid decrease in numbers of organisms present

on the HA discs was considered due to the inability of the discs to physically maintain such large numbers of organisms.

Since fluoride is only released upon sufficient acid production which in turn is produced by large numbers of organisms, it is perhaps paradoxical that this potential beneficial effect of fluoride in hard tissue can only be attained after considerable plaque formation. Further investigations are definitely in order to substantiate the above outlined hypothesis as well as to look at possible other alternative methods of fluoride effect.

The possibility of immunization against dental caries has long been attractive, but results of previous attempts at immunization by the production of humoral antibodies have been inconsistent. This inconsistency probably has been due in part to failure to ascribe specific components of the caries process to specific components of the oral flora at specific loci on the teeth and to the variability in animal behavior consequent to institution of immunizing regimens, variability which can result in reduced caries scores. Two facts, however, made it feasible to test critically if animals can be protected immunologically against caries: First, certain plaque-forming streptococci named Streptococcus mutans have been implicated in multisurface caries in rodents and primates; and second, certain animal models, such as the NIH specific pathogen free Osborne-Mendel rat caries model, develop smooth surface caries only when infected by S. mutans. Using this model system and monitoring animal weights in order to exclude the possibility of artifactual caries reductions, studies were carried out to test 1) whether high humoral antibody titers are elicited in rats in response to antigenic challenge by formalin-killed S. mutans, 2) whether salivary antibody response is so elicited and 3) whether the immunization regimen confers immunologic protection against smooth surface caries resulting from infection by plaque-forming S. mutans.

Rats were infected with Streptomycin-resistant S. mutans strain 6715 after formalin-killed cells of strain 6715 were injected subcutaneously distant from the salivary glands. High humoral agglutinin titers were elicited and salivary antibody response was observed both by the agglutination and by the indirect fluorescent antibody techniques. No differences could be detected in the recoveries of S. mutans from the teeth or in the amount of dental plaque on the teeth between infected-immunized and infected-non-immunized animals. Although smooth surface caries was essentially dependent on infection by strain 6715, sulcal caries, while not dependent upon infection by strain 6715, was augmented by it. Immunization provided an apparent although variable protection of rats against caries associated with S. mutans infection. It resulted not only in reduction of smooth surface caries scores but also in reduction of sulcal caries scores when the contribution of S. mutans to the sulcal disease was most prominent. Absence of weight variations between animal groups suggests that the observed caries reductions could not be ascribed to alteration in food intake resulting from the immunization regimen.

Each of the areas of investigation will be continued and if successful will be intensified during the coming year. The studies concerning the identification and quantitation of oral actinomycetes should be at a point where meaningful information can be gathered. Hopefully at least one mode of action of fluoride will be substantiated and other perhaps more useful fluoride containing com-

pounds will be screened as to their usefulness. The project concerning immunization has been completed but like all good studies, it has engendered numerous other questions, several of which our group intends to investigate during the coming year. Finally, investigations into the structure and importance of certain oral bacteria polysaccharides will be continued.

Summary Report of the Laboratory of Microbiology and Immunology
National Institute of Dental Research

It has always been our practice in this Laboratory to review, reassess and update the goals and directions of our research programs in microbiology and immunology. With the abolition of the Virology Section and the establishment of the Laboratory of Oral Medicine during fiscal year 1973 it was reasoned that this Laboratory could best serve the mission of NIDR by expanding its involvement in immunobiology. Probably the most important event in FY 1973 affecting the future of this Laboratory was the decision to expand our immunology programs in building 30 and to create a clinical immunology section in our clinical investigations area in building 10. Accordingly, high priority was given to this reorganization by the Director of NIDR so as to more effectively translate our fundamental approaches and findings into more applied studies on human subjects with the eventual hope of better understanding the contributions of autoimmune, allergic and hypersensitivity reactions to periodontal disease and other oral soft tissue diseases as well as systemic diseases such as Sjorgren's syndrome, Behcet's syndrome and immune deficiency states. This change is viewed with considerable excitement and is occuring at a time when our increased knowledge of disease mechanisms can be translated into more rational clinical approaches to treatment and prevention of oral disorders. Thus, for the first time in a Laboratory setting we can realistically view basic and applied studies as a continuum.

During the past year a contract was initiated with Bionetics Laboratories to define more clearly the nature of antigenic stimulation in periodontal disease. With newer knowledge on the role of delayed hypersensitivity in this disease it is anticipated that this project will identify the microbial antigens responsible for triggering sensitized lymphocytes to produce the biologically-active molecules that are responsible for the chronic inflammatory response.

While research programs here and at academic institutions are guided to fruition by experienced scientists, it is of considerable consequence that NIH per se has served as a training base for imaginative and energetic young investigators. This Institute like others at NIH has flourished because of these young trainees who contribute consistently and meaningfully to the research programs while at a later date they assume positions of leadership in biomedical research institutions. It is for these reasons that the recent decisions to curtail support for the training of young scientists is indeed unfortunate. Like other biomedically-oriented programs the achievement of our research goals in this Laboratory will unquestionably suffer because of the drastic cut back in financial support of trainees. A number of these Fellows have made notable contributions to our programs during their training period at NIDR.

Summary Report of the Microbial Physiology Section
Laboratory of Microbiology and Immunology
National Institute of Dental Research

The oral cavity represents an immensely complex ecosystem; complex not only in the diversity and numbers of the individual elements (both biotic and abiotic) that comprise it, but also in the diversity and extreme variability of the environmental pressures acting upon these elements. Two of the most prevalent and costly afflictions of man, dental caries and periodontal disease, must with our present state of knowledge, be regarded as consequences of the evolutionary process that lead to the establishment of this particular ecosystem. In the most general terms it seems reasonable to think of these pathological states as a response of the host, or a lack of one, to one or more of the elements that comprise this ecosystem.

The Microbial Physiology Section is engaged in a program to study systematically the most prevalent biotic element of the oral ecosystem, the microbial flora. The broad objective of this program is to establish fundamental principals that will serve as a basic for formulating a rational approach to the control of those pathological states resulting from interactions of the oral cavity and its mircoflora. The major research efforts of this Section over the past year can best be summarized under two categorial areas; 1)Taxonomy and Ecology of Microorganisms, and 2) Microbial Metabolism and its Regulation.

Taxonomy and Ecology of Microorganisms.

An obvious and necessary requisite for studying the oral microflora is the establishment of reliable means for identifying and characterizing individual microorganisms. Because of some abiguity in the assignment of strain designation to organisms of several genera, all strains of Lactobacillus, Pediococcus, and Leuconostoc now in the American Type Culture Collection were checked for authenticity by generally accepted tests. In collaboration with Drs. M. Mandel and F. Gasser, guanosine and cytosine content of the DNA from type, neotype, and important reference strains of the above three genera were also determined and updated. In addition, certain hitherto unrecognized phenotypic traits were discovered and proved to be of great differential value in categorizing certain organisms in these genera. These tests involved assaying for the presence of an enzyme that converts malate to lactate and CO_2 without the intermediate formation of pyruvate (5), determination of the ability of certain strains to grow on gluconate, (7), glucosamine, turanose, or tagatose, and in the case of the pediococci, an assessment of the ability to grow on certain amino acids in the absence of exogenously supplied carbohydrates.

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*This report is based upon progress achieved during the year in Project Nos. NIDR-LMI010b66, NIDR-LMI012b72, NIDR-LMI013b61, NIDR-LMI014b66, and NIDR-LMI019b72.

While classical taxonomic approaches based on similar phenotypic characteristics have served as well in the identification of organisms that comprise any ecological niche, another approach to this problem was introduced last year which had even broader goals. A project was initiated to explore the possibility that techniques could be devised to establish the actual phylogenetic relationship among various microorganisms to gain insight into their evolution. These studies employed the technique of utilizing antiserum prepared against a purified enzyme from one organism to determine the degree of structural homology of the same enzyme from other organisms. The feasibility of this approach has been experimentally documented (6) and this year the project was expanded.

The enzyme, FDP aldolase was purified to homogeneity from cell extracts of Streptococcus faecalis strain MR and used as an antigen to prepare aldolase specific antiserum in rabbits. Employing the anti S.faecalis aldolase serum to detect structural relatedness among the various aldolases of homolactic bacteria, it was established that the three major genera which comprise the lactic acid bacteria; namely, Streptococcus, Lactobacillus and Pediococcus, are related to one another through a common evolutionary ancestor. A phylogenetic map of the homofermentative lactic acid bacteria based on the extent of immunological cross reactivity among the respective aldolases and the antialdolase serum was assembled. Each of the genera represent a separate and distinct line of evolution; however, in addition to the three major diverging lines, four minor branches were also detected.

In general, the immunological methodology readily circumscribed those species of lactic acid bacteria which have been extensively characterized by conventional phenotypic means. For example, strains of oral streptococci such as Streptococcus salivarus or Streptococcus sanguis clustered together as discreet evolutionary groups in the large phylogenetic map. In sharp contrast to this pattern, strains of Streptococcus mitis and Streptococcus mutans do not cluster together. Except for cell wall antigen analysis (1) and DNA homology studies (4), it is impossible to distinguish the various strains of S. mitis or S. mutans by conventional phenotyping procedures. This study has revealed that each of the above species is composed of phylogenetically disparate groups of organisms which are physiological "look-alikes". The species S. mutans can be divided into four distinct evolutionary groups while the species S. mitis is actually comprised of at least three evolutionarily distinguishable groups. From these data, one must conclude that there are at least seven species of streptococci which are concurrently erroneously classified under two specific epithets.

It would appear, that the oral cavity imposes rather stringent physiological constrains on its indigenous microflora and, in doing so, produces a superficial type of convergence among evolutionarily diverse microbes. The results of these experiments suggest that there may be a natural reservoir for the "S. mutans" and "S. mitis" types outside of the oral cavity and that this highly selective environment requires that its new inhibitants modify themselves for ultimate survival. However, until the natural source or reservoir of the organisms capable of establishing themselves in the oral cavity is discovered and studied, the preventive measures used to

insure proper oral hygiene may always lag behind the evolutionary processes which are constantly producing new inhabitants for the various oral surfaces.

Microbial Metabolism and Its Regulation.

Identification of the various members of the oral microflora and the establishment of their phylogenetic relationships, even when finally resolved, still leaves us with the very complex problems of their relationships, even when finally resolved, still leaves us with the very complex problems of their relationship with one another, with their environment and with their host. Limited answeres to some of these fundamental problems are being sought through our continuing studies on the biochemical activities of some of the oral microorganisms.

The synthesis of insoluble extracellular polysaccharides by certain of the oral streptococci is generally accepted as one of the key factors in the microbial colonization of the tooth surface. The enzyme responsible for dextran formation, dextransucrase, has been purified about 50-fold from the culture supernatant of Streptococcus mutans 6715 grown on mannitol or sorbitol. The K_m of the enzyme for sucrose was 5.0 mM when assayed in imidazole buffer at pH 6.3. Several other buffers caused a decreased reaction velocity as well as a shift in the pH optimum. Tris buffer almost completely inhibited dextransucrase activity at rate limiting concentrations of sucrose, although it had little effect at saturating levels of the substrate. EDTA (10 mM) was without effect on dextransucrase activity and certain metals had varying effects. For example, Mg^{++} stimulated activity significantly, while Ag^{+}, Zn^{++}, and Cu^{++} were rather potent inhibitors.

The 50-fold purified enzyme is associated with a high molecular weight carbohydrate of unknown composition. The dextransucrase-carbohydrate complex was retained (with 77% of the catalytic activity) on membranes that exclude molecules of approximately 300,000 molecular weight. Chemical analysis of the purified enzyme showed it contained 39% carbohydrate, of which 18% assayed as fructose. The glycoprotein nature of the enzyme was further suggested by the fact that major protein bands of disc gel electrophoresis, which showed dextransucrase activity, also showed a positive periodic acid Shiff strain for glycoprotein. The carbohydrate associated with dextransucrase appears to be required for catalytic activity. Treatment of the purified enzyme with dextranase from several sources resulted in a time-dependent inactivation of the dextransucrase. The loss of activity was associated with the appearance of compounds that had an Rf of thin layer chromatography which corresponded to glucose and fructose (3).

A new project was undertaken this year which is designed to resolve the basic mechanism by which carbohydrates are transported into the cells of cariogenic streptococci. Prior studies from another laboratory indicated that certain facultative anaerobes utilized a complex phosphoenolpyruvate (PEP)-phosphotransferase system for this purpose (8). Since the pathway for mannitol and sorbitol catabolism has been resolved for S. mutans (2), initial studies have dealt with the transport of these hexitols in S.mutans 6715-14.

An assay system was designed to measure the amount of mannitol-1-phosphate (MIP) formed when crude cell extracts of mannitol-grown S. mutans were incubated with an MIP generating solution (MGS) containing mannitol, PEP, and Mg^{++}. The amount of MIP formed was determined spectrophotometrically by measuring the maximum absorbance obtained at 340 nm for the nicotinamide adenine dinucleotide (NAD)-dependent conversion of MIP to fructose-6-phosphate by mannitol-1-phosphate dehydrogenase. Under assay conditions where a crude extract containing 8 milligrams total protein was incubated with MGS for 60 minutes at 37°, approximately 300 nanomoles of MIP were formed per milligram of protein. The formation of MIP by S. mutans was specific for cell extracts prepared from mannitol-grown cultures. Clearly one or more of the components of the mannitol transport system are inducible. Several mutants of S. mutans have been obtained that fail to grow on mannitol but which will grow on other carbohydrates. These mutants will be used to characterize more completely the number and nature of the components in the complete mannitol transport system.

Fructose-1,6-diphosphate (FDP) has been shown to be an important compound in the regulation of carbohydrate metabolism in streptococci by serving as an activator for both pyruvate kinase (11) and lactate dehydrogenase (9) and as an inhibitor for the 6-phosphogluconate dehydrogenase from S. faecalis (10). The present study showed that FDP is a competitive inhibitor of mannitol-1-phosphate dehydrogenase from S. mutans with respect to MIP. On the other hand, preliminary data indicated that FDP stimulated MIP formation by the S. mutans phosphotransferase system. The role of FDP in the control of hexitol transport and its subsequent catabolism remains unclarified at present. However, future experiments may show that FDP is intimately involved in controlling the intracellular levels of PEP, NAD, or the phosphorylated substrate. Future objectives of this project will be to seek information concerning the mechanism by which cariogenic bacteria transport carbohydrates into the cell and how the subsequent metabolism fermenting only a limited variety of carbohydrates. Thus it would be of interest to determine if other carbohydrates are also transported by a PEP-dependent phosphotransferase system, and to study the comparative biochemistry of the transport of carbohydrates by other microorganisms comprising the oral flora.

Relevant Bibliography

1. Bratthal, D. 1970. Demonstration of five serological groups of streptococcal strains resembling Streptococcus mutans. Odontol. Revy., 21: 143-152.

2. Brown, A. T., and C. L. Wittenberger. 1973: Mannitol and Sorbitol catabolism in Streptococcus mutans. Arch. Oral Biol. 18: 117-126.

3. Ciardi, J., and C. L. Wittenberger: Studies on dextransucrase from Streptococcus mutans. Abstracts of the Annual Meeting of the American Society for Microbiology. p. 284. 1973.

4. Coykendal, A. L. Genetic heterogeneity in Streptococcus mutans. J. Bacteriol. 106: 192-196, 1971.

5. Kaufman, S., S. Korkes, and A. del Campillo. Biosynthesis of dicarboxylic acids by carbon dioxide fixation. V. Further study of the "malic enzyme" of Lactobacillus arabinosus. J. Biol. Chem., 301-312, 1951.

6. London, J. Detection of phylogenetic relati9nships between streptococci and lactobacilli by a comparative biochemical and immunological study of isofunctional malic enzymes. J. Dental Res. 50: 1083-1093, 1971.

7. Rogosa, M. Characteristics used in the classification of lactobacilli. International J. Systematic Bacteriol., 20: 519-533, 1970.

8. Roseman, S. The transport of carbohydrates by a bacterial phosphotransferase system. J. Gen. Physiol., 54: 1385-1805, 1969.

9. Wittenberger, C. L., and N. Angelo. Purification and properties of a fructose-1,6-diphosphate-activated lactate dehydrogenase from Streptococcus faecalis. J. Bacteriol., 101: 717-724, 1970.

10. Wittenberger, C. L., M. P. Palumbo, R. B. Bridges,and A. T. Brown. Mechanisms for regulating the activity of constitutive glucose degradative pathways in Streptococcus faecalis. J. Dental Res., 50: 1094-1103, 1971.

11. Wittenberger, C. L., M. Palumbo, and I. Klein. Two forms of pyruvate kinase from Streptococcus faecalis. Abstracts of the Ninth International Congress of Biochemistry. (In press) 1973.

Summary Report of the Immunology Section
Laboratory of Microbiology and Immunology
National Institute of Dental Research

The Immunology Section investigates the mechanisms by which cells and their products are recruited, participate in, and contribute to immunological reactions. Currently immunology provides a variety of techniques with which our defense mechanisms to pathogenic stimuli can be assessed and manipulated. However, our basic understanding of the regulation of these factors must be increased to eventually permit therapeutic manipulation of inflammatory and other immune reactions.

It is now clear that there are two major types of immunological reactions. Those produced by bone marrow derived lymphocytes (B cells) which eventually differentiate into plasma cells that produce specific antibodies directed against foreign antigens. When these antibodies bind the antigens against which they are directed they activate the enzymes in the complement cascade and this produces acute inflammatory as well as anaphylactic reactions. In contrast, thymic dependent lymphocytes (T cells) react directly with antigens and produce nonspecific "mediators" that result predominantly in mononuclear cell inflammation characteristic of delayed hypersensitivity reactions and direct contact lysis of cells as in allograft and tumor rejection. Interactions of these two immune populations serves to modulate the overall immune function of the host.

Dr. H. Kirchner and Dr. J. J. Oppenheim, in collaboration with Dr. M. Blaese (NCI) have continued their studies of B and T cell interactions utilizing chickens. When chickens are chemically bursectomized they become B cell deficient and agammaglobulinemic. This therefore provides a source of pure T cells whose contribution to various immunological reactions have been evaluated. They can be stimulated to produce mediators and delayed hypersensitivity reactions. In contrast, their proliferative and antibody reactions to antigens (acute inflammatory responses) are impaired. Attempts at reconstituting these deficiencies have as yet been unsuccessful, but we have recently obtained enriched populations of chicken B cells by using specific cytotoxic anti-T cell antisera. Thus, the reactions of isolated B cells and their reconstituting effects are now being studied.

Interactions of B and T cells in allergic humans are also being studied by Drs. J. J. Oppenheim and W. Hook in collaboration with Dr. H. Brown of the George Washington Medical School. The in vitro lymphocyte reactivity of untreated and hyposensitized subjects to animal dander allergens is being correlated with their in vitro histamine release and immediate cutaneous reactions. This represents an effort to develop more quantitative methods for detecting and evaluating induction of blocking antibodies that reduce immunological reactions. Coincidentally it was found by Drs. W. Hook and J. Oppenheim that hamster mast cells and human basophils are stimulated to

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This report is based upon progress achieved during the year in Project Nos. NIDR-LMI002c71, NIDR-LMI003b69, NIDR-LMI004b69, NIDR-LMI006b71, NIDR-LMI008b71, NIDR-LMI020b72, NIDR-LMI021b72, NIDR-LMI022b72, NIDR-LMI023b72, NIDR-LMI024b72, and NIDR-LMI025b72.

release histamine by lymphocyte mitogens such as phytohemagglutinin and concanavalin A. This effect was not found to be due to a lymphocyte mediator, but presumably represents a direct interaction of these lectins with receptors on the basophil surface. We are currently pursuing this promising means of investigating the mechanisms of histamine release.

Drs. J. J. Oppenheim, A. Sandberg, W. Hook and L. Altman, are studying multiple immunological effects of saline extracts of walnuts. Most nuts that have been studied contain factors mitogenic for lymphocytes. In the case of Black walnuts the mitogenic activity is resistant to boiling and enzymatic degradation. The extract releases histamine from the leucocytes of atopic subjects. It also induces production of a mediator chemotactic for mononuclear cells suggesting that T cells are responsible for the proliferative response to walnut extracts. In contrast, extracts of the pellicle of English and Black walnuts have been found to contain other substances which are toxic to tissue cultures of leucocytes and fibroblasts. Materials extracted from pellicles such as ellagitannins (extracted from pellicle of California walnuts by Dr. Jurd, USDA, Albany, Calif.) and juglone (from Black walnut pellicles) are similarly toxic. Normal subjects who are fed a 100 gm of fresh walnuts develop a transient marked suppression of *in vitro* lymphocyte reactivity. A few of them also develop aphthous ulcers several days later. In collaboration with Drs. J. Marquardt (NEI, NIH) and R. Snyderman, patients with Behcet's syndrome were found to experience a similar depression of lymphocyte reactivity and temporary exacerbation of their symptoms for up to two weeks after eating 100 gm of walnuts. The exact relationship between the mitogenic and toxic factors present in walnuts to the pathogenesis of these diseases is being studied.

Doctor J. Pincus continued his studies of solubilized murine histocompatibility antigens ($H\text{-}z^{0}$) from L1210 leukemic tissue culture cells (supplied on contract by Associated Biomedic Systems, Inc., Buffalo, N.Y., #NIDR-71-2385). Dr. R. Gordon and subsequently Dr. D. Ranney administered the solubilized antigens to allogeneic mice and induced production of agglutinating humoral antibodies, delayed hypersensitivity, suppressed graft versus host reactions and produced significant acceleration or prolongation (from 10 to 13 days after multiple doses) of skin graft rejection. Since these biological effects were not very dramatic and Dr. J. Pincus left to join the transplantation group at Northwestern University Medical School, Chicago, Ill., these studies were terminated and the contract cancelled in February 1973.

Drs. D. Ranney and J. J. Oppenheim, in collaboration with Dr. A. Quattrone (Lab. Biochem., NIDR) have had some success in studying a pronase resistant noncytotoxic inhibitor of B as well as T cell proliferation and cell division. This factor is produced by rat spleen cell cultures and in some respects resembles a lymphocytic chalone. However, unlike previously described chalones, it has been found to be less than 1000 M.W. We are currently producing it in large quantities for purpose of purification and identification. This material may play an important role in regulation of cell-mediated immune responses.

Drs. J. Horton, J. J. Oppenheim and S. Mergenhagen in collaboration with Dr. L. Raisz (Univ. Rochester Med. School, Rochester, N.Y.) are pursuing

their studies of osteoclast activation factor (OAF). This factor is generated in supernatants of leucocyte cultures stimulated with mitogens, or when the sensitized leucocytes from subjects with periodontal disease (P.D.) are stimulated by plaque antigens. This material may therefore be responsible for the bone resorption associated with P.D. Chromatographic studies reveal it to be approximately 17,000 M.W. It is produced in vitro only when macrophages as well as lymphocytes are present. Collaborative studies with Dr. D. Buell (NCI) have also revealed OAF to be present in the supernatants of long-term B but not T cell lymphoid cell lines. The cell source and nature of this important product of inflammatory cells is being further defined.

The mechanism by which lymphocytes are activated to produce mediators that are chemotactic for mononuclear cells (MNL CTX) are being studied by Drs. L. Altman, S. Wahl, S. Mergenhagen and J. J. Oppenheim. The attraction of MNL to sites of lymphocyte activation by this mediator probably reflects mechanisms of in vivo accumulation of macrophages at inflammatory sites. The M.W. of the mediator has been found to be very similar and approximately 12,000 by Sephadex chromatography in guinea pigs, chickens and man. However, the MNL CTX from these different species do not cross react. Agammaglobulinemic chickens produce normal amounts of the MNL CTX indicating it to be a T cell product. Dr. L. Altman in collaboration with Dr. M. Blaese (NCI) is also studying the role of this mediator by investigating its function in humans with a variety of abnormalities such as chronic mucocutaneous candidiasis, Wiskott-Aldrich syndrome, ataxia-telangiectasia, chronic lymphocytic leukemia, Hodgkin's disease, and lymphomas.

Drs. D. Rosenstreich and S. Mergenhagen in collaboration with Dr. A. Nowotny (Temple Univ. Med. School, Phila., Pa.) have made some fascinating studies of the activation of lymphocytes by endotoxic lipopolysaccharides. The LPS was found to be a B cell mitogen in guinea pigs and mice. By utilizing mutant strains as the source of endotoxin, the protein and carbohydrate-free lipid moiety was found to stimulate lymphocytes to the same degree as intact lipopolysaccharide suggesting that receptors for lipids may be present on surface of B cells. A direct correlation between biological toxicity and ability to activate lymphocytes was found suggesting that B cell activation may contribute to the in vivo toxic effects of endotoxin.

Dr. D. Rosenstreich is also studying the immune response to a potentially oncogenic herpes-like virus infection in the guinea pig. Guinea pigs infected with low doses of virus develop short-lived lymphoproliferative reactions and persistent antibodies to the virus. In contrast, high infective doses of virus do not elicit any immune reaction at all. The relationship of these disparate reactions to consequent effects of persistent replicating virus on the host are being studied.

The role of complement in mediation of the inflammatory response with special emphasis on the newly defined alternate complement pathway is being investigated by Dr. A. L. Sandberg. In vitro lysis of rabbit platelets with resultant release of vasoactive amines and clotting factors provides an excellent model of cell injury produced by nonplatelet related immune complexes and complement. Current studies have demonstrated that the classical

and alternate complement pathway are equally effective in platelet lysis. Several other aspects of the alternate complement pathway currently under investigation in conjunction with Drs. S. Mergenhagen, A. Notkins and Mrs. J. Phillips are: 1) the participation of antibody in the activation of the alternate complement pathway, 2) the antigenic configuration required for activation of this pathway as opposed to the classical pathway, and 3) the role of this pathway in host defense mechanisms.

We look forward to the arrival of Dr. M. Iverson who will be joining our section on May 1, 1973. He will be pursuing studies of the role of "armed" macrophages in inflammation and tumor immunity, as well as the contribution of T and B cells to autoimmune reactions. His interaction with the section will enrich the activities of our group considerably.

Relevant Bibliography

1. Allen, J. C., Oppenheim, J. J., Brody, J. and Miller, J.: Labile inhibitory factor of lymphocyte transformation in SSPE plasma. Infection and Immunity. In press.

2. Altman, L. C., Snyderman, R., Oppenheim, J. J. and Mergenhagen, S. E.: A human mononuclear chemotactic factor: characterization, specificity and kinetics of production by homologous leukocytes. J. Immunol. 110: 801-810, 1973.

3. Oppenheim, J. J., Blaese, R. M., Horton, J. E., Thor, D. E. and Granger, G. A.: Production of macrophage migration inhibitory factor and lymphotoxin by leucocytes from normal and Wiskott-Aldrich syndrome patients. Cell Immunol. In press.

4. Marquardt, J. L., Snyderman, R. and Oppenheim, J. J.: Depression of lymphocyte transformation and exacerbation of Behcet's syndrome by ingestion of English walnuts. Cell. Immunol. In press.

5. Ranney, D. F. and Oppenheim, J. J.: Inhibition of in vitro proliferation of rat thymocytes by isologous spleen cells and supernatants. Proc. of the 7th Leucocyte Culture Conference. F. Daquillard (Ed.): Acad. Press.

6. Kirchner, H., Oppenheim, J. J. and Blaese, R. M.: The contribution of B cells to the in vitro proliferative reaction of chicken lymphocytes to nonspecific mitogens and antigens. Proc. of the 7th Leucocyte Culture Conference. F. Daquillard (ed.). Acad Press. In press.

7. Anderson, J. A., Lane, S. R., Howard, W. A., Leikin, S. and Oppenheim, J. J.: The effect of a "blocking" plasma factor induced by hyposensitization alternaria induced lymphocyte transformation. Cell. Immunol. In press.

8. Horton, J. E., Oppenheim, J. J. and Mergenhagen, S. E.: Elaboration of lymphotoxin by cultured human peripheral blood leucocytes stimulated with dental plaque-deposits. Clin. Exp. Immunol. In press.

9. Horton, J. E., Raisz, L. G., Simmons, H. A., Oppenheim, J. J. and Mergenhagen, S. E.: Bone resorbing activity in supernatant fluid from cultured human peripheral blood leukocytes. Science 177: 793, 795, 1972.

10. Blaese, R. M., Weiden, P., Oppenheim, J. J. and Waldmann, T. A.: Phytohemagglutinin as a skin test for the evaluation of cellular immune competence in man. J. Lab. Clin. Med. In press.

11. Souther, S. G., Gordon, R. O., Oppenheim, J. J. and Stinson, E. B.: In vivo and in vitro studies of cardiac allografts in the rat: immunological enhancement. Arch. Surg. 105: 756-760, 1972.

12. Kirchner, H., Altman, L. C., Fridberg, A. P. and Oppenheim, J. J.: Dissociation of in vitro lymphocyte transformation from production of a mononuclear leucocyte chemotactic factor in agammaglobulinemic chickens. Cell. Immunol. In press.

13. Hook, W. A., Snyderman, R. and Mergenhagen, S. E.: Further characterization of a factor from endotoxin-treated serum which releases histamine and heparin from mast cells. Infection and Immunity 5: 909-914, 1972.

14. Rosenstreich, D. L. and Rosenthal, A. S.: Peritoneal exudate lymphocyte II. In vitro lymphocyte stimulation induced by brief exposure to antigen. J. Immunol. In press.

15. Rosenthal, A. S. and Rosenstreich, D. L.: The lymphocyte uropod: A specialized surface site for immunologic recognition. In Biomembranes. In press.

16. Shevach, E. M., Rosenstreich, D. L. and Green, I. The distribution of histocompatibility antigens on T and B cells in the guinea pig. Transplantation. In press.

17. Rosenthal, A. S., Johnson, J. S., Rosenstreich, D. L., and Blake, J. T.: A nonprecipitating antibody in the guinea pig. Int. Arch. Aller. and Immunol. In press.

18. Osler, A. G., and Sandberg, A. L.: Alternate complement pathways. Progress in Allergy. In press.

19. Raisz, L. G. and Horton, J. E.: Pathogenesis of localized bone loss. In Proceedings of Intr. Symposium on Clinical Aspects of Metabolic Bone Disease. Henry Ford Hospital, Detroit, Mich. Excerpt. Medica. In press.

20. Altman, L. C., Kirchner, H.: The production of a monocyte chemotactic factor by agammaglobulinemic chicken spleen cells. J. Immunol. 109: 1149-1151, 1972.

21. Snyderman, R., Altman, L. C., Frankel, A., Blaese, R. M.: Defective Mononuclear leukocyte chemotaxis: A previously unrecognized immune dysfunction. Studies in a patient with chronic mucocutaneous candidiasis. Annals. of Internal Medicine. 78: 509-513, 1973.

22. Snyderman, R., Altman, L. C., and Mergenhagen, S. E.: Human mononuclear leukocyte chemotaxis: definition of two chemotactic factors and a previously unrecognized immune dysfunction. In Proceedings of a Conference on the Role of Host Factors in Nonspecific Resistance. In press.

Summary Report of the Virology Section
Laboratory of Microbiology and Immunology
National Institute of Dental Research

Over the last year the Virology Section has concentrated on four areas of research: (1) immunological factors involved in the recovery from acute infection with herpes simplex virus (HSV); (2) in vitro studies on the cell-mediated immune response to HSV and extension of this technique to patient material; (3) development of sensitive and rapid assays for the detection of HSV antigens and anti-HSV and (4) the role of viruses in diseases of the pancreas and salivary glands.

For many years, the host's ability to defend itself against HSV infection was analyzed almost exclusively in terms of its ability to make neutralizing antibody. Recently, it has become quite clear that neutralization of virus by antiviral antibody is only one component of the host's multifacet immunological defense against this infection. Over the last year we have attempted to learn how the different immunological defense mechanisms interact to stop the spread of HSV infection.

It is well known from clinical observations that HSV infections reoccur in the presence of neutralizing antibody. Moreover, in vitro studies have shown that HSV can destroy a monolayer of cells even when high concentrations of neutralizing antibody are present in the medium. Presumably this occurs because the virus spreads from cell-to-contiguous cell and thereby avoids extracellular exposure to neutralizing antibody. These observations strongly indicated that something more than antibody was needed to stop the cell-to-cell spread of the infection.

Several years ago we found that monolayers of cells that had been infected with HSV could be destroyed by anti-HSV antibody and complement, whereas neither anti-HSV antibody or complement alone had any destructive effect. This raised the possibility that antiviral antibody and complement might not only contribute to the destruction of infected cells, but in some cases might be an important factor in stopping the cell-to-cell spread of the infection. Data obtained over the last 12 months showed, however, that antibody and complement did not stop the spread of the infection. This raised a second possibility i.e., the virus was being transferred to contiguous cells before the cell in which it was originally synthesized was destroyed by antibody and complement. Detailed studies on the relationship between the time of appearance of viral antigens on the surface of infected cells, the susceptibility of these cells to immune lysis by antibody antiviral and complement, and the actual time at which the virus was transferred to adjacent cells, supported this hypothesis. Thus, viral spread was always one step ahead of immune lysis and the infection persisted.

The fact that viral spread could not be stopped by antiviral antibody plus complement raised a third possibility; i.e., that leukocytes which are often seen at the site of the herpetic lesion might be needed to stop the infection. It is known that leukocytes, especially activated macrophages, can non-specifically destroy cells. We reason that if activated macrophages were

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This report is based upon progress achieved during the year in Project Nos. NIDR-LMI015b67, NIDR-LMI016b71, NIDR-LMI017b70, and NIDR-LMI018b71.

attracted to the site of the herpetic lesion, they might aid in stopping the spread of the virus by either destroying infected cells or the surrounding uneffected cells.

To test this hypothesis monolayers of primary rabbit kidney cells were infected with HSV. Peritoneal exudate cells from rabbits, containing approximately 80% macrophages, then were added to the monolayers and the number of plaques that appeard at 72 hours after infection was determined. Our experiments showed that the macrophages inhibited the formation of viral plaques and decreased the amount of infectious virus. Theoretically, at least, the cell-to-cell spread of the infection can be stopped at any one of three different sites. First, macrophages might non-specifically destroy or inhibit viral replication in the infected cells. Second, if macrophage toxicity broke the connection or bridges between adjacent cells this would stop the infections because the virus could not be transferred to contiguous cells without being exposed extracellularly to neutralizing antibody. Third, if macrophages non-specifically destroyed the adjacent uneffected cells this also could stop the spread of the infection by eliminating potential target cells from viral replication. Detailed experiments indicated that the macrophages acted at all three sites. However, when the macrophages were removed from the monolayers, viral plaques often reappeared. Thus the macrophages only temporarily suppressed the development of the plaques. This suggested that in vivo leukocytes were needed, but might not be sufficient to cure the infection. The possibility that the interaction of the various immunological responses (i.e. antiviral antibody, complement, and leukocytes) acting in conjunction were required to effectively eradicate the infection was next investigated. Our experiments indicated that all three components were required to permanently suppress the development of plaques.

On the basis of these and other observations we have proposed a new hypothesis to explain how the immune response stops viral infections such as HSV which spread by a cell-to-cell route. The immunological defense against HSV consists of two phases, one specific and the other non-specific. The specific phase consists of (a) the interaction of antiviral antibody and complement with virus or virus-infected cells; and (b) the stimulation of immune lymphocytes by viral antigens. This results in the generation of a variety of biological mediators, some of which are chemotactic for polymorphonuclear and mononuclear leukocytes. The non-specific phase of the defense against HSV consists of the attraction of leukocytes by these mediators to the site of the infection where they exert their toxic effect non-specifically on the infected and surrounding uneffected cells. This results in the destruction of some of the infected cells and some of the surrounding uneffected cells to which the virus would have spread. In addition, leukocytes can break or interrupt cell-to-cell contact and thereby prevent the virus from spreading directly to adjacent cells without being exposed to antiviral antibody. Infected cells which survive the leukocyte attack may be destroyed by antiviral antibody and complement before cell-to-cell contact is reestablished. Once the infected cells are eliminated the stimuli for the generation of chemotactic factors no longer exists, the leukocyte infiltrate disappears and the cells regenerate. Thus, it appears that antibody, complement, immune lymphocytes, biological mediators, and inflammatory cells acting non-specifically all work in conjunction and contribute to the cure of the infection.

This hypothesis suggests that better ways of getting leukocytes to the site of the infection might prove useful in treating viral infections that spread by a cell-to-cell route. It is hoped that these and future studies will lead to a more rational approach to the immunotherapy of HSV infections.

