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ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF DENTAL RESEARCH
FISCAL YEAR 1979
PART III

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

PART III

United States

NATIONAL INSTITUTE OF DENTAL RESEARCH

ANNUAL REPORT of program activities

EXTRAMURAL PROGRAMS

October 1, 1978 - September 30, 1979

Edited By

Special Assistant for Program Coordination

Extramural Programs

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

Compiled By

Dental Research Data Officer

National Institute of Dental Research

National Institutes of Health

Bethesda, Maryland

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REPORT OF THE EXTRAMURAL PROGRAMS

NATIONAL INSTITUTE OF DENTAL RESEARCH

October 1, 1978 - September 30, 1979

by

Dr. Clair L. Gardner
Associate Director for Extramural Programs

The Extramural Programs of the National Institute of Dental Research support a wide spectrum of research ranging from laboratory investigations on the basic causes of oral diseases to clinical trials of public health measures. This broad array of scientific activity is divided into five categorical programs, each supporting a specific area of dental research, and a non-categorical program which consists of five university-based Dental Research Institutes and Centers conducting research in the entire field or oral biology. The health scientist administrators who develop and manage these extramural programs encourage a high level of interest in oral biology throughout the scientific community. In the planning and implementation of specific research programs, they carefully consider advice from advisory committees, special consultants and other NIH staff. As a result of this collaboration, the NIDR Extramural Programs (NIDR-EP) have been able to maintain strong basic research programs. These programs have set the stage for current efforts to develop and expand coordinated clinical research programs in the fields of periodontal disease, oral soft tissue diseases, dental implantology, behavioral science and dental pain. The progress achieved in these areas is reflected in this report.

PERSONNEL AND ADMINISTRATION

During FY 1979, there were no changes in the organization of the NIDR-EP and only one change in full-time personnel. Dr. Matthew Kinnard left his position as Program Officer in the Soft Tissue Stomatology and Nutrition Branch to accept an appointment with the Veterans Administration Hospital System. It should also be mentioned that Mr. Nelson E. Lyttle discontinued his services as a special consultant for revision of the NIDR-EP Operations Manual for Scientist Administrators.

Continuing efforts are made to maintain a favorable work environment at NIDR-EP for all employees, including those with physical handicaps as well as other problems. Routine observations indicate that the EP staff has been successful in this area of personnel management. Employees work together in a friendly manner and seem to have a genuine personal interest in their fellow workers. Both individually and in small groups, they show a spirit of cooperation which is especially evident when short deadlines must be met and smooth teamwork is necessary.

During FY 1979, all positions were audited and position descriptions updated as a part of the DHEW Three-Year Classification Review. Included was a comprehensive review of secretarial jobs, a task which required review and modification of a basic position description to make it applicable to all program areas. No downgrading was found to be necessary, but during the review only a small number of promotions were found to be appropriate.

NIDR-EP again offered opportunities for work experience in science administration to NIH Grants Associates as a part of their training program, and to university-based professionals under such programs as the Intergovernmental Personnel Act (IPA) Program and the new Extramural Associates Program initiated during FY 1978. The Associate Director, NIDR-EP, served as Chairman of the review committee which selects candidates for the Extramural Associates Program and served as advisor to one of the associates. During the past year, one grants associate conducted an analysis to determine the impact of the 1976 Ad Hoc Scientific Evaluation Panel on subsequent periodontal diseases program activities, and a university professor accepted a five-month assignment under IPA sponsorship to refine program objectives and develop evaluation criteria for the Pain Control and Behavioral Studies Program.

The informal accountability sessions begun several years ago to review the progress of each categorical program at least once a year were continued during FY 1979. In these small meetings, program staff and the Associate Director discussed elements related to program balance and developed specific recommendations. The main topics considered were the current status of training within each Program, and staff use of HPR designations, scientific workshops, contracts, and RFAs.