The second phase of our work was concerned with a detailed analysis of the interaction of viral antigens with immune lymphocytes. This lymphocyte stimulation assay (which measures the incorporation of ^{3}H-thymidine into acid-insoluble material) is thought to be an in vitro correlate of cell mediated immunity and was described in last years reports. The assay utilized both spleen cells and peripheral leukocytes from rabbits. It is virus specific and capable of detecting differences in antigenicity between strains of the same virus. This year we have applied this technique to the study of HSV infections in man. Our experiments showed that lymphocytes from patients who had a history of HSV infections could be significantly stimulated in vitro by exposure to HSV antigens. In contrast, lymphocytes from subjects with no antibody or previous history of exposure to HSV could not be stimulated in vitro by HSV antigen. The ability to stimulate immune lymphocytes seemed to be greatest during the acute phase of the viral infection. In addition, we found that the supernatant fluids from the stimulated cultures contained certain biological mediators. These included chemotactic factors for mononuclear leukocytes and lymphotoxin, a factor which is capable of destroying both infected and uninfected cells. At the present time these studies are being extended to patients with known immunological deficiencies and it is hoped that these studies will provide new insights as to why certain patients are unable to effectively handle an HSV infection.

The third phase of our work was concerned with the detection of viral antigens on the surface of infected cells and the measurement of antiviral antibody by means of radioimmunoassays. This work was described in great detail in last years report. Over the last 12 months we have extended these studies and refined our techniques. We have shown that immune complexes on the surface of virus-infected cells cannot only be detected by the binding of ^{125}I-labeled anti-immunoglobulin, but also by the binding of ^{125}I-labeled rheumatoid factor and ^{125}I-labeled anti-C-3. In addition, a method for the purification and concentration of antiviral antibody was worked out, taking advantage of the fact that antiviral antibody binds to the surface of infected cells and can be eluted by lowering the pH.

Recently, we have used radiolabeled antibody not only to detect viral antigens on the surface of infected cells, but to detect viral antigens within the sytoplasma of infected cells. For example, by this technique we were able to show that cells infected with Coxsackie virus had no viral antigens on their surface while substantial amounts of viral antigens could be detected within the cells. In contrast cells infected with HSV had substantial amounts of antigens both on the surface and within the infected cell. This technique offers a very sensitive and quantitative method for detecting viral antigens and might prove useful in detecting HSV antigens in normal and malignant tissues.

The fourth phase of our work was concerned with viral infections which effect both the exocrine and endocrine systems. Encephalomyocarditis (EMC)

virus was used as a model. Our studies showed that infection of DBA mice with EMC virus resulted in a diabetes-like syndrome characterized by hyperglycemia, glycosuria, hypoinsulinenia, polydipisa, and polyphagia. Blood glucose levels were elevated within four days after infection and reached a maximum mean level of 320 milligrams per cent within 12 days. Approximately 60 to 80% of the animals developed a transient hyperglycemia while 10 to 15% of the animals remained hyperglycemic for well over 6 months. The remaining animals failed to become hyperglycemic but many had abnormal glucose tolerance curves. Hyperglycemia was most pronounced when animals were allowed free access to food, and the incidence of hyperglycemia was related to both the strain and sex of the animals with few females developing hyperglycemia. The amount of immune-reactive insulin in the plasma of infected hyperglycemic mice was significantly lower than appropriate controls and injection of exogenous insulin resulted in a rapid drop in blood glucose levels. Despite the fact that certain animals were hyperglycemic for many months, virus could not be recovered from the pancreas after the first ten days of the infection. By immunofluorescence we are able to show that the virus replicated in the beta cells of the pancreas and that there was little or no replication in the acinar cells. In contrast -infection of mice with Coxsackie virus did not result in diabetes but resulted in pancreatitis acinar cell destruction and elevated levels of serum anylase. Both viruses infected the salivary glands and detailed histologic studies are underway.

The demonstration that at least EMC virus can produce a picture that metabolically resembles diabetes adds credence to the hypothesis that viral infections might be a cause of some forms of diabetes in man. It is evident from our experiments, however, that the development of diabetes is not only dependent on the tropism of the particular virus but the degree of beta cell damage produced by the complex interaction between the virus and the host. At the present time studies are in progress to compare the effects of different viruses such as EMC and Coxsackie in terms of their ability to selectively destroy beta cells as compared to acinar cells and their effects on the pancreas versus the salivary glands. It is hoped that these animal models will provide new insight into both the etiology and pathogenesis of disease of the pancreas and salivary glands.

Bibliography July 1972 - July 1973

1. Lodmell, D. L., A. Niwa, K. Hayashi, and A. L. Notkins: Prevention of cell-to-cell spread of Herpes simplex virus by leukocytes. J. Exp. Med. 137: 706, 1973.

2. Notkins, A. L., Chairman. Workshop on the treatment and prevention of herpes simplex virus infection. J. Inf. Dis. 127: 117, 1973.

3. Rosenberg, G. L., C. Wohlenberg, A. J. Nahmias, and A. L. Notkins: Differentiation of type 1 and type 2 herpes simplex virus by in vitro stimulation of immune lymphocytes, J. Immunol. 109: 413-414, 1972.

4. Rosenberg, G. L., and G. J. Elfenbein: In vitro proliferation of rabbit bone marrow-derived and thymus-derived lymphocytes in response to vaccinia virus. Cellular Immunology, June 1973 in press

5. Snyderman, R. C., Wohlenberg, and A. L. Notkins: Inflammation and viral infections: Chemotactic activity resulting from the interaction of antiviral antibody and complement with cells infected with herpes simplex virus. J. Inf. Dis.,126: 207, 1972.

6. Rosenthal, J. D., K. Hayashi, and A. L. Notkins: Rapid micro-radioimmunoassay for the measurement of antiviral antibody. J. Immunol. 109: 171-173, 1972.

7. Rosenthal, J. D., K. Hayashi, and A. L. Notkins: Virus antigens on the surface of infected cells: Binding and elution of ^{125}I-labelled anti-virus antibody. J. Gen. Virol. 18: 195-199, 1973.

8. Hayashi, K., D. Lodmell, J. Rosenthal, and A. L. Notkins: Binding of ^{125}I-labelled anti-IgG, rheumatoid factor and anti-C3 to immune complexes on the surface of virus-infected cells. J. Immunol. 110: 316-319, 1973.

9. Rosenthal, J. D., K. Hayashi, and A. L. Notkins: Comparison of direct and indirect solid phase microradioimmunoassay for the detection of viral antigens and antiviral antibody. Applied microbiology, in press.

10. Yamazaki, S., and A. L. Notkins: Inhibition of replication of lactic dehydrogenase virus by actinomycin. J. Virology 11: 473, 1973.

11. Niwa, A., S. Yamazaki, J. Bader, and A. L. Notkins: Incorporation of label precursors into the ribonucleic acid and proteins of lactic dehydrogenase virus. J. Virology. in press.

12. Allison, A. C., J. East, H. Koprowski, A. C. Mims, A. L. Notkins, and J. Van Loghem. Virus associated immunopathological conditions: Animal models and implications for human disease. Bull. World Health Organization, August 1972.

13. Boucher, D. W., K. Hayashi, and A. L. Notkins: Virus-induced diabetes mellitus in mice. Amer. J. Path. In press.

14. Boucher, D. W., and A. L. Notkins: Virus-induced diabetes mellitus. I. hyperglycemia and hypoinsulinemia in mice infected with encephalomyocarditis virus. J. Exp. Med. In press.

15. Notkins, A. L., and H. Koprowski: How the immune response to a virus can cause disease. Scientific American. 228: 22, 1973.

Report of the Dental Services Branch
National Institute of Dental Research
Summary Statement

The Dental Services Branch provides collaborative clinical support to all NIDR intramural programs and extends necessary dental care to the NIH-wide research patient population at the Clinical Center. The Dental Clinical Associate program offers career development opportunities to young clinicians who have research interests.

Intramural Support and Clinical Programs:

The Branch contributes to intramural programs directly and through collaborative activities with other units of the Institute as follows:

Caries - A study of differences in occurrence of dental caries in Caucasian and Negro children is under continuing investigation. Differences in enamel fluorosis between these two groups is being studies.[1]

Dental Materials - A collaborative study with the National Bureau of Standards is being concluded which demonstrates the effects of the gamma 2 phase on the physical properties of dental amalgam.[2]

Sjögren's Syndrome - Lower lip biopsies from patients with Sjögren's syndrome are analyzed both histologically and with radio-immunoelectrophoretic methods to evaluate lymphoid infiltration and mechanisms for synthesis of specific immunoglobulins.[3] Extracts from normal salivary glands are examined and analyzed for antigenic groups against which patients with Sjögren's syndrome can be shown to have antibody activity.

Tissue healing - Post-operative stability of the dental occlusion and the temporomandibular joints of patients following various techniques of maxillary or mandibular osteotomy continues under study.[4] Factors associated with the development of post-extraction localized osteitis are also being studied.[5]

Tooth Transplant Study - Patients with previously placed transplants are being followed to determine the extent of any immunologic rejection phenomenon and to determine other factors influencing clinical success.

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1. NIDR-DSB-73-002-(c)(68)
2. NIDR-DSB-73-006-(a)(72)
3. NIDR-OMS-73-008-(c)(72)
4. NIDR-DSB-73-005-(c)(67)
5. NIDR-DSB-73-004-(c)(67)

Primate Implant Study - A radiographic and histopathological evaluation of the effect of occlusal loading on the longevity of acrylic dental implants in Rhesus monkeys has been initiated. Initial emphasis is toward development of appropriate surgical and analytical techniques.

Fracture Visibility Study - The detectability of skull fractures using routine radiographic methods is being determined in a controlled study of carefully induced post mortem lesions. Such studies are intended to demonstrate the functional limitations of existing diagnostic techniques.[6]

Inter-Institute Collaborative Services:

The Branch provides dental services to the clinical programs of all the categorical Institutes.[7] The following examples are cited:

National Heart and Lung Institute - The Branch heavily supports the clinical programs of the NHLI Cardiology and Surgery Branches. Cardiac surgical candidates with congenital or acquired heart defects who require a cardiovascular prosthesis are provided comprehensive dental care to control the oral enviroment as a focus of postoperative bacteremias.

National Cancer Institute - Excellent liaison with the NCI Surgery Branch continues. Our prosthetic and oral surgery services receive an increasing number of consultations to aid in the management of head and neck oncologic surgical cases. Dental evaluation at the critical preoperative workup of these patients has come to be appreciated by the NCI staff.

National Institute of Arthritis and Metabolic Diseases - Submaxillary saliva is still being collected for the purpose of immunologic and biochemical studies of the products of accessible secretory glands such as salivary glands in patients with cystic fibrosis.

Dental Clinical Associateship Program:

This program offers a combination of research and clinical opportunities to the young clinicians of the Dental Services Branch. During the past year, Branch Clinical Associates have been collaborating with various laboratories on the following projects.

Gamma 2 phase in dental amalgams.
Research Division, American Dental Association
National Bureau of Standards, Department of Commerce.

Primate implant study.
Oral Medicine and Surgery Branch, NIDR

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6. NIDR-DSB-73-007-(c)(73)
7. NIDR DSB-73-001-(c)(72)

Report of the Oral Medicine and Surgery Branch
National Institute of Dental Research
Summary Statement

The diversity of the program of the Oral Medicine and Surgery Branch is exemplified in the research projects of the six investigators included in this summary. The projects are arbitrarily grouped under the following headings and will be discussed in this same sequence: (1) Sjögren's syndrome, (2) Aphthous stomatitis, (3) Salivary studies, (4) Oral physiology, and (5) Oral roentgenology.

Sjögren's Syndrome - Autoimmunity.[1-9]

The studies were focused on the overall problem of autoimmunity, both in human patients - particularly those with Sjögren's syndrome - and in NSB/W mice, an excellent animal model that spontaneously develops diseases analogous to human systemic lupus erythematosus and Sjögren's syndrome. Specific areas of inquiry included:

1. The nature of the mononuclear cell infiltrating the salivary gland in Sjögren's syndrome.

   A technique was developed for use with human frozen tissue sections and it showed that most but not all the cells infiltrating the minor salivary glands of the lip are derived from bone marrow cells.

2. The role of suppressor thymic derived (T) lymphocytes in autoimmunity.

   Evidence is accumulating that T cells can regulate bone marrow cells (B) and prevent them from making an antibody response. The removal of T cells with antithymocyte serum (ATS) increases the response to a T dependent antigen, synthetic RNA, in NZB/W mice. This result supports the concept that autoimmune disorders may result from the loss of suppressor T cells.

3. Grafts versus host GVH reactivity in NZB/W mice.

   The GVH activity of the spleen cells of NZB/W mice increases with age to about 6 months then rapidly decreases. The addition of inactive young cells to highly active six month cells decreased their reactivity,

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1. NIDR-OMS-73-008-(c)-(72)
2. NIDR-OMS-73-004-(c)-(72)
3. NIDR-OMS-73-005-(c)-(72)
4. NIDR-OMS-73-023-(c)-(73)
5. NIDR-OMS-73-024-(c)-(73)
6. NIDR-OMS-73-025-(c)-(73)
7. NIDR-OMS-73-026-(c)-(73)
8. NIDR-OMS-73-027-(c)-(73)
9. NIDR-OMS-73-028-(c)-(73)

suggesting a positive control over GVH activity that was lost with age in NZB/W mice. This was the first demonstration of suppressor cell activity in a GVH, or T cell mediated, system.

Sjögren's Syndrome - Salivary studies.[10-12]

1. Immunoglobulins

Parotid saliva was collected from 17 patients with Sjögren's syndrome and subjected to polyacrylamide disc electrophoresis. More anode-migrating protein was found with Sjögren's syndrome patients as well as less salivary amylase than in the control group. However, there was only borderline significance to this finding. No significant difference in the concentration of immunoglobulins, IgG, IgA and IgM was found between the parotid saliva of the controls and those with Sjögren's syndrome. Collaborative studies with Dr. Larry Anderson at Massachusetts General Hospital demonstrated that the immunoglobins (IgM and IgG) and rheumatoid factor in labial salivary glands was distinctive for Sjögren's syndrome. Forty-three percent of the Sjögren's patients demonstrated rheumatoid factor in the labial salivary glands compared to six percent in controls.

2. Antisalivary duct antibody (ASDA)

In patients with Sjögren's syndrome (SS), lymphocytic infiltration of minor salivary glands in lip biopsy material was correlated with the humoral response of antisalivary duct antibody (ASDA). It was found that patients with SS and ASDA-positive sera had significantly less cellular infiltration than those who lacked the antibody. The ASDA-negative patients, as a group, had greater destruction of salivary glands and more severe xerostomia. The negative direct immunofluorescence of affected glands suggests that possible role of ASDA as a blocking antibody in this connective tissue disease.

Aphthous Stomatitis - Etiology.[13]

The new approach during the past year in the investigation of the etiology of this disease was centered around establishing a relationship between the degree of skin reactivity to streptococcal antigens and the recurrent oral and genital ulcers which are characteristic of this disease. The skin reactions to both alpha and beta hemolytic streptococcal antigens are arthus type reactions, similar in appearance to skin lesions produced in guinea pigs

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10. NIDR-OMS-73-008-(c)-(72)
11. NIDR-OMS-73-018-(c)-(72)
12. NIDR-OMS-73-021-(c)-(73)
13. NIDR-OMS-73-020-(c)-(73)

sensitized to these antigens. The aphthous patients' skin reactions are much larger than those in the controls and the difference in the reactions was found to be statistically significant. This finding indicates that aphthous patients are hypersensitive to streptococcal antigens. Further studies are being done to identify the specific streptococcal antigen responsible for the skin reaction and to determine if it is related to the formation of the oral and genital ulcers in this disease.

Aphthous Stomatitis - Auto-antibodies.[14]

It has been found that some of the patients with aphthous stomatitis or Behcet's syndrome have antibodies against an extract of genital tissue from a newborn infant. This humoral response is detected in general only after the patients had been skin tested with commercially prepared streptococcal antigen. The serum reaction to genital tissue extract was absorbed out with streptococcal antigens, indicating these are cross-reacting antibodies. The significance of these findings is: (1) It provides clues to the potential role bacteria may play in primary or secondary pathogenesis in these syndromes (2) it helps establish the concept of disordered immunity involving these diseases; and (3) it may further unify the concept of a cross-reacting humoral ("autoimmune") response to various antigens in the pathogenesis of a wide variety of diseases with ulcerative lesions of both oral and genital tissue.

Salivary Studies - Amylase[15-16]

Of all the proteins in parotid saliva, amylase is in the greatest concentration. Collaborative studies with Dr. L. Taussig, Childrens Hospital, Montreal, Canada, have shown that the human salivary glands start to produce amylase at about 17 weeks *in utero*. Amniotic fluid amylase was proved to be of fetal origin rather than of maternal origin as some had postulated. Future efforts will be directed at *in utero* diagnosis of cystic fibrosis of the pancreas by determining the isoamylases in the amniotic fluid rather than duodenal fluid.

Oral Physiology - Blood flow studies.[17]

Photoelectric plethysmography has been designed to monitor pulsatile blood flow in teeth. The technique may be of considerable value in testing for vitality of teeth in oral diagnosis.

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14. NIDR-OMS-73-009-(c)-(68)
15. NIDR-OMS-73-022-(c)-(73)
16. NIDR-OMS-73-019-(c)-(72)
17. NIDR-OMS-73-002-(c)-(70)

Oral Physiology - Motor mechanisms.[18]

Using another device, the biting pressures of edentulous patients were monitored at varying degrees of jaw opening. The expected reduction of biting force at opened positions beyond the normal occlusal level was not found. Rather, the patients were able to generate a relatively constant level of force at jaw positions up to 10 mm greater than the normal occlusion. These studies should be helpful in understanding the neuromuscular dynamics in normal and dentally abnormal individuals. Studies in patients with rheumatoid arthritis of the temporomandibular joint revealed that the biting forces gererated were decreased considerably during acute inflammation of the joints. In cases of symmetric TMJ involvement it was noted that the acutely involved joint demonstrated an increased temperature after exercise over the more normal joint.

Oral Roentgenology[19-20]

Previous work has demonstrated the importance of mode of display in the interpretation of radiographic information. Recent findings confirm that interpretability of radiographic displays can be enhanced by means of analog preprocessing of video images having relatively low channel capacities.

Since comparatively little information is of interest when performing a specific diagnostic task, these findings indicate that with appropriate processing it should be possible to produce useful displays with much lower radiation doses than are currently employed. This possibility has led to experiments designed to investigate the absolute minimum radiation required to produce a diagnostically meaningful radiographic image. Spin off from such experiments is expected to find its way into the development of a new type of dental radiographic system, which couples an intraoral source of radiation to an extraoral transducer. The system output is a preprocessed video display which is available "on line" and can be stored both electronically and photographically.

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18. NIDR-OMS-73-003-(c)-(65)
19. NIDR-OMS-73-016-(c)-(72)
20. NIDR-OMS-73-017-(c)-(72)

Report of the Oral and Pharyngeal Development Section
Oral Medicine and Surgery Branch
National Institute of Dental Research
Summary Statement

The activities of this Section continue to be concerned with the development of structure and the development of function in the oral and pharyngeal area.

This is the fourth year of gross anatomical studies of the face, pharynx and cranium of the human fetus at term.[1] Representative matching crania have been illustrated in general views, in coronal and in transverse sections by Keiko Moore. The anatomical sections are each matched to a tomoradiograph. This atlas project is currently being expanded by a series of drawings, by Howard Bartner, of anatomical sections in sagittal plane through the ear region. The pending book is an Atlas of Cranial Anatomical Sections and Tomoradiographs of the Human Fetus and Term, authored by Robert Pierce, Michael Mainen and James Bosma.

Forty two illustrations of selected areas of term fetal crania and of individual bones have been completed by Beverly Etter.[2] These, supplemented by portions of the section illustrations noted above and a small number of radiographs and detail photographs, comprise the materiel of a prospective general book, The Head Skeleton of the Human Fetus at Term, by J. Bosma. These two descriptions of the fetal head skeleton correspond in approach and in demonstration with the pending book, Development of the Rat Skull, under authorship of Melvyn Baer, James Bosma and James Ackerman. Publication of this book is prospectively a collaborative publication project of the NIDR and the National Library of Medicine.

We continue to engage in a small number of clinical anatomical studies, in relevance to this dissection and radiographic work. The most notable current studies are of two subjects, age 9 and 29 years, who have marked hypoplasia of the nasal composite. In each, the internal nasal skeleton (ethmoid and vomer bones) is markedly diminished in vertical and anteroposterior diameter, per tomoradiography. But there is no cleft or demonstrable absence of skeletal elements. These studies have occasioned an interpretation of the genesis of this abnormality which differs from previous interpretation, and designation, of "arrhinia" and "cleft palate". These studies are collaborative with Richard Christiansen, of the NIDR, Robert Henkin of the National Heart and Lund Institute, and Jean Herdt, of the Clinical Center Diagnostic Radiology Department.

Detailed histological studies of the human fetal, infantile and mature lip have been completed by Dr. Bradley Thach, and are reported in the pending Fourth Symposium on Oral Sensation and Perception.[3] The mucosa and

1. NIDR-OPD-73-001-(c)(72)
2. NIDR-OPD-73-003-(c)(72)
3. NIDR-OPD-73-004-(c)(73)

associated vasculature in the fetus and infant are distinctive, and are the basis for classification of zones within the lip. The prominent capillary loops in the "pars villosa", and their associated neural network have been particularly described. Dr. Thach has extended this study to the living infant, employing photographic techniques demonstrating the labial zones. In dark-skinned infants, pigment is differentially concentrated in the pars villosa.

In the current year, our physiological studies have concentrated upon elicitation of reflexes in the tongue of the human infant. The filament elicitation of lateral tongue reflex has been further calibrated by Drs. Weiffenbach and Thach and has been extended to the premature infant. This response is found consistently in viable prematures, at low sensory thresholds. Report of these studies is pending in the Fourth Symposium.

The filament touch elicitation of the lateral tongue reflex has been extended to liquid touch, by a 5 microliter drop delivered by a micropipete. It was found that the reflex response to the liquid touch diminished by adaptation, during periodic application of stimuli. But a change of solute again evoked the response. This paradigm of different response has now been developed into a novel test of linqual taste discrimination in the newborn human.

The transverse tongue reflex has also been found in adults having certain forms of oral dyskinesia. These observations were during studies of oral and pharyngeal functions in adult patients in a continuing NIMH (Section on Experimental Therapeutics, Laboratory of Clinical Science) study of patients having certain dystonic and athetotic disorders which can be neuropharmacologically modified. During these modifications, the oral, pharyngeal and laryngeal functions are studied in routines standard in our Section, including cinephotography, photovideotaping, cineradiography, sound recording and spectrographic analysis, and oral sensory and reflex testing. These routines afford unique and sensitive criteria of the effect of neuropharmacologic medications.

These intramural activities of the Section have continued in integration with the design, arrangement and publication of the Symposia on Oral Sensation and Perception. The Third Symposium, subtitled: "The Mouth of the Infant", held under joint NIDR-Fogarty International Center sponsorship in November 1970, was publiched by Thomas Company in September, 1972. The Fourth Symposium, subtitled: "Development in Infancy", was held November 20-22, 1972 and will be in press (the Government Printing Office) in July 1973. This Symposium reports recent advances in embryology of the oral and pharyngeal area, in mucosal sensory histology, in the physiology and experimental psychology of suckle feeding and of vocalization, and in the reflex testing and other clinical evaluations of the oral and pharyngeal area of the infant.

As an extension of these Symposia, and a further implementation of our interactions with clinicians and investigators, we are preparing a cinema and brochure demonstration, "Examination of the Mouth and Pharynx of the Infant". This is intended for use by persons familiar with the area. A modification of this cinema for medical school teaching is in design.

NIDR ANNUAL REPORT

RESEARCH PROJECTS AND INDEXES

INTRODUCTION

This section of the Annual Report--one of the innovative changes referred to in the Preface--replaces the Individual Project Reports that appeared in earlier issues. Since much of the information concerning the individual projects is identical to that requested on the PHS-166 form for the Smithsonian Science Information Exchange, the two efforts were consolidated insofar as possible on one form which is then reproduced to become a page in this section of the Report.

The project reports are classified, in part, by the principal investigator and again by the Scientific and Technical Communications Officer who also does some editing and all of the indexing. Keywords are converted, when possible, to conform with Medical Subject Headings (MesH), the fixed vocabulary most universally used for this purpose and developed by the National Library of Medicine. However, in order to meet certain unique requirements that the Institute has for the Annual Report, and because the front of research is often ahead of the published literature, other subject headings (non-MeSH) have been added. Likewise, the categories of subject headings have been tailored to suit the purposes of NIDR and do not necessarily coincide with those in MesH.

The following list describes our six categories and the code letter (in parantheses) identifying each.

(A) Diseases and Disorders
(B) Fields of Science and Related Subjects
(C) Technics and Equipment
(D) Biomaterials, Chemicals and Drugs
(E) Anatomical Terms
(F) Organisms and Named Groups of People

A complete list of subject headings within each of these categories will be found following the Indexes.

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RESEARCH PROJECTS BY COOPERATING UNITS

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RESEARCH PROJECTS BY INVESTIGATOR

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

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(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

(1) As determined by the principal investigator.

Principal investigators are shown in capital letters.

Total Number of Investigators 155

Total Number of Principal Investigators 80

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RESEARCH PROJECTS BY NUMBER

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RESEARCH PROJECTS BY SUBJECT AREA

( ) Denotes subject category - see page ii

SUBJECT AREA PAGE

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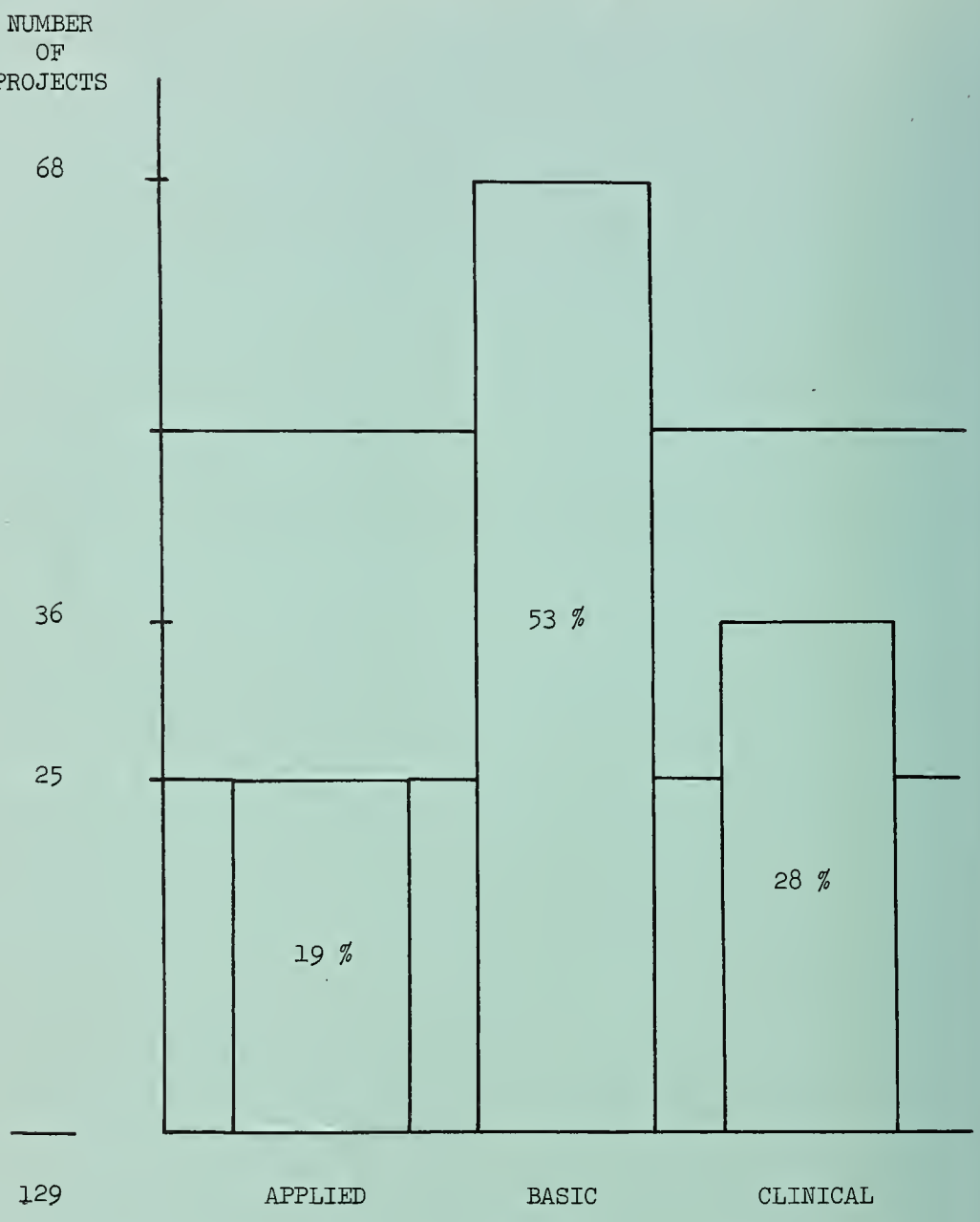
RESEARCH PROJECTS BY TYPE OF RESEARCH

As defined by the Principal Investigator

RESEARCH PROJECTS BY TYPE OF RESEARCH



The type of research is designated by the principal investigator.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Classification Guide

CATEGORIES and SUBJECTS

CATEGORY A

DISEASES AND DISORDERS

Amloidosis
Autoimmune Diseases
Behcet's Syndrome
Carcinoma
Caries, Dental
Caries, Root
Clefts
Connective Tissue Diseases
Cystic Fibrosis
Deafness
Dental Calculus
Dental Caries
Dental Enamel Hypoplasia
Dental Plaque
Diabetes
Diabetes Mellitus
Endocrine Diseases
Etiology
Fluorosis
Fibrous Dysplasia of Bone
Genetic Disorders
Gingival Hyperplasia
Gingivitis
Hereditary Diseases
Herpes Simplex
Hyperplasia
Hypersensitivity, Delayed
Hypophasphatemia, Familial
Inflammation
Keratosis
Leukemia
Leukodema
Malformations
Malocclusion
Metabolic Diseases
Monocytes
Neoplasms
Nutrition Disorders
Odontogenic Tumor
Osteitis
Pain
Periodontal Diseases
Precancerous Conditions
Pseudohypoparathyroidism
Root Caries
Salivary Gland Neoplasms
Sarcoidosis
Sjogren's Syndrome
Skull Fractures
Stomatitis, Aphthous
Submucous Fibrosis
Teratoid Tumor
Trigeminal Neuralgia
Uremia
Virus Diseases
Xerostomia

CATEGORY B

FIELDS OF SCIENCE AND RELATED SUBJECTS

Aging
Amino Acid Sequence
Anatomy
Anesthesiology
Anthropology
Antibody Formation
Biochemistry
Biological Transport
Biology
Biomedical Engineering
Biophysics
Biosynthesis
Blood Coagulation
Cell Division
Chemistry
Chemistry, Physical
Chemistry, Protein
Chemotaxis
Classification
Coagulation
Computer Science
Crystallization
Cytology
Delivery of Health Care
Dental Occlusion
Dentistry, Preventive
Development
Discrimination
Electrophoresis, Disk
Electron Spin Resonance
Embryology
Enzymology
Epidemiology
Flow Rates
Evolution
Fermentation
Gestation
Genetics
Graft vs Host Reaction
Growth
Hemodynamics
Heuristics
Histamine Liberation
Histochemistry
Histocompatibility
Histology
Histology, Comparative
Histopathology
Immune Tolerance
Immunity
Immunochemistry
Immunology
Inhibition
Lipolysis
Lymphocyte Transformation
Kinetics
Maternal-fetal Exchange
Medicine
Metabolism
Microbiology
Motor Activity
Neurology
Neurophysiology
Nidation
Nuclear Magnetic Resonance
Nutrition
Odors
Orthodontics
Pathology
Perception
Periodontics
Pharmacology
Physiology
Rheumatology
Segregation
Sensation
Speech
Stomatology
Surgery, Oral
Taste
Teratology
Virology
Wound Healing
Zoology

CATEGORY C

TECHNICS AND EQUIPMENT

Analysis
Anesthesia
Antigen-Antibody Reactions
Atlases
Autoanalysis
Automation
Bacteriology
Biochemistry
Biometry
Biopsy
Biosynthesis
Cannulation
Cephalometry
Characterization
Chemistry
Chemistry, Physical
Chemotography
Chromatography
Chromatography, Gas
Classification
Complement Fixation Tests
Computers
Cytochemistry
Cytodiagnosis
Demography
Densitometry
Dental Implantation
Dental Prophylaxis
Diagnosis
Diet
Dissection
DMF Index
Drug Therapy
Electrocardiography
Electroencephalography
Electron Probe Microanalysis
Examination
Exodontia
Flow Rates
Fixation
Fluorescent Antibody Technic
Fluoridation
Fluorometry
Freeze-etching
Hemodynamics
Histamine Liberation
Histocompatibility Testing
Histocytochemistry
Histological Technics
Hypersensitivity
Immunoassay
Implantation
Information Retrieval Systems
Lymphocyte Transformation
Medical Illustration
Microincineration
Microradiography
Microscopy
Microscopy, Electron
Microscopy, Fluorescence
Models, Theoretical
Motion Pictures
Osteotomy
Perfusion
Photography
Physics
Polygraph
Precipitation
Psychiatric Consultation
Purification
Radioautography
Radiography
Radiography, Dental
Radioimmunoassay
Scintillation
Spectrophotometry
Spectrum Analysis
Statistics
Stereognosis
Surgery
Tactile Stimulation
Therapeutics
Tomography
Tongue Tracking
Transducer
Transplantation
Vaccination
X-Ray Diffraction

CATEGORY D

BIOMATERIALS, CHEMICALS, AND DRUGS

Adhesive Sealants
Agar
Amino Acids
Amylase
Amyloid
Antibodies
Antigens
Antihistaminics
Anti-Infective Agents
Antimicrobial
Antiserotonin
Antithymocyte Serum
Apatites
Calcium Phosphates
Carbohydrates
Carcinogens
Ceramics
Complement
Collagen
Conconavalin
Corticosterone
Cryoglobulins
Dental Amalgam
Dextran
Elastin
Electrolytes
Endotoxins
Enzymes
Fluorides
Folic Acid
Gamma Globulin
Hemoglobin
Hexitols
Hormones
Hydroxyapatite
Immune Serums
Inhibitors
Ions
Isoenzymes
Lecithins
Lectins
Mannitol
Metals
Minerals
Mouthwashes
Mucopolysaccharidss
Peptides
Phosphates
Phosphofluoridate
Phospholipids
Pilocarpine
Polymyxin
Polysaccharides
Polysaccharides, Bacterial
Proteins
RNA
Steroids
Sucrose
Tablets
Teratogens
Tetracycline
Tissue Factor
Tooth, Artificial
Walnuts
Zirconium

CATEGORY E

ANATOMICAL TERMS

Ameloblasts
Amniotic Fluid
Basophiles
Blood
Bone and Bones
Cartilage
Cells, Cultures
Central Nervous System
Chemoreceptors
Chick Embryo
Connective Tissue
Dental Enamel
Dental Pulp
Dentin
Dentition
Embryo
Enzymes
Face
Face, Embryonic
Fetus
Genitalia
Gingiva
Hair
Head
Intercellular Junctions
Jaw
Leukocytes
Lip
Lymphocytes
Lysosomes
Macrophages
Mandible
Mast Cells
Monocytes
Mouth
Mouth Mucosa
Muscle
Newborn
Palate
Pancreas
Periodontium
Peripheral Nervous System
Pharynx
Pleural Fluid
Ribosomes
Saliva
Salivary Glands
Semen
Skull
Spleen
Synapses
Temporamandibular Joint
Tendons
Thymus Gland
Tissue, Connective
Tissue, Differentiating
Tissue, Mineralized
Tongue
Tooth
Trigeminal Nerve
Velum

CATEGORY F

ORGANISMS AND NAMED GROUPS OF PEOPLE

Adult
Bacteria
Chickens
Cats
Child
Clinical Center Patients
Dogs
Family
Hamsters
Hawaiians
Human
Human, Females
Human, Infants
Human, Infants, Newborn
Human, Males
Hydra
Indians, North American
Indians, South American
Infant
Mammals
Mice
Microorganisms
Mollusca
Monkeys
Negroes
Protozoa
Rabbits
Rats
Rodents
Taenia
Viruses

NATIONAL INSTITUTE OF DENTAL RESEARCH

Prepared for the Science Information Exchange.

Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

rev. Ser. No. CPR-72-001-(a)-(70)

NIDR-CPR001a70

TITLE OF PROJECT

he efficacy of an adhesive resin in preventing occlusal caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigators: Herschel S. Horowitz
Other Investigators: Stanley B. Heifetz and Sven Poulsen
Cooperating Units: Dental Division of the Montana State Health Department

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Community Programs
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

n May 1970, baseline dental examinations were conducted on 700 grade school children, indergarten and grades 1, 6 and 7, in Kalispell, Montana, a non-fluoridated community. total of 450 children were found to have one or more sound pairs of homologous ermanent teeth. Only these children were included in the study. One-half of the outh of each subject was randomly designated as the test side and the other half erved as the control side. On the test side sound occlusal surfaces were conditioned nd sealed with the bisphenol A material developed by Buonocure. Teeth were cleaned n preparation for the sealant by a dental hygienist. However, Public Health Service entists did the actual conditioning and application. The physical properties of the ealant including the extent of loss will be evaluated periodically during the three-ear investigation; follow-up examinations for dental caries increment will be made nnually.

otal Man Years: 1 1/4
Professional: 1/2
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Herschel S Horowitz	March 23, 1973

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

UPPORTING AGENCY

ETHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

UNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

HS-166
EV. 5-70

Prepared for the Science Information Exchange.

Not for publication or publication reference.

NATIONAL INSTITUTE OF DENTAL RESEARCH
U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. CPR-002-(a)-(68)

NIDR-CPR002a68

TITLE OF PROJECT

The effect of school water fluoridation on dental caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Stanley B. Heifetz
Co-Investigator: Herschel S. Horowitz
Other Investigator: Frank E. Law
Cooperating Units: Division of Water Hygiene, Environmental Protection Agency, and Dental Health Division, North Carolina State Board of Health

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Community Programs
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

In 1968, fluorides were added to the well water supply of a consolidated school (grades 1-12) in Seagrove, North Carolina at 6.3 ppm, seven times the optimal level recommended for community fluoridation in the geographic area. Children attending the Seagrove School live in an area without a central water supply and where the various drinking waters contain negligible levels of fluoride. Children are exposed to the higher concentration of fluoride at school in an attempt to duplicate the total fluoride intake of children who drink optimally fluoridated water on a full-time basis. Prior to the installation of fluoridation equipment, dental examinations using the DMF surface index were conducted on approximately 1100 children to determine base line caries prevalence. Follow-up examinations will be conducted at four-year intervals to measure caries protective benefits as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering in the first grade. On the eight-year, follow-up examinations an assessment of the prevalence of fluorosis, if any, will be made along with the regular examinations for dental caries.

Total Man Years: 1 1/2
Professional: 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Stanley B. Heifetz	March 23, 19

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

PHS-166
REV. 5-70

Prepared for the Science Information Exchange.

Not for publication or publication reference.

NATIONAL INSTITUTE OF DENTAL RESEARCH
U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-CPR003a69

Prev. Ser. No.: CPR-003-(a)-(69)

TITLE OF PROJECT

ects of acidulated phosphate-fluoride chewable tablets on dental caries in school children

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: William S. Driscoll
Other Investigators: Stanley B. Heifetz and David C. Korts
Cooperating Units: Dental Health Division, North Carolina State Board of Health and the Public Schools of Wayne County, North Carolina

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Community Programs
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forworded to investigators who request such information. Your summary is to be used for these purposes.

Baseline dental examinations were conducted in September 1969 on approximately 1000 first- and second-grade school children in Wayne County, North Carolina. Children assigned to one test group chew an acidulated phosphate-fluoride (APF) tablet containing 1 mg. F., rinse for 30 seconds with the resulting solution and then swallow the material. Another test group follows the same procedure except that they repeat the procedure at least 3 hours later using a second APF tablet also containing 1 mg. F. A third group using a placebo tablet serves as the controls. The treatments are carried out each day in school under supervision of the classroom teacher. Treatments will continue until the participants have completed the eighth grade. Follow-up dental caries examinations will be conducted at 2-3 year intervals. The first follow-up evaluation was conducted in April 1972.

Total man years: 1 3/4
Professional: 1 1/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
N/A	William S. Driscoll	March 23, 1973

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR- CPR 004a72

TITLE OF PROJECT

Amine Fluoride Gel Study

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Roald J. Shern
Other Investigators: Dr. O. Joly, Mr. R. Senning, Dr. L. Duany, Dr. D. Zinner
Cooperating Units: University of Miami, Miami, Florida

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Preventive Methods Development
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

An amine fluoride is being compared with an acidulated phosphate fluoride gel both for fluoride uptake and for caries prevention. Five treatments are applied for four minutes using a custom tray as follows:

Group A - Acidulated phosphate fluoride gel applied once daily

Gr up B - Amine fluoride gel applied once daily

Group C - Amine fluoride gel applied weekly

Groups D_1 and D_2 - Controls, suitably placebo treated.

The age range of the proband is from six through thirteen years. Definitive results will be available at the end of the second year (1974).

Total Man Years: 1 1/2
Professional: 1/2
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Roald J. Shern	

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-CPR 005a72

TITLE OF PROJECT

Amine Fluoride Mouth Rinse Study

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Roald J. Shern
Other Investigators: Dr. D. Korts, Dr. O. Joly, Mrs. B. Rundell
Cooperating Units: University of Detroit, Mount Clemens Community College, Detroit, Michigan

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Preventive Methods Development
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

An amine fluoride has been shown to be caries-restrictive when used as a dentrifice. On the basis of concurrent and antecedent laboratory studies, a new formulation of amine fluorides (oleyl and cetylamine hydrofluoride) is being tested as a mouthrinse for safety and efficacy in short clinical trials. The purpose of the study is to gain evidence concerning the advisability of undertaking long-term clinical tests on inhibiting caries and periodontal disease.

The agent, when used twice daily for thirty seconds has demonstrated the ability to reduce plaque extent and plaque viability, both on cleaned teeth and on those with pre-existing plaque. There was no evidence of toxicity. Shifts on the microbial population have not been found.

Total Man Years: 1/4
Professional: 1/2
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Roald J. Shern	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-CPR 006b72

TITLE OF PROJECT

A Study of the Mode of Action of Fluoride Treatment in Experimental Dental Caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Rachel H. Larson
Cooperating Units: The Kendall Company,
Barrington, Ill.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Preventive Methods Development Sec.
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The ultimate goal of this work is to determine the minimum fluoride exposure associated with maximum caries protection in humans. This can be achieved only as the mode of action of fluoride is better understood. As a result, a series of caries studies are run in the rat and supported by bacteriological studies and chemical analysis in an attempt to determine the conditions associated with the maximum uptake of fluoride by the tooth, the fluoride exposure associated with maximum caries protection and to determine any relationship which may exist between these two factors.

Total Man Years: 2
Professional: 1
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Rachel H Larson	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR CPR 007672

Prev. Ser. No.: NIDR-CPR-007-(b) (70)

TLE OF PROJECT

Plaque Microbiology and Familial Aggregation

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: H. M. Stiles, Senior Staff Fellow
Other Investigators: M. L. Ringelberg
Cooperating Units: John Hopkins University School of Hygiene and Public Health

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
Location: NIH, NIDR, Bethesda, Md. 20014

IMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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Individuals representing high and low caries risk groups that have remained so over a period of time have been identified in a relatively stable population. The children and grandchildren of these individuals (index cases) have been surveyed to determine the numbers of 5 streptococcal species in the dental plaque, for DMFS and other factors such as residential and familial background which might be pertinent to a familial aggregation.

A test of independence based on a two-way classification (DMFS and number of plaque microorganisms) were used to assess the two methods of distribution.

Total Man Years: 1
Professional: 1/2
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	H. M. Stiles	

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UPPORTING AGENCY

ETHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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Prev. Ser. No.: None

U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

NIDR CPR 008b72

TITLE OF PROJECT

Transmission of Oral Streptococci in Children

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: H. M. Stiles, Senior Staff Fellow
Other Investigator: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

A pilot study is being conducted to ascertain age and population number parameters needed for a longitudinal study of the problem of presence and time of appearance of Streptococcus mutans and Streptococcus sanguis in the mouths of children. Included in this analysis are oral and fecal samples. Of particular significance is the variability of dental eruption patterns and time of occurrence of some of the oral streptococci.

Laboratory preparation for this study involves carrying out several small short range studies dealing with transportation and storage of specimens. These included supression of other flora (i.e., preventing overgrowth by gram negative flora)without suppressing the streptococcal population.

Total Man Years: 1 1/4
Professional: 1/2
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	H. M. Stiles	

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotioted Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No.: None

NIDR-CPR 009b72

TITLE OF PROJECT

Identification of S.mutans and other Dental Plaque Streptococci Using Cultural, Morphological and Immunofluorescent Methods.

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigators: L. Ariel Thomson
Janet Brunelle
Richard Facklam CDC, HSMA,DHEW
William Cherry CDC, HSMA,DHEW

Cooperating Units: Center for Disease Control HSMA, DHEW

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
Location: NIDR,NIH, Bethesda, Md.,20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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The purpose of the present project is to provide identification criteria (with supporting data) for certain streptococci considered to be potentially cariogenic. In addition, the immunofluorescent capabilities of several laboratories have been joined in colaborative research designed to develop FA reagent grade conjugates specific for certain plaque streptococci.

Numerical taxonomic methods, other computer techniques, as well as conventional methods are being used to analyse the data from over 700 strains to develop differentiating criteria. Colored plates are being prepared to portray representative colonial types.

Fluorescent antibody research has attempted to build on the work of both Jablon and Bratthall. Improved methods for obtaining IgG fractions, labeling, and conjugate evaluation with subsequent improvement have been adopted to improve S. mutans conjugates. Additionally the optimal F/P ratio, antigen preparation methods, immunization schedule, and specificity are under study.

Total Man Years: 33/4
Professional: 2¾
Other: 1½

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No.: None

NIDR CPR 010c72

TITLE OF PROJECT

Analysis of dental caries prevalence data in Danish children of preschool age

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Sven Poulsen,
Guest Worker

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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Data collected during a semilongitudinal survey on dental caries prevalence in the decid dentition of Danish children of preschool age are being analyzed. An attempt to develop a method to estimate the condition of deciduous surfaces which are missing at the time o examination is being done.

Man Years:

Professional: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Sven Poulsen	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No.: None

NIDR CPR 011a73

TITLE OF PROJECT

The effect of fluoridated milk on the development of dental caries in rats

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Sven Poulsen, Guest Worker
Co-Investigator: Rachel Larson, Section Chief

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
Location: NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Milk fluoridated to 50 ppm is being compared to water with the same fluoride content, non-fluoridated milk and non-fluoridated water in an animal caries-test system.

The fluoridated solutions are being administered to each animal in a 3 ml volume in separate drinking cups, all other drinking solutions being withheld until the rats have finished the above mentioned volume.

Total Man Years:

Professional: 1/4
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Sven Poulsen	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-CPR 022b70

Prev. Ser. No. NIDR-CPR-22-(B)-(70)

TITLE OF PROJECT

A Study of the Modes of Action of Fluoride on the development of Dental Caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Rachel H. Larson
Other Investigators:
Cooperating Units: The Kendall Co., Barrington, Illinois

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Caries Prevention & Research
Section: Preventive Methods Development
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The ultimate goal of this work is to determine the minimum fluoride exposure associated with maximum caries protection in humans. This can be achieved only as the mode of action of fluoride is better understood. As a result, a series of caries studies have been run in the rat supported by bacteriological studies and chemical analysis in an attempt to determine the conditions associated with maximum caries protection and to determine any relationship which may exist between these two factors.

Total Man Years:
Professional:
Other:

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Rachel H. Larson	4/16/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NATIONAL INSTITUTE OF DENTAL RESEARCH
U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

Prepared for the Science Information Exchange.

Not for publication or publication reference.

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-CPR-026C73

Prev. Ser. No. None

TITLE OF PROJECT

Evaluation of a combination of self-administered measures of fluoride exposure for the control of dental caries in a non-fluoride area

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Herschel S. Horowitz
Other Investigators: Stanley B. Heifetz, Rhea Meyers and William S. Driscoll
Cooperating Units: Virginia Department of Health, Division of Dental Health and Albemarle-Charlottesville-Nelson Health District, and Nelson County Public School System

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Community Programs
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK — *(200 words or less — Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Base line dental examinations were conducted in October 1972 on approximately 2200 first- through twelfth-grade children in Nelson County, Virginia. All study participants in grades 1-6 chew daily in school under supervision of the classroom teacher an acidulated phosphate-fluoride (APF) tablet containing 1 mg. F, rinse for 30 seconds with the resulting solution and then swallow the material. Once a week in school the same children also swish 10 milliliters of a 0.2 percent sodium fluoride solution for 60 seconds and then empty the contents of the mouth into a cup. A fluoride-containing dentifrice is distributed to the same children for use at home, and they receive toothbrushes periodically to take home. County health authorities and the county public school system are responsible for developing an oral health education program designed for elementary grades. These combined preventive procedures will continue in the elementary schools for a minimum of ten years. Follow-up dental examinations will be carried out biannually in all schools.

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Herschel S Horowitz	March 26, 1973

DO NOT WRITE BELOW THIS LINE — FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. None

NIDR-CPR-027C73

TITLE OF PROJECT

Evaluation of a combined program of school water fluoridation and sodium fluoride mouthrinsing for the control of dental caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Olga G. Joly
Other Investigators: Dr. George von Mohr and Dr. Douglas Jackson
Cooperating Units: North Carolina State Board of Health, Fayetteville, N.C.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Community Programs
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

About 2000 children attending grades 1 through 12 in three schools of Robeson County, North Carolina, are exposed to 1. fluoridation of the school water supply at four and one-half times the level estimated to be optimum for community fluoridation in the same geographic area, and 2. weekly mouthrinsing with 0.2% NaF solution. Teachers dispense 10 milliliters of solution to each participant-child and supervise the one-minute rinsing procedures which are carried out in the classroom. To evaluate the long-term benefits of the combined treatments on the prevalence of dental caries, pre-treatment clinical data will be compared with the data obtained every two years until the completion of the program in 1984. Base line examinations using the DMF tooth and surface index were conducted in September 1972. The data are currently being processed.

Total Man Years: 4
Professional: 3
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Olga G. Joly	March 26,

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-CPR-028C73

Prev. Ser. No. None

TITLE OF PROJECT

Prevention of Smooth Surface and Pit and Fissure Caries.

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigators: L. Ariel Thomson
Other Investigators: Donald Boggs
Cooperating Units: Indian Health Service, HSMA, D.H.E.W.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Caries Prevention and Research
Section: Etiology
Location: NIDR, NIH, Bethesda, Md., 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Preventive measures affecting both smooth surface and fissure decay were studied in a school population. Two groups of 400 fifth and sixth grade students were assigned randomly for either routine maintenance dental care or care plus two preventive measures. Both the "routine care group" and the "preventive group" had all decayed teeth restored. The preventive regime consists of application of tooth sealant (bisphenol A material developed by Buonocore) to decay-free chewing surfaces and 10 fluoride treatments using custom-fitted trays.

Beginning April, 1972 and continuing during 1973, students will be examined (baseline) by classes and radiographs taken. Specially trained dental auxiliary workers will provide the preventive services as rapidly as possible for those designated and record the time required. All children will receive dental restorative care during the three years observation after the baseline examinations. Annual examinations will be conducted to determine the dental caries increment. Teeth observed to require sealant will be sealed. The data will be analyzed to determine the effectiveness, the time required, and the cost-benefit of combining preventive treatments of tooth sealant and repeated topical fluoride in the population.

Total Man Years: 2½
Professional: ½
Other: 2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-1

NIDR-DIR001a68

TITLE OF PROJECT

Laboratory Automation

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Mr. John J. Wilson, Chief, Computer Processing and Analysis Se
Other Investigators: Mr. Claude K. Jackson, Computer Programmer
Mr. Terry P. Medlin, Computer Programmer
Cooperating Units: Computer Systems Laboratory, Division of Computer Research and Technology, NIH

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Computer Processing and Analysis Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This project is a joint collaborative endeavor on the part of NIDR and of the Division of Computer Research and Technology, NIH.

It is a laboratory automation project which is involved in the concurrent on-line acquisition and real time processing of data from a number of analytical instruments and experiments. The project also involves the control of bacteria growth experiments. The computer complex (Honeywell H-316 and H-516) operates under the OLERT multiprogramming operating system. The system is interfaced with an amino acid analyzer, liquid scintillation counters, a spectrophotometer, a gas chromatograph, X-ray diffractometer, a fermenter-pumping system and neurophysiology microelectrode experiments. Telecommunication with the NIH central IBM 370 and PDP-10 computers has been established.

Total Man Years: 5
Professional: 2
Other: 3

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	John J. Wilson	4/1 73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-DIR002a72

TITLE OF PROJECT

Laboratory Automation Software

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Mr. William I. Wood, Senior Assistant Health Services Officer
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Computer Processing and Analysis Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

1. Multiprogramming Systems Software for the NIDR Laboratory Automation System: Maintenance of the OLERT multiprocessor real-time operating system. Modification of OLERT for addition of a fixed head disk.

2. Neurophysiological Data Analysis Software: Design and implementation of a series of programs to process and graph neurophysiological data. These programs run on the NIDR OLERT computer system.

3. Binary Synchronous Communications Software: Design and implementation of IBM compatible communications systems software for telephone communications between the NIDR OLERT computer system and the IBM 360/370 computer at the Division of Computer Research and Technology, NIH.

Total Man Years: 1
Professional: 1
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	John J Wilson	4/18/73

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR008b68

NIDR-DIR101b68

TITLE OF PROJECT

Control of Aggregation and Differentiation in Dictyostelium Discoideum

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Bruce M. Chassy, Research Chemist
Other Investigators: Mr. Leslie L. Love, Biological Laboratory Technician
Ms. Emily V. Palumbo, Chemist
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Adenyl cyclase activity was identified in the slime mold. It was found to be stimulat by thiamin pyrophosphate. Purification and further characterization of this controlling enzyme will be continued.

A new metabolite arising from cyclic AMP has been detected. Its identity and physiological function are being studied.

Folic acid inhibits aggregation and controls feeding behavior in D. discoideum.

Total Man Years: 1 3/4
Professional: 1/2
Other: 1 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Bruce M Chassy (PD)	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR009b72

NIDR-DIR102b72

TITLE OF PROJECT

Time Lapse Photographic Studies of Aggregation and Differentiation in Dictyostelium Discoideum

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Mr. Leslie L. Love, Biological Laboratory Technician
Other Investigators: Dr. Micah I. Krichevsky, Chief, Environmental Mechanisms Section
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

We have been able to grow five strains Dictyostelium discoideum in axenic liquid culture. Also, these strains can be grown on agar surfaces, but slowly. Thus, stock cultures of axenically grown strains can be maintained without the short term liquid cultures previously required.

The axenic strain (AX-1) responds chemotactically to cyclic AMP, almost like NC4 strain. However, the concentration required is both lower and critical. Too much cyclic AMP does not show chemotaxis even though the cells differentiate. We found that folic acid causes chemotactic responses at the same concentrations in both strains.

Folic acid, at the proper concentration, inhibited differentiation in the axenic strain without losing its chemotactic properties. The cells retained their motility and normal appearance although they do not differentiate at any time on agar.

Time lapse cinematography has shown that folic acid and cyclic AMP act at different stages of development. A cell suspension, placed on agar containing both chemotactic agents shows two concentric rings of cells. The outer ring has the same diameter change with time as that with folic acid alone. The inner ring corresponds to that obtained with cyclic AMP alone.

Total Man Years: 1 1/2
Professional: 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Leslie L. Love	4-18-73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR011a70

NIDR-DIR103a70

TITLE OF PROJECT

Kinetics of Growth and Acid Production by Dextran-Forming Streptococci

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jacob A. Donkersloot, Senior Staff Fellow
Other Investigators: Mr. Lawrence A. Pareles, Biologist

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The kinetics of growth and acid production by Streptococcus mutans in glucose, sucrose, or fructose-containing media have been measured. Data were acquired in real-time by a digital computer. Afterwards, a PDP-10 computer and MLAB (a powerful modeling program with visual display capabilities) were used to evaluate various mathematical models to describe the results. A simple differential equation was found to accurately represent the acid production data during logarithmic growth. The developed model also allows the calculation of the specific growth rate from the acid production data. The results obtained agreed with these obtained by conventional methods.

Presently transient states, such as those occurring after a downward pH shift or a sodium fluoride addition, are studied. The effects of a fluoride addition on the growth and acid production rate are more pronounced at lower pH. For instance, at pH6 a significant decrease occurs with 40 ppm F, while at pH 5.5 a similar decrease is observed with 20 ppm.

Total Man Years: 1
Professional: 1/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Gerard Donkersloot	4/18/7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR012a71

NIDR-DIR104a71

TLE OF PROJECT

On-line Control of, and Data Acquisition from Fermentations by a Digital Computer

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jacob A. Donkersloot, Senior Staff Fellow
Other Investigators: Mr. Frederick J. Brown, Electronic Engineer
Mr. Claude K. Jackson, Computer Programmer

AME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The fermentor-computer system has been expanded and tested. Turbidity and fluoride ion activity data are now also collected in real-time, in addition to pH, acid addition and base addition. Consequently, the data acquisition and control program was updated to account for these changes. The program was modified to allow the user to shift the pH up or down at a specified rate.

Both hardware and software were extensively tested and performed to specification. The actual pH control observed was within ± 0.02 of the setpoint during the entire course of a fermentation or during pH shift experiments.

Total Man Years: 1
Professional: 1/4
Other: 3/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Jacob Donkersloot	4/18/73

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PORTING AGENCY

THOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR01367

NIDR-DIR10571

TITLE OF PROJECT

Extracellular Polysaccharide Formation by Streptococcus Mutans

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jacob A. Donkersloot, Senior Staff Fellow
Other Investigators: Dr. Bruce M. Chassy, Research Chemist
Dr. H. A. deLeon, Visiting Fellow
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

One of the characteristics of Streptococcus mutans is the formation of elevated colonies on Mitis-Salivarius agar. It is generally believed that polysaccharide production from sucrose contributes to this special colonial morphology. Certain S. mutans strains produce, in addition, a clear exudate which can be seen either as a bubble on top of the colony or as a puddle around the colony. Our present aim is to analyze this exudate. Preliminary results indicate that the major constituent of the exudate is a polyglucan. Minor amounts of sucrose, glucose, fructose and lactic acid have also been detected.

Total Man Years: 1
Professional: 1/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Jacob A. Donkersloot	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR014b71

NIDR-DIR106b71

TLE OF PROJECT

Sucrose Metabolism by Streptococcus Mutans

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Bruce M. Chassy, Research Chemist
Other Investigators: None
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

In order to study the various enzymatic systems responsible for the primary attack on the sucrose molecule by cariogenic streptococci, we are purifying the polysaccharide producing enzymes. The invertase activities are also being isolated for characterization since this is the primary route of sucrose metabolism.

Total Man Years: 1/2
Professional: 1/4
Other: 1/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Bruce M Chassy (PD)	

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THOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PROJECT NO. (DO NOT USE THIS SPACE

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-DIR015a71

NIDR-DIR107a71

TITLE OF PROJECT

In situ Measurement of Turbidity in Microbial Cultures

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jacob A. Donkersloot, Senior Staff Fellow
Other Investigators: Dr. Micah I. Krichevsky, Chief, Environmental Mechanisms Sec
Mr. Leidy Zern, Physical Science Technician
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The prototype autoclavable light probe to measure bacterial growth in situ (described in the previous year report) has been tested for long- and short-term stability. The probe has also been used to monitor the growth of Streptococcus mutans. It performed well over a wide range of bacterial concentrations (comparable range of absorbancy measurements at 600 nm is 0.01-10). Mathematical functions to convert the output, a recorded by the computer, into absorbancy units have been evaluated and a cubic polynomial choosen for preliminary calibration purpose.

As a result of the encouraging results obtained with the prototype, four new light probes with different design parameters are being built.

Total Man Years: 1 1/2
Professional: 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Jacob A. Donkersloot	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR016a71 NIDR-DIR108a71

E OF PROJECT

Handling of Microbial Strain Information by Computers

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER
ESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Micah I. Krichevsky, Chief, Environmental Mechanisms Section
Other Investigators: Mr. Morrison Rogosa, Research Microbiologist, Microbial Physiology Section, Laboratory of Microbiology, NIDR
Cooperating Units: Dr. L. Norton, Heuristics Laboratory, Division of Computer Research and Technology
Dr. R.R. Colwell, Biology Department, Georgetown University
Americal Type Culture Collection
Professor V. Skerman, Univ. of Queensland, Brisbane, Australia

AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

ARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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o-sciences and are forwarded to investigators who request such information. Your summary is to be used for those purposes.

finition and standardization of the questions that are asked about microbial strains in computer-compatible form is the first goal. Programs are being developed to enter data d retrieve it in a variety of ways for epidemiological, diagnostic, taxanomic, ological, etc. uses. The long term goal is to establish a world-wide data bank at a ries of cooperating centers.

data file of primary data on a large number of bacteria found in the oral cavity and lated types is being established. This file will provide a resource for asking both ological and epidemiological questions of interest in dental research.

tal Man Years: 3/4
Professional: 1/2
Other: 1/4

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH
THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR
Micah I. Krichevsky (KRS)

DATE

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ORTING AGENCY

OD OF SUPPORT *(Check one)*

gency Staff (ntramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-DIR017a72

NIDR-DIR109a72

TITLE OF PROJECT

Use of an On-Line Computer as a Laboratory Instrument

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Bruce M. Chassy, Research Chemist
Other Investigators: None
Cooperating Units: Mr. L. Freeman, Division of Computer Research and Technology

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Environmental Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Under the operating system of the NIDR computer, procedures were developed to acquire data from the gas chromatograph and calculate areas under the curves. This system now operates in conjunction with the central PDP-10 using an interactive graphics package.

The Gilford spectrophotometer was interfaced with the NIDR computer. A complex set of modular programs including calculations of enzyme kinetics and scanning densitometry have been implemented. Other application are under development.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Bruce M Chassy (RO)	

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DAT

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

, Ser. No. NIDR-DIR-006-(b)-(66) NIDR-DIR201b66

OF PROJECT

omical Studies of the Organization of the Main Sensory and Spinal V Nuclei

AMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER SSIONAL PERSONNEL ENGAGED ON THE PROJECT.

cipal Investigator: Dr. Stephen Gobel, Senior Dental Surgeon
Co-Investigator: Ms. Marlene Purvis, Biological Laboratory Technician
Cooperating Units: None

ANO ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

RY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

he Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

project involves light and electron microscopical studies of the neuronal morphology organization of the neuropil of the main sensory and spinal V nuclei in rats, cats and eys. The objectives of these studies are to broaden our understanding of orofacial ation and the mechanisms of pain.

l Man Years: 2
ofessional: 1
Other: 1

SSIONAL SCHOOL *(medical, dental, etc.)* WITH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Stephen Gobel	4/18/73

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TING AGENCY

OF SUPPORT *(Check one)*

ency Staff tramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PROJECT NO. (DO NOT USE THIS SPA

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-DIR-003-(b)-(70)

NIDR-DIR202b70

TITLE OF PROJECT

The Activity of Trigeminal Afferent Fibers of the Monkey in Response to Noxious and Innocuous Stimuli

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Rhyuji Sumino, Visiting Scientist
Co-Investigators: Dr. Ronald Dubner, Chief, Neural Mechanisms Section
Dr. Sidney Starkman, Research Associate

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Under barbiturate anesthesia, thin nerve bundles are dissected from the infraorbi and inferior alveolar nerves, and their electrical activity recorded and stored o magnetic tape. Thermal and noxious heat stimuli are delivered with a precisely-controlled thermode which has a temperature range of 20°C to 60°C and can produce temperature change of 10°C at the thermode-skin junction within one second. Dat analyzed with the aid of an on-line, real-time computer system.

Total Man Years: 2
Professional: 1 3/4
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	R. Sumino	April 1

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-DIR-005-(b)-(65)

NIDR-DIR203b65

E OF PROJECT

The Response Properties of Neurons in the Trigeminal Brain-stem Nuclear Complex of the Monkey to noxious and innocuous stimuli

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Ronald Dubner, Chief, Neural Mechanisms Section
Co-Investigator: Dr. Ralph Beitel, Staff Fellow
Cooperating Units: None

AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

ARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in o-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Microlectrode recordings from the rostral and caudal divisions of the trigeminal brain-stem nuclei are performed in anesthetized monkeys. Mechanical, thermal, and noxious stimuli are applied to the face and oral mucosa and the response properties of single neurons are recorded and stored on magnetic tape. Data is analyzed with the aid of an on-line, real-time computer system.

Total Man Years: 1 1/4
Professional: 3/4
Other: 1/2

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR

Ronald Dubner

DATE

4/12/73

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D OF SUPPORT *(Check one)*

☐ gency Staff ntramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-DIR-007-(a)-(69)

NIDR-DIR204a69

TITLE OF PROJECT

Neurophysiologic Instrumentation, Laboratory Instrumentation and Computer Interfacing of Laboratory Instrumentation

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Mr. Frederick J. Brown, Electronic Engineer
Co-Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This work involves the development of suitable electronic and electromechanical instrumentation to be used in neuro-physiological and behavioral research. It involves the adaptation and interfacing of these and other instruments to a multipurpose computer installation.

Total Man Years: 1
Professional: 1
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Frederick J. Brown	4-18-7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-DIR205b72

OF PROJECT

Correlation of anatomically identified oro-facial skin receptors in the monkey with the physiological properties of mechanosensitive trigeminal afferent fibers

IAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER SSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Sidney Starkman, Research Associate
Co-Investigators: Dr. Rhyuji Sumino, Visiting Scientist
Dr. Ronald Dubner, Chief, Neural Mechanisms Section
Dr. Stephen Gobel, Senior Dental Surgeon
Ms. Joan Binck, Biologist
Cooperating Units: Dr. Bryce Munger, Department of Anatomy, Hershey Medical Center, Hershey, Pennsylvania

AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

RY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The oral-facial skin receptive fields of mechanosensitive trigeminal afferent nerve fibers, dissected from the infraorbital nerve in the monkey, are studied. At the termination of the experiment, these skin areas are surgically dissected for histological study with light and electron microscope techniques.

Total Man Years: 1
Professional: 1/2
Other: 1/2

SSIONAL SCHOOL *(medical, dental, etc.)* WITH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Sidney Starkman

DATE: 4/18/73

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RTING AGENCY

D OF SUPPORT *(Check one)*

lency Staff tramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

NIDR-DIR206a72

Prev. Ser. No. None

TITLE OF PROJECT

Behavorial measurement of pain and temperature discrimination in monkey and human

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Ralph Beitel, Staff Fellow
Co-Investigators: Dr. Ronald Dubner, Chief, Neural Mechanisms Section
Ms. Joan Binck, Biologist
Mr. Frederick Brown, Electronic Engineer
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Office: Director of Intramural Research
Section: Neural Mechanisms Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Monkeys are trained to detect and recognize warming or cooling stimuli applied to their face in order to receive a liquid reward. They are also trained to avoid potentially noxious or escape from noxious stimuli. Measurement of warm, cold, and escape thresholds are made. A corresponding study of pain and temperature thresholds in humans, utilizes the same stimulus parameters.

Total Man Years: 1 3/4
Professional: 1
Other 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Ralph E. Beitel	4-18-7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

. Ser. No. DS-72-001-(c)-(72)

NIDR-DSB001c72

E OF PROJECT

ntal Care for the Clinical Center Patient Population

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER FESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Dr. Samuel Kakehashi, Chief, Dental Services Branch, NIDR
Co-Investigators: Dr. John Folio, Dental Services Branch, NIDR
Dr. Patrick Looney, Dental Services Branch, NIDR
Dr. William Beck, " " " "
Dr. Stephen Fred, " " " "

E AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

ARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in io-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

nsultations and necessary dental care are extended to the research patient population the Clinical Center. Services are limited to dental treatment germane to the medical re and research requirements of patients undergoing active study in one of the intra-ral clinical research programs at the National Institutes of Health.

Man Years: 1 3/4
rofessional: 1 1/4
Other: 1/2

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		4/17/73

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OD OF SUPPORT *(Check one)*

Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PROJECT NO. (DO NOT USE THIS SPA

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. DS-72-002-(c)-(68)

NIDR-DSB002c68

TITLE OF PROJECT

A Study of Differences in Occurrence of Dental Caries in Caucasian and Negro Children

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Samuel Kakehashi, Chief, Dental Services Branch, NIDR
Co-Investigator: Dr. Norman W. Littleton, Richmond, Virginia
Other Investigators: None
Cooperating Units: Forsythe Dental Clinic, Boston, Massachusetts

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Studies to determine the dental caries experience and clinical evidence of fluorosis in samples of Caucasian and Negro children have been undertaken. An analysis of enamel biop data is currently in progress.

Total Man Years: 7/8
Professional: 3/8
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		4/17/73

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. DS-72-004-(c)-(67)

NIDR-DSB004c67

LE OF PROJECT

ssue Healing Following Oral Surgical Procedures

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Incipal Investigator: Dr. Patrick Looney, Dental Services Branch, NIDR
Other Investigators: None
Cooperating Units: None

AE AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

MARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

study to delineate the relationship of localized osteitis following oral surgical ractions to local, systemic, constitutional and operative factors.

rtinent pre and postoperative data are recorded and patients are kept under close servation until surgical sites are asymptomatic postoperatively.

l Man Years:
rofessional: 1/4
Other: 0

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		4/17/73

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HOD OF SUPPORT *(Check one)*

Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Previous Ser. No. DS-72-005-(c)-(67)

NIDR-DSB005c67

TITLE OF PROJECT

Roentgenographic Study of the Temporomandibular Joint Following Osteotomy of the Mandibular Rami

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Patrick Looney, Dental Services Branch, NIDR
Co-Investigator: Dr. John Folio, Dental Services Branch, NIDR
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

There continues to be many unanswered questions as to what happens to the temporomandibul joints following bilateral osteotomy of the mandible. To date, only sparse long-term postoperative data are available.

This study utilizes several different surgical techniques and the patients are followed for as long as possible. X-ray and clinical examinations are undertaken each year to evaluate changes in the occlusion or temporomandibular joint symptoms.

Total Man Years: 1 1/4
Professional: 1/4
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Patrick J Looney	4/17/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DAT

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U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

. Ser. No. DS-72-006-(a)-(72)

NIDR-DSB006c72

E OF PROJECT

termination of the Presence and Location of Gamma 2 Phase in Dental Amalgams
Related to Tarnish and Corrosion

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER
FESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

cipal Investigator: Dr. Stephen R. Fred, Dental Services Branch, NIDR
Co-Investigator: Mr. Richard Waterstrat, A.D.A. Research Associate, NBS, Gaithersburg, M
ther Investigators: Dr. Edward D. Eanes, Chief, Molecular Structure Section, LBS, NIDR
Dr. David Greenfield, Molecular Structure Section, LBS, NIDR
Cooperating Units: National Bureau of Standards, Gaithersburg, Md.

E AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

ARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in
o-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

ttempt is being made to identify the gamma 2 phase (mercury-tin) and its geography
malgams. This phase, thought to be responsible for amalgam tarnish and corrosion
be found concentrated in certain areas of the final amalgam filling. Six amalgam
ys are being studied, two of which are reported to have little or no gamma 2 phase
he final amalgam.

tron microprobe and x-ray diffraction studies of these amalgam surfaces may lead to
ideas of the nature of gamma 2 phase location and its role in tarnish and corrosion.

Man Years: 7/8
rofessional: 7/8
Other: 0

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Stephen R. Fred	4/17/73

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ORTING AGENCY

OD OF SUPPORT *(Check one)*

☐ gency Staff Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

NIDR-DSB007c73

Prev. Ser. No. None

TITLE OF PROJECT

Radiographic Detection of Induced Skull Fractures

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. John Folio, Dental Services Branch, NIDR
Other Investigators: Dr. Richard Webber, Oral Medicine and Surgery Branch, NIDR
Dr. John Doppman, Diagnostic Radiology Department
Cooperating Unit: Diagnostic Radiology Department, The Clinical Center, NIH

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Dental Services Branch
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This investigation is to study the ability to diagnose fractures of the occipital and tempro-parital regions of the skull, by conventional radiographic interpretive procedu Preliminary evidence suggest that many types of non-displaced fractures are not detecta This study will involve a double blind interpenetration of radiographs containing indu skull fractures.

Total Man Years: 3/4
Professional: 3/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	John Folio	4/17/7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Ser. No. NIDR-EPB-72-001-(b) (64)

NIDR-EPB001b64

OF PROJECT

istochemical and chemical studies of normal and diseased tissues

AMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER
SSIONAL PERSONNEL ENGAGED ON THE PROJECT.

ipal Investigator: Dr. W. A. Gibson
her Investigators: None
Cooperating Units: Dr. H. Spencer, Hines V.A. Hospital, Hines, Ill.

AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology Branch
Section: Histochemistry Section
Location: NIDR, NIH, Bethesda, Maryland 20014

RY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

ochemical and chemical studies carried out in normal and diseased tissues to determine basic mechanisms involved in the pathogenesis of various oral diseases. Emphasis is ed on qualitative and quantitative changes in the various enzyme activities reflecting ges in basic metabolic activities. The periodontium of human and experimental animals, n oral mucosa in folic acid deficiency and polyoma induced salivary gland tumors in are currently being investigated.

l Man Years: 4
rofessional: 1
Other: 3

SSIONAL SCHOOL *(medical, dental, etc.)* WITH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	William A. Gibson	4/5/73

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RTING AGENCY

D OF SUPPORT *(Check one)*

ency Staff tramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. NIDR-EPB-72-002-(b) (66)

NIDR-EPB002b66

TITLE OF PROJECT

Histochemical and biochemical studies of connective tissues

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: J. F. Goggins
Other Investigators: L. G. Raisz, A. R. Severson
Cooperating Units: G. S. Johnson and I. Pastan, NCI

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology Branch
Section: Histochemistry Section
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The purpose of this study is to examine the biosynthesis and degradation of connective tissue acid mucopolysaccharides in the normal and diseased states. In vitro methods are used to examine these processes and the factors that affect them.

Total Man Years: 4
Professional: 1
Other: 3

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	John F. Goggins	4/5/7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-EPB003a63

rev. Ser. No. NIDR-EPB-003-(a)-63

TLE OF PROJECT

linico-pathologic Studies of Human Oral Mucosa

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Howell O. Archard, DDS, Chief, Diagnostic Pathology
Other Investigators: None
Cooperating Units: National Cancer Institute, Laboratory of Pathology
National Cancer Institute, Dermatology Branch

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology
Section: Diagnostic Pathology
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Human oral mucosal lesions of a keratotic nature are studied clinically and histologically over their duration for changes occurring in them. Lesions studied include hyperplastic, pre-malignant, and inherited mucosal disorders. Each case undergoes a detailed environmental analysis, appropriate laboratory studies are performed, and a record of the clinical and histologic changes is obtained in order to document the clinicopathologic progression of the disease.

Where indicated, appropriate correlative studies, such as histochemistry, electron microscopy, biodynamic (turnover) studies, immunofluorescent procedures, etc., are employed to provide additional significant data.

otal Man Years: 1-1/4
Professional: 1/2
Other: 3/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	H. O. Archard DDS	4/6/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-EPB-72-004-(a)-66

NIDR-EPB004a66

TITLE OF PROJECT

Clinical and Morphologic Studies of the Human Dentition in Acquired and Inherited Metabol Diseases

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Howell O. Archard, DDS, Chief, Diagnostic Pathology
Other Investigators: None
Cooperating Units: National Heart and Lung Institute, Endocrinology Branch
National Institute of Arthritis and Metabolic Diseases, Metabolic Diseases Branch
National Institute of Neurological Diseases and Strokes, Section on Child Neurology

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology
Section: Diagnostic Pathology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Clinical and morphologic characteristics of the deciduous and permanent teeth of individuals affected with a wide variety of inherited or acquired metabolic diseases are studied. The gross and microscopic changes in the teeth are recorded, and correlations with the developmental events and general metabolic changes are undertaken.

Teeth removed from patients with metabolic disease who have been studied at the Clinical Center of the National Institutes of Health, comprise the major source of material, although specimens from outside sources are studied as well. Disorders currently under study include: Hereditary hypophosphatemia (Vitamin D-resistant rickets), hypophosphatasia, pseudohypoparathyroidism, pseudo-pseudohypoparathyroidism, Hurler's syndrome, diabetes insipidus, ectodermal dysplasia, chronic Vitamin D intoxication, infantile hypercalcemia, primary hyperoxaluria, porphyria, the Fanconi syndrome, cystinuria, acatalasia, etc.

Total Man Years: 1-1/4
Professional: 1/2
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	H. O. Archard DDS	4/6/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-EPB005a70

ev. Ser. No. NIDR-EPB-72-005-(a)-70

TLE OF PROJECT

linicopathologic Studies of Minor Salivary Glands

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Thomas M. Tarpley, Jr., DDS
Other Investigators: Norman A. Cummings, Thomas M. Chused, and Robert O. Wolf
Cooperating Units: National Institute of Arthritis and Metabolic Diseases, Arthritis and Rheumatism Branch

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology
Section: Diagnostic Pathology
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forworded to investigators who request such information. Your summary is to be used for these purposes.

Major and minor salivary glands from human and animal sources are studied by routine histological techniques and correlative histochemical, immunofluorescent, and electron microscopic procedutes. Clinicopathologic studies of salivary glands in various disease states are undertaken. Diseases currently under study include Sjögren's syndrome, sarcoidosis, adenoid cystic carcinoma, lupus erythematosus and related connective tissue disorders.

Total Man Years: 2¼
Professional: 1
Other: 1¼

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas M. Tarpley	4/6/73

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THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-EPB-72-006-(c)-(66)

NIDR-EPB006c66

TITLE OF PROJECT

Oncological Research

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. James E. Hammer, III , Chief, Section of Oncology, EPB, NIDR

Other Investigators: None

Cooperating Units: Dr. Jens J. Pindborg, Chairman, Dept. of Oral Pathology
The Royal Dental College, Copenhagen, Denmark
Tata Institute of Fundamental Research
The Royal Dental College
Dr. Fali S. Mehta, Tata Institute Fundamental Research, Bombay,

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Experimental Pathology Branch
Section: Oncology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Scientific investigations underway or planned for the immediate future by the Section of Oncology include: (A) continued accumulation of fibro-osseous study cases; (B) continued 10 year follow-up of oral cancer and oral premalignant conditions in 65,000 rural Indian villagers with Dr. Mehta and Dr. Pindborg; (C) collaboration as a Collecting Center for oral precancerous conditions with other World Health Organization designated centers; and (D) clinical and basic studies of fibro-osseous lesion and oral carcinoma cases within the Clinical Center framework. Basic investigations of the carcinogenic potential of the ingredients of betel quid are being conducted on baboons (Papio cynocephalus) under contract arrangement with the Southwest Foundation for Research & Education in San Antonio, Texas by Dr. Hammer.

Total Man Years: 1
Professional: 1/2
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	James E. Hammer III	4/5/73

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SUPPORTING AGENCY

METHOD OF SUPPORT (Check one)

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-HG-001-(a)-(54)

NIDR-HGB001a54

TITLE OF PROJECT

Genetics of Chemoreception Thresholds

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: K. S. Brown, Director, Human Genetics Branch, NIDR

Other Investigators: C. J. MacLean, Mathematical Statistician, Human Genetics Branch, NIDR

W. C. Leyshon, Biologist, Human Genetics Branch, NIDR

Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Developmental Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this study are to describe experimentally the variation between individuals in their detection thresholds for odors and tastes and to analyze the variation genetically and statistically to determine the nature of the components that contribute to this variation.

Several methods are being utilized. A statistical study of the components of variation in the detection thresholds of a variety of odors in a group of normal individuals is directed to identifying chemical groups that show common patterns of odor threshold. Family and twin studies are directed at detecting the contribution of genetic components of variability and at the study of any specific segregating traits.

Preliminary results have indicated that the components of variation include; a general factor which accounts for about 30 percent of variance and is positively correlated to all odors, specific factors related to ionization as acids or bases, and a residual factor which seems to rank unionized molecules according to molecular weight. Examination of the correlation between the thresholds of sibs, parents and children, do not provide evidence for a measurable genetic control of the thresholds of any of the tested pure compounds.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Kenneth S. Brown	4/9/73

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METHOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

NIDR-HGB002a62

Prev. Ser. No. NIDR-HG-002-(a)-(62)

TITLE OF PROJECT

Discrimination and Segregation Analysis of Hereditary Deafness in the Students of the Clarke School for the Deaf

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: K. S. Brown, Director, Human Genetics Branch, NIDR
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Developmental Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are to study the distribution of cases of profound childhood deafness in families and in relationship to history of trauma or disease. By analysis of the segregation of deafness in families of different mating types the genetic contributions to deafness in childhood can be estimated.

The preliminary genetic analysis, treating the deafness as a clinically homogenous entity, has given results similar to other workers. Further studies are underway attempting to use clinical and genetic discrimination of different specific entities that the general analysis has shown to exist.

Techniques for detection of defects in the cochlear microphonic part of the hearing mechanism as developed in animals are being applied to humans.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Kenneth S. Brown	4/9/

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-HG-003-(b)-(67)

NIDR-HGB003b67

TITLE OF PROJECT

Developmental Processes in Genetically Controlled Malformations

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: K. S. Brown, Director, Human Genetics Branch, NIDR
Other Investigators: M. C. Johnston, Visiting Scientist, Human Genetics Branch, NIDR
H. A. Gross, Research Associate, Human Genetics Branch, NIDR
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Developmental Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are to describe the development of experimental animals with genetically produced congenital malformations, particularly those of the oral region, and to examine the interaction between the tissues of these animals in the developmental processes in order to determine the nature of the mechanism producing the genetic defect.

Several methods will be employed. A survey of mouse mutants is being undertaken to evaluate their oral structures in relation to those of the lines from which they arose. Mutants of specific interest are being collected, and colonies of these animals are to be developed as a basis for experimental study. Timed matings are being made to produce animals of known gestational age. Serial sacrifice and histological study will produce systematic documentation of the developmental processes. Comparative differences in rate of cleft palate and cleft lip and palate in inbred strains of mice are being studied as a response to changes in maternal environment during different stages of pregnancy.

Assays of hormonal levels during pregnancy and during teratogenic stimulation are being carried out to evaluate the effects of hormones on development of defects.

Total Man Years: 3
Professional: 1/2
Other: 2 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Kenneth S. Brown

DATE: 4/9/73

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REPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PROJECT NO. (DO NOT USE THIS SP

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-HG-004-(b)-(70)

NIDR-HGB004b70

TITLE OF PROJECT

Embryological Studies of Facial Development

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: M. C. Johnston, Visiting Scientist, Human Genetics Branch, NI

Other Investigators: A. Bhakdinaronk, Visiting Fellow; Y. C. Reid, Biologist; M. A. Larsen, Microbiology Tech.; and K. S. Brown, Director, Human Genetics Branch, NIDR. R. M. Pratt, Sr. Staff Fellow, and J. Hassel, Staff Fellow, Biol. Structure Lab., NIDR

Cooperating Units: R. D. Hazelton, Hospital for Sick Children, Toronto; D. M. No University of Mass., A. J. Steffek, American Dental Associati and R. Peach, University of North Carolina

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Developmental Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resea the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are to study the migrations, interactions and derivativ of neural crest and other primordia involved in normal embryological facial developmen and to use the information for the study of facial malformations. The normal aspects of the study are conducted primarily on avian and rodent embryos, the latter developi in organ culture. Various histological and biochemical techniques are used. These include radioautography (and other procedures for following the migrations of cells) and histochemistry, as well as fluorescence, interference contrast and electron microscopy. The embryogenesis of spontaneous and induced malformations, particularly cleft lip and palate, is being studied in the mouse. Efforts are being made to influence the abnormal processes leading to "spontaneous" clefts through manipulation of environmental factors.

Total Man Years: 3 3/4
Professional: 2
Other: 1 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Malcolm C Johnston

DATE: Apr 19

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. NIDR-005-(a)-(72)

NIDR-HGB005a72

ITLE OF PROJECT

adioimmunoassay of Hormones

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: H. A. Gross, Research Associate, Human Genetics Branch, NIDR
Co-Investigators: K. S. Brown, Director, Human Genetics Branch, NIDR
W. C. Leyshon, Biologist, Human Genetics Branch, NIDR
Cooperating Units: M. Ebert, Unit on Clinical Pharmacology, Laboratory of Clinical Science, NIMH

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Developmental Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in ie bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

sing a radioimmunoassay for plasma corticosterone we have been studying ie phenomenon of stress. Stress as defined biochemically as an adrenal cortisol esponse has many complex interrelationships in that stress has the capability o cause many biological phenomena. Corticosterone is the major glucocorticoid n rodents and the second most prevalent glucocorticoid in man after cortisol.

n the A/Jax mouse, stress or a pharmacologically elevated plasma corticosterone as been shown to be correlated with an elevated incidence of cleft palate. e are investigating this phenomena and in addition carrying out studies to orrelate the mutally interdependent hormonal systems of the adrenal-thyroid xis. We are measuring the plasma thyroid hormones with radioimmunoassays.

n studies on manic-depressive patients, in correlation with Dr. Michael Ebert, e are measuring plasma cortisol, corticosterone, T_4 and T_3 under baseline onditions and while the patients are being treated with lithium carbonate. ithium carbonate has been shown to inhibit pituitary TSH and thyroidal release f T_4 and T_3 in 1/3 of the patients given the drug. We are currently investigating his phenomenon in mice and man.

otal Man Years: 2
Professional: 1 3/4
Other: 1/4

ROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH HICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Howard Allen Gross, M.D.	4/5/73

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PPORTING AGENCY

ETHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

INDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-HG-006-(b)-(67)

NIDR-HGB006b67

TITLE OF PROJECT

Genetic Analyses in Human Populations

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: C.J. MacLean, Mathematical Statistician, Human Genetics Branch
Other Investigators: W.C. Leyshon, Biologist, Human Genetics Branch, NIDR
B.Y. Iba, Geneticist (Human Medical), Human Genetics Branch, NI
R. Singleton, Computer Systems Analyst, Human Genetics Branch,
Co-investigator: J.D. Niswander, Dental Director, Human Genetics Branch, NIDR
Cooperating Units: M.S. Adams, University of Rochester
P.L. Workman, University of Massachusetts

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Population Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are: (1) to determine which particular factors are influencing the pattern of genetic variation in any population at a given time. Of special interest are such factors as assortative mating, selection, population intermixt and population size; (2) to develop suitable methods for the analysis of intra- vs inter-population differences. Particularly important are those techniques which permit an assessment of the relative stability of genotypic distributions over space and time. (3) To investigate the relationship of birth and death rates to population size and age structure. This work concentrates on the differences within and between various populations with particular emphasis on the American Indian.

Total Man Years: 3/4
Professional: 3/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	C.J. MacLean	4/9/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. NIDR-HG-007-(a)-(58)

NIDR-HGB007a58

TITLE OF PROJECT

Congenital Malformation and Birth Characteristics of American Indians

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: J. D. Niswander, Dental Director, Human Genetics Branch, NIDR
Other Investigators: B. Y. Iba, Geneticist (Human Medical), Human Genetics Branch, NIDR
R. Singleton, Computer Systems Analyst, Human Genetics Branch, NIDR
Cooperating Units: M. V. Barrow, J. Hillis Miller Health Center, University of Florida

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Population Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This study is based on approximately 45,000 clinical newborn records from forty-six Public Health Service Hospitals to ascertain the total (at birth) frequency of congenital malformation among American Indians as well as frequencies of specific major defects. These data are compared to those obtained from other Mongoloid and Caucasoid populations. A major objective of this study is to examine the effect of tribal background and Caucasion admixture on variation in malformation rates. Several medical, culture and socio-economic variables are included as indicators of environmental effects. Birth weight and twinning rates are examples of other birth characteristics being analyzed.

Total Man Years: 1 1/4
Professional: 1/2
Other: 3/4

PROFESSIONAL SCHOOL (medical, dental, etc.) WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Jerry D. Niswander	4/9/73

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SUPPORTING AGENCY

METHOD OF SUPPORT (Check one)

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SP

NIDR-HGB008a63

Prev. Ser. No. NIDR-HG-008-(a)-(63)

TITLE OF PROJECT

Genetic Studies of Oral Diseases, Anomalies and Development

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: J. D. Niswander, Dental Director, Human Genetics Branch, NIDR
Cooperating Units: J. A. Sofaer, Department of Genetics, University of Cambridge
C. S. Chung, School of Public Health, University of Hawaii

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Population Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Studies are being conducted on Hawaiian school children and among various Amerindian tribes in an attempt to clarify the relative role of genetic and environmental factors in certain oral conditions. These studies have as their objective: (a) to determine the effects of inbreeding and hybridization on malocclusion and dental caries; (b) to assess the role of environment in malocclusion--specifically has the frequency, type and severity of malocclusion changed over time, and if so, can the changes be related to socioeconomic status and changing cultural patterns; (c) to assess the correlation between relatives in malocclusion; and (d) to elucidate genetic mechanisms involved in morphological variations of the teeth.

Total Man Years: 3/4
Professional: 1/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Jenny D. Niswander

DATE: 4/9/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. NIDR-HG-009-(a)-(58)

NIDR-HGB009a58

TLE OF PROJECT

haracteristics of Families with Oral Clefts

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: J. D. Niswander, Dental Director, Human Genetics Branch, NIDR
Co-investigator: M. C. Johnston, Visiting Scientist, Human Genetics Branch, NIDR
Other Investigators: B. Y. Iba, Geneticist (Human Medical), Human Genetics Branch, NIDR
R. Singleton, Computer Systems Analyst, Human Genetics Branch, NIDR
K. Kurisu, Visiting Scientist, Human Genetics Branch, NIDR
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Population Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

IMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

his project is designed to determine 1) if there are physical stigmata particularly hanges in facial morphology associated with oral clefts, which are familial in ature and may be useful in clarifying the role of genetic (and environmental) factors n the etiology and pathogenesis in these anomalies. Of particular interest is the tudy of various characteristics obtained from cephalometric radiographs of affected ndividuals as well as their "normal" relatives including twins. Another objective) is to develop methods to identify and classify heterogeneous subtypes of oral clefts nd to identify individuals who are genetically at high risk.

otal Man Years: 1 1/2
Professional: 3/4
Other: 3/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH IICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Jerry D. Niswander

DATE: 4/9/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. NIDR-None

NIDR-HGB010a73

TITLE OF PROJECT

Maternal Factors in Congenital Malformation

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: J. D. Niswander, Dental Director, Human Genetics Branch, NIDR
Other Investigator: B. Y. Iba, Geneticist (Human Medical), Human Genetics Branch, NI
Cooperating Units: H. J. Sommers, Division of Biometrics, Office of S.G., USAF
W. Wertelecki, Dept. of Pediatrics, Medical University S.C.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Human Genetics
Section: Population Genetics
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This project utilizes computorized data from U.S. Air Force hospitals for the years 1965 through 1971. Records of approximately 350,000 maternities (mother and child) are being analyzed for association between epilepsy, diabetes, thyroid and other endocrine defects, and nutritional deficiencies in mothers and the occurrence of birth anomalies in their infants.

Total Man Years: 1 1/4
Professional: 1/4
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Jerry D. Niswander	4/9/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LB-101-(b)-52

NIDR-LBC101b52

TITLE OF PROJECT

Structural Studies on Collagen

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Karl A. Piez, Chief, Laboratory of Biochemistry, NIDR
Other Investigators: Dr. Alfred Quattrone, Staff Fellow, Protein Chem. Sec., LB, NIDR
Dr. Dennis Torchia, Guest Worker, " " " " "
Cooperating Units: National Bureau of Standards
Laboratory of Molecular Biophysics, Oxford University

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biochemistry
Section: Protein Chemistry
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

It is the long range purpose of this project to study the structure of collagen various levels and correlate with function. The topics of present interest are:

The role of the amino-terminal nonhelical regions of the α chains of collagen fibrillogenesis. Linear and lateral associations of collagen molecules in the collagen fibril must be controlled by structural features of the molecule. The role of the amino-terminal regions is being examined by studying interactions of peptides containing these regions with collagen.

The conformation of collagen. Nuclear magnetic resonance studies on well characterized peptides from collagen should contribute to an understanding of several structural features such as side chain interactions and hydrogen bonding.

The three-dimensional structure of collagen. Computer analysis of the primary structure of the polypeptide chains in collagen is being done to understand the origins of molecular packing.

The structure of elastin. Nuclear magnetic resonance is being used to measure the mobility of the polypeptide chains in elastin and elucidate the mechanism of elasticity of elastin fibers.

Total Man Years: 3 1/2
Professional: 2 1/2
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Karl A Piez/BSH	4/13/73

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REPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

DOLLARS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. LB-102-(b)-62

NIDR-LBC102b62

TITLE OF PROJECT

The Chemistry of Collagen

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Karl A. Piez, Chief, Laboratory of Biochemistry, NIDR
Other Investigators: Dr. Herbert Evans, Staff Fellow, Protein Chem. Sec., LB, NIDR
Dr. Alan Nicholls, Visiting Fellow, " " " " "
Dr. Gowri Chandrakasan, Visiting Fellow, Protein Chem. Sec., L
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Protein Chemistry
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The broad aim of this project is to determine the covalent structure of collagen including both amino acid sequence and covalent interchain cross-links. Differences that can be related to tissue, species, developmental stage and connective tissue pathology are of particular interest. Currently active aspects of the study include the isolation and characterization of multichain peptides containing cross-links, the development of methods to scan small samples of collagen for differences, and the characterization of collagen from lower species of animals including ascaris cuticle collagen and codfish skin collagen.

Total Man Years: 7 1/2
Professional: 3 1/2
Other: 4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Karl A Piez	4/13/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LB-201-(b)-62

NIDR-LBC201b62

TITLE OF PROJECT

The Chemistry and Biosynthesis of Connective Tissue

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. G. R. Martin, Chief, Connective Tissue Section, LB, NIDR

Other Investigators: Dr. Peter Byers, Research Associate; Dr. Geoffrey Herring, Guest Worker; Dr. D. J. Lichtenstein, Guest Worker; Dr. Thomas Lustberg, Research Associate; Dr. David Rowe, Clinical Associate; Dr. Barbara Smith, Research Chemist; Dr. Michael Sussman, Guest Worker; Dr. Larry Wahl, NIDR Postdoctorate Fellow

Cooperating Units: Genetics Department and Department of Orthopedic Surgery, Johns Hopkins Medical School

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Connective Tissue
Location: NIDR, NIH, Bethesda, Md.

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Collagen and elastin are two of the major constituents of connective tissues. The purpose of this project is to study their metabolism in normal and disease states and the cellular control of their formation. Currently active aspects of the project include the isolation and characterization of procollagen, elucidation of the conversion of procollagen to collagen, the study of inherited diseases of connective tissue utilizing cells in culture and examination of the effect of inflammation on collagen metabolism.

Total Man Years: 13 1/4
Professional: 8 1/2
Other: 4 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Geo R Martin	4-17-73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LB-202-(b)-61

NIDR-LBC202b61

TITLE OF PROJECT

Studies on Chemotaxis

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Elliott Schiffmann, Chemist, Connective Tissue Section, LB,
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Connective Tissue
Location: NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with gavernment and private agencies supporting research in the bio-sciences and are forworded to investigators who request such information. Your summary is to be used for these purposes.

It is proposed to gain insight into the biochemical processes of cell migration along a chemical gradient. Cells such as leucocytes, bacteria, and the slime mold have the property of responding to their environment in this manner. Some progress has been achieved in the isolation and characterization of compounds exuded from an E. coli strain which are chemotactic for leucocytes. The material from E. coli appears to compete for the cell with a chemotactic substance derived from complement. With the aid of various inhibitors some information has been obtained on the requirements of the leucocyte for chemotaxis. It is hoped that by a study of the direct interaction of chemotactic factors with white cells some understanding of the directed movement of cells would be gained.

Total Man Years: 2
Professional: 1
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Elliott Schiffmann	4/9/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

PHS-166
REV. 5-70

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Not for publication or publication reference.

NATIONAL INSTITUTE OF DENTAL RESEARCH
U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

v. Ser. No. LB-301-(b)-72

NIDR-LBC301b72

TLE OF PROJECT

Comparative Aspects of the Mammalian Transglutaminases

ME NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Dr. S. I. Chung, Senior Staff Fellow, Enzyme Chemistry Section, LB NIDR

Other Investigators: Dr. J. E. Folk, Chief, Enzyme Chemistry Section, LB, NIDR

Cooperating Units: None

ME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Enzyme Chemistry
Location: NIDR, NIH, Bethesda, Md.

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

As an extension of a project carried out over the past few years, studies will be ntinued on the isolation and characterization of an important group of enzymes termed ansglutaminases that catalyzed the formation of covalent cross-links between protein lecules. Three distinct classes of these enzymes in guinea pig and human tissues d organs have been defined in this laboratory. Additional classes of enzymes that are volved in mammalian fertilization are in the process of characterization. Of rticular interest are the roles of the individual transglutaminases in blood coagulation, und healing, seminal plasma coagulation during fertilization, cell membrane formation, oss-linking of hair proteins, and covalent attachment of biological amines in proteins.

Biological control mechanisms, essential metal ion requirements, the immunochemical d the structural aspects of zymogen activation, active site and substrate specificities e under investigation. The biosynthesis and hormonal regulations of plasma pro-ansglutaminase are under consideration to be investigated.

tal Man Years: 3 1/2
Professional: 1 1/2
Other: 2

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Soo I. Chung	4/13/73

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

DS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

NIDRLBC302b52

Prev. Ser. No. LB-302-(b)-52

TITLE OF PROJECT

Chemical, Stereochemical and Conformational Aspects of Transglutaminases

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. M. A. Gross, Senior Staff Postdoctoral Fellow, Enzyme Chemistry Section, LB, NIDR

Other Investigators: Dr. J. E. Folk, Chief, Enzyme Chemistry Section, LB, NIDR

Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Enzyme Chemistry
Location: NIDR, NIH, Bethesda, Md.

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Active site mapping studies on transglutaminases using model substrates will continue with special emphasis on the blood coagulation transglutaminases (plasma and platelet factor XIII). Active site mapping of transglutaminases using alkyl isocyanates which irreversibly inhibit the enzymes is in progress. Investigations of the γ-glutamyl esterase and ester synthetase activities of guinea pig liver transglutaminase are also currently in progress. It is the purpose of these studies to relate the structures and mechanisms of these enzymes to their catalytic function.

Total Man Years: 1-1/2
Professional: 1-1/2
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	M. A. Gross	4/12/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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REV. 5-70

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LB-401-(b)-62

NIDRLBC401b62

TITLE OF PROJECT

Cell Growth Studies in Normal and Abnormal Subjects

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. H. L. Cooper, Chief, Cell Biology Section, LB, NIDR
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biochemistry
Section: Cell Biology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Biochemical processes which regulate the growth of human lymphocytes in vitro are being studied. The RNA metabolism of resting and growing lymphocytes is being investigated in order to clarify growth-related alterations in the synthesis, processing and degradation of various RNA species.

Messenger RNA will be isolated from lymphocytes by specific binding to polyadenylate sequences. Size distribution, kinetics of synthesis and degradation and movement among intracellular compartments will be studied. Differences between resting and growing lymphocytes for these characteristics will be sought.

Activity of appropriate fractions in cell free systems will be examined.

Total Man Years: 3
Professional: 3/4
Other: 2-1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		4/12/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LB-402-(b)-67

NIDRLBC402b67

TITLE OF PROJECT

RNA Synthesis and Degradation in Animal Cells

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. R. Stern, Medical Officer, Cell Biology Section, Laboratory of Biochemistry, NIDR
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Cell Biology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The enzyme in serum which hydrolyzes double-stranded RNA will be further purified and characterized. The enzyme will be used as a tool to study the structure of the intermediates in the replicative cycle of RNA viruses. A comparable enzyme from macrophages, particularly of pulmonary origin, is also being investigated. Macrophages may be the origin of the serum enzyme.

Total Man Years: 1/2
Professional: 1/4
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Robert Stern	4/12/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDRLBC404b72

Prev. Ser. No. LB-404-(b)-72

TITLE OF PROJECT

Studies of Ribosomal Proteins in Mammalian Cells

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. S. Jakoby, Senior Staff Fellow, Cell Biology Section, LB, NIDR
Other Investigators: Dr. H. Cooper, Chief, Cell Biology Section, LB, NIDR
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Cell Biology
Location: NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Initial stages of this project will involve isolation and partial characterization of proteins from ribosomes and ribosomal subunits of HeLa cells and normal human lymphocytes. Methods for isolation of specific ribonucleoprotein particles will be developed.

Total Man Years: 2-1/2
Professional: 1-1/4
Other: 1-1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Sheldon B Jakoby	4/12/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PROJECT NO. (DO NOT USE THIS SP

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. LB-405-(b)-72

NIDRLBC405b72

TITLE OF PROJECT

In Vitro Synthesis of Collagen and Characterization of the Messenger RNA for Collagen

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. R. Stern, Medical Officer, Cell Biology Section, LB, NIDR
Other Investigators: Dr. Kathy Benveniste, Staff Fellow, Cell Biology Section, LB,
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biochemistry
Section: Cell Biology
Location: NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The purpose of this project is to synthesize collagen in a cell-free system usi heterologous sources of ribosomes and protein synthesizing factors. Messenger RNA for collagen is being isolated and characterized. Tissue specific factors for optimal collagen synthesis are being sought including tRNA and protein initiation factors. In vitro synthesis of hemoglobin and viral proteins are being used for comparative analysis.

The acylation of proline, glycine and arginine to their respective tRNAs from collagenous and non-collagenous tissues is being studied. The isoacceptor tRNAs fo these amino acids are being separated and their interaction with their respective amino acyl synthetases is being examined. The RNA codewords for each tRNA will be established by ribosome binding assays. Results will be coordinated with the cell-free synthesis of collagen.

Total Man Years: 2 1/2
Professional: 1 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	R Stern	4/13

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. NIDR-(FY68)38(63)

NIDR-LBS101b63

TLE OF PROJECT

xperimentally Induced Enamel Defects

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigators: Dr. Marie U. Nylen, Chief, Laboratory of Biological Structure
Dr. Jytte Westergaard, Visiting Associate

ther Investigators : None

ooperating Units : None

AME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section : Experimental Morphology
Location : NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

: is the purpose of the study to (1) establish which dose levels of tetracycline ydrochloride consistently give rise to gross hypoplastic lesions in the incisors id molars of 4 day old rats; (2) compare the effect of a given dosage of tetra- ycline on incisors in rats of various ages, and (3) determine blood concentrations the antibiotic in the experimental animals.

itraperitoneal injections of varying dosages of tetracycline hydrochloride are lministered to Sprague-Dawley rats ranging in age from 4 to 75 days old. The imals are sacrificed 6-14 days after the injection and ground sections of teeth e examined for the presence of gross hypoplastic enamel lesions using uorescence microscopy and contact microradiography. Blood concentrations e determined spectrofluorometrically 1, 2, 6, 24 and 48 hours as well as days after administration of the antibiotic.

tal Man Years: 2 3/4
Professional: 1 1/4
Other: 1 1/2

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
ional Institute of Dental Research	Marie U. Nylen	4/5/73

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PORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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Not for publication or publication reference.

U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS102b72

Prev. Ser. No. LBS-001-(b)-(72)

TITLE OF PROJECT

Ultrastructural Studies of Enzymes in Secretory and Post-Secretory Ameloblasts in Incisors of Young Rats

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jytte Westergaard, Visiting Fellow
Other Investigator: Dr. Marie U. Nylen, Chief, Laboratory of Biological Structure
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Morphology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The distribution and localization of acid phosphatase and thiamine pyrophosphatase in secretory and post-secretory ameloblasts are under investigation. Continuously growing incisors from 4 day old rats are fixed by vascular perfusion with aldehyde solutions. The teeth, some of which are decalcified first, are cut into small pieces and incubated in appropriate media. Acid phosphatase activity is demonstrated through incubation using cytidine monophosphate as substrate and thiamine pyrophosphatase activity through incubation using thiamine pyrophosphate as substrate. Localization of reaction product is determined by observation in the electron microscope.

Total Man Years: 1 1/4
Professional: 3/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Jytte Westergaard	4/4 - 73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

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Not for publication or publication reference.

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LBS-003-(b)-(68)

NIDR-LBS111b68

TLE OF PROJECT

Ultrastructure and Cytochemistry of Salivary Glands

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Arthur R. Hand
Other Investigators : Dr. Margit Hamosh, Laboratory of Nutrition and Endocrinology, NIAMDI
Cooperating Units : None

ME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section : Experimental Morphology
Location : NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The ultrastructure and cytochemistry of the rat parotid gland are being studied. Emphasis is placed upon the basic mechanisms involved in exocrine secretory phenomena, and a comparison with other exocrine glands is being made.

The biochemistry, ultrastructure, and cytochemistry of developing and adult von Ebner's glands are also being examined in the light of the recent discovery of a potent lipolytic activity in this gland and its possible role in nutrition and growth.

Total Man Years: 1 1/4
Professional : 1/2
Other : 3/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
tional Institute of Dental Research	Arthur R Hand	4/30/73

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PORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. LBS-005-(b)-(7I) NIDR-LBS112b71

TITLE OF PROJECT

Ultrastructure of Salivary Glands of Maturing and Aging Hamsters (Mesocricetus auratus)

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Helen Flon, USPHS Special Research Fellow, Guest Worker, N
Other Investigators : None
Cooperating Units : None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section : Experimental Morphology
Location : NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objective of this continuing study is to investigate the form, content, and distribution of organelles, granules, and inclusions of (1) the acinar cells of the sublinguial, submandibular, and parotid glands, (2) the convoluted granular tubules of the submandibular gland, and (3) intercalated duct and demilune cells during cytodiffer entiation, maturation, and aging. The ultrastructure of these cells will be studied following the application of cytochemical procedures used for the demonstration of mucosubstances and specific enzymes associated with those organelles involved in secret granule synthesis (peroxidase, thiamine pyrophosphatase). The process of mucus secreti (exocytosis) will be studied using a variety of salivary secretion stimulators.

Total Man Years: 1 1/4
Professional : 1
Other : 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Helen Flon	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

Prepared for the Science Information Exchange.

Not for publication or publication reference.

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. None

NIDR-LBS121b73

TLE OF PROJECT

iochemical and Ultrastructural Studies of Tissue Fixation by Protein Crosslinking Reagents

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigators: Dr. John R. Hassell and Dr. Arthur R. Hand
ther Investigators : None
ooperating Units : None

ME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section : Experimental Pharmacology and Experimental Morphology
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

An attempt is currently being made to develop useful fixation procedures with the ifunctional protein crosslinking reagent dimethyl suberimidate (DMS). Selection of ptimum crosslinking conditions is achieved by using an assay based on the assumption hat intermolecular crosslinking increases the proportion of water insoluble protein in issue blocks. The degree of crosslinking obtained with DMS is similar to that obtained ith glutaraldehyde. In addition ultrastructural and cytochemical studies of liver locks fixed with DMS indicate acceptable fine structure and good retention and localzation of enzymatic activity. Quantitative biochemical studies of the effects of DMS nd other fixatives on the activity of various enzymes are now in progress. The ability f DMS to preserve tissue fine structure with minimal alteration of the properties of ne proteins, and without the introduction of Schiff-positive aldehyde groups suggest hat it may prove superior to gluraraldehyde for certain cytochemical studies.

tal Man Years: 1 3/4
ofessional : 3/4
her : 1

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
tional Institute of Dental Research	John Hassell Arthur R Hand	8/14/73

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

DS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LBS-010-(b)-(70)

NIDR-LBS200670

TITLE OF PROJECT

Electron Microscopic Study of the Formation and Conversion of Amorphous Calcium Phosphates

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. E. David Eanes, Chief, Molecular Structure Section
Other Investigators: Dr. John D. Termine, Dr. Marie U. Nylen
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Studies are currently underway to establish the morphology of amorphous calcium phosphate (ACP) during its formative stage and the alterations in this morphology during its conversion to crystalline apatite. Particular attention is being directed to elucidating the spatial relationships between the ACP precursor and the final crystalline phase, and to the structural state of ACP in aqueous solution. Since the ACP must be dry for electron microscopy, procedures are being investigated that will minimize the shrinkage and distortion that may accompany the drying of ACP.

The formation of ACP as an intermediate step in the growth of seed crystals of apatite in supersaturated solutions is also being studied, as is the role of ACP as an intermediate phase in the incongruent hydrolysis of acidic calcium phosphates to apatite in basic media.

Total Man Years: 3/4
Professional: 1/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	E. David Eanes	4/4/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS201b73

r. Ser. No. None

LE OF PROJECT

The Reactivity of Phosphorofluoridate with Calcium Phosphate Salts

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER FESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Incipal Investigator: Dr. E. David Eanes, Chief, Molecular Structure Section
Other Investigators: None
Cooperating Units: None

E AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

MARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

n the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

e chemical interaction of phosphorofluoridate (PO_3F^{2-}) with amorphous calcium phosphate CP) and crystalline apatite (CA) is being studied. The extent of PO_3F^{2-} adsorption on ' and CA, the stability of adsorbed PO_3F^{2-}, and the inhibition of adsorption by other ionic species are being investigated. PO_3F^{2-} reactivity will be correlated with the mposition, texture and prior chemical history of ACP and CA.

al Man Years: 1 1/2
Professional: 1/2
Other: 1

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
l Institute of Dental Research	E. David Eanes	4/4/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

ORTING AGENCY

DD OF SUPPORT *(Check one)*

gency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. LBS-014-(b)-70

NIDR-LBS202b70

TITLE OF PROJECT

X-ray Diffraction Study of the Influence of Electrolytes on Lamellar Lecithin Mesophases

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. E. David Eanes, Chief, Molecular Structure Section
Other Investigators: None
Cooperating Units: Dr. Melvin H. Gottlieb, Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The effect of added electrolytes on the thickness of phospholipid bylayers in lecithin-water mixtures is being investigated by small-angle x-ray diffraction techniques. Electrolytes being investigated are LiCl, NaCl, Na_2SO_4, CsCl, KCl, $CaCl_2$, and HCl at 1.0 N concentrations. The effect of electrolytes on the maximal amount of solution that can be mixed with lecithin before phase separation occurs is also being determined.

Non-isothermal crystalline-liquid crystalline transitions in lecithin-water mixtures is also currently under investigation.

Total Man Years: 1/4
Professional: 1/4
Other: None

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

National Institute of Dental Research

SIGNATURE OF PRINCIPAL INVESTIGATOR

E. David Eanes

DATE

4/4/7

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS203b73

Prev. Ser. No. None

TITLE OF PROJECT

Vibrational Spectroscopy of Amorphous and Apatitic Calcium Phosphates

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. John D. Termine
Other Investigator: Dr. Donald R. Lundy
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK — *(200 words or less — Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The submolecular environments of the hydroxide and carbonate ions in both hydrazine-deproteinated bone specimens and synthetic apatites prepared under solution conditions approximating physiological fluids is being studied by infrared spectroscopy. In this study, these synthetic and biological samples are being subjected to various thermal as well as solution recrystallization treatments in order to establish the relationships, if any, existing between their native submolecular configurations and those found in idealized apatite crystal models.

Infrared, far infrared and laser Raman spectroscopy studies are being conducted to ascertain the average submolecular configuration of the major ions present in synthetic amorphous calcium phosphate and related non-crystalline compounds. In particular, the influence of the magnesium, copper, carbonate and pyrophosphate ions on amorphous calcium phosphate structure is being investigated under various experimental and spectroscopic conditions, including temperature variation.

Total Man Years: 1
Professional: 1
Other: None

PROFESSIONAL SCHOOL (medical, dental, etc.) WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	John D Termine	4/4/73

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SUPPORTING AGENCY

PERIOD OF SUPPORT (Check one)

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

AMOUNT OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-LBS204b(73)

TITLE OF PROJECT

X-ray Diffraction Studies of Bone Mineral in Human Disease

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. John D. Termine
Other Investigators: None
Cooperating Units: Dr. Louis V. Avioli and Dr. Jean R. Russell, Department of Medic
Jewish Memorial Hospital, Washington University School of Medici
St. Louis, Missouri

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research i the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Hydrazine extraction is being used to prepare bone mineral specimens that are both free of protein and unaltered in physicochemical properties compared to native tissue. The deproteinated bone specimens are then examined by quantitative x-ray diffraction procedures measuring diffraction intensity and broadening. Conventional diffraction equipment is being automated and computerized in order to time-average these data. The x-ray diffraction measurements accurately estimate both the quantity and average size (and/or perfection) of the apatite present in the biological specimen relative to synthetic standards of similar composition and crystal texture. Thus, the relative level of average mineral maturation is being assessed for each deproteinated bone sample examined. These new experimental procedures are being used to study bone mineral degeneration in untreated and treated chronic uremia. Tetracycline effects on developing bone mineral maturation are also being examined.

Total Man Years: 1/2
Professional: 1/4
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	John D Termine	4/4/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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ev. Ser. No. None

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS205b73

ITLE OF PROJECT

Physicochemical Studies of Protein-Mineral Interactions

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

'rincipal Investigator: Dr. John D. Termine
Other Investigators: Dr. E. David Eanes, Chief, Molecular Structure Section
Cooperating Units: Dr. Ira Pullman, Dr. Ray Peckauskas, Department of Radiology, New York Medical College, New York, N. Y.

AME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

JMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded t. investigators who request such information. Your summary is to be used for these purposes.

The structural interaction of mineral ions and mineral solids with synthetic nd biological macromolecules is being studied by a number of physicochemical echniques including infrared spectroscopy, laser Raman spectroscopy, electron aramagnetic resonance spectroscopy and electron microscopy. The mineral ions ncompass the alkaline earth metals and the HPO_4^{-2}, $H_2P_2O_7^{-2}$ and HCO_3^- anions, hile the mineral solids include the synthetic amorphous and apatite calcium hosphates. The macromolecules include cationic, neutral and anionic proteins, oth simple and complex, as well as model mono- and polyfunctional biopolymers. acromolecular entropic, electrostatic and conformational influences on mineral on/solid binding are under study. Mineral solid-protein fiber quaternary tructure relationships are also being investigated.

otal Man Years: 1/4
Professional: 1/4
Other: None

IOFESSIONAL SCHOOL *(medical, dental, etc.)* WITH TIICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
al Institute of Dental Research	John D. Termine	4/4/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LBS-015-(b)-(63)

NIDR-LBS206b63

TITLE OF PROJECT

Infrared and Raman Spectroscopic Studies of Teeth and Bones and Related Synthetic Compounds

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Mr. Bruce O. Fowler
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Molecular Structure
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forworded to investigators who request such information. Your summary is to be used for these purposes.

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared (4000-60 cm^{-1}) and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for preparation of synthetic calcium apatites of controlled physical properties (crystal size and perfection) and chemical composition containing biologically relevant constituents, e.g., hydroxyl, 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 dependency and polarization) are utilized to establish compositional and structural details of the apatites which include the type and geometry of ions, the site or number of sites occupied by ions, orientation of ions, chemical bonding and interactions of ions and semi-quantitative estimations of constituents present. The results of findings for the controlled apatite system are related to the inorganic phase in calcified tissues.

Total Man Years: 1
Professional: 1
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Bruce O. Fowler	2 April 73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. None

NIDR-LBS301b73

TLE OF PROJECT

olysaccharide Production by Oral Organisms

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Dr. Howard A. Bladen, Chief, Structural Interactions Section
her Investigators : Dr. Jan D. DeStoppelaar
Dr. Robert M. Pratt
operating Units : None

ME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section : Structural Interactions
Location : NIDR, NIH, Bethesda, Maryland 20014

AMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Numerous oral organisms are being surveyed for similarities or differences in the lecular composition of their soluble and insoluble (plaque associated) polysaccharides the use of concanavalin A. Concanavalin A binds specifically to branched chained lymers of glucose, fructose or mannose. Therefore a reaction with concanavalin A veals the presence of these saccharides. Caries-active streptococci will be surveyed r specific similarities which may reflect some important factor essential to the ganisms caries activity. Lectins other than concanavalin A which bind specifically to fferent saccharides will be investigated as to their potential usefulness.

tal Man Years: 2 1/4
ofessional : 1
ner : 1 1/4

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
tional Institute of Dental Research		

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-LBS302b73

TITLE OF PROJECT

In vitro Effect of Hydroxyapatite Bound Fluoride on Oral Organisms

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Howard A. Bladen, Chief, Structural Interactions Section
Other Investigators : Dr. John D. Termine, Mr. Bruce O. Fowler, Dr. E. David Eanes, Chief, Molecular Structure Section
Cooperating Units : None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section : Structural Interactions
Location : NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The effects of hydroxyapatite bound fluoride on growth, adhesion and acid productio by specific oral organisms are being studied utilizing pressed discs of hydroxyapatite containing bound fluoride in various concentrations. The objective is to use such discs in an in vitro plaque forming system in order to mimic closely the in vivo tooth surface plaque relationship. Numbers of organisms shall be monitored by usual plating procedure Results should reflect the effects of fluoride release from the hydroxyapatite due to th presence of the various organisms. Such parameters as concentration of fluoride in the disc, crystal size, accumulation of fluoride in the plaque and numbers of organisms necessary before fluoride has an effect are being studied.

Total Man Years: 1 1/2
Professional : 1/2
Other : 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Howard A. Bladen	4/1/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. LBS-007-(b)-(69)

NIDR-LBS320b69

TLE OF PROJECT

udies on Oral Filamentous Microorganisms Implicated in Periodontal Disease

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Dr. George J. Hageage, Jr.
her Investigators : Dr. Howard A. Bladen, Chief, Structural Interactions Section
Dr. L. Ariel Thompson, Caries Prevention Branch
Dr. Paul H. Keyes, Oral Medicine and Surgery Branch
operating Units : Dr. Jason M. Tanzer, VA Hospital, Newington, Conn.

ME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section : Structural Interactions
Location : NIDR, NIH, Bethesda, Maryland 20014

IMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The general objectives of this project involve the structure, taxonomy, epidemiology, munology and pathogenicity of gram-positive oral filamentous bacteria of rodent and man origin implicated in the initiation of periodontal pathosis. Present studies involve e application of (1) fluorescent antibody technique, and (2) solid phase microradio-unoassay as methods for the detection and rapid identification of these microorganisms plaque.

al Man Years: 1 3/4
fessional : 3/4
ier : 1

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED

ional Institute of Dental Research

SIGNATURE OF PRINCIPAL INVESTIGATOR

DATE 4/4/73

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. None

NIDR-LBS321b73

TITLE OF PROJECT

Immunization of Rats Against Streptococcus Mutans-associated Dental Caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. George J. Hageage, Jr.
Other Investigators : Dr. Rachel H. Larson, Chief, Preventive Methods Development Se
Cooperating Units : Dr. Jason M. Tanzer, VA Hospital, Newington, Conn.

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section : Structural Interactions
Location : NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are 1) to determine whether high humoral antibody titers are elicited in rats in response to antigenic challenge by caries-active strepto cocci, 2) whether salivary antibody response is to elicited, and 3) whether the immuniz tion regimen confers immunologic protection against smooth surface caries produced by Streptococcus mutans.

Total Man Years: 3/4
Professional : 1/4
Other : 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	George J. Hageage, Jr.	7/1/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. LBS-016-(b)-(63)

NIDR-LBS401b63

ITLE OF PROJECT

Exogenous and Endogenous Factors Affecting Normal Development of the Oral Facial Region

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Dr. Cecil T. G. King, Chief, Experimental Pharmacology Section
Other Investigators: Miss Ann L. Wilk
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

ne congenital malformation of cleft palate is being induced at will with a variety f chemical agents (Lathyrogens; Benzhydrilpiperazines. Vitamin A, etc.) in a mber of different mammalian species. However, this malformation is associated ith other malformations of the skeletal system. Investigations are under way in n effort to induce cleft palate alone in order to better delineate the etiology f the malformation.

nterspecies differences in the metabolism, placental transfer and fetal binding f the chemicals used are under investigation. In addition fetal outcome from olonged versus acute drug administration is being studied to determine drug nzyme activation or the effects of drug-binding in the fetus.

otal Man Years: 1 1/2
Professional: 3/4
Other: 3/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
al Institute of Dental Research	Cecil T. G. King	4/4/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NATIONAL INSTITUTE OF DENTAL RESEARCH

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Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. None

NIDR-LBS321b73

TITLE OF PROJECT

Immunization of Rats Against Streptococcus Mutans-associated Dental Caries

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. George J. Hageage, Jr.
Other Investigators : Dr. Rachel H. Larson, Chief, Preventive Methods Development Se
Cooperating Units : Dr. Jason M. Tanzer, VA Hospital, Newington, Conn.

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section : Structural Interactions
Location : NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The objectives of this project are 1) to determine whether high humoral antibody titers are elicited in rats in response to antigenic challenge by caries-active streptococci, 2) whether salivary antibody response is to elicited, and 3) whether the immunization regimen confers immunologic protection against smooth surface caries produced by Streptococcus mutans.

Total Man Years: 3/4
Professional : 1/4
Other : 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research		4/4/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LBS-016-(b)-(63)

NIDR-LBS401b63

TITLE OF PROJECT

Exogenous and Endogenous Factors Affecting Normal Development of the Oral Facial Region

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Cecil T. G. King, Chief, Experimental Pharmacology Section
Other Investigators: Miss Ann L. Wilk
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The congenital malformation of cleft palate is being induced at will with a variety of chemical agents (Lathyrogens; Benzhydrilpiperazines. Vitamin A, etc.) in a number of different mammalian species. However, this malformation is associated with other malformations of the skeletal system. Investigations are under way in an effort to induce cleft palate alone in order to better delineate the etiology of the malformation.

Interspecies differences in the metabolism, placental transfer and fetal binding of the chemicals used are under investigation. In addition fetal outcome from prolonged versus acute drug administration is being studied to determine drug enzyme activation or the effects of drug-binding in the fetus.

Total Man Years: 1 1/2
Professional: 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Cecil T. G. King	4/4/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. LBS-018-(b)-70

NIDR-LBS411b70

TITLE OF PROJECT

Changes in the Macromolecular Constituents of Embryonic Connective Tissue in Experimentally Induced Cleft Palate of the Rat

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Robert M. Pratt, Jr.
Other Investigators: Dr. Cecil T. G. King, Dr. John R. Hassell and Dr. Malcolm C. Johnston
Cooperating Units: Dr. Gary R. Smiley, Dental Research Center University of North Carolina

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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The malformation of cleft palate is being induced by specific agents, such as Beta-aminopropionitrile and diazo-oxo-norleucine, in order to delineate the etiology of the malformation. The development of the malformation is being followed in vivo and in vitro in order to determine the role of extracellular macromolecules such as collagen and hyaluronic acid. The appearance and changes during development in glycoproteins on the surface of palatal shelf epithelium prior to fusion are being followed using the lectins, concanavalin A and wheat germ agglutinin.

Total Man Years: 1 1/2
Professional: 1
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Robert M. Pratt, Jr.	4/4/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS421b72

Rev. Ser. No. LBS-019-(b)-(72)

TITLE OF PROJECT

Quantitative Changes in Macromolecular Components in Palatal Shelf Tissue During Palatal Growth and Epithelial Fusion in the Rat Fetus

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. John R. Hassell

Other Investigators: Dr. Cecil T. G. King, Chief, Experimental Pharmacology Section
Dr. Robert M. Pratt

Cooperating Units: Dr. Stanley Cohen, Department of Biochemistry, School of Medicine
Vanderbilt University, Nashville, Tennessee 37203

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Current studies are designed to provide insight into the biochemical processes involved in palatal shelf growth and fusion. Growth will be measured by determining the rates of synthesis, accumulation and breakdown of DNA, RNA and protein in palatal shelf tissues obtained from control animals as well as animals treated with teratogenic agents. Epithelial fusion and breakdown will be evaluated at the ultrastructural level and ferritin labeled lectins will be used to determine the distribution of carbohydrates on the surface of the epithelium. In addition, experiments will be conducted to determine the mechanisms by which the Epidermal Growth Factor (provided by Dr. Stanley Cohen) blocks epithelial cell breakdown in cultured palatine shelves.

Total Man Years: 1
Professional: 3/4
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	John R. Hassell	4/4/73

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. NO LBS-017-(b)-(63)

NIDR-LBS431b63

TITLE OF PROJECT

Norchlorcyclizine Teratogenesis; Mechanism of Action and Molecular Effects Leading to Cleft Palate Production

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Miss Ann L. Wilk
Other Investigator: Dr. Cecil T. G. King, Chief, Experimental Pharmacology Section
Cooperating Units : None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Investigations are being carried out to define the role of acid mucopolysaccharides in the growth and development of the palatine shelves. Preliminary studies indicate that norchlorcyclizine alters the synthesis of acid mucopolysaccharides in the palatine shelves prior to rotation. The information gained from these studies will be used to further detail the synthesis, distribution and alteration of hyaluronic acid and chondroitin sulphate(s) in the palate at different stages of development in normal and cleft palate fetuses.

Total Man Years: 1 1/2
Professional: 1
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
National Institute of Dental Research	Ann Louise Wilk	4/13/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D.

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LBS441b73

rev. Ser. No. None

ITLE OF PROJECT

The Effect of Prolonged Gestation or Delayed Inplantation on Reproductive Capacity and Oral Facial Development and Growth

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Dr. Cecil T. G. King, Chief, Experimental Pharmacology Section
Other Investigators: None
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Biological Structure
Section: Experimental Pharmacology
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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ıe normal gestational period of 21 days in the rat is being extended to periods) to 44 days and in the mouse from 19 up to 24 days by the administration of ɔecific phenothiazine derivatives at critical stages of gestation. Investigations ?e underway to determine whether the prolongation of gestation is due to a delay ı inplantation or disturbances in the normal processes of organogenesis and growth. ıe teratogenic properties of the compounds used are also being studied.

)tal Man Years: 3/4
Professional: 1/4
Other: 1/2

)FESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
:al Institute of Dental Research	Cecil T. G. King	4/4/73

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'PORTING AGENCY

'HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotioted Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. LMI-002-(c)-(71)

NIDR-LMI002c71

TITLE OF PROJECT

Bone Resorption in Periodontal Disease by a New Mediator Elaborated by Mononuclear Cells: Osteoclast Activating Factor

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. John E. Horton
Other Investigators: Dr. Joost J. Oppenheim, Dr. Stephan E. Mergenhagen, Mrs. Cynthia Fischler
Cooperating Units: University of Rochester, School of Medicine and Dentistry, Dr. L. G. Raisz

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Osteoclast Activating Factor (OAF) is generated with supernatants of in vitro mononuclear cell cultures when stimulated by lectins, or when sensitized leukocytes from periodontally-diseased subjects are stimulated by plaque-antigens. OAF causes bone resorption in organ cultures of fetal rat bone shafts as measured by the increase release of calcium - 45, and also induces the appearance of large numbers of activated osteoclasts in such bone. It has a similar dose response cruve to parahormone. OAF is distinguishable from prostaglandin by its dose-response cruve and also by the fact it is heat labile. Fractionation studies with Sephadex G-100 column chromatography reveal its MW to be between 13,500 and 25,000. It is produced in vitro within 6 hours following cell activation. OAF may well be responsible for localized bone resorption near inflammation, as in periodontal disease, or even adjacent to neoplasma. The cell source and nature of this new important product of inflammatory cells is being further defined.

Total Man Years: 1 3/4
Professional: 1 1/2
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	John E. Horton	4/13/

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. LMI-003-(b)-(69)

NIDR - LMI003b69

ITLE OF PROJECT

Immunological Mechanisms Involved in Histamine Liberation

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. William A. Hook
Other Investigators: Dr. Joost J. Oppenheim, Mr. Julian Washington, Jr., Mrs. Sue Doughe
Cooperating Units: George Washington University, Dr. Halla Brown

AME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Immunology
Location : NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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The release of histamine and other pharmacologically active substances from mast cells and blood basophiles is being studied. The interaction of bacterial endotoxin with the serum complement system to generate histamine liberating activity was reported. Histamine release is being studied as one of the immunological effector mechanisms by which antigens, allergens, mitogens or endotoxins may interact with serum components or leukocytes to cause inflammation. Histamine release by mitogenic plant proteins, by extracts of walnuts, and by extracts of animal danders is currently under investigation in relation to immediate hypersensitivity.

otal Man Years: 2
Professional: 3/4
Other 1 1/4

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	William A Hook	April 13, 1973

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

NIDR-LMI004b69

Prev. Ser. No. LMI-004-(b)-(69)

TITLE OF PROJECT

Relationship of cell-mediated and Bursal Dependent Reactions to Delayed Hypersensitivity and Lymphocyte Transformation

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Holger Kirchner
Other Investigators: Dr. Joost J. Oppenheim, Mr. Andrew Fridberg, Mrs. Sue Dougher
Cooperating Units: National Cancer Institute, Dr. Michael Blaese

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resea the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Mechanisms of interaction of different classes of immune cells (B and T) are being studied. Agammaglobulinemic chickens depleted of B cells and splenic B cell suspensions which have been treated with specific cytotoxic anti T cell antisera are being used for these studies. B cell deprived chickens have defective in vitro proliferative reactions to antigens, but still make normal amounts of mediators of cellular immunity in response to antigens.

Total Man Years: 1 3/4
Professional: 1 1/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Dr Holger Kirchner	4/13/-

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. LMI-006-(b)-(71)

NIDR- LMI006b71

TITLE OF PROJECT

Isolation, Characterization and Biological Effects of Murine Histocompatibility Antigens

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Jack Pincus
Other Investigators: Dr. David Ranney, Mrs. Nina Chace and Mr. Thom Atchison
Cooperating Units: Associated Biomedic Systems, Inc., Buffalo, New York

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Solubilized H-2^d antigens were obtained from cultured murine leukemia cell line, L1210. The biological effects of administering these solubilized antigens to allogeneic mice were investigated. Single doses in adjuvant produced delayed hypersensitivity reactions, humoral antibody production, and accelerated graft rejection whereas multiple doses suppressed GVH reactions and prolonged skin graft rejection by about 30%. The effects on immune responses of these solubilized H antigens are being further manipulated

Total Man Years: 1 1/2
Professional: 3/4
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Dr. Jack Pincus cgj	4/13/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NATIONAL INSTITUTE OF DENTAL RESEARCH

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HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LMI-008-(b)-(71)

NIDR-LMI008b71

TITLE OF PROJECT

Chemotactic Factors Produced by Human and Animal Leukocytes and their Role in Mediating Acute and Chronic Inflammatory Reactions

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Leonard C. Altman
Other Investigators: Dr. Sharon M. Wahl, and Mr. John B. Kennedy, Dr. Stephan Mergenhag
Cooperating Units: National Cancer Institute, Dr. Michael Blaese

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The work presently underway and/or planned for study in my laboratory involves the investigation of soluble mediators of inflammation. Specifically lymphocyte products which are chemotactic for homologous monocytes and/or polymorphonuclear leukocytes are presently being characterized in humans, guinea pigs and chickens. A primary goal of this research is to study the role of this mediator in vivo and its biologic significance in certain human diseases including chronic lymphatic leukemia, Hodgkin's disease, intestional lymphangectasia, chronic mucocutaneous candidiasis and Wiskott-Aldrich syndrome. In addition the production of this mediator has been and is being used as a tool to study the role of T and B lymphocytes in lymphokine synthesis. Finally studies of phagocytosis by macrophages and factors responsible for promoting this function and macrophage activation are planned.

Total Man Years: 3 1/4
Professional: 2 1/4
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Leonard C. Altman MD	04/13/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LMI-010-(b)-(66)

NIDR-LMI010b66

TITLE OF PROJECT

Phylogenetic Mapping of the Homofermentative Lactic Acid Bacteria

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Jack London
Other Investigators : Miss Kimberly Kline and Mrs. Sandra Kulczyk
Cooperating Units : None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology and Immunology
Section : Microbial Physiology
Location : NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The enzyme, FDP aldolase of Streptococcus faecalis strain MR has been purified to homogeneity and used as an antigen to prepare aldolase specific antisera in rabbits. Using the anti S. faecalis aldolase sera to detect structural relatedness among the various aldolases of homolactic bacteria, it was established that the three major genera which comprise the lactic acid bacteria, namely, Streptococcus, Lactobacillus and Pediococcus, are related to one another through a common evolutionary ancestor. A phylogenetic map of the homofermentative lactic acid bacteria based on the extent of immunological cross reactivity between the respective aldolases and the anti-aldolase sera was assembled. Each of the genera represent a separate and distinct line of evolution; however, in addition to the three major diverging lines, four minor branches were also detected.

Total Man Years: 2 3/4
Professional : 1
Other : 1 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Jack London

DATE: 4/13/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

NIDR- LMI012b72

Prev. Ser. No. LMI-012-(b)-(72)

TITLE OF PROJECT

Carbohydrate Metabolism and Regulation in Caries Producing Microorganism

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Joseph E. Ciardi
Other Investigators: Dr. Charles L. Wittenberger and Mr. Alfred Beaman
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology and Immunology
Section: Microbial Physiology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Dextransucrase from Streptococcus mutans, which catalyzes the formation of insoluble, adhesive polysaccharide from sucrose, is being studied. Various kinetic properties of the "enzyme" as well as the mechanism of polymer production are under investigation in partially purified enzyme preparations. Purification to homogeneity is underway. Continuing experiments on inhibition of dextran synthesis and on the elucidation of the role of protein-associated carbohydrate in dextran-sucrose activity involve both chemical and enzymatic methods. The relativeness of this enzyme from S. mutans to dextran producing enzymes from other oral microorganisms will be investigated.

Total Man Years: 1 3/4
Professional: 1 1/4
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Joseph E Ciardi Ph.D.

DATE: 4/10/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. LMI-013-(b)-(61)

NIDR-LMI013b61

ITLE OF PROJECT

Studies on the Regulation of Carbohydrate Metabolism and Lactic Acid Production in Oral Microorganisms

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: C. L. Wittenberger, Ph. D.
Other Investigators: Mrs. M. P. Palumbo and Ms I. Klein
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology and Immunology
Section: Microbial Physiology
~~Location: NIDR, NIH, Bethesda, Maryland 20014~~

IMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The mechanisms by which cariogenic and noncariogenic Streptococci regulate the istribution of substrate carbon among multiple catabolic pathways is under investigation. everal enzymes, which function in the constitutive pathways that lead to lactic acid, ave been identified as specific control sites. The means by which the catalytic function f these enzymes is modulated by their interaction with specific low molecular weight igands is being studied. These comparative studies with cariogenic and noncariogenic treptococci are designed to gain insight into a possible biochemical basis for patho-enicity and ultimately to formulate a rational approach for its control.

otal Man Years: 2 1/2
Professional: 1/2
Other: 2

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	C. L. Wittenberger	4/13/73

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THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. LMI-014-(b)-(66)

NIDR-LMI014b66

TITLE OF PROJECT

Systematic Microbiological Taxonomic Studies

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator : Morrison Rogosa
Other Investigators : Dr. Rita Colwell, Dr. Micah Krichevsky, Dr. Irwin Lessel and Mr. Charles Mills
Cooperating Units : University of Maryland, American Type Culture Collection, Bergey's Manual Trust, and International Subcommittee on Bactobacilli and Related Organisms.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology and Immunology
Section : Microbial Physiology
Location : NIDR, NIH, Bethesda, Md. 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Integrated studies will be continued on common reactions, utilization of substrates and metabolic pathways in the lactic acid bacteria representing chiefly Lactobacillus, Leuconostoc, and Pediococcus. New differntial tests and comparative enzyme functions in the lactic acid bacteria will be studied further. The definition of the term "lactobacilli and related organisms"is being expounded. Computer storage and informati programming of microbial characteristics will continue.

Total Man Years: 1 1/2
Professional: 1
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Morrison Rogosa	4/13/73

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. LMI-015-(b)-(67)

NIDR-LMI015b67

TITLE OF PROJECT

Persistent Viral Infections and Virus-Induced Immunopathology

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Abner Louis Notkins
Other Investigators: William Burns, Tony Walz and Akira Niwa
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Virology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Factors responsible for the persistence and activation of herpes simplex virus in animals is being investigated. The ability of various components of the immune system to inhibit virus infections in vitro is being examined. Correlates are being made with in vivo findings in which animals are depleted of the ability to make antibody or mount a cell-mediated immune response.

Total Man Years: 3 1/4
Professional: 2 1/4
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Abner Louis Notkins, M.D.	Sept 10, 70

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. LMI-016-(b)-(71)

NIDR-LMI016b71

TITLE OF PROJECT

Detection of Viral Antigens and Antiviral Antibody

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Abner Louis Notkins
Other Investigators: Kozaburo Hayashi and Joel Rosenthal
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Virology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Efforts are being made to develop sensitive and rapid methods for detecting Herpes Simplex Virus (HSV) antigens and antibody to HSV. Emphasis is on solid phase micro-radioimmunoassay. The sensitivity of this technique is being compared with standard techniques such as immunofluorescence.

Total Man Years: 4 1/4
Professional: 2 1/4
Other: 2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	[signature] M.D.	[illegible]

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

. Ser. No. LMI-017-(b)-(70)

NIDR-LMI017b70

E OF PROJECT

Cellular Immunity and Viral Infections

NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER
ESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

ipal Investigator: Abner Louis Notkins
her Investigators: Gary L. Rosenberg
Cooperating Units: Duke University Medical Center, Durham, North Carolina,
Ralph Snyderman

E AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Virology
Location: NIDR, NIH, Bethesda, Maryland 20014

ARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in
o-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Cell mediated immunity to Herpes Simplex Virus (HSV) in animals and man is under
. In vitro proliferation of peripheral blood lymphocytes in response to HSV
ing utilized to detect and quantitate immune recognition of viral antigen by
tized T lymphocytes. The elaboration of biologically active soluble products,
lled mediators of cellular immunity (e.g., lymphotoxin, interferon, and human
uclear cell chemotactic factor), by these stimulated lymphocytes is being studied
effector mechanism leading to an inflammatory response and elimination of the
infection. In vivo studies on the relationship between the cellular and
al immune responses to viral infections are currently under investigation.

Man Years: 2 1/4
ofessional: 1 1/4
Other: 1

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Abner Louis Notkins	Sept 10, 73

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D OF SUPPORT *(Check one)*

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. LMI-018-(b)-(71)

NIDR-LMI018b71

TITLE OF PROJECT

Viral Diseases of the Salivary Glands and Pancreas

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Abner Louis Notkins
Other Investigators: D. Wark Boucher, Michael Ross
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Virology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The ability of viruses to infect the exocrine and endocrine glands of animals is under investigation. Viruses under study include Encephalomyocarditis and Cytomegalovirus. Chemical, pathologic and histologic techniques are being employed in an attempt to elucidate the factors involved in the development of diabetes, pancreatitis and sialadenitis.

Total Man Years: 3 1/4
Professional: 2 1/4
Other: 1

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR

DATE

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LMI019b72

r. Ser. No. None

LE OF PROJECT

Transport and Metabolism of Hexitols in Streptococcus mutans

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Dr. James H. Maryanski
Other Investigators: Dr. C. L. Wittenberger
Cooperating Units: None

IE AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Microbial Physiology
Location: NIDR, NIH, Bethesda, Maryland 20014

MARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

n the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in io-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The transport of mannitol in Streptococcus mutans by a phosphoenolpyruvate-pendent phosphotransferase system has been demonstrated, and the properties of is enzyme system are being studied in relation to similar transport systems ported for other bacteria. Mutants of Streptococcus mutans unable to grow on nnitol are being studied to elucidate the enzymatic reactions involved in xitol transport and metabolism. The effect of various metabolic intermediates the regulation of the phosphotransferase system and on mannitol-1-phosphate hydrogenase is currently under investigation.

tal Man Years: 1 1/4
Professional: 1 1/4
Other:

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE

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IOD OF SUPPORT *(Check one)*
Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

NIDR-LMI020b72

Prev. Ser. No. None

TITLE OF PROJECT

Immunological Activation of the Alternate Complement Pathway and its Relationship to the Inflammatory Response

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Ann L. Sandberg,
Other Investigators: Mrs. Judy Bechtold, Mrs. Jean Phillips, Dr. Stephan Mergenhagen
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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Certain immunoglobulin classes as well as non specific substances such as endotoxin activate the alternate complement pathway with resultant production of an inflammatory response. The participation of and requirement for immunoglobulins in activation of this pathway by endotoxin is currently under investigation. The antigenic configurations required for activation of this alternate pathway as opposed to the classical pathway are also currently being examined. The biological function of the alternate complement pathway is also being evaluated in terms of its ability to neutralize herpes simplex virus.

Total Man Years: 5 1/2
Professional: 2 1/2
Other: 3

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: Ann L. Sandberg

DATE: 4/13/-

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. None

NIDR-LMI021b72

TITLE OF PROJECT

Isolation and Identification of Inhibitor of Lymphocyte Proliferation

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. David Ranney
Other Investigators: Dr. A. Quattrone, Dr. Joost J. Oppenheim and Mr. Thom Atchison
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Rat spleen cells have been found to produce a dialysable pronase resistant inhibitor by both B and T lymphocyte in vitro DNA synthesis and mitotic rate. This inhibitor is not species specific, but is generally maximally effective on lymphoid tissue and therefore resembles a chalone. This potential regulatory factor is being further characterized.

Total Man Years: 1 1/4
Professional: 1
Other: 1/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR David Ranney M.D.

DATE 4-13-23

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. None

NIDR-LMI022b72

TITLE OF PROJECT

Immunological Effects and Possible Effects on Aphthous Stomatitis and Behcet Syndrome of Ingestion of Walnuts

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Joost J. Oppenheim
Other Investigators: Dr. Ann Sandberg, Mrs. Sue Dougherty, Dr. William Hook, Dr. Leonard Altman and Mrs. Judy Bechtold
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Saline extracts of walnuts have been found to be mitogenic for lymphocytes, stimulate production of mediators by them, and release histamine from basophils of atopic subjects. In contrast extracts of pellicles of walnut were found to be cytotox for tissue culture cells. Ingestion of walnuts (>100 gms) temporarily markedly suppresses ability of lymphocytes to transform for a few days in normals and patients. Some subjects develop aphthous ulcers and some Behcet patients symptoms are exacerbate after eating walnuts. The relationship between these disparate findings is being studied.

Total Man Years: 1 3/4
Professional: 1
Other: 3/4

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
		4/13/

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METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION D

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LMI023b72

ev. Ser. No. None

TLE OF PROJECT

Investigation of the Role of Cell Mediated Immunity in the Host-Response to a Latent Herpes Virus Infection

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. David L. Rosenstreich
Other Investigators: Miss Lynda Weedon
Cooperating Units: None

ME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Guinea pigs experimentally injected with a guinea pig herpes-like virus (GPHLV) evelop cell mediated immunity to this virus as measured by in vitro lymphocyte roliferation and production of lymphokine. Despite this immune response, viral rowth continues in the infected host. At higher infective doses of GPHLV, no mmunity to the virus develops at all. Current investigations involve the mechanism f this viral-induced loss of immunity and the role this plays in viral persistence nd possible viral-related pathology in the host.

otal Man Years: 1
Professional: 1/2
Other: 1/2

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	David L. Rosenstreich	4/13/73

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

OS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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U. S. Department of
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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. None

NIDR-LMI024b72

TITLE OF PROJECT

Investigation of the Mechanism of Action of Lymphocyte Stimulation by Bacterial Endotoxins

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. David Rosenstreich
Other Investigators: Dr. Stephan E. Mergenhagen, Dr. Thomas Chused, Miss Lynda Wee
Cooperating Units: Temple University, Department of Microbiology, Dr. Alois Nowo

NAME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology and Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Purified lipid fractions have been found to be responsible for the mitogenic activity of bacterial endotoxin on mouse bone-marrow derived (B) lymphocytes. Any modification of endotoxin that decreases its biological toxicity or complement-activat ability has been found to also decrease its mitogenic activity suggesting an interesti correlation between all these activities. Current studies involve further characteriz of the in vitro potential of endotoxin-stimulated lymphocytes.

Total Man Years: 1
Professional: 1/2
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR

DATE 4/13

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-LMI025b72

ev. Ser. No. None

ITLE OF PROJECT

Role of Complement in Increasing the Vascular Permeability in the Inflammatory Response

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Dr. David Small
Other Investigators: Dr. Stephan E. Mergenhagen
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION Laboratory: Microbiology & Immunology
Section: Immunology
Location: NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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The role of the fifth componenet of complement as a mediator of early vascular permeability in response to endotoxin is being studied in inbred mice. A radiolabeled assay for permeability has been developed and the effects of polymyxin on the activity of endotoxin in the inflammatory response is being studied.

otal Man Years: 1
Professional: 1
Other:

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	D. Small	4/13/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. OMS-002-(c)-(70)

NIDR-OMS002c70

TITLE OF PROJECT

Hemodynamics of Oral-Facial Tissues

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Richard L. Christiansen, Dental Director
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting resear the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

A system was previously developed to control blood perfusion to the mandible and mandibular teeth. The mandibular artery and vein were isolated and cannulated on 8 dogs. The influence of various drugs on hemodynamic parameters was monitored. It was found that catecholamines and serotonin produced a 25% increase in vascular resistance to mandibular blood flow while histamine decreased resistance by 15%. The data produced a curvilinear relationship between perfused flow and arteriovenous pressure difference with the convexity toward the pressure axis. This relationship is consistent with reports from studies of nonosseousvascular beds. It is proposed to study the hemodynamics of oral tissues under normal conditions and under the impact of environmental changes.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Richard L. Christiansen	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. OMS-003-(c)-(65)

NIDR-OMS003c65

ITLE OF PROJECT

tudy of Oral Area Motor Mechanisms by Use of Displacement and Pressure Transducers

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Dr. Richard L. Christiansen, Dental Director
Other Investigators: None
Cooperating Units: Instrument Fabrication Division, NIH
Dr. K. Moller, School of Dentistry, University of Minnesota
School of Dentistry, Georgetown University

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

IMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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Certain groups of patients, such as cleft palate, exhibit varying degrees of rticulation errors and an overall perceptible hypernasality. Velar motion was nstantaneously monitored via a displacement transducer; the transducer output also rovided a visual feed-back to assist in modification of velar movement by increased levation and increased velopharyngeal closure. Increased elevation was produced uring conditioning sessions and during post-conditioning speech. It is proposed to etermine patterns of velar elevation at other locations with multichannel instru-entation.