A special project in FY 1979 was the preparation by the Dental Research Data Officer of an Orientation Handbook for new members of the National Advisory Dental Research Council (NADRC). Adapted from a manual developed for similar purposes by the National Cancer Institute, the NIDR Handbook contains information on the history and organizational structure of NIH and NIDR as well as descriptions of funding mechanisms, explanations of NIH review procedures, and a section on the specific responsibilities of the NADRC. It is hoped that this document will be helpful to Council members in carrying out their vital role in furthering the mission of the NIDR.

Another special project involved updating the Operations Manual for Scientist Administrators at NIDR. Since the field of manpower development in the biological sciences has undergone drastic alterations recently, it was necessary to make major revisions in the chapter on Research Training Programs for dental research.

The Scientific Review Branch, established three years ago to separate review from program activities, continued to function in an effective manner. In FY 1979, staff of this Branch conducted 11 project site visits and convened 3 meetings of the NIDR Special Grants Review Committee, which

reviewed 12 Institutional National Research Service Award (NRSA) applications requesting $8.6 million and 3 Dental Research Institute/Center applications requesting $20.6 million. The Scientific Review Branch also conducted 6 No-Study-Section reviews. In addition, staff prepared summary statements of these 21 initial reviews for secondary review by the National Advisory Dental Research Council.

The Office of Centers and Special Programs provided fiscal and administrative support for the five Dental Research Institutes and Centers and provided staff to aid in implementing the NIDR Cooperative Agreement with the Division of Research Resources relative to the Minority Biomedical Support (MBS) Program. The Chief of this office also served as NIDR representative to DRG to resolve problems of assignment and review.

STAFF ACTIVITIES

The Special Assistant for Program Coordination served as editor of the annual reports and of written material prepared for various purposes such as the Congressional Budget Justification, the NIDR Research Plan, the NIH Research Highlights and responses to Congressional inquiries, and drafted an NIDR position paper on research training. He also conducted staff meetings and represented the Institute as a member of the Diabetes Mellitus Coordinating Committee. In addition, he presented seminars to graduate students at Georgetown University School of Dentistry and Eastman Dental Center.

The Special Assistant for Research Manpower served as executive secretary of the Dental Research Institute and Special Programs Advisory Committee when it reviewed institutional fellowship grant applications, conducted site visits for program projects, center applications and research grants, and represented the Institute at meetings of the NIH Extramural Training Advisory Committee. He also served as Chairman of the Staff Executive Committee, which provides secondary review of Fellowship applications, and of the Fellowship Advisory Committee, which advises the Director, NIDR, on NRSA Fellowship policies and practices. In addition, he participated in a Howard University workshop on "Research and Program Development."

Extramural staff made 55 visits to institutions and participated in 35 different scientific meetings to keep abreast of scientific developments, monitor research progress, and maintain close liaison with the scientific community. They also attended 7 courses for professional development. Several workshop reports were published and three scientific exhibits were shown at national meetings to facilitate technology transfer. Information on program priorities for federal funding was again disseminated by staff at the annual meeting of the American Association of Dental Schools, and at the joint meeting of the International Association for Dental Research (IADR) and the American Association for Dental Research (AADR) in New Orleans. At the AADR meeting, staff maintained a consultation room to inform scientists of research opportunities, resolve problems, and encourage young investigators to develop research plans.

NIDR-sponsored workshops and meetings included five staff-initiated workshops or planning meetings, and four meetings or conferences supported in part by grant, contract, or other funds. These funds were made available primarily for travel expenses incurred by participants. A total of 14 such meetings had been held the previous fiscal year. The FY 1979 series included meetings relevant to periodontal diseases, craniofacial anomalies, behavioral sciences and restorative materials. Two were devoted to the planning of an NIH consensus development conference on the removal of third molars. The development of this conference follows current NIH policy of applying technical expertise to new or debatable areas of therapy. In these conferences, participants review available data and by common agreement, develop recommendations on specific modes of treatment. FY 1979 meetings are listed below.

Staff-Initiated Meetings

1) Periodontal Therapy Workshop Bethesda	June 1979
2) Workshop to Refine the Research Objectives and Approaches for Acquired Craniofacial Disfigurement, Bethesda	Dec. 1978
3, 4) Planning Sessions for the "NIH Consensus Development Conference for Removal of Third Molars," to be held November 28-30, 1979, Bethesda	Apr. 1979 June 1979
5) Conference on "Oral Motor Behaviors," Bethesda (co-sponsored by NIDR, NICHHD, and NIA)	May 1979

NIDR-Supported Meetings

1) Phenytoin Induced Teratology & Gingival Pathology Seminar Chapel Hill, N.C. (Grant to University of N.C.)	May 1979
2) Workshop on the Etiology of Facial Clefting Warrenton, Virginia (Grant to Indiana University)	Dec. 1978
3) 36th Annual Meeting of the American Cleft Palate Assoc. San Diego, California	Feb. 1979
4) Society for Biomaterials Annual Conference Clemson, S.C. (Grant)	Apr. 1979

PROGRAM EVALUATION ACTIVITIES

During FY 1979, formal program evaluation activities were conducted in the Craniofacial Anomalies Program and in the Pain Control and Behavioral Studies Program, and an analysis of the impact of the previous evaluation of periodontal disease research activity was prepared. The challenging Craniofacial Anomalies Program effort, begun prior to FY 1979, made significant progress during the year. Although the Pain Control and Behavioral Studies Program evaluation activity was not initiated until the closing weeks of the fiscal year, it has already gained considerable momentum.

To prepare for its Program evaluation, the Craniofacial Anomalies Program held three workshops to review and refine program objectives: one on congenital anomalies, one on malocclusion, and one on acquired craniofacial defects. The first two were held in FY 1978 and the third in FY 1979. The objectives and approaches developed at these meetings were then circulated to approximately 40 experts for comment. Subsequently, material from the three workshops and the mail review panels was summarized and reduced to a set of program objectives and approaches with appropriate criteria for assessing progress. After further staff discussion, a preliminary Director's charge to the evaluating panel was prepared and all of the documents further refined. It is anticipated that an RFP will soon be issued and a contract for the actual evaluation initiated during FY 1980.

To accelerate its preparations for a formal evaluation, the Pain Control and Behavioral Studies Program invited Dr. Donald Kruper, Chairman of the Department of Behavioral Science, University of Pittsburgh School of Dental Medicine, to accept a five-month NIDR assignment to aid program staff in formulating and refining program objectives and in developing evaluation criteria. Since Dr. Kruper's arrival late in FY 1979, several meetings have been held and a draft outlining goals, objectives and evaluative criteria has been prepared. Early in FY 1980, this draft, together with other background materials, was mailed to expert consultants who will soon be convened in two groups (a Pain Control Panel and a Behavioral Studies Panel) to review, modify and prioritize the goals and objectives presented in the staff document.

From his analysis of current periodontal diseases research activity following the recent formal evaluation, Dr. David Wolff found that the Periodontal Program has closely followed the recommendations of the Ad Hoc Evaluation Panel of 1976. Research in both microbiology and in the clinical aspects of periodontal disease have increased, and coordinated laboratory and clinical studies are now underway, especially at the three recently initiated periodontal clinical research centers. Dr. Wolff also concluded that the objectives developed earlier should now be simplified and broadened.

CENTERS

The NIDR now supports eight university-based centers. Five of these are non-categorical centers initiated during the 1960s and three are specialized centers recently initiated to accelerate clinical research on periodontal diseases.