In the area of intraoral pressure measurement lateral pressure of the relaxed ongue was monitored with sensitive pressure transducers. The relaxed pressure was ound to be of low magnitude (< 0.5 g/mm^2). It is proposed to study the complex atterns of tongue muscular activity with pressure measurement as it relates to ongue displacement and confinement.

otal Man Years: 1/2
Professional: 1/2
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Richard L. Christiansen	4/3/73

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THOD OF SUPPORT *(Check one)*

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PROJECT NO. (DO NOT USE THIS SPA

NIDR-OMS004c72

Prev. Ser. No. OMS-004-(c)-(72)

TITLE OF PROJECT

Effect of Antiserotonin and antihistamine agents on the Immune Complex of NZB/W F_1 Hybrid Mice

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Thomas M. Chused, M.D.
Other Investigators: None
Cooperating Units: Alfred D. Steinberg, M.D., A&R, NIAMDD

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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It has been shown that methysergide and cyproheptadine, both antagonists of serotonin, delay the onset of proteinuria and prolong life in NZB/W hybrid mice. Studies are in progress to determine the age at which therapy must be started and the effect of combining the drug with other agents.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas M. Chused, M.D.	4-24-

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. OMS-005-(c)-(72) NIDR-OMS005c72

ITLE OF PROJECT

Functional Capacity of Thymus Derived Cells in New Zealand Mice

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Thomas M. Chused, Sr. Staff Fellow
Other Investigators: John A. Hardin, M.D.
Cooperating Units: Leroy M. Parker, M.D.

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

JMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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It has been noted that 6-month old New Zealand mice have a decreased capacity to reject skin grafts. We are investigating the functional capacity of their thymus-derived (T) cells by in vivo graft versus host reaction, and in vitro response to phytohemaglutinin, conconavalin and allogenic lymphocytes, as well as sensitivity to anti-θ and anti-immunoglobulin antibodies. We wish to determine the life span, migratory behavior and antigenic properties of the deficient population.

Total Man Years: 3/8
Professional: 3/8
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	T. Thomas McChused M.D.	4/20/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. OMS-007-(c)-(68) NIDR-OMS007c68

TITLE OF PROJECT

Mechanisms of Cryoprecipitation in Cryoglobulins

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Norman A. Cummings, Medical Officer
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Mechanisms to explain cold insolubility in cryoglobulins are explored by immunochemical and physicochemical techniques. Peptide mapping of appropriate (CRYO) gamma globulin fragments will be emphasized in terms of chromatographic behavior and amino acid content. Energetics of cryoprecipitability are studied.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	N. A. Cummings, M.D.	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DAT

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

ev. Ser. No. OMS-008-(c)-(72)

NIDR-OMS008c72

ITLE OF PROJECT

Immunochemical Studies of Sjögren's Syndrome

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Norman A. Cummings, Medical Officer
Other Investigators: J. Hardin, Surgeon
T. M. Chused, Sr. Staff Fellow
T. M. Tarpley, Jr., Dental Surgeon
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

IMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The immunochemical aspects of salivary gland antigens will be assessed in Sjögren's syndrome. Ability to inhibit anti-salivary gland antibody in fluorescence or autoradiographic techniques will be used to identify the antigens isolated from the gland by column chromatography. Protein fractions from the gland will also be evaluated for their ability to stimulate transformation of lymphocytes from patients.

otal Man Years: 3/8
Professional: 3/8
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	N. A. Cummings MD	4/3/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. OMS-009-(c)-(68)

NIDR-OMS009c68

TITLE OF PROJECT

Study of Behcet's Syndrome

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Dr. Norman A. Cummings, Medical Officer
Other Investigators: Dr. B. R. Thach, Clinical Associate, Oral Pharyngeal Developmen
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting researc the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Patients with Behcet's syndrome will be studied with regard to the natural history of their clinical course. Their immunologic response to various antigens, the incidence and extent of neurologic involvement, and the response to various therapeutic modalities will also be assessed. Comparison to patients with aphthous stomatitis will also be made.

Total Man Years: 1/8
Professional: 1/8
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Norman A. Cummings, M.D.	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. OMS-010-(c)-(70) NIDR-OMS010c70

TITLE OF PROJECT

Evaluation of Dental Implants

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: George E. Garrington, D.D.S.
Other Investigators: Patrick Looney, D.D.S.
Cooperating Units: Philip M. Lightbody, D.D.S., Winter Park, Florida

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Sixteen ceramic implants were placed in New Zealand white rabbits, 6 subcutaneously, 8 in the femur, and 2 in the body of the mandible. Twenty-one dental implants were placed in adult Rhesus monkeys in extraction sites. Eight of the implants in monkeys were embedded as roots only, 12 were complete teeth including crowns, and one was a root with a glazed cylindrical collar protruding through the gingiva. All of the implants were ground to duplicate the size and shape of the extracted tooth that they replaced. Materials used were porous calcium aluminate ceramic (15), porous titanium (4), pyrolytic carbon (1), and porcelain coated gold (1). Pore sizes of the ceramic implants ranged from 50 microns to 200 microns. Surgical procedures were well tolerated by the animals and there were no operative or anesthetic deaths. All the rabbits and 4 monkeys have now been sacrificed with implants having been in place up to 2 years. The other implants in the remaining 8 monkeys are being removed en bloc to retain the animals for other studies. There has been no gross of evidence of toxicity or carcinogenesis, all materials appearing clinically to be well tolerated biologically. Specimens recovered are being prepared for histologic evaluation.

Total Man Years: 1
Professional: 1/2
Other: 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	George E. Garrington	4/5/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPA

Prev. Ser. No. OMS-016-(c)-(72) NIDR-OMS016c72

TITLE OF PROJECT

Development of an Image Preprocessing Dental Radiographic System

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Richard L. Webber, Senior Dental Surgeon
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

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A new type of radiographic system is being developed which couples an intraoral source of radiation to an extraoral transducer. The output of the device is an immediate display which can be stored both photographically and electronically. The latter capability also permits the output to be preprocessed in ways which have been demonstrated to assist in the detection of caries. The design provides for the production of images using a theoretical minimum of exposure to ionizing radiation.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Richard L. Webber	4/3/73

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METHOD OF SUPPORT *(Check one)*

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PROJECT NO. (DO NOT USE THIS SPACE)

NOTICE OF RESEARCH PROJECT

Prev. Ser. No. OMS-017-(c)-(72)

NIDR-OMS017c72

TITLE OF PROJECT

Selection and Clinical Testing of Techniques for Preprocessing Visual Images Containing Diagnostic Information.

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Richard L. Webber, Senior Dental Surgeon
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Research is being directed toward the selection and testing of heuristics designed to restrict the domain of promising transformations available for manipulating the display of diagnostic information. Related studies are underway which demonstrate the degree to which selected preprocessing techniques improve diagnostic performance obtainable in various clinical situations. Transformations of particular interest are currently being related to the interpretation of radiographs.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Richard L. Webber	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NATIONAL INSTITUTE OF DENTAL RESEARCH

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U. S. Department of
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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

NIDR-OMS018c72

Prev. Ser. No. OMS-018-(c)-(72)

TITLE OF PROJECT

Sjögren's Syndrome Saliva

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Robert O. Wolf, Dental Director
Other Investigators: Steven M. Herzberg, Clinical Associate
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Using the NIH Sjögren's Syndrome population we are investigating the disc acrylamid electrophoretic protein patterns, cytology and flow rates of the parotid saliva. The values are being compared to non-Sjögren's Syndrome values. More evidence concerning the etiology of this disorder is expected to result from this project.

Total Man Years: 1/2
Professional: 1/2
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Robert O. Wolf	4/3/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DA

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. OMS-019-(c)-(72)

NIDR-OMS019c72

ITLE OF PROJECT

Salivary Gland Studies

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Robert O. Wolf, Dental Director
Other Investigators: Steven M. Herzberg, Clinical Associate
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in he bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Human amniotic fluid and human pleural fluid isoamylases are being investigated for possible diagnostic value. Salivary gland disease is being studied by sequential salivary scintigraphy sialography, parotid flow rate, cytology and saliva analysis.

otal Man Years: 1/2
Professional: 1/2
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Robert O Wolf	4/3/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPAC

Prev. Ser. No. None

NIDR-OMS020c73

TITLE OF PROJECT

Streptococcal Skin Test for Aphthous Stomatitis

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Edward A. Graykowski, D.D.S., M.D.
Other Investigators: Takashi Fujibayashi, D.D.S., D.D.Sc.
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

A procedure to test the skin reactivity to streptococcal antigen in aphthous stomatitis patients was developed. The relationship of the skin reaction to the disease is being evaluated. The reliability of this procedure as a diagnostic test will be determined.

Total Man Years: 3
Professional: 1 1/2
Other: 1 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Edward A. Graykowski	4/20/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

v. Ser. No. None

NIDR-OMS021c73

ITLE OF PROJECT

Baseline Studies of the Effect of Pilocarpine on Parotid Gland Function in Sjögren's Syndrome

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

ncipal Investigator: Robert O. Wolf, Dental Director
Other Investigators: Dr. Steven Herzberg, Dr. John Hardin
Cooperating Units: L. G. Anderson, M.D., J. Sydney Stillman, M.D., Department of Medicine
Robert B. Brigham Hospital, Boston, Massachusetts.
Gerald S. Johnston, M.D., Chief, Nuclear Medicine Dept., CC, NIH.

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

UMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in ie bio-sciences and are forwarded : investigators who request such information. Your summary is to be used for these purposes.

The therapeutic effect of pilocarpine on xerostomia of Sjögren's syndrome ill be examined. Affected individuals will be compared to control individuals efore and after a short course of pilocarpine therapy. Parotid gland function nd saliva quality will be monitored by sialometry, scintigraphy and chemical nalysis of the secretion.

tal Man Years: 3/4
Professional: 3/4
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH ICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Robert O. Wolf	4/4/73

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PPORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

NDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-OMS022c73

Prev. Ser. No. None

TITLE OF PROJECT

Amylase Isoenzymes in Cystic Fibrosis

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Robert O. Wolf, Dental Director
Other Investigators: None
Cooperating Units: Lynn M. Taussig, M.D., Richard Deckelbaum, M.D., and Mimi M. Belmonte, M.D.
Montreal Children's Hospital, Montreal, Canada

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Separation of serum and urine amylase into pancreatic and salivary isoenzymes may be of diagnostic benefit. Pancreatic insufficiency, particularly patients with cystic fibrosis, are being assessed. The isoamylase patterns of duodenal fluid, serum, urine, along with their total amylase values are being evaluated. Other pancreatic enzymes and stool fat are also being evaluated.

Total Man Years: 3/8
Professional: 3/8
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Robert O. Wolf	4/4/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-OMS023c73

Prev. Ser. No. None

TITLE OF PROJECT

Purification of Murine Thymic Derived (T) and Bone Marrow Derived (B) Cells.

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Thomas M. Chused, Sr. Staff Fellow
Other Investigators: None
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Immunologic functions are mediated by separate populations of lymphocytes, T and B, which have been shown to interact with each other.

A method is needed to separate these populations for study separately. It has been found that anti-immunoglobulin antisera and complement, in the presence of sodium azide, kills all B cells. Anti theta antibody and complement kills all T cells, as shown by the response to mitogens.

Total Man Years: 1/8
Professional: 1/8
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas M. Chused	4/5/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-OMS024c73

TITLE OF PROJECT

Identification of cell type in mononuclear infiltrates

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Thomas M. Chused, M.D.
Other Investigators: None
Cooperating Units: Dr. Ira Green, NIAID

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Many pathological states are characterized by mononuclear infiltrates in the affected tissue. It is important to determine whether these cells are thymus derived (T), bone marrow derived (B) or phagocytic monocytes (M). We have utilized 9S antibody and complement coated sheep erythrocytes (E) to detect B cells in tissue sections and 7S antibody coated E to detect M. Studies are in progress to develop a method of identifying T. We have shown that most of the cells infiltrating salivary glands in Sjogren's syndrome are B.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas M. Chused M.D.	4/20/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

rev. Ser. No. None

NIDR-OMS025c73

TLE OF PROJECT

Specific Antigen Response of Murine Lymphocytes

IVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Thomas M. Chused, M.D.
Other Investigators: Dr. David Rosenstreich
Cooperating Units: None

AME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

We are establishing a system in which mouse lymphocytes will respond to pecific antigens in vitro and plan to study the effect of macrophages in the ystem and the behavior of New Zealand mice.

tal Man Years: 1/4
Professional: 1/4
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Johnson M. Chused M.D.	4/20/73

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PORTING AGENCY

THOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-OMS026c73

TITLE OF PROJECT

Specificity of Natural Thymocytotoxic Antibody (NTAJ) in NZB Mice

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Thomas M. Chused, M.D.
Other Investigators: None
Cooperating Units: Leroy M. Parker, M.D.

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Immunofluorescent studies have shown that NTA is directed against the theta antigen on murine thymocytes but not to the same determinant as conventional anti theta C3H. NTA was found not to induce cap formation alone.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas M. Chused M.D.	4/20/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. None

NIDR-OMS027c73

TLE OF PROJECT

Lymphocyte Surface Antigen and Receptors in New Zealand Mice

/E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

incipal Investigator: Thomas M. Chused, Sr. Staff Fellow
Other Investigators: None
Cooperating Units: None

ME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

MARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Studies are in progress of the surface immunoglobulin, complement receptor, mmune complex receptor and theta antigen in New Zealand and normal mice.

tal Man Years: 1/8
Professional: 1/8
Other: 0

FESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	T. Thomas M. Chused, M.D.	4/20/73

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PORTING AGENCY

HOD OF SUPPORT *(Check one)*

☒ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. None

NIDR-OMS028c73

TITLE OF PROJECT

In vitro immune response of New Zealand mice

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: Thomas M. Chused, M.D.
Other Investigators: Dr. Michael Iverson
Cooperating Units: None

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Location: NIDR, NIH, Bethesda, Maryland 20014

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

New Zealand mice show tolerance abnormalities in vivo. We are going to dissect their immune system in vitro to determine the abnormal cell population.

Total Man Years: 1/4
Professional: 1/4
Other: 0

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	Thomas McBurd M.D.	4/20/73

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SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

ev. Ser. No. OPD-001-(c)-(72)

NIDR-OPD001c72

TLE OF PROJECT

ymposia on Oral Sensation and Perception

VE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: James F. Bosma
Other Investigators : None
Cooperating Units : Fogarty International Center

ME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Med. & Surg.
Section: Oral and Pharyngeal Development
Location: NIH, Bethesda, Maryland 20014

MMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in e bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

e Third Symposium on Oral Sensation and Perception: The Mouth of the Infant is in int since October, 1972, by CC Thomas, Springfield.

e Fourth Symposium on Oral Sensation and Perception: Development in the Fetus and fant was held November 20-22, 1972. This is now being accumulated into a 24 apter book, scheduled for submission to the Government Printing Office in May.

tal Man Years: $\frac{1}{4}$
Professional: $\frac{1}{4}$
Other: 0

OFESSIONAL SCHOOL *(medical, dental, etc.)* WITH CH THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR: James F. Bosma

DATE: Apr 133

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PORTING AGENCY

THOD OF SUPPORT *(Check one)*

Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Prev. Ser. No. OPD-003-(c)-(72)

NIDR-OPD003c72

TITLE OF PROJECT

Anatomical Studies of Head of Fetus at Term

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Principal Investigator: James F. Bosma, M.D., Chief, Oral Pharyngeal Development Section

Other Investigators: Beverly Etter, Illustrator, Oral Pharyngeal Development Section,

Cooperating Units: Robert Pierce, Clinical Center, NIH; St. Anthony's Hospital, Rockford, Ill.

Michael Mainen, M.D., USPHS Hospital, Baltimore, Maryland

Howard Bartner, Chief, Medical Illustrations Section, MAPB, NIH

Keiko Moore and Beverly Etter, Medical Illustrators, Washington,

NAME AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery

Section: Oral Pharyngeal Development

Location: NIDR, NIH, Bethesda, Maryland 20015

SUMMARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

This is the fourth year of gross anatomical studies of the face, pharynx and cranium of th human fetus at term. Successive dissections with 40 corresponding illustrations of the head have been completed. Also 9 coronal cross-section illustrations. A portion of these dissection illustrations were included in a brief anatomical description for the Third Symposium on Oral Sensation and Perception: The Mouth of the infant, now in print.(Thomas

Representative matching crania have been illustrated in general views, in coronal and in transverse sections. Each of the anatomical sections matches a tomoradiograph. This material is now in final stage of preparation as an Atlas of Cranial Anatomical Sections and Tomoradiographs of the Human Fetus at Term, authored by Robert Pierce (former Associate in Radiology at the Clinical Center),Michael Mainen (former Clinical Associate in this Section) and James Bosma.

In an additional study of the fetal cranium, 46 illustrations of selected areas of crania a individual bones have been completed. These, supplemented by fractions of the section illustrations noted above and a small number of radiographs and detail photographs, compri the material of a prospective general book, the Head Skeleton of the Human Fetus at Term, by J. Bosma.

Total Man Years: 2
Professional: 1/2
Other: 1 1/2

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
	James F. Bosma	4-3-

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

PHS-166
REV. 5-70

NATIONAL INSTITUTE OF DENTAL RESEARCH

Prepared for the Science Information Exchange.
Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

NIDR-OPD004c73

ev. Ser. No. None

LE OF PROJECT

Microscopic Anatomy of the Fetal and Newborn Human Lip

E NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

rincipal Investigator: Bradley T. Thach
Other Investigators: James F. Bosma
Cooperating Units: None

E AND ADDRESS OF APPLICANT INSTITUTION

Branch: Oral Medicine and Surgery
Section: Oral & Pharyngeal Development
Location: NIDR, NIH, Bethesda, Maryland 20014

MARY OF PROPOSED WORK – *(200 words or less – Omit Confidential data.)*

n the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in io-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Tissue specimens from the tongue of 15 to 20 human fetuses and 8 to 12 adults will be studied. The histology will be compared. Emphasis will be given to superficial innervation.

tal Man Years:
Professional: 1/2
Other: 0

ESSIONAL SCHOOL *(medical, dental, etc.)* WITH H THIS PROJECT SHOULD BE IDENTIFIED

SIGNATURE OF PRINCIPAL INVESTIGATOR

DATE 4/19/73

DO NOT WRITE BELOW THIS LINE – FOR OFFICE USE ONLY

ORTING AGENCY

OD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

S OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED BEYOND CURRENT FISCAL YEAR	BEGINNING DATE	ESTIMATED COMPLETION DATE

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NIDR ANNUAL REPORT

RESEARCH CONTRACTS AND INDEXES

INTRODUCTION

The growth and development of contracts as a mechanism for the support of research within the National Institute of Dental Research has necessitated some changes in the Annual Report. Unlike intramural research projects, which are reported to the Smithsonian Science Information Exchange on an annual basis, Contracts and Interagency Agreements are reported as they are executed. This can occur at any time throughout the year. For the purpose of this Report, which is prepared before the close of the fiscal year, we have arbitrarily chosen the period of time between April 1 of the last fiscal year and March 31 of the current fiscal year as the "Report Year."

A second major change in the reporting of contracts has been one of format. The NIH form (1688) for reporting contracts to SSIE is not as well suited to reproduction for the Report as is the one for intramural research projects. In this case, however, the time element works in our favor and we obtain all of the basic information, as well as any salient results, directly from the Division of Research Grants in a format more suitable for printing and much easier to read.

NIDR ANNUAL REPORT - FY 73

(4/1/72 - 3/31/73)

INDEX

RESEARCH CONTRACTS BY CONTRACTOR (1)

(1) Includes Interagency Agreements

Total Number of Contractors 49

(1) Includes Interagency Agreements

NIDR ANNUAL REPORT - FY 73

(4/1/72 - 3/31/73)

INDEX

RESEARCH CONTRACTS BY CONTRACT NUMBER (1)

(1) Includes Interagency Agreements

NIDR ANNUAL REPORT - FY 73

(4/1/72 - 3/31/73)

INDEX

RESEARCH CONTRACTS BY TRANSACTION NUMBER

NIDR ANNUAL REPORT - FY 73

(4/1/72 - 3/31/73)

INDEX

RESEARCH CONTRACTS BY PROJECT DIRECTOR

Includes Interagency Agreements

Total number of Project Directors 59

NIDR ANNUAL REPORT - FY 73

(4/1/72 - 3/31/73)

INDEX

RESEARCH CONTRACTS BY NIDR PROJECT OFFICER

Includes Interagency Agreements

Total number of NIDR Project Officers 27

INTERAGENCY AGREEMENT

Agreement Number NIDR-01
Party/Agency ..... U. S. COAST GUARD
Address ..... GOVERNORS ISLAND, NEW YORK
Initial Start Date 06/01/67
Expiration Date 06/30/72
Class Code DE-1-01
Project Director ..... WOOLRIDGE E D
NIDR Project Officer .. ENGLANDER H R

TITLE:

ANTICARIES EFFECT OF REPEATED 27,000 TOPICAL FLUORIDE TREATMENTS ON THE DECIDUOUS DENTITION.

OBJECTIVES:

The population of Governors Island has been used to conduct the following projects:

1. The effect of frequently repeated fluoride toplications on caries in the deciduous dentition.

2. Epidemiologic studies on the occurrence and distribution of cariogenic streptococci.

3. Studies on adhesive sealants.

4. Prevalence of root surface caries in adults.

INTERAGENCY AGREEMENT

Agreement Number NIDR-02
Party/Agency ..... NATIONAL BUREAU OF STANDARDS
Address ..... WASHINGTON, D.C.
Initial Start Date 07/01/64
Expiration Date 06/30/73
Class Code DE-2-01
Project Director ..... CASSEL J M
NIDR Project Officer .. GREULICH R C

TITLE:

CHARACTERIZATION OF THE TOOTH.

OBJECTIVES AND WORK SCOPE:

The study will be continued to obtain new knowledge on the properties and interactions of oral structures and restorative materials. Areas upon which research emphasis will be placed are as follows:

1. Crystallography and structural chemistry.

2. Surface (dentin, enamel, dental materials) characterization by water adsorption.

3. Mechanism of the attachment and grafting to dentin and enamel.

4. Physical, mechanical and physical chemical properties of hard and soft tissues and of dental materials.

5. 3D Panoramic X-Ray.

SALIENT RESULTS TO DATE:

For better comprehension of processes such as mineralization and demineralization (bone resorption and development, caries, fluoride incorporation), precise crystallographic structure data were obtained on pertinent Ca/P minerals, e.g. $Ca_4(PO_4)_2O$, β Ca_3PO_4, the component of tooth calculus, and others. The water vapor adsorptive characteristics of tooth components and a number of dental materials have been measured with emphasis on an interpretation (or prediction) of hysteresis paths exhibited by these materials. The mechanism of polymer grafting to collagenous tissue surfaces via a redox reaction has been studied, using both free amino acids and synthetic polymers and evidence provided that the serine residues offer a preferred site for reaction. Dilatometric measurement of oriented specimens show

that human dentin exhibits much less anisotropic variation in thermal expansion than does ivory, perhaps as a result of more uniform tubule orientation in the latter. Measurement of water gain or loss by mineralized collagenous tissue as a function of temperature indicates that the time dependent "thermal" dimensional changes in such materials are dominated by this exchange of water. Precision calorimetry-determined enthalpy values of 16-17 cal/g for the melting of bovine achilles tendon and bovine dental collagen versus 13-15 cal/g for rat-tail or kangaroo tail tendon have provided supporting evidence of greater crosslinking in the former collagen species. Three monomers, the bis(2 methacryloxyethyl) ethers of hydroquinone, resorcinol and catechol, were prepared as crystalline solids, mixtures of which will be examined for potential use as sealants and as dental composite resin matrix.

INTERAGENCY AGREEMENT

Agreement Number NIDR-05
Party/Agency ..... INDIAN HEALTH SERVICE, HSMHA
Address ..... ROCKVILLE, MARYLAND
Initial Start Date 10/01/71
Expiration Date 06/30/73
Class Code DE-1-01
Project Director ..... BUTTS J
NIDR Project Officer .. THOMSON L A

TITLE:

CLINICAL CONTROL OF SMOOTH SURFACE AND PIT AND FISSURE CARIES.

OBJECTIVES AND WORK SCOPE:

Purpose of Project is to determine the dental caries preventive benefit of a combined program of tooth sealant and repeated fluoride application.

Two groups of American Indian 5th and 6th graders will be randomly assigned to receive maintenance care or preventive measures with maintenance care. Both groups will have all accumulated carious lesions restored before the study commences and receive additional care as required. The Public Health significance of the preventive measures will be determined by contrasting the care required during a three year observation period to estimate the cost benefit.

INTERAGENCY AGREEMENT

Agreement Number NIDR-06
Party/Agency ..... NAVAL DENTAL RESEARCH INSTITUTE
Address ..... GREAT LAKES, ILLINOIS
Initial Start Date 10/16/71
Expiration Date 06/30/73
Class Code DE-1-01
Project Director ..... KEENE H J
NIDR Project Officer .. ENGLANDER H R

TITLE:

QUANTIFICATION OF CALCIUM AND FLUORIDE IN TOOTH ENAMEL SAMPLES.

OBJECTIVES AND WORK SCOPE:

To provide the personnel, equipment, and expendable supplies to provide analytical procedures per year principally to provide accurate quantification of calcium and fluoride in tooth enamel samples.

INTERAGENCY AGREEMENT

Agreement Number NIDR-07
Party/Agency ..... FEDERAL HEALTH PROGRAM SERVICE, HSMHA
Address ..... SAN FRANCISCO, CALIFORNIA
Initial Start Date 11/17/71
Expiration Date 06/30/73
Class Code DE-2-01
Project Director ..... MOFFA J
NIDR Project Officer .. WACHTEL L W

TITLE:

SUPPORT FOR RESEARCH PROJECT TO DETERMINE STATISTICAL RELIABILITY OF DENTAL EXAMINATIONS WITH DIFFERENT EXAMINERS.

OBJECTIVES AND WORK SCOPE:

To continue to provide support to train non-professional dental examiners (auxiliary personnel) and professional dental examiners (dentists) to continue research project designed to test hypothesis that non-professional dental examiners will agree with each other with at least 85% reliability when adequately trained and calibrated by professional dentists.

SALIENT RESULTS TO DATE:

Professional and non-professional examiners were trained in the evaluation of dental restorative materials. They have shown that non-professional examiners can achieve a satisfactory 85% agreement level with themselves and each other in some of the evaluation criteria. This is a promising demonstration of the potential of using non-professionals in these evaluation technics.

INTERAGENCY AGREEMENT

Agreement Number NIDR-08
Party/Agency ..... DIVISION OF DENTAL HEALTH, BHME
Address ..... SAN FRANCISCO, CALIFORNIA
Initial Start Date 10/20/71
Expiration Date 06/30/72
Class Code DE-1-01
Project Director ..... CVAR J F
NIDR Project Officer .. WACHTEL L W

TITLE:

OBTAINING DATA PROCESSING AND STATISTICAL ANALYSIS SERVICES ON SEALANTS.

OBJECTIVES AND WORK SCOPE:

Scope of services includes data processing and statistical services beginning with preparation of examination forms through the selection and preparation of statistical tables to be used in a publication, statistical testing of results, and preparation of written statistical interpretation of findings.

a. Study of four sealants initiated in Maracaibo, Venezuela in April, 1970.

b. Study of four sealants utilizing a half-mouth design, initiated in October, 1970.

c. Study of one sealant in a high fluoride area initiated in September, 1970.

d. Study of several sealants initiated in February, 1972.

INTERAGENCY AGREEMENT

Agreement Number NIDR-09
Party/Agency ..... NATIONAL BUREAU OF STANDARDS
Address ..... WASHINGTON, D. C.
Initial Start Date 09/01/72
Expiration Date 02/28/73
Class Code DE-2-01
Project Director ..... CASSEL J M
NIDR Project Officer .. WACHTEL L W

TITLE:

LABORATORY AND CLINICAL STUDY FOR DEVELOPMENT OF IMPROVED POLYCARBOXYLATE DENTAL CEMENT.

OBJECTIVES AND WORK SCOPE:

A study will be made of the mechanism involved in bonding to tooth surfaces and the clinically controllable variables important for optimum cementing potential will be determined. Experimental evidence will be sought for the mode of attachment by which the zinc-containing polycarboxylate cement bonds to tooth structure.

INTERAGENCY AGREEMENT

Agreement Number NIDR-10
Party/Agency ..... NATIONAL BUREAU OF STANDARDS
Address ..... WASHINGTON, D. C.
Initial Start Date 09/01/72
Expiration Date 06/30/73
Class Code DE-2-01
Project Director ..... FRANKLIN A D
NIDR Project Officer .. FOWLER B O

TITLE:

MASS TRANSPORT AND PHYSICAL PROPERTIES OF LARGE CRYSTALS OF CALCIUM APATITES.

OBJECTIVES AND WORK SCOPE:

Prepare and furnish to NIDR large single crystals of pure hydroxyapatite, $Ca_5(PO_4)_3OH$. These crystals are to be prepared from fluorapatite using electrochemical techniques to be developed by the Bureau. They are to be approximately one millimeter thick in the crystallographic c - axis direction and have lateral dimensions in both crystallographic a - axis directions of the order of a centimeter.

Contingent upon satisfactory preparation of pure crystals, the Bureau shall determine the OH^- diffusion coefficients, the ionic conductivity, and the OH^- transference numbers of hydroxyapatite as a function of temperature and crystallographic direction.

INTERAGENCY AGREEMENT

Agreement Number NIDR-11
Party/Agency ..... CENTER FOR DISEASE CONTROL, HSMHA
Address ..... ATLANTA, GEORGIA
Initial Start Date 11/01/72
Expiration Date 06/30/73
Class Code DE-1-01
Project Director ..... CHERRY W B
NIDR Project Officer .. THOMSON L A

TITLE:

IDENTIFICATION AND CHARACTERIZATION OF STREPTOCOCCUS MUTANS AND OTHER PLAQUE BACTERIA: DEVELOPMENT OF SPECIFIC FLUORESCENT ANTIBODY REAGENTS.

OBJECTIVES AND WORK SCOPE:

CDC shall provide the personnel, equipment, and expendable supplies to conduct a research project to: (1) develop, evaluate, and improve to "reagent quality" FA conjugates designed to be specific for certain plaque organisms considered important in dental caries etiology (e.g., Streptococcus mutans, serotypes a-e); (2) research improved methods for preparing reagent conjugates specific for plaque organisms; and (3) determine and recommend optimal conditions for the utilization of FA conjugates in identifying plaque bacteria.

INTERAGENCY AGREEMENT

Agreement Number NIDR-12
Party/Agency ..... CENTER FOR DISEASE CONTROL, HSMHA
Address ..... ATLANTA, GEORGIA
Initial Start Date 11/01/72
Expiration Date 06/30/73
Class Code DE-1-01
Project Director ..... PICKARD L G
NIDR Project Officer .. HAGEAGE G J

TITLE:

DEVELOPMENT AND EVALUATION OF FLUORESCENT ANTIBODY REAGENTS FOR FILAMENTOUS ORAL ORGANISMS OF THE GENERA: ACTINOMYCES, ARACHNIA, AND ROTHIA.

OBJECTIVES AND WORK SCOPE:

CDC shall provide the personnel, equipment, and expendable supplies to conduct a research project to: (1) develop and evaluate specific FA conjugates for A. viscosus serotypes 1 and 2, A. naeslundii serotype 2, A. odontolyticus, Arachnia propionica serotypes 1 and 2, and Rothia dentocariosa; (2) define the optimal conditions for the application of these conjugates; and (3) formulate protocols to be followed for the large-scale production of the approved conjugates.

RESEARCH CONTRACT

Transaction Number 2N01DE81262-07
Contract Number PH-43-68-1262
Name of Contractor .... KENDALL COMPANY
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-28-68
Expiration Date 10-31-73
Class Code DE-1-01
Project Director ..... MELLBERG JAMES R
NIDR Project Officer .. ENGLANDER HAROLD R

TITLE: MICROCHEMICAL ANALYSIS OF FLUORIDE UPTAKE IN TOOTH ENAME

OBJECTIVES AND WORK SCOPE:

The contractor will perform a microchemical analysis of the fluoride uptake in tooth enamel. Specifically, the contractor will: Remove thin layers of tooth enamel by emersion in perchloric acid by the method of Ohmori, Brudevold, and Gron; Conduct fluoride determinations for each of the tooth layers removed; Conduct experiments to determine whether artifically acquired enamel fluoride can be washed out in artificial saliva; and Perform fluoride and calcium assays on tooth enamel specimens supplied by the Project Officer.

SALIENT RESULTS TO DATE:

Progress has been very satisfactory, and over 15,000 Fluoride (F) analyses have been performed. The Contractor has produced useful enamel F data from the NIDR Clinical studies in Stickney, Illinois, and has demonstrated that initially high concentration of F are acquired by the enamel of deciduous and permanent teeth from a short series of intensive F toplications. The F slowly leeches out of the enamel for about eight months but remains significantly higher than the controls. There has been research resulting in refinement and improvement of the abrasive biopsy technic. Kendall has conducted hundreds of biopsies for the U.S. Health and Nutrition Survey, and F analysis from teeth and biopsies in the Kentucky mouth-rinse study, the NIDR clinical trial in Puerto Rico, and other NIDR studies in Stickney, Illinois, and on hundreds of rat teeth supplied by the NIDR. The Contractor has also performed miscellaneous tooth and water analyses for the NIDR and other study groups.

RESEARCH CONTRACT

Transaction Number 2N01DE71475-08
Contract Number PH-43-67-1475
Name of Contractor .... SOUTHWEST FOUNDATION FOR RES & EDUC
Address ..... SAN ANTONIO TEXAS
Initial Start Date 06-28-67
Expiration Date 06-27-73
Class Code DE-2-01
Project Director ..... REED O M
NIDR Project Officer .. HAMNER JAMES E

TITLE: STUDY OF BETEL QUID CARCINOGENESIS

OBJECTIVES AND WORK SCOPE:

The main objective of this study is to produce carcinoma in the baboon's cheek pouch, with a secondary objective of studying the carcinogenic potential of each of the ingredients of betel quid, namely: betel leaf, areca nut, lime, and tobacco, and also to study their interplay. The baboons are divided into two groups, i.e. plain betel quid amd betel quid plus tobacco. They are maintained on a protein deficient diet. Cheek pouches are created surgically, and the betel quid inserted three times per week. Note: This is a long term study and the current year effort will parallel closely the research done over the past 3 years. The existing Scope of Work is amended to add: After 48 months of treatment, half of the animals in each group will be placed on a normal diet, and the betel quid treatment of their buccal pouches will cease; At 1, 3, 6, 9, and 12 month intervals, biopsies will be taken to determine if these buccal pouches can return to normal when the suspected carcinogen is removed. The other animals will be continued under the original regimen. Note: This is a long term study and the current year effort will parallel closely the research done over the past three years.

SALIENT RESULTS TO DATE:

Clinical ulcerations have appeared in the cheek pouches of all animals in both groups after three years of treatment. The group which has tobacco in the quid also is the group with the greatest degree of epithelial atypia. It is anticipated that during another year of continued treatment, gross tumors will appear. It would be quite an achievement to be able to chemically induce a carcinoma in a subhuman primate, using the very ingredients which we suspect from NIDR P.L. 480 study in India as causing the high rate of oral cancer in South India where the habit of chewing betel quid is so prevalent.

RESEARCH CONTRACT

Transaction Number 2N01DE71476-08
Contract Number PH-43-67-1476
Name of Contractor .... SOUTHWEST FOUNDATION FOR RES & EDUC
Address ..... SAN ANTONIO TEXAS
Initial Start Date 06-28-67
Expiration Date 06-26-73
Class Code DE-2-01
Project Director ..... REED O M
NIDR Project Officer .. HAMNER JAMES E III

TITLE: REIMPLANTATION OF BABOON TEETH

OBJECTIVES AND WORK SCOPE:

The objectives of this research effort are to observe in time sequence the response of the surrounding periodontium to the implantation of ceramic, polymethylmethacrylate, prolyte carbons, titanium, and other possible artificial materials and to the heterogenous transplantation of natural teeth in adult baboons.