The Dental Research Institutes and Centers (DRICs) at the Universities of Alabama, Michigan, North Carolina, Pennsylvania, and Washington supported 94 collaborative research projects during FY 1979. Although their activities have been reduced due to increased costs, these Centers continue to maintain an outstanding record of scientific achievement. During the past year, their senior investigators published 235 scientific papers (excluding abstracts) and had an additional 185 papers accepted for publication. Research training was provided for 35 research associates through direct participation in the research activities. Center investigators also served as preceptors for 108 fellows, many of whom conducted their research in the DRICs.

During this year, renewal applications from the Centers at the Universities of Michigan, North Carolina, and Pennsylvania were reviewed and approved for an additional five years. As part of its five-year renewal, the University of Michigan center has initiated a novel Associate Membership Program, in which a special collaborative effort is developed between a young center investigator and an established university scientist not previously active in dental research. During the past year, five excellent projects of this type were begun in relevant areas of immunology, biochemistry, microbiology, and neurology. This novel Program, which is partially funded by the University, is expected to foster continuing cooperation between center investigators and other university scientists at all levels. A recent site visit found this program highly effective in utilizing outside-center expertise on meritorious projects. It is hoped that this program will serve as a model for other centers.

The University of Washington center recently submitted a five-year renewal application, and a project site visit was conducted. The site visit team found that significant changes had occurred at this center since the last visit. The Nutrition and Pain projects, and a substantial part of the Salivary Secretion project had been phased out; and greater emphasis was being placed on the microbiology and immunology of periodontal diseases as well as on clinical studies of salivary changes in cancer chemotherapy patients.

Research highlights of the DRIC Program are described elsewhere in this report and also in the categorical reports which follow this report. Thus, only administrative activities have been included here.

The three specialized Periodontal Clinical Research Centers recently initiated at Forsyth in Boston, SUNY at Buffalo, and Virginia Commonwealth in Richmond continued to undergo maturation during FY 1979. In accord with advice from advisory committees and the Ad Hoc Evaluation Panel of 1976, these centers were established to develop coordinated laboratory and clinical research approaches to the diagnosis, treatment and prevention of periodontal diseases. Funding was modest initially and the budgets were not increased until the centers showed overt progress in organizing competent research teams and in establishing study populations.

In the short span of two years since the first two were initiated, the centers have already made significant progress in microbiology and immunology, and have begun to develop research in prevention, particularly in chemotherapeutic approaches. The current third year budget for each of the centers approximates $500,000. Since the three centers have similar goals and objectives, NIDR staff encourages them to communicate freely with each other and with investigators elsewhere, and to form collaborations when indicated. Thus, investigators from all three centers meet as a group at least once annually and exchange visits more frequently during the year.

RESEARCH FUNDING

During FY 1979, the NIDR Extramural Programs awarded more than $33 million for research. Of this amount, $24.4 million was awarded for research grants and career awards, $1.3 million for contract research by the five categorical programs, and $7.6 million was awarded by the Dental Research Institutes and Centers Program.

Grants

Table 1 presents data on the FY 1979 distribution of research grant funds by program and by type of grant. It does not include grants by the National Caries Program. Altogether, the Extramural Programs made 360 grant awards: 312 for research projects, 5 for scientific conferences, 5 for the university-based dental research institutes and centers, 3 for periodontal clinical research centers, 31 for research career development awards, 1 for research career awards, and 3 for minority programs. Of the 312 project awards, 250 were made for regular research grants (R01), 9 for program projects (P01), and 53 for the new investigator awards (R23).

Compared to last year, the total research grant funds awarded by NIDR-EP in FY 1979 increased numerically by $2.5 million, or 8.3%, but because of inflation, there was actually a slight decrease in the buying power of these funds. In FY 1979, 29% of the research grant funds was awarded for new grants and competing renewals, and 71% was awarded for noncompeting continuations and supplemental grants. The new research awards included 51 regular grants, one program project, 22 special dental awards, and 5 conference grants. The competing renewals included 29 regular grants, 1 program project, 2 university-based center grants, and 2 MBS projects.