SALIENT RESULTS TO DATE:

Nine animals have had 38 ceramic implants inserted. Thirteen (13) of these implants have been evulsed, and two surgical block sections have been removed and are in the decalcification process now. As of this date, 23 ceramic implants are remaining in place. Four of these implants are full bicuspids, and nineteen are buried root fragments. All of the full tooth implant (crown and root) are being maintained with stabilization, and all demonstrate deep periodontal pockets. They have been in place from five months to one week. Twelve plastic implants have been placed in two animals. Three implants have been evulsed. Opex (15%) has been used in seven implants. Five implants are polymethylmethacrylate withouth the foaming agent. Holes have been drilled through the anterior teeth plastic implant roots. Some difficulty has been experienced because certain types of ceramic implants break easily. It was decided to begin burying the ceramic implants to test for tissue compatibility and possible periodontal attachment. Work is now beginning on the histology. Grossly, the ceramic seems compatible (it was compatible in the University of Tennessee toxicity profile), and most of the implants upon sectioning appear to have some form of attachment by the periodontium.

RESEARCH CONTRACT

Transaction Number 2N01DE22003-03
Contract Number ..... NIH -72-2003
Name of Contractor .... WORLD HEALTH ORGANIZATION
Address ..... GENEVA SWITZERLAND
Initial Start Date 10-01-71
Expiration Date 10-31-73
Class Code DE-1-01
Project Director ..... BARMES D E
NIDR Project Officer .. SENNING R S

TITLE: ETIOLOGY OF DENTAL CARIES IN PAPUA-NEW GUINEA

OBJECTIVES AND WORK SCOPE:

The Contractor will perform: A thorough and complete comparative evaluation, in young human subjects, of the test slab lesions as contrasted to natural lesions, utilizing all available procedures for measurement; A thorough and complete comparative evaluation, in young human subjects, of the microbial and biochemical compositions of test slab versus natural plaque; After the intraoral cariogenicity system has been sufficiently validated, in the opinion of the Project Officer, a series of test variables may be imposed upon the model system to determine their relation to the promotion or inhibition of caries development.

SALIENT RESULTS TO DATE:

A reconnaissance trip was made to Papua-New Guinea early in the contract year for the specific purpose of identifying a suitable population for the investigation and to develop methods and a suitable design for obtaining soil and food samples. Oral examinations were made on villagers living in three geographic areas of the Territory and a population living in villages on a single river system having a range of caries experience from nil through moderate to high was located. This will permit the investigation to be carried out on a different population but one similar to that on which the preliminary associations between caries and alkaline earth elements were noted. Recommendations on possible methods for sampling foods and soil have been made after onsite visits to Papua-New Guinea. The Project Officer and Co-Project Officer participated in the reconnaissance visit. The survey team is spending the months of April and May making oral examinations and taking plaque, saliva, and enamel specimens. Progress has been most satisfactory and the team judged to be very competent to carry out the project.

RESEARCH CONTRACT

Transaction Number 2N01DE22005-02
Contract Number ..... NIH -72-2005
Name of Contractor .... UNIVERSITY OF AARHUS
Address ..... AARHUS DENMARK
Initial Start Date 09-01-71
Expiration Date 09-30-73
Class Code DE-1-01
Project Director ..... LOE H
NIDR Project Officer .. CARLOS JAMES P

TITLE: LONGITUDINAL STUDY OF CHLORHEXIDINE

OBJECTIVES AND WORK SCOPE:

The Contractor shall conduct a controlled clinical study to: Test the long term effect of chlorhexidine on the development of dental plaque, caries, and periodontol changes; Examine the microflora of the saliva, gingiva, and tooth surfaces during such treatment; and Assess possible systemic and local side effects of long term use of chlorhexidine. The following was added to the existing Scope of Work: Gingival biopsy samples will be obtained from study subjects and examined microchemically and microscopically to determine any effect of chlorhexidine on gingival metabolism, architecture, and keratin-producing mechanisms.

SALIENT RESULTS TO DATE:

Results of clinical findings on Caries and Gingivitis after one year are being processed. Microbiologic analyses have demonstrated reduction of total salivary microflora, no overgrowth of gram negative rods, and a slight reduction in relative proportion of S. Mutans in the Chlorhexidine treated group. No undesirable side reactions, except the expected mild tooth staining, have been observed.

RESEARCH CONTRACT

Transaction Number 3N01DE12013-05
Contract Number ..... NIH -71-2013
Name of Contractor .... UNIVERSITY OF MIAMI
Address ..... CORAL GABLES FLORIDA
Initial Start Date 08-15-70
Expiration Date 08-14-73
Class Code DE-1-01
Project Director ..... ZINNER DORAN D
NIDR Project Officer .. SENNING R S

TITLE: STUDIES OF ANTICARIES AGENTS

OBJECTIVES AND WORK SCOPE:

Contractor shall provide the necessary staff, facilities and populations to conduct clinical studies of potential anti-caries agents encompassing the following objectives: To test the hypothesis that certain strains of dextran-forming streptococci are etiologically involved in the induction of human dental caries; To study the feasibility of the use of the enzyme dextranase as an anti-plaque and caries-inhibitory agent in humans; To study the effect of long-term use of dextranase on the oral streptococcal flora; To study the effect of long-term use of dextranase on gingival status.

SALIENT RESULTS TO DATE:

Baseline examiniations, daily treatment regimens with test enzyme, and year-end examinations are complete for the approximately 500 participants. Microbiologic analyses of plaque specimens from every subject are nearing completion. Analyses of clinical data will take place later this year.

RESEARCH CONTRACT

Transaction Number 3N01DE12022-05
Contract Number ..... NIH -71-2022
Name of Contractor .... UNIVERSITY OF UTAH
Address ..... SALT LAKE CITY UTAH
Initial Start Date 11-17-70
Expiration Date 08-16-73
Class Code DE-2-01
Project Director ..... WILLIAMS M L
NIDR Project Officer .. WACHTEL L W

TITLE: ENGINEERING EVALUATION OF DENTAL ADHESIVES

OBJECTIVES AND WORK SCOPE:

The Contractor shall concentrate its efforts in providing a significantly large sample size, including statistical evaluation, of the "blister test" to establish its reproduceability as a test method for dental application, as well as for a comparison to other adhesive test methods.

SALIENT RESULTS TO DATE:

This group showed that the two new tests were indeed superior to other tests for studying dental adhesives. Their data from the new tests are more reliable and quantitative than those from other commonly used tests. They noted that the necessary materials for the "blister" test can be obtained for less than $100.00. The adhesive strength and other physical properties of barnacle cement have been determined in a cooperative study with the Navy Research Laboratory at Mare Island, California. A few commercial dental adhesives have also been evaluated. Good progress has been made during the first year. They have proved the feasibility of these tests for studying dental adhesives.

RESEARCH CONTRACT

Transaction Number 2N01DE22030-03
Contract Number ..... NIH -72-2030
Name of Contractor .... UNIVERSITY OF ALABAMA BIRMINGHAM
Address ..... BIRMINGHAM ALABAMA
Initial Start Date 11-15-71
Expiration Date 10-14-73
Class Code DE-1-01
Project Director ..... KOULOURIDES THEODORE
NIDR Project Officer .. IKARI N S

TITLE: EVALUATE INTRAORAL TEST OF HUMAN CARIOGENICITY

OBJECTIVES AND WORK SCOPE:

The Contractor will perform: A thorough and complete comparative evaluation in young human subjects, of the test slab lesions as contrasted to natural lesions, utilizing all available procedures for measurement; A thorough and complete comparative evaluation, in young human subjects, of the microbial and biochemical compositions of test slab versus natural plaque; After the intraoral cariogenicity system has been sufficiently validated, in the opinion of the Project Officer, a series of test variables may be imposed upon the model system to determine their relation to the promotion or inhibition of caries development.

SALIENT RESULTS TO DATE:

The Contractor has evaluated the potential usefulness of the Intraoral Cariogenicity Test (ICT). This system involved incorporation of enamel slaps into acrylic flanges of prosthetic devices. The criterion of cariogenicity was the degree of demineralization of slaps maintained in the human mouth for selected time intervals. Incipient demineralization was determined by measuring microhardness changes. Comparative evaluations of slab versus natural lesions and microbial as well as biochemical composition of slab versus natural plaque have been undertaken. If evaluation demonstrates that the ICT is a valid model, it could be utilized to investigate effects of the many variables thought to promote or inhibit caries development.

RESEARCH CONTRACT

Transaction Number 2N01DE22039-02
Contract Number ..... NIH -72-2039
Name of Contractor .... EASTMAN DENTAL CENTER
Address ..... ROCHESTER NEW YORK
Initial Start Date 10-26-71
Expiration Date 10-25-73
Class Code DE-1-01
Project Director ..... BIBBY B G
NIDR Project Officer .. ROGERS W E

TITLE: CARIOGENICITY OF SNACK FOODS

OBJECTIVES AND WORK SCOPE:

Independently and not as an agent of the Government, the Contractor shall exert its best efforts to define which food stuffs are more harmful or less harmful to the teeth in regard to caries. Tests shall be carried out in rats to determine the effect of using the less harmful ones. In addition, the Contractor intends to delineate snack materials to replace those of high cariogenicity. Specifically, the Contractor shall perform, but not necessarily be limited to, the following efforts: Test various human snack foods and confections for their effects on enamel dissolution, food retention, and plaque pH; and Test human foods considered to be most and least cariogenic utilizing rats.

SALIENT RESULTS TO DATE:

Performance of work has been directed mainly toward laboratory-type studies. Various foodstuffs were tested for their influence on enamel dissolution, food retention, and plaque pH. An "artificial mouth" was developed and brought to a working level. This interesting, unique new system permits quick, easy evaluation of the effects of various foods on enamel dissolution. The Contractor has not yet assembled and analyzed sufficient data to delineate what foods are more or less cariogenic (based on laboratory testing). But this is imminent and will, no doubt, be accomplished before the periodic rat feeder arrives, is assembled, and brought to a working level.

RESEARCH CONTRACT

Transaction Number 2N01DE12041-03
Contract Number ..... NIH -71-2041
Name of Contractor .... HOWARD UNIVERSITY
Address ..... WASHINGTON DIST OF COL
Initial Start Date 11-25-70
Expiration Date 01-24-74
Class Code DE-1-01
Project Director ..... WHITEHURST VIRGIL P
NIDR Project Officer .. STILES HORACE M

TITLE: EVALUATION OF AN ADHESIVE SEALANT MATERIAL

OBJECTIVES AND WORK SCOPE:

Approximately 1,000 children between the ages of 6 and 14 will have one or more sites of contralaterally matched teeth covered with an adhesive sealant material. These sites will be observed and examined over a period of four years to assess the durability of the sealant material, the caries preventive ability of the sealant material, the fate of the tooth site in the event of sealant loss, whether dental hygienists can effectively apply the sealant under the supervision of a dentist, and the time and difficulties involved in applying the material in field-clinical conditions.

SALIENT RESULTS TO DATE:

Of the 1,000 children, 913 have received sealant material in one or more sites on one of a pair of contralaterally matched teeth. These placements were begun in November, 1960, and completed in February, 1970. The first six month examinations were completed and submitted to the NIDR, where they were processed and analyzed. Since the sealant placement extended over a period of four months, the six month evaluations follow the same schedule. The one year evaluations and examinations are currently in progress. The results of the six month examinations show that the adhesive sealant remained in place (either partially or completely) in over 38% of the treated sites. No significant differences were noted in the incidence of dental caries between the experimentally treated sites and the contralaterally matched untreated control sites.

RESEARCH CONTRACT

Transaction Number 2N01DE22045-01
Contract Number ..... NIH -72-2045
Name of Contractor .... AMERICAN DENTAL ASSOCIATION
Address ..... CHICAGO ILLINOIS
Initial Start Date 11-01-71
Expiration Date 10-31-73
Class Code DE-1-01
Project Director ..... HEFFERREN JOHN J
NIDR Project Officer .. KAKEHASHI SAMUEL

TITLE: DETECTION OF DENTAL CARIES IN CYSTIC FIBROSIS PATIENTS

OBJECTIVES AND WORK SCOPE:

In the studies and investigations relative to early detection and characterization of dental caries in cystic fibrosis patients, the Contractor's effort during this twelve-month period of renewal shall be limited to continuing the study of the initial test and control populations to provide evidence that "altered areas" progress into clinical caries.

SALIENT RESULTS TO DATE:

The Contractor spent approximately 1/3 to 1/2 time of the first contract year acquiring and calibrating the necessary instrumentation. In the remaining contract time, he has accumulated a mzdest base of data comparing caries detection by way of a normal clinical (visual-tactual) examination, radiographic examination, and ultraviolet light examination. Tabulated results show twice as many "altered areas" of tooth surface detected by ultraviolet examination as by clinical examination. The ultraviolet fluorescent data was obtained using color film and the human eye as detectors.

RESEARCH CONTRACT

Transaction Number 2N01DE12062-04
Contract Number ..... NIH -71-2062
Name of Contractor .... UNIVERSITY OF PUERTO RICO MED SCI CAMPUS
Address ..... SAN JUAN PUERTO RICO
Initial Start Date 12-30-70
Expiration Date 01-31-74
Class Code DE-1-01
Project Director ..... HARRIS NORMAN O
NIDR Project Officer .. FREW RALPH A

TITLE: EVALUATION OF AN ADHESIVE SEALANT MATERIAL

OBJECTIVES AND WORK SCOPE:

This project is designed to evaluate the anticaries potential of an adhesive sealant in a dental public health program. The sealant is placed in children's teeth by various types of dental auxiliary personnel under a variety of field conditions in the Virgin Islands. Baseline and follow up examinations will define the efficacy and persistance of the agent under the operant conditions. the project is entering its third year. The first year involved baseline examinations and treatment of the study population. The activity for subsequent years is primarily involved with the reexamination of the study population.

SALIENT RESULTS TO DATE:

Plastic sealant was placed in the occusal surfaces of the posterior molars of three different school populations in the Virgin Islands. In St. Thomas, a contralateral control technic was used for both permanent and deciduous teeth; in St. Croix, only permanent teeth were used on the contralateral basis. In St. John, sealant was used on all permanent posterior teeth. Plastic was also flowed over small pit and fissure lesions. The applications were made by dentistry technicians, hygienists, and a dentist. Results differed to some extent between islands, but in general the retention was poor in buccal grooves, poor but slightly better in the distal grooves, and best in the lower first molars. In St. Thomas, the retention rate for one year was approximately 46% and in St. Croix, 77% for six months. In St. Johns, the retention rate was 73% for six months where it was placed in sound teeth, and 79% when placed over small lesions. Problems experienced included early failure in performance of the ultra violet guns in non air conditioned environments in a tropical climate.

RESEARCH CONTRACT

Transaction Number 2N01DE92066-10
Contract Number ..... NIH -69-2066
Name of Contractor .... TEMPLE UNIVERSITY
Address ..... PHILADELPHIA PENNSYLVANIA
Initial Start Date 06-27-69
Expiration Date 06-26-73
Class Code DE-1-01
Project Director ..... MUMMA RICHARD D JR
NIDR Project Officer .. BRUNELLE J

TITLE: DEVELOPMENT OF ROOT-CARIES PROCEDURES

OBJECTIVES AND WORK SCOPE:

A clinical and radiographic description of root caries lesions will be made and criteria established for differential diagnosis. An examination procedure will be developed together with a scoring system suitable for epidemiological and clinical studies. The contractor will use the procedure in examining severfal hundred subjects for evidence of root caries. Data will be recorded in a systematic fashion and submitted to the Government for analysis.

SALIENT RESULTS TO DATE:

During the first nine months of the contract period, the Contractor accomplished the following. 500 employees of an insurance company in Hartford, Conn., were examined clinically and radiographically for the presence of root surfaces caries. The results indicated that 39.4% of those examined had at least one lesion and that the incidence increased directly with age. 355 teeth obtained from Coast Guard personnel and patients at Temple University School of Dentistry were examined, using visual, radiographic, and histopathologic techniques. The lesion was present in 103 teeth (49 patients). Most lesions were on the buccal/labial surface and the largest percentage fell into a category described as dentinal tubular matrix destruction. Two manuscripts have been submitted for publication. They are: "The Problem of Root Caries, I. Literature Review and Clinical Description", with Drs. Hazen, Chilton, and Mumma as authors (Journal of the American Dental Association); and "Root Surface Caries. Clinical and Histopathologic Classification", with Drs. Miller, Westbrook, Chilton, and Mumma as authors (Journal of Caries Research). Three papers on the subject have been prepared and presented at the annual meeting of the International Association of Dental Research in Las Vegas, Nevada, on March 23-26, 1972.

RESEARCH CONTRACT

Transaction Number 2N01DE12093-04
Contract Number ..... NIH -71-2093
Name of Contractor .... NEW YORK STATE DEPARTMENT OF HEALTH
Address ..... ALBANY NEW YORK
Initial Start Date 05-17-71
Expiration Date 04-30-73
Class Code DE-1-01
Project Director ..... CONS NAHAM C
NIDR Project Officer .. FREW RALPH A

TITLE: ADHESIVE SEALANT FIELD TRIAL

OBJECTIVES AND WORK SCOPE:

The workscope remains the same, except that portion dealing with tests on 100 children using fiberoptics has been deleted, i.e., item 6 of the Description of Work.

SALIENT RESULTS TO DATE:

During the initial period of the project, the following items were accomplished: Parents or guardians of 11,500 first and second grade children in Rochester, N. Y., were invited to permit their children to participate in the study; Oral examinations were completed for the approximately 4500 children with parental consent for participation in the program; and Sealant applications were completed on 2,000 children (approximately 3,000 teeth treated) who met the oral criteria and the residency requirements of the study. This progress accomplishes the major objective of the initial period of the project.

RESEARCH CONTRACT

Transaction Number 2N01DE02135-03
Contract Number ..... NIH -70-2135
Name of Contractor .... STICKNEY TOWNSHIP PUBLIC HEALTH DISTRICT
Address ..... OAK LAWN ILLINOIS
Initial Start Date 05-12-70
Expiration Date 06-25-73
Class Code DE-1-01
Project Director ..... FRANCHI GENE L
NIDR Project Officer .. ENGLANDER HAROLD

TITLE: EFFECT OF FLUORIDE IN OUTER ENAMEL ON DENTAL CARIES

OBJECTIVES AND WORK SCOPE:

Contractor will perform study to determine whether children who have relatively high caries experience in a flurodiated area (caries increments of 2 DMFT/year), show significant anticaries benefits after the outer 5 microns of their enamel have acquired at least 3,000 pp, F. from an intensive regimen of topical fluoride therapy.

SALIENT RESULTS TO DATE:

Fluoride uptake by children ages 10-11 participating in separate but related clinical trials of APF gel (1.2% F, 0.1 M PO4, pH 3.2) applications by toplicator was determined by analysis of exfoliated deciduous teeth. The first trial consisted of 25 fifteen-minute gell applications on consecutive school days while the second trial employed 10 tenminute gel applications. Eight thin layers reaching a dept of 250 um were etched from each of the 612 teeth analyzed. Enamel give 25 APF gel applications had 2991 ppm. Control of enamel contained 1016 ppm F at 5 um. A gradual loss of fluoride from teeth in both F gel groups began shortly after treatments stopped. The rate of fluoride loss was 5 ppm F per day at 5 um. Teeth given 25 gel applications continued to lose fluoride for 8 months, at which time 2650 ppm F remained. No further loss was noted during the subsequent 8 months. Teeth from the group that received 10 gel applications were completed but were still losing fluoride. Fluoride penetrated beyond 250 um in teeth given 25 applications and to approximately 150 um in teeth given 10 applications.

RESEARCH CONTRACT

Transaction Number 3N01DE92230-06
Contract Number ..... NIH -69-2230
Name of Contractor .... UNIVERSITY OF WASHINGTON
Address ..... SEATTLE WASHINGTON
Initial Start Date 06-30-69
Expiration Date 12-29-72
Class Code DE-1-01
Project Director ..... BORNSTEIN PAUL
NIDR Project Officer .. PIEZ K A

TITLE: DETERMINATION OF CYANOGEN FRAGMENT STRUCTURE

OBJECTIVES AND WORK SCOPE:

The Contractor will exert its best efforts to determine the primary structure of 1-CB8 from rat skin collagen. Specifically, the Contractor will: Obtain one chain of collagen by chromatography from purified rat skin collagen; Partial hydrolysis will be performed and resulting enzymatically-produced peptides will be separated by ion exchange chromatography; and Assign the sequence of tryptic peptides in 1-CB8 by combination of specific enzymatic and chemical cleavage.

SALIENT RESULTS TO DATE:

In the first two years hydroxylamine cleavage and enzymatic cleavage was used to prepare peptides. The peptides have all been separated and the order of many of them has been determined. The sequencing of these peptides has progressed to where about one-third of the sequence is complete. The remaining sequence will proceed much faster since the small peptides are available and the techniques are well established. Progress has been approximately as originally planned. Two publications based on this work have already appeared.

RESEARCH CONTRACT

Transaction Number 2N01DE02237-03
Contract Number ..... NIH -70-2237
Name of Contractor .... BATTELLE MEMORIAL INSTITUTE
Address ..... COLUMBUS OHIO
Initial Start Date 06-26-70
Expiration Date 08-25-73
Class Code DE-2-01
Project Director ..... HILLMAN ROBERT E
NIDR Project Officer .. PECORA L J

TITLE: STUDY OF ADHESIVE MECHANISM OF THE SEA MUSSEL

OBJECTIVES AND WORK SCOPE:

The workscope of the existing contract was amended as follows. The Contractor shall maintain mussel and barnacle colonies. The cement and/or its components shall be chemically and physically identified before secretion, immediately after secretion, and after aged cement has been attached for periods of time. After the components of the cement which are critical to hardening and adhesion in the aqueous environment have been defined and purified, the Contractor shall synthesize products having similar adhesive properties which will be used for dental research. The Contractor shall furnish quantities of cement(s) to the Government for testing at regular intervals when they believe they have a cementitious product ready for dental testing.

SALIENT RESULTS TO DATE:

Colonies of both Mytilus edulis and M. californianus continue to be maintained at both the Duxbury and Columbus facilities. Work was primarily aimed at further characterization of the adhesive secretion. Material was collected from the mussels in the usual manner. The protein components of M. edulis were completely excluded from Sephadex 6-50 and G-75, indicating the molecular weight above 30,000. Protein was eluted at pH2 from DEAE-Sephadex at 1 M NaCL and above, indicating binding to the dextran through the acidic aspartate and glutamate residues. Two major fractions absorbing at 208mm can be obtained, only one of which is retained upon dialysis. Electrophoresis is currently being carried out on this fraction. Electrophoresis of secretions from both species show a small portion of the material remaining at the origin, with a large amount of material migrating toward the anode. This fraction may contain the large proportion of glutamate and aspartate residues.

RESEARCH CONTRACT

Transaction Number 2N01DE02238-05
Contract Number ..... NIH -70-2238
Name of Contractor .... FRANKLIN INST OF THE ST OF PENN
Address ..... PHILADELPHIA PENNSYLVANIA
Initial Start Date 06-26-70
Expiration Date 07-31-73
Class Code DE-2-01
Project Director ..... THELEN E
NIDR Project Officer .. PECORA L J

TITLE: DEVELOP DENTAL CEMENT FROM A MARINE SOURCE

OBJECTIVES AND WORK SCOPE:

Independently and not as an agent of the Government, the Contractor shall exert its best efforts to determine the chemical and physical properties of the cementum of marine organisms including the mussel and barnacle in both the hardened and unhardened state, for use in synthesizing similar products which will be useful in dentistry. The Contractor shall also maintain mussel and barnacle colonies and other organisms as required by the study. The Contractor shall furnish small quantities of cement(s) to the Government for testing at regular intervals when they believe they have a cementitious product ready for dental testing.

SALIENT RESULTS TO DATE:

Barnacle cement has been temporarily shelved in favor of the arca zebra because the latter produces much more cement. Performic acid and 5 percent sodium hydroxide solutions have been studied. Thin layer chromatography is also being used to help focus in on protein segment composition. A rubbery byssal percursor appears promising for study. Some preliminary work with trypsin shows that some protein digestion is taking place. Both tensile and compressive strengths of the cementitious material being studied are made as needed. Electron and scanning electron microscopy is done as needed both for viewing cement components and animal tissues. Secreting glands and the cement are also being observed as it starts in the glands and along the ducts to setting externally. Histologic studies have revealed additional help ful new information on how the cement is produced and excreted. The latest technics and instrumentation are being utilized to solve the problem.

RESEARCH CONTRACT

Transaction Number 2N01DE12329-01
Contract Number ..... NIH -71-2329
Name of Contractor .... BECKMAN INSTRUMENTS
Address ..... FULLERTON CALIFORNIA
Initial Start Date 06-30-71
Expiration Date 06-29-73
Class Code DE-1-01
Project Director ..... ISENBERG D L
NIDR Project Officer .. ROGERS W E

TITLE: PURIFICATION OF MICROBIAL DEXTRANASES

OBJECTIVES AND WORK SCOPE:

Contractor will exert best efforts to produce enzymes, with specific ability to degrade the branched polysaccharide dextrans present in human plaque. Utilize an in vitro system to assay dextranase enzymes produced through the efforts described above.

SALIENT RESULTS TO DATE:

An enrichment/screening procedure was developed which was used to isolate in pure culture approximately 1500 microbial strains which produced enzymes capable of hydrolyzing the insoluble dextrans of cariogenic streptococcal strains HS-7, OMZ-61, and OMZ-176. These cultures were characterized on the basis of a dextran solubilization assay. Preliminary data obtained on the linkage character of selected dextrans suggest a more complex structure than the common linear dextran (B-512) from Leuconostoc. The results, so far, also suggest the possiblity that these polymers may in fact be glycoproteins.

RESEARCH CONTRACT

Transaction Number 2N01DE12330-03
Contract Number ..... NIH -71-2330
Name of Contractor .... SOUTHERN ILLINOIS UNIVERSITY EDWARDSVL
Address ..... EDWARDSVILLE ILLINOIS
Initial Start Date 06-30-71
Expiration Date 11-30-73
Class Code DE-1-01
Project Director ..... BAHN ARTHUR N
NIDR Project Officer .. IKARI NORMAN

TITLE: IMMUNIZATION WITH ENZYMES FROM CARIOGENIC STREPTOCCI

OBJECTIVES AND WORK SCOPE:

Contractor will exert best efforts to isolate and purify dextransucrases, levansucrases, and glycosidic hydrolases, from cariogenic streptococci, which will be investigated for their effectiveness as immunizing agents to reduce experimental dental caries.

SALIENT RESULTS TO DATE:

Results suggestive of caries reduction have been obtained from the two rat experiments completed during the first contract year. Efforts thus far were centered on the following: Elucidation of optional conditions for growth of S. mutans FA-1 to obtain maximal enzyme production; Selection of the best method for extraction and purification of enzymes with the highest possible yield and purity; and The completion of two and the near completion of two additional enzymeimmunized rat experiments and the initiation of one monkey experiment. Glycoside hydrolase-immunized rats showed significantly reduced mean caries scores as compared to a non-immunized, cariogenic bacterially challenged control group. However, a sham-immunized group showed mean caries scores similar to the enzyme-immunized group. Dextransucraseimmunized rats showed a 30% reduction of caries compared to sham-immunized controls of the second of two experiments. Data from the one levansucrase-immunized rat experiment which was completed on July 26, 1972, are not yet available. A glycosidic hydrolase mixture was injected into ten monkeys on January 4, 1972. Cariogenic challenge was initiated one month later, and as of June, 1972, some dental plaques were observed but no caries lesions had developed.

RESEARCH CONTRACT

Transaction Number 2N01DE12331-01
Contract Number ..... NIH -71-2331
Name of Contractor .... UNIVERSITY OF MINNESOTA MINNEAPOLIS
Address ..... MINNEAPOLIS MINNESOTA
Initial Start Date 06-29-71
Expiration Date 06-28-73
Class Code DE-1-01
Project Director ..... SCHACHTELE CHARLES F
NIDR Project Officer .. ROGERS W E

TITLE: AFFECTING CARIOGENIC MICROORGANISMS

OBJECTIVES AND WORK SCOPE:

Contractor will exert best efforts to investigate unique features of the membrane-associated phosphotransferase sugar uptake system of oral cariogenic bacteria and to analyze for antimicrobials which could inhibit this system.

SALIENT RESULTS TO DATE:

Glucose transport has been studied in cariogenic and noncariogenic oral streptococci. These organisms utilize a phosphoenolpyruvate (PEP)-dependent phosphotransferase system which results in phosphorylation of glucose at carbon-6. This enzyme system can be measured in decryptified (frozen and thawed) cells and is not sensitive to fluoride. However, glucose uptake into resting cell suspensions is sensitive to fluoride. The utilization of sucrose by a cariogenic strain of Streptococcus mutans was studied. The soluble and cell-bound sucrose-dependent, polymer-forming sucrase activities constitutively produced by the bacteria during growth on glucose were measured. About eight times more dextransucrase activity was present than levansucrase activity. During various states of growth on sucrose, S. mutans accumulated two to five times more insoluble and water-soluble dextran than levan. Sucrose labeled in the fructosyl (3H) and glucosyl (14C) moieties was used to quantitate extracellular polysaccharide production and degradation by cariogenic and noncariogenic oral streptococci. All of the strains produced glucan and fructan. Streptococcus salivarius produced primarily fructan, whereas S. mutans and S. sanguis produced more glucan than fructan. The cariogenic streptococci could degrade the fructan produced by noncariogenic strains.

RESEARCH CONTRACT

Transaction Number 2N01DE12332-01
Contract Number ..... NIH -71-2332
Name of Contractor .... MEDICAL COLLEGE OF GEORGIA
Address ..... AUGUSTA GEORGIA
Initial Start Date 06-29-71
Expiration Date 06-28-73
Class Code DE-1-01
Project Director ..... BURNETT GEORGE W
NIDR Project Officer .. SHERN R J

TITLE: TESTS OF ANTICARIES EFFECACY OF CERTAIN AGENTS

OBJECTIVES AND WORK SCOPE:

Contractor will exert its best efforts to search for and obtain potential anticaries agents from pharmaceutical companies and conduct in vitro and in vivo tests of the anticaries efficacy of these agents.

RESEARCH CONTRACT

Transaction Number 3N01DE12333-03
Contract Number ..... NIH -71-2333
Name of Contractor .... FORSYTH DENTAL CENTER
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-28-71
Expiration Date 06-27-74
Class Code DE-1-01
Project Director ..... TAUBMAN MARTIN
NIDR Project Officer .. IKARI N S

TITLE: EFFECT OF SALIVARY ANTIBODY DENTAL CARIES

OBJECTIVES AND WORK SCOPE:

Contractor will exert best efforts to investigate the effects of specifically induced salivary antibody to Streptococcus mutans upon plaque bacteria and dental caries in gnotobiotic rats and conventional hamsters.

SALIENT RESULTS TO DATE:

Experiments were initiated in germfree rats to determine effective modes of immunization producing a salivary antibody response. These experiments have recently been completed and the results are being tabulated. Polyvalent rabbit antisera to hamster serum and whole saliva are being prepared. Immunochemically pure hamster lgG was isolated from serum by chromatography on DEAE-cellulose.

RESEARCH CONTRACT

Transaction Number 2N01DE12375-06
Contract Number ..... NIH -71-2375
Name of Contractor .... FORSYTH DENTAL CENTER
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-28-71
Expiration Date 12-31-73
Class Code DE-2-01
Project Director ..... DE PAOLA PAUL F
NIDR Project Officer .. JOLY OLGA

TITLE: STUDY TOPICAL USE OF ACIDIFIED NH4F VS ACIDIFIED NAF

OBJECTIVES AND WORK SCOPE:

The Contractor will compare the relative caries inhibitory effect of supervised, daily, one-minute mouth rinses in school with 5 ml of NH4F, pH4.4 containing 5 mg of F ion, and determine fluoride uptake in subjects receiving the foregoing treatments and to relate fluoride uptake to caries experience on a group basis.

SALIENT RESULTS TO DATE:

During the last funded period, the Contractor accomplished: Toxicity studies of the test agents; The obtainment of the necessary IND's from the Food and Drug Administration; Selection of a test community; Calibration of the examiner; Implementation of a community public relations program; Distribution of permission slips; The incorporation into the study of 578 subjects who received an enamel biopsy, dental prophylaxis, bitewing radiographic survey, clinical examination, and topical treatment; Comprehensive reports to the local school and health authorities; Chemical analysis of the enamel biopsy samples (in progress); Interpretation of the dental radiographs (in progress); and Statistical evaluation of the baseline data (to be carried out in the fall).

RESEARCH CONTRACT

Transaction Number 2N01DE12376-03
Contract Number ..... NIH -71-2376
Name of Contractor .... UNIVERSITY OF MINNESOTA MINNEAPOLIS
Address ..... MINNEAPOLIS MINNESOTA
Initial Start Date 06-30-71
Expiration Date 06-29-73
Class Code DE-1-01
Project Director ..... FOLKE LARS E
NIDR Project Officer .. IKARI N S

TITLE: INFLUENCE OF DIET OF HUMAN DENTAL PLAQUE

OBJECTIVES AND WORK SCOPE:

The scope and nature of this clinical research study is directed toward achieving the following objectives: 1. The development of a precise clinical method or procedure for evaluating the effects of diet variations upon microbial and biochemical composition of human dental plaque. 2. The estimation of the relative effects of sucrose upon plaque formation in human subjects. 3. The measurement of effects of sodium trimetaphosphate upon plaque formation in the presence as well as the absence of sucrose.

SALIENT RESULTS TO DATE:

Sixteen young men with different caries experiences were maintained for 2 weeks on a "normal diet" or on a nutritionally balanced menu which provided either high or low levels of sucrose. Subjects abstained from all oral hygiene procedures during the feeding periods. Following a 12-hour fasting period, 4 or 12 day old plaque samples were collected from all lingual and facial surfaces of their teeth, placed in cold sterile diluent, and dispersed by sonication. Aliquots of plaque were diluted, plated, and analyzed microbiologically and biochemically. Level of sucrose in the diet did not significantly alter the quantity of plaque produced during each diet series. The quantity of plaque recovered after 4 vs. 12 days was also unaltered. The high sucrose diet increased the number of Streptococcus mutans and Lactobacillus species per mg of plaque, and increased the density of total viable microorganisms. The density was 40-60% higher in plaque collected after 4 days than 12 days while being fed compatible diets. Reductions of Streptococcus sanguis populations always preceeded increases in S. mutans. Levan and dextran sucrase specific activities were not dependent on dietary sucrose level. Dextran sucrase activity was generally 2-3 times higher than levan sucrase activity. However, less than 2% of plaque total saccharide was composed of fructose. Thus, plaque levans were very low in these fasted subjects.

RESEARCH CONTRACT

Transaction Number 3N01DE12377-03
Contract Number ..... NIH -71-2377
Name of Contractor .... UNIV OF TEXAS DENTAL BRANCH HOUSTON
Address ..... HOUSTON TEXAS
Initial Start Date 06-30-71
Expiration Date 06-29-73
Class Code DE-1-01
Project Director ..... DREIZEN SAMUEL
NIDR Project Officer .. STILES H M

TITLE: EFFECT OF XEROSTOMIA ON HUMAN ORAL MICROFLORA

OBJECTIVES AND WORK SCOPE:

Prime objectives are: 1. Clarify the saliva-microbial-dental caries relationship by: a. Delineating the general characteristics of the oral microbial population changes in patients with radiation induced xerostomia b. Identifying the specific changes in the occurence and numbers of those microorganisms which have been implicated in the caries process. c. Elucidating the effect of caries preventive agents and procedures onf the oral microflora in patients with radiation induced xerostomia. 2. Establish a human model system for the rapid screening of anticaries agents in the presence of an unusally potent and ever present caries challenge.

SALIENT RESULTS TO DATE:

The study group has more than doubled in size since the last report. It now consists of 11 head and neck cancer patients with radiation induced xerostomia. Nine have completed the entire course of radiotherapy ranging from 5000 to 7000 rada and are now in the post-radiation phase. Each developed xerostomia within 2 to 3 weeks after the start of treatment. Three of the 9 have passed the first 3-month post-radiation examination point without any detectable increase in salivary flow. The drastic reductions in salivary flow have been paralleled by marked qualitative and quantitative shifts in the composition of the plaque, salivary, and mucosal microflora. The most conspicuous manifestations have been a pronounced and persistent increase in Streptococcus mutans and Lactobacillus spp. and a precipitous decline in neisseria and fusobacteria.