Only one categorical program showed significant growth during FY 1979. The Pain Control and Behavioral Studies Program registered a 45% increase in research grant funding. New awards in this program included a program project award for coordinated clinical and laboratory studies on the management of human pain.

To expedite early utilization of research monies by successful applicants, the Department of Health, Education, and Welfare had requested that all NIH Institutes award at least 70% of the research grant funds during the first three quarters of the fiscal year. NIDR more than fulfilled this request; 90% of the Institute's funds were expended within the first three quarters.

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N.I.D.R. EXTRAMURAL RESEARCH GRANTS FUNDED FY 79
DATA AS OF SEPTEMBER 30, 1979 - BY TYPE & CODE

TYPE	CODE	PERIODONTAL NO.	PERIODONTAL AMOUNT	CRANIOFACIAL NO.	CRANIOFACIAL AMOUNT	RESTORATIVE NO.	RESTORATIVE AMOUNT	STOMATOLOGY NO.	STOMATOLOGY AMOUNT	PAIN CONTROL NO.	PAIN CONTROL AMOUNT	INSTITUTES NO.	INSTITUTES AMOUNT	TOTALS NO.	TOTALS AMOUNT
1	K04	0	$0	0	$0	0	$0	2	$47,294	0	$0	0	$0	2	$47,294
	P01	0	$0	0	$0	0	$0	0	$0	1	$231,796	0	$0	1	$231,796
	R01	11	$768,792	17	$1,133,087	4	$189,455	9	$594,174	10	$585,950	0	$0	51	$3,271,458
	R13	2	$84,632	1	$1,680	1	$8,500	0	$0	1	$47,625	0	$0	5	$142,437
	R23	2	$52,557	5	$137,983	4	$112,673	8	$219,547	3	$82,376	0	$0	22	$605,136
	TYPE TOTALS	15	$905,981	23	$1,272,750	9	$310,628	19	$861,015	15	$947,747	0	$0	81	$4,298,121
2	P01	0	$0	1	$251,581	0	$0	0	$0	0	$0	0	$0	1	$251,581
	P50	0	$0	0	$0	0	$0	0	$0	0	$0	2	$2,748,182	2	$2,748,182
	R01	6	$444,696	7	$493,482	4	$230,995	9	$736,511	3	$115,788	0	$0	29	$2,021,472
	S06	1	$43,105	1	$31,172	0	$0	0	$0	0	$0	0	$0	2	$74,277
	TYPE TOTALS	7	$487,801	9	$776,235	4	$230,995	9	$736,511	3	$115,788	2	$2,748,182	34	$5,095,512
3	R01	0	$0	0	$81,713	0	$57,807	0	$0	0	$51,592	0	0	***	$191,112
	R13	0	$0	0	$0	0	$10,433	0	$0	0	$0	0	0	***	$10,433
	TYPE TOTALS	0	$0	0	$81,713	0	$68,240	0	$0	0	$51,592	0	0	***	$201,545
4	K06	0	$0	0	$0	0	$0	1	$32,454	0	$0	0	$0	1	$32,454
	TYPE TOTALS	0	$0	0	$0	0	$0	1	$32,454	0	$0	0	$0	1	$32,454
5	K04	7	$265,583	8	$292,446	6	$222,375	6	$203,324	2	$66,146	0	$0	29	$1,049,874
	P01	1	$518,138	5	$1,934,491	0	$0	1	$178,053	0	$0	0	$0	7	$2,630,682
	P50	3	$1,635,752	0	$0	0	$0	0	$0	0	$0	3	$4,803,818	6	$6,439,570
	R01	32	$2,305,943	45	$3,350,472	29	$1,792,697	49	$3,061,838	15	$988,717	0	$0	170	$11,499,667
	R23	10	$288,462	7	$186,578	5	$110,398	7	$127,030	2	$47,023	0	$0	31	$759,491
	S06	1	$37,050	0	$0	0	$0	0	$0	0	$0	0	$0	1	$37,050
	TYPE TOTALS	54	$5,050,928	65	$5,763,987	40	$2,125,470	63	$3,570,245	19	$1,101,886	3	$4,803,818	244	$22,416,334
	GRAND TOTALS	76	$6,444,710	97	$7,894,685	53	$2,735,333	92	$5,200,225	37	$2,217,013	5	$7,552,000	360	$32,043,966

THIS REPORT DOES NOT INCLUDE THE NATIONAL CARIES PROGRAM.

TYPES:
- 1 NEW AWARDS
- 2 COMPETING CONTINUATION AWARDS
- *** 3 SUPPLEMENTAL AWARDS
- 5 NON-COMPETING CONTINUATION AWARDS

CODES:
- P01 PROGRAM PROJECTS
- P50 INSTITUTE AWARDS
- K04 CAREER DEVELOPMENT AWARDS
- K06 CAREER AWARDS
- R01 REGULAR RESEARCH GRANTS
- R13 CONFERENCE GRANTS
- R23 SPECIAL DENTAL AWARDS
- S06 MINORITY PROGRAMS

Contracts

Collaborative research funded by contract and by interagency agreement during FY 1978 totalled $1.3 million, which was distributed among three categorical extramural programs as shown in Table 2. Three of the projects supported by the Craniofacial Anomalies Program had received multi-year awards from the FY 1978 budget, and therefore did not require FY 1979 funds. Thus, the level of contract activity during FY 1979 was actually higher than Table 2 indicates.

Table 2

FY 1978 DISTRIBUTION OF CONTRACT SUPPORT BY PROGRAM

Program	No. of Contracts and Interagency Agreements	Amount ($000,s)	Percent of Total
Craniofacial	6*	176	13.8
Restorative	3	563	44.1
Stomatology	8	537	42.1
Totals	17	$1,276	100.0

*Includes 3 projects which were active but did not receive FY 1979 funds

TRAINING

Table 3 summarizes the distribution of training funds awarded in FY 1979. The NIDR Extramural Programs (NIDR-EP) awarded a total of nearly $3 million for 43 individual fellowships and 25 institutional fellowship grants. All awards were made under the National Research Service Awards Program. Table 3 does not include funds of $325,694 awarded by the National Caries Program for eight individual fellowships and two institutional grants. Training funds expended in FY 1979 closely approximated FY 1978 expenditures. For the second year, no new institutional fellowship grants were awarded because of prevailing fiscal conditions, even though the mandatory budget for individual fellowships surpassed the newly established 15% level.

Although the training budget is expected to remain level, steps have been taken to provide a broader range of training opportunities, and thus to provide more flexibility in meeting the various needs of the future. The scope of the National Research Service Awards has been expanded to include Senior Fellowship Awards and short-term training programs, and there have been increases in fellowship stipends and institutional allowances. These measures are expected to result in a significant increase in the number of applications and to enable the Institute to develop an overall training activity appropriate for the 1980s.

The NIDR position paper on research training, prepared by Dr. Rizzo, was presented to the NADRC in May 1979 for discussion and critique, and comments were also solicited from other advisory committee persons. This paper is now being revised and will be resubmitted at a subsequent Council meeting for further consideration.

Table 3

Distribution of FY 1979 Research Training Funds ($ in thousands)

Type of Award	Perio.		Cranio.		Restor.		Stomat.		Pain		Total	
	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.	No.	Amt.
Fellowships	16	$185	10	$125	2	$ 30	9	$105	6	$ 76	43	$ 520
Institutional Fellowship Grants	6	$722	5	$549	6	$397	6	$592	2	$184	25	$2,444
Totals		$907		$674		$427		$697		$260		$2,965

RESEARCH HIGHLIGHTS

In research related to periodontal diseases, investigators have intensified efforts to identify and characterize microorganisms associated with periodontal disease. In current studies scientists are not only identifying the predominant organisms throughout the periodontal pocket, but they are also attempting to identify all organisms in soft attached plaque in deep portions of the pockets. In this deep plaque scientists have identified and characterized 1,105 isolates from 25 sites in 19 patients and found 141 different species, 61% of which had not been described before. Eubacterium and spirochetes were abundant.