RESEARCH CONTRACT

Transaction Number 2N01DE12379-05
Contract Number ..... NIH -71-2379
Name of Contractor .... FORSYTH DENTAL CENTER
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-28-71
Expiration Date 12-31-73
Class Code DE-2-01
Project Director ..... DE PAOLA PAUL F
NIDR Project Officer .. JOLY OLGA

TITLE: NH4F MOUTH RINSE VERSUS HIGH-POTENCY FLUORIDE RINSE

OBJECTIVES AND WORK SCOPE:

The Contractor will study the relative effectiveness of semi-annual 3-minute topical application of 0.06M NH4f, pH4.4 preceeded by .05M H3P04 (1 minute) and NaF, 1.2% in 0.1M HoP04, pH 3.2, in the control of human dental caries; and fluoride uptake in teeth of subjects receiving the foregoing treatments and to relate fluoride uptake to caries experience on a group basis.

SALIENT RESULTS TO DATE:

During the last funded period, the Contractor accomplished: Toxicity studies of the test agents; The obtainment of the necessary IND's from the Food and Drug Administration; Selection of a test community; Calibration of the examiner; Implementation of a community public relations program; Distribution of permission slips; The incorporation into the study of 578 subjects who received an enamel biopsy, dental prophylaxis, bitewing radiographic survey, clinical examination, and topical treatment; Comprehensive reports to the local school and health authorities; Chemical analysis of the enamel biopsy samples (in progress); Interpretation of the dental radiographs (in progress); and Statistical evaluation of the baseline data (to be carried out in the fall).

RESEARCH CONTRACT

Transaction Number 2N01DE12380-02
Contract Number ..... NIH -71-2380
Name of Contractor .... UNIVERSITY OF ALABAMA BIRMINGHAM
Address ..... BIRMINGHAM ALABAMA
Initial Start Date 06-28-71
Expiration Date 06-27-73
Class Code DE-1-01
Project Director ..... FINN SIDNEY
NIDR Project Officer .. FREW RALPH A

TITLE: STUDY OF TRIMETAPHOSPHATE ON PLAQUE

OBJECTIVES AND WORK SCOPE:

Objective of study is to establish and measure anticaries activity of Sodium Trimetaphosphate (STMP) when topically applied to the dentition of children in a chewing gum vehicle. The gum will be distributed to approximately 600 children participants on a systamatic basis with oral examinations to be conducted prior to the study and at 6 month intervals thereafter. Both clinical and radiographic results will be observed and recorded by the Contractor, and transmitted to NIDR for processing and analysis of the data in-house. All children will receive a dental prophylaxis prior to each examination.

SALIENT RESULTS TO DATE:

Base-line mirror and explorer dental examinations augmented with bite-wing radiographs were completed in November on 544 children. The second clinical examination was completed in May 1972, approximately 5 months after the start of the chewing regime. The second examinations, as was done with the first, are presently being sent to Dr. Ralph Frew of NIDR for tabulation and analyses. The results will be expressed in increments of decay experience. Pooled plaque samples were collected on approximately 100 children in October and again in December prior to treatment. They were obtained again in January, approximately 1 month after treatment, and again in May, after 5 months of treatment. All samples were analyzed for total streptococci, total bacteria, total lactobacilli, and Streptococcus mutans. These samples were done within 48 hours of collection. The data obtained from all 4 samplings are being analyzed at NIDR by Dr. Frew. Counts will be expressed as total bacteria and percent of total for the differential count. The gum chewing has been carefully supervised and well accepted by the participating children. Plans have been made for home visitations. No adverse effects of the gum have been observed or reported.

RESEARCH CONTRACT

Transaction Number 2N01DE12381-02
Contract Number ..... NIH -71-2381
Name of Contractor .... EASTMAN DENTAL CENTER
Address ..... ROCHESTER NEW YORK
Initial Start Date 06-29-71
Expiration Date 06-28-73
Class Code DE-1-01
Project Director ..... LOSEE F L
NIDR Project Officer .. JOLY O

TITLE: TRACE-ELEMENT CONTENT OF TEETH AND DENTAL CARIES

OBJECTIVES AND WORK SCOPE:

Contractor will attempt to relate trace elements of teeth to their caries susceptibility by: 1. Selecting human teeth from geographic areas of high and low caries prevalence to determine the approximate concentrations in enamel and dentin of the major trace elements, together with calcium and phosphorus. 2. Analyses from residents of Colorado, Texas, New York, Ohio, Maine, and California will be reported. 3. Sufficient samples will be analyzed to provide information on intra-tissue distribution of elements so that a relationship can be established between trace element composition and resistance to caries. The following was added to the existing Scope of Work: Compare dietary factors with the trace element contents of teeth obtained from areas of high or low caries prevalence. Food, water, and other environmental sources of trace elements will be collected from areas where a dietary reconnaissance visit is made. Analysis of the trace element contents of these samples will be performed.

SALIENT RESULTS TO DATE:

Established workable procedure for obtaining bicuspids was established. Data received with the tooth includes the DMFT status, age, sex, and water supply of the individual contributing the tooth. Critical examination of the enamel to be sampled was determined to be extremely important. It was found that with the inclusion of altered, stained (after pumice prophylaxis), or carious enamel would increase the concentration of several elements depending on the proportion of included sample. The F and Sr levels in enamel and dentin are both associated with the levels found in the water supplies. Fluoride and zinc concentrations are greatest in the surface enamel, whereas Sr tends to be evenly distributed. Spark-source mass spectrographic technique appears to be a desireable means to look for 74 elements in a sample of only 1 mg. By using the Laser for

excitation t present a plume of evaporated material to standard electricarc emission spectrophotometry it was shown that teeth from two different locations in California had differences in the enamels compositions of Al, Ag, Ba, Be, Cr, Cu, Ni, Pb, Sn, and Zn. A method for the determination of density has been worked out for enamel samples weighing 10 to 120 mg.

RESEARCH CONTRACT

Transaction Number 2N01DE12382-03
Contract Number ..... NIH -71-2382
Name of Contractor .... HARVARD UNIVERSITY
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-29-71
Expiration Date 06-28-73
Class Code DE-1-01
Project Director ..... SHAW JAMES H
NIDR Project Officer .. ROGERS W E

TITLE: ROLE OF FOOD IN INHIBITING CARIES

OBJECTIVES AND WORK SCOPE:

Contractor will exert best efforts to estimate caries-producing potentials of human foods and their potential role as caries of possible caries-inhibiting agents by assays with experimental rodents. Contractor will: Develop in rodents an assay which will utilize programmed feeder technique to measure caries-producing potential of human foods. Utilize assay to estimate caries-producing potentials of commonly used human foodstuffs. Evaluate efficacy of adding foodstuffs with high caries potential, agents believed to be caries inhibitors, such as sodium trimetaphosphate, calcium sucrose phosphate, & any others which may eventually be utilized in human diets. The following was added to the existing Scope of Work: Evaluate the caries-producing potential in rodents, of no more than five dietary products as may be provided to the Contractor by the Government.

SALIENT RESULTS TO DATE:

The first experiment was conducted with Harvard caries-susceptible rats to test the design of the assay proposed for the evaluation of the cariogenic potential of human foods. The experiment had two similar parts: the one conducted in regular cages, the other in cages on the periodic feeder. A nutritious diet of low caries-producing potiential was fed in alternation with sucrose, which was the substance for which the caries-producing potential was to be determined inn this trial. The results indicated that this step in the design of a suitable assay had been successful. A second experiment of similar design, with some refinements, is almost complete with mutant albion caries-susceptible rats as the next step in the refinement of the assay. The third experiment is being conducted with a similar design in which the sucrose as the test food is compared with either a breakfast cereal alone, with the same breakfast cereal moistened with whole milk as would be used typically for human consumption, and with a vanilla flavored cookie to determine how the cariogenic potential of these human foods compares with that of sucrose.

RESEARCH CONTRACT

Transaction Number 3N01DE12383-03
Contract Number ..... NIH -71-2383
Name of Contractor .... MIDWEST RESEARCH INSTITUTE
Address ..... KANSAS CITY MISSOURI
Initial Start Date 06-30-71
Expiration Date 06-30-73
Class Code DE-1-01
Project Director ..... BREED L W
NIDR Project Officer .. WACHTEL L W

TITLE: STUDY OF ELASTOMERIC LINERS FOR DENTAL RESTORATIONS

OBJECTIVES AND WORK SCOPE:

Various experimental polymers shall be prepared and screened as dental restorative materials potentially useful as elastomeric liners or composite filling base and shall also perform as an adhesive. The contractor shall select a series of monomers for exploratory copolymerization with the higher alkyl acrylates. The copolymers shall be characterized and screened for their elasticity or toughness, stability in aqueous media, and their adhesion to tooth structure. As the work progresses, suitable modifications shall be made in the monomer composition or selection of monomers to bring about improvement in the properties on the basis of correlations that can be established. When the best polymer system with respect to these requirements has been found, polymerization variables shall be optimized to further improve polymer properties such as tensile strength, elongation, and hardness. The following was added to the original work statement: When optimal polymers are obtained, preliminary screening for adhesion to dentin and enamel both in vitro and in vivo shall be initiated.

SALIENT RESULTS TO DATE:

In work on the development of an acrylate or methacrylate-based elastomeric material for use as an adhesive liner for dental restorations, several new monomers were prepared and copolymerized with butyl methacrylate. In addition, a series of copolymers of butyl methacrylate and methyl acrylate with maleic anhydride was prepared and hydrolyzed. All these comonomers should provide sites in the polymer structure for the formation of calcium complexes with high stability constants. The new monomers include N-acryloyliminodiacetic acid, N-2-hydroxy-3-methacroyloxypropyl-Nmethylglycine, and N-2-hydroxy-3-methacroyloxypropyliminodiacetic acid. Copolymers of butyl methacrylate and 10 mole percent of the experimental monomers were prepared with chain transfer agents to provide materials with molecular weights of 10,000 to 20,000. A second

series of copolymers of butyl methacrylate and methyl acrylate with 5, 10, and 20 mole percent of maleic anhydride in the same molecular weight range has also been prepared and hydrolyzed. Compositizns containing the experimental copolymers, butyl methacrylate, a glycol dimethacrylate, an initiator, and an accelerator have been screened to evaluate the effects of the vzrious compositional parameters on the properties of the system containing the modified polymers. In order to provide the maximum number of active sites and to reduce cure shrinkage, the experimental polymers were incorporated in concentrations of 50-60%. Compositions which provided the toughest cured materials with elastic properties were defined. Although several compositions have been screened on phosphoric acid-treated dentin, systematic screening of the new compositions has not been initiated.

RESEARCH CONTRACT

Transaction Number 2N01DE12384-01
Contract Number ..... NIH -71-2384
Name of Contractor .... FORSYTH DENTAL CENTER
Address ..... BOSTON MASSACHUSETTS
Initial Start Date 06-28-71
Expiration Date 12-27-73
Class Code DE-1-01
Project Director ..... GLASS ROBERT L
NIDR Project Officer .. JOLY O

TITLE: EFFECT OF TRACE METALS ON CARIES

OBJECTIVES AND WORK SCOPE:

Contractor will analyze for presence of various trace elements samples obtained from two groups of people having marked differences in caries prevalence not due to fluoride. He will attempt to associate presence of these elements with resistance to caries to determine if certain trace elements in diet may potentiate anti-cariogenic effect of fluoride. Select for analysis samples of water, soil, food, saliva, and teeth which have been obtained from Colombia, S.A. Analyze these samples for presence of as many of the major trace elements as possible. Relate these elements to caries prevalence in these two communities. The existing workscope is amended to add: Conduct a nutritional survey of the populations from Heliconia and Don matias in order to establish the dietary intake of various trace elements and other nutrients in the population of these two communities; Examine the association of the dietary intake of these elements with differences in the caries prevalence in these communities.

SALIENT RESULTS TO DATE:

All samples intended for analyses for trace metals have been completed or are in progress. Spectroscopic analyses are in progress for 25 teeth, the last of those available. Initial findings from the analyses of the first series of teeth show higher levels of manganese in teeth from the village with the higher caries prevalence. Lower mean levels of manganese were observed in teeth from the low caries village. Concentrations of iron were higher in the teeth from the low caries village. Spectroscopic analyses of the last samples of food and saliva have just been completed and the findings are being summarized. Brief summaries of data from those food samples first completed do not show the clear cut differences observed in samples of water and soil. This suggests that the source of the trace metal effect, whatever it may be, might be drinking water. The results of analyses continue to reveal definite differences in the environment of trace metals between these two villages. Except for the foods, differences are obvious in the various samples collected for analyses.

RESEARCH CONTRACT

Transaction Number 2N01DE12385-01
Contract Number ..... NIH -71-2385
Name of Contractor .... ASSOCIATED BIOMEDIC SYSTEMS
Address ..... BUFFALO NEW YORK
Initial Start Date 06-24-71
Expiration Date 06-23-73
Class Code DE-2-01
Project Director ..... MAUER BRUCE
NIDR Project Officer .. OPPENHEIM J J

TITLE: PRODUCTION OF CELL LINES AND ISOLATATION OF ANTIGENS

OBJECTIVES AND WORK SCOPE:

This contract effort will provide for the production and origination of cell lines and the isolation of histocompatibility antigens from lymphoid cell lines. It will include furnishing cell lines derived from human and mouse cells, certification as to percent viability, establishment of new cell lines, preparation and supplying of histcompatibility antigens including purity certifications, and providing mouse lymphocytes and tumor cells as requested by the Government Project Officer.

SALIENT RESULTS TO DATE:

Studies were conducted to produce the following information: What are the optimum conditions of cell maintenance, propagation, and harvesting of cultured mouse L1210 lymphocytes for the extraction of H-2d antigens (3, 4, 8, and 31); and What procedures are most efficient in the extraction and evaluation of these antigens. Seventeen batches of L1210 cells involving approximately 200 x 10-9 cells were used in studies in which viability, cell concentration, method of production, i.e., cytogenerators and spinner flasks, pH of medium, pretreatment, i.e., settling of 4oC., were evaluated for their qualitative and quantitative effect on H-2d antigens and their extraction. Procedural details of a 3M KCl extraction procedure, i.e., methods of centrifugation, diluent for the KCl, and duration of dialysis, were evaluated. These variations were evaluated using direct cytotoxicity, cytotoxic inhibition, and quantitative absorption tests. These studies have resulted in the successful extraction of mouse H-2d antigens (3, 4, 8, and 31) from L1210 lymphocytes. Large amounts (approximately 20 x 10-9 cells) of cells with high viability and in a logarithmic growth stage extracted without storage with 3M KCl (15 ml/10 cells) in PBS without Ca++ and Mg++ and dialyzed for 12-18 hours against this salt solution yielded significant quantities of active antigens.

RESEARCH CONTRACT

Transaction Number 2N01DE12386-02
Contract Number ..... NIH -71-2386
Name of Contractor .... PACIFIC NORTHWEST LABORATORIES
Address ..... RICHLAND WASHINGTON
Initial Start Date 06-30-71
Expiration Date 06-29-73
Class Code DE-2-01
Project Director ..... MARSHALL ROBERT P
NIDR Project Officer .. PECORA L J

TITLE: DEVELOPMENT OF ARTIFICIAL DENTAL ANCHORS

OBJECTIVES AND WORK SCOPE:

The contractor will study at least four materials as dental implants. These will include Titanium, void metal composite; Titanium, powder metallurgy product; Alumina porcelain; and Magnesium aluminate spinel. These materials will be characterized by physical tests and implants designed to optimize desirable characteristics will be implanted in swine jaws and subjected to clinical and microscopic examinations to determine their clinical efficacy. A special scanning electron microscope examination of frozen-fractured specimens of the bone material interface will be performed. of implant stability.

SALIENT RESULTS TO DATE:

Four basic materials, two ceramic and two forms of porous titanium alloy, have been fabricated into dental anchors and implanted into the mandibles of miniature swine. The ceramic materials, alumina-porcelain and magnesium aluminate spinel, have been formed into anchors of varying porosity by slip casting techniques. The metal anchors, all Tri-6Al4V alloy, have been made from Battelle-developed Void Metal Composite and gravity-sintered spherical particles. Two basic anchor designs have evolved; oblong and cylindrical. The oblong anchors are primarily intended for fresh socket implantation, though they have been inserted into trephined sockets as well. The cylindrical anchor concept arose from recognition of the need for a tightly fitting implant, and the concomitant requirement of close anchor/hole dimensional control. Three animals containing 18 implants were sacrificed at 4 to 12 week post operative. Low grade infections were found around most of the oblong anchors, evidenced by a thick-fibrous tissue capsule and bone resorption.

Eight of the twelve test pins were lost entirely. The last anchors implanted (two animals) were cylindrical, with chisel points, and were driven into sub-sized (8-12%) pilot holes. At four week post operative the anchors were still firmly in place, with no visible bone resorption. These are very encouraging results, and indicate strong likelihood of future success.

RESEARCH CONTRACT

Transaction Number 3N01DE12387-01
Contract Number ..... NIH -71-2387
Name of Contractor .... COLUMBIA UNIVERSITY
Address ..... NEW YORK NEW YORK
Initial Start Date 06-30-71
Expiration Date 12-31-72
Class Code DE-1-01
Project Director ..... FERTIG JOHN W
NIDR Project Officer .. SENNING RICKLEY S

TITLE: DEVELOP MANUAL FOR DENTAL CARIES FIELD TRIAL

OBJECTIVES AND WORK SCOPE:

contractor shall exert best efforts in studying analytic procedures for data of the type collected by NIDR during dental caries clinical trials and in reporting the results of these studies. This will include the testing of existing data for degree of adherence to any model assumptions, and identification of the most appropriate analytic method under several variations in experimental design. The ultimate result will be the methodologies as well as standardized methods of data presentation including any appropriate charts and tables.

RESEARCH CONTRACT

Transaction Number 3N01DE12388-02
Contract Number ..... NIH -71-2388
Name of Contractor .... UNIVERSITY OF UTAH
Address ..... SALT LAKE CITY UTAH
Initial Start Date 06-16-71
Expiration Date 08-15-72
Class Code DE-1-01
Project Director ..... BROWN W S
NIDR Project Officer .. WACHTEL L W

TITLE: ENVIRONMENTAL STRESS ON TEETH

OBJECTIVES AND WORK SCOPE:

Contractor will perform temperature cycling tests on teeth filled with specifically shaped fillings designed to stimulate commonly used filling configurations. Teeth will be inspected for cracks by several methods with the fluorescent dye technique. Calculations of time-dependent temperature and stress distribution will be related using the appropriate computer systems. The results of both the experimental and analytical programs will be analyzed to determine if improvement can be made in cavity preparation and choice of material with emphasis on thermal considerations.

RESEARCH CONTRACT

Transaction Number 1N01DE12389-00
Contract Number ..... NIH -71-2389
Name of Contractor .... RESEARCH TRIANGLE INSTITUTE
Address ..... RESEARCH TRIANGLE NORTH CAROLINA
Initial Start Date 06-29-71
Expiration Date 06-28-72
Class Code DE-1-01
Project Director ..... FINKNER A L
NIDR Project Officer .. SENNING RICKLEY S

TITLE: DENTAL CARIES FIELD TRAILS

OBJECTIVES AND WORK SCOPE:

This project has three prime objectives which are to: 1. study alternative approaches to the analysis of the data on dental caries of the type collected by NIDR under several variations in experimental designs which include simple and stratified random sampling in the selection of control and treatment groups. 2. Identify and provide detailed expositions of the most appropriate analytic methods. 3. To consider new methods for the analysis of caries data so that the selective effect of treatment by surface type may be investigated and the possibility of recording data from selected surfaces and/or teeth may be explored.

RESEARCH CONTRACT

Transaction Number 2N01DE22400-01
Contract Number ..... NIH -72-2400
Name of Contractor .... UNIVERSITY OF MIAMI
Address ..... CORAL GABLES FLORIDA
Initial Start Date 01-01-72
Expiration Date 12-31-73
Class Code DE-1-01
Project Director ..... ZINNER DORAN D
NIDR Project Officer .. SHERN RONALD J

TITLE: FLUORIDE UPTAKE IN TOOTH ENAMEL

OBJECTIVES AND WORK SCOPE:

Contractor shall provide all necessary staff, facilities, and populations to carry on a field trial of a topical fluoride study to: Assess the level of enamel fluoride attained after a series of treatments of topical gel to teeth of children consuming fluoride deficient water; Assess the rate of loss of fluoride from enamel after treatments have been completed; and Assess the residual anti-caries effect of 3 short treatment regimens.

SALIENT RESULTS TO DATE:

Seven hundred thirty children received five applications of a fluoride gel. The following responses are being monitored as a result of these applications: Assessment of the level of enamel fluoride attained after a series of treatments of topical gel to teeth of children consuming fluoride deficient water; Assessment of the rate of loss of fluoride from enamel after treatments have been completed; and Assessment of the residual anticaries effect of the three short treatment regimens, i.e., amine fluoride vs. acidulated phosphate fluoride and the clinical significance of the labile fluoride reservoir (five weekly applications contrasted with five daily applications). Increment DMF-S exams and F decrement exams for the first twelve months were also accomplished.

RESEARCH CONTRACT

Transaction Number 2N01DE22401-01
Contract Number ..... NIH -72-2401
Name of Contractor .... PENNSYLVANIA STATE UNIVERSITY
Address ..... UNIVERSITY PARK PENNSYLVANIA
Initial Start Date 02-27-72
Expiration Date 02-26-74
Class Code DE-1-01
Project Director ..... MUNGER B L
NIDR Project Officer .. BOSMA JAMES F

TITLE: STUDY OF DEVELOPMENT OF ORAL SENSORY RECEPTORS

OBJECTIVES AND WORK SCOPE:

The contractor with his laboratory staff and appropriate associates from other Departments of his University will select and breed monkeys, obtain fetuses at selected gestational ages by caesarian section, evaluate the stage of their development by appropriate qualitative and quantitative criteria, and obtain oral tissues at standard sites. Will prepare sample tissues for microscopic and ultramicroscopic study, employing methods standard in that laboratory. Will study and describe the histological progressions of development, as evidenced in the specimens prepared in this project.

RESEARCH CONTRACT

Transaction Number 1N01DE22402-00
Contract Number ..... NIH -72-2402
Name of Contractor .... DUKE UNIVERSITY
Address ..... DURHAM NORTH CAROLINA
Initial Start Date 05-17-72
Expiration Date 05-16-73
Class Code DE-2-01
Project Director ..... DANIELS CHARLES A
NIDR Project Officer .. NOTKINS ABNER L

TITLE: RHEUMATOID FACTOR AND INFECTIOUS ANTIBODY COMPLEXES

OBJECTIVES AND WORK SCOPE:

The contractor proposes to test the ability of different concentrations of rheumatoid factor, complement, rheumatoid factor plus complement, Ctg and cryoglobulins to neutralize infectious virus-antibody complexes. Herpes simplex, vaccinia influenza and hepatitus virus will serve as models. Fixation of complement by the alternate pathway and lysis of sensitized virus by complement also will be studied.

RESEARCH CONTRACT

Transaction Number 1N01DE22403-00
Contract Number ..... NIH -72-2403
Name of Contractor .... UNIVERSITY OF ALABAMA IN BIRMINGHAM
Address ..... BIRMINGHAM ALABAMA
Initial Start Date 06-05-72
Expiration Date 06-04-73
Class Code DE-1-01
Project Director ..... NAVIA JUAN M
NIDR Project Officer .. ROGERS W E

TITLE: POTENTIAL FOR SUGAR-SUBSTITUTED FOODS WITH LOW CARIES

OBJECTIVES AND WORK SCOPE:

The Contractor shall formulate, manufacture, and test in the laboratory a group of snack foods which have been modified by substitution of the sucrose with other carbohydrates to reduce its caries promoting properties. Specifically, the Contractor shall: Evaluate by conventional rat caries tests the relative cariogenicity of various sweetening agents when substituted for various levels of sucrose in the diet; Design and develop taste-acceptable (to humans) snack foods and dessertslwhich would contain sucrose substituted sweetening agents of low cariogenicity to the rat; and Test for cariogenicity (in rats) snack foods in which low cariogenic sweetening agents have been used.

RESEARCH CONTRACT

Transaction Number 1N01DE22404-00
Contract Number ..... NIH -72-2404
Name of Contractor .... UNIVERSITY OF FLORIDA GAINESVILLE
Address ..... GAINESVILLE FLORIDA
Initial Start Date 05-16-72
Expiration Date 05-15-73
Class Code DE-1-01
Project Director ..... OLSON GERALD A
NIDR Project Officer .. IKARI N S

TITLE: STUDY OF CARIES-INHIBITORY ACTIVITY IN HUMAN SALIVA

OBJECTIVES AND WORK SCOPE:

The Contractor shall investigate the existence and delineate the prevalence of naturally occurring caries inhibitory factors in human saliva. Specifically, he shall perform the following: Saliva shall be collected from at least 100 subjects; Information relative to caries experience shall be recorded for each human subject which shall include oral examinations for DMFT, and other pertinent data as required for clinical evaluation of present and past caries activity; All saliva samples shall be subjected to quantitative in vitro assay for adherence inhibitory activity of saliva as tested against at least six strains of Streptococcus mutans.

RESEARCH CONTRACT

Transaction Number 1N01DE32405-00
Contract Number ..... NIH -72-2405
Name of Contractor .... CENTRAL INSTITUTE FOR EXP ANIMALS
Address ..... TOKYO JAPAN
Initial Start Date 09-14-72
Expiration Date 09-13-73
Class Code DE-2-01
Project Director ..... NISHIMURA HIDEO
NIDR Project Officer .. GRIFFO ZORA

TITLE: PRENATAL DEVELOPMENT OF HUMAN ORAL AND FACIAL STRUCTURES

OBJECTIVES AND WORK SCOPE:

A Manuscript entitled "Prenatal Development of the Human with Specific Reference to Oral-Facial Structures" is aimed at publication of a monograph under the authorship of Dr. Hideo Nishimura on complete descriptive study of the normal and abnormal development of human embryo and fetus with special reference to the head and oral-facial complex. This will furnish dentists, oral surgeons, and other biomedical scientists with the most reliable standard for their studies on the related subjects. The manuscript will be for disposition by the National Institute of Dental Research.

RESEARCH CONTRACT

Transaction Number 3N01DE22406-01
Contract Number ..... NIH -72-2406
Name of Contractor .... STATE UNIVERSITY NEW YORK ALBANY
Address ..... ALBANY NEW YORK
Initial Start Date 06-12-72
Expiration Date 08-31-75
Class Code DE-1-01
Project Director ..... ENGLISH JAMES A
NIDR Project Officer .. JOLY O

TITLE: DEGREE OF EFFECTIVENESS OF CARIES PREVENTIVE PROCEDURES

OBJECTIVES AND WORK SCOPE:

Effectiveness of a combined series of caries-preventive measures will be evaluated in 1500 students (6-7-8th grades) in Buffalo, New York. All will undergo standard preventive measures: fluoridated drinking water, restorative dental care, oral hygiene instruction, and prophylactic treatment. Additionally, Test Groups will get adhesive sealant applied to pit and fissure tooth areas followed by topical application of acidulated fluoride phosphate solution. One Test Group will also be given special oral hygiene instruction and unusual encouragement to seek appropriate dental care; selected individuals, moreover, will receive specific behavioral procedures designed to emphasize motivational incentives to change oral hygiene behavior.

RESEARCH CONTRACT

Transaction Number 1N01DE22407-00
Contract Number ..... NIH -72-2407
Name of Contractor .... UNIVERSITY OF MINNESOTA
Address ..... MINNEAPOLIS MINNESOTA
Initial Start Date 05-02-72
Expiration Date 11-01-74
Class Code DE-1-01
Project Director ..... LILJEMARK WILLIAM F
NIDR Project Officer .. IKARI N S

TITLE: AGENTS AFFECTING ADHERENCE OF CARIOGENIC MICROORGANISMS

OBJECTIVES AND WORK SCOPE:

The Contractor shall perform the following efforts: (1) Isolate and characterize the "fuzzy coats" of Streptococcus salivarius, Streptococcus sanguis, Streptococcus miteor, and Streptoccocus mutans. (2) Use appropriate radiostopic tags to isolate and characterize the soluble and insoluble dextrans from the same organisms. (3) Utilize the "fuzzy coats", radiolabeled dextrans, and radiolabeled bacteria to analyze the site-specific interactions which result in the selective adherence of oral streptococci to enamel powder and hydroxyapatite. (4) Analyze the effectiveness of a variety of agents including polysacchaharases, proteases, lipases, low molecular weight dextrans, and detergents which might inhibity the development of sucrose-caused plaque by blocking specific sites of the components involved in adherence.

RESEARCH CONTRACT

Transaction Number 1N01DE32408-00
Contract Number ..... NIH -73-2408
Name of Contractor .... ROYAL COLLEGE OF SURGEONS
Address ..... LONDON UNITED KINGDOM
Initial Start Date 12-04-72
Expiration Date 12-03-73
Class Code DE-1-01
Project Director ..... COHEN BERTRAM
NIDR Project Officer .. LARSEN RACHEL

TITLE: STUDY OF EFFECT OF DIETARY ADDITIVES ON PLAQUES & CARIES

OBJECTIVES AND WORK SCOPE:

Plaque chemistry and caries incidence will be determined in 12-16 month old monkeys maintained on a cariogenic diet, with and without supplements of potential anti-cariogenic substances: pyridoxine and sodium inositol phosphate. Plaque samples obtained before and during the feeding experiments will be examined for numbers of microorganisms producing extracellular and intracellular polysaccharides, and also for the quantities of calcium phosphorus, magnesium, carbohydrate, and protein. The identity of the acids produced in situ by plaque from sugars in the different diets will be determined.

RESEARCH CONTRACT

Transaction Number 1N01DE22409-00
Contract Number ..... NIH -72-2409
Name of Contractor .... HUNTINGDON RESEARCH CENTER
Address ..... BALTIMORE MARYLAND
Initial Start Date 06-12-72
Expiration Date 06-11-73
Class Code DE-1-01
Project Director ..... WILSNAK ROGER
NIDR Project Officer .. HAGEAGE G J

TITLE: ANTIBODY INDENTIFICATION OF CARIOGENIC S. MUTANS

OBJECTIVES AND WORK SCOPE:

Research shall be conducted to develop technology which will permit comparison of the fluorescent antibody method with cultural procedures for identification and quantitation of cariogenic Streptococcus mutans from oral samples. Specific tasks will include: Prepare antisera against the cariogenic serotypes of S. mutans; Conjugate antisera with fluorescent dye; Develop technology for qualitative and quantitative assay of oral samples for S mutans by the fluorescent antibody technique; and Process known samples utilizing the technology developed.

RESEARCH CONTRACT

Transaction Number 1N01DE22410-00
Contract Number ..... NIH -72-2410
Name of Contractor .... UNIVERSITY OF MICHIGAN AT ANN ARBOR
Address ..... ANN ARBOR MICHIGAN
Initial Start Date 06-30-72
Expiration Date 06-30-76
Class Code DE-1-01
Project Director ..... BAGRAMIAN ROBERT A
NIDR Project Officer .. WILLIAM DRISCOLL

TITLE: PREVENTIVE PROCEDURES FOR STUDY OF CARIES

OBJECTIVES AND WORK SCOPE:

The Contractor shall complete a three year study to determine the degree of effectiveness of a combination of several caries-preventive measures in a population of children in a Michigan community. A total of 1200 students shall be selected from elementary schools in the optimally fluoridated city of Ypsilanti by utilizing 600 each from Grades 1 and 6. At various intervals of time the children will receive prophylaxis, application of adhesive sealants, application of phosphate-fluoride gel, oral hygiene education and technique instruction, and restorative dental care. Interim technical reports, summaries, and a final report is required.

RESEARCH CONTRACT

Transaction Number 3N01DE22411-02
Contract Number ..... NIH -72-2411
Name of Contractor .... FAMILY HEALTH FOUNDATION
Address ..... NEW ORLEANS LOUISIANA
Initial Start Date 06-30-72
Expiration Date 11-30-75
Class Code DE-1-01
Project Director ..... BUTLER BRUCE B
NIDR Project Officer .. HEIFETZ STANLEY B

TITLE: PREVENTIVE PROCEDURES FOR STUDY OF CARIES

OBJECTIVES AND WORK SCOPE:

The Contractor shall complete a three year study to determine the degree of effectiveness of a combination of several caries-preventive measures in a population of children consisting of approximately 1020 subjects from three similar neighborhoods in the City of New Orleans. Both test and control groups of children shall be used at ages six and eleven. A final report embodying the results is to be furnished.

RESEARCH CONTRACT

Transaction Number 1N01DE22412-00
Contract Number ..... NIH -72-2412
Name of Contractor .... CANCER RESEARCH CENTER
Address ..... COLUMBIA MISSOURI
Initial Start Date 06-30-72
Expiration Date 06-29-75
Class Code DE-1-01
Project Director ..... GUERRA OSCAR N
NIDR Project Officer .. IKARI N S

TITLE: STUDY OF ORAL FLUIDS IN HIGH-INCIDENCE CARIES

OBJECTIVES AND WORK SCOPE:

Study the causal factors of dental caries by evaluating the interaction and interrelationship between oral flora and the biochemical changes undergone by oral fluid during radiation therapy. Specifically, the Contractor shall select approximately 30 subjects each year who are receiving at least 4,000 rads of radiation treatment for head and neck cancer, and who will receive initially no antibiotic or fluoride therapy. Scrapings from teeth of the subjects shall be used for biochemical analysis to be obtained prior to treatment, during therapy, and after discharge from the Hospital.

RESEARCH CONTRACT

Transaction Number 1N01DE22413-00
Contract Number ..... NIH -72-2413
Name of Contractor .... UNIVERSITY OF PENNSYLVANIA
Address ..... PHILADELPHIA PENNSYLVANIA
Initial Start Date 06-29-72
Expiration Date 06-28-73
Class Code DE-1-01
Project Director ..... CAGAN ROBERT H
NIDR Project Officer .. ROGERS W E

TITLE: EVALUATION OF THE SWEET-TASTING PROTEIN MONELLIN

OBJECTIVES AND WORK SCOPE:

Conduct studies to biochemically characterize a sweet-tasting protein (Monellin) which has been isolated from the berries of a tropical plant, Dioscoreophyllum cumminsii, as possible application as a substitute for sucrose, particularly in snack-type foods. Contractor shall isolate the protein, determine the molecular weight and isoelectric point, ascertain the UV and fluorescent spectra, determine the amino acid composition including cysteine, cystine, tryptophan, and amide moiety, and perform appropriate degradation studies.

RESEARCH CONTRACT

Transaction Number 3N01DE22414-02
Contract Number ..... NIH -72-2414
Name of Contractor .... LITTON BIONETICS
Address ..... BETHESDA MARYLAND
Initial Start Date 06-30-72
Expiration Date 06-29-75
Class Code DE-2-01
Project Director ..... MACKLER BRUCE F
NIDR Project Officer .. MERGENHAGEN STEPHAN E

TITLE: ISOLATION OF ANTIGENS FROM HUMAN DENTAL PLAQUE

OBJECTIVES AND WORK SCOPE:

Independently, and not as an agent of the Government, the Contractor shall exert its best efforts to conduct studies, as necessary, to isolate and identify antigens in human dental plaque which have the potential to transform lymphocytes from patients with periodontal disease and to characterize biologically-active effector molecules such as lymphotoxin and bone resorbing factors, produced in tissue culture cell lines or by lymphocytes when stimulated by plaquederived or other antigens or mitogens.

RESEARCH CONTRACT

Transaction Number 1N01DE22415-00
Contract Number ..... NIH -72-2415
Name of Contractor .... UNIVERSITY OF MINNESOTA
Address ..... MINNEAPOLIS MINNESOTA
Initial Start Date 06-29-72
Expiration Date 01-31-76
Class Code DE-1-01
Project Director ..... LILJEMARK WILLIAM F
NIDR Project Officer .. IKARI N S

TITLE: SUSCEPTIBILITY TO STUDY OF DENTAL PLAQUE AND CARIES

OBJECTIVES AND WORK SCOPE:

The major purpose of this research is to evaluate specific microorganisms and sucrose activity of plaque samples as an indicator of future caries potential.

RESEARCH CONTRACT

Transaction Number 1N01DE32416-00
Contract Number ..... NIH -73-2416
Name of Contractor .... GENERAL MILLS INC.
Address ..... MINNEAPOLIS MINNESOTA
Initial Start Date 11-15-72
Expiration Date 11-14-73
Class Code DE-1-01
Project Director ..... ANDERSON RAY H
NIDR Project Officer .. ROGERS W E

TITLE: STUDY OF SUCROSE SUBSTITUTES

OBJECTIVES AND WORK SCOPE:

The Contractor shall exert its best efforts to conduct feasibility studies and investigations to achieve the following objective: Formulate, manufacture, and taste-test a variety of foods normally containing high contents of sucrose but which have been modified by substitution of the sucrose with other carbohydrates to reduce their caries-producing potential. The re-formulated products shall have the lowest level of sucrose feasible consistent with retention of properties that would still render the new products acceptable for human consumption. Analyze, interpret, and report the data resulting from performance of this effort.

DATE DUE

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