The evidence against B. asaccharolyticus, A. actinomycetem-comitans (Y4), and B. melaninogenicus has been strengthened by new findings. Numerous B. asaccharolyticus are found in periodontal pockets and the patients show specific antibody to this organism. Moreover, the organism produces collagenase, fibrinolysin, and other destructive agents, and its capsule prevents leukocytes from engulfing it. The Y4 organism is abundant in patients with localized juvenile periodontitis (LJP), many of whom show specific antibody against the organism and the leucotoxin it makes.

In pregnancy gingivitis B. melaninogenicus ss intermedius increased when the levels of estradiol and progesterone increased. This organism also utilized more estradiol and progesterone in vitro than controls.

Host response research included studies on plaque-induced polymorphonuclear cell (PMN) lysosome release, polyclonal activators, and PMNs. PMNs exposed to 21-day human plaque released more lactoferrin than PMNs exposed to 3-day plaque; presumably concomitant release of destructive enzymes also occurred. Factors released by PMNs also stimulated a 10-fold increase in mononuclear cell proliferation.

The abundance of lymphocytes and plasma cells in advanced periodontal lesions may be due to polyclonal activators, which stimulate B-cells to divide, differentiate and produce nonspecific antibodies and destructive lymphokines, such as osteoclast activating factor (OAF). Potent polyclonal activators have been found in many plaque organisms including B. melaninogenicus and A. viscosus.

Investigators have extended previous findings that PMN chemotaxis and phagocytosis are impaired in LJP. Since this impairment persisted in patients treated for LJP, it is considered an intrinsic trait.

Treatment. Frequent removal of plaque by professionals coupled with strict oral hygiene prevented periodontal disease. Patients given prophylaxis and hygiene instructions every 2 or 3 months for 3 years had healthier tissues than control patients given prophylaxis only once a year with no oral hygiene instructions.

Monitoring the flora by quantitative darkfield microscopy was helpful in determining the status of patients being treated for periodontitis. Before treatment motile rods, curved rods, and spirochetes predominated in diseased sites, but after treatment, the flora showed a decrease in these organisms, and an increase in coccoid cells.

Using a new assay for tetracycline in crevicular fluid, scientists showed that after systemic administration, crevicular levels peaked at 6-20 mg/ml whereas blood levels only reached 1.5-2.5 mg/ml.

Collagen and bone research. Scientists discovered that mononuclear cells secrete a protein factor which inhibits collagen synthesis by fibroblasts. They also showed that proteoglycans influence the rate of collagen fibrillogenesis; some accelerate the process, whereas others retard it. The basement membrane was found to contain collagen secreted by the epithelial cells rather than by fibroblasts, the expected cellular source.

Craniofacial research included studies on the migration and differentiation of neural crest cells, which give rise to the skeletal and connective tissue structures of the craniofacial region as well as to certain muscular, neural, and endocrine elements. Recent studies indicate that neural crest cells from different levels are not preprogrammed for their ultimate function; instead, each level can provide a wide spectrum of cells. Studies also suggest that the process of embryonic morphogenesis sets up preferential migration routes for the neural crest cells.

Studies of vascular tissue formation indicate that during normal development, endothelial cells are highly invasive, but are carefully regulated. Nonvascularized cartilage was shown to contain a factor which selectively inhibits endothelial cell proliferation but does not inhibit smooth muscle cells or fibroblasts.

Studies in mice indicate that specific genes may inhibit the initial fusion of the palatal shelves by affecting cell adhesiveness. Other basic findings in mice suggest that steroids interfere with programmed cell death in the medial edge of the palatal shelves and thereby prevent fusion. The mechanism of drug action here is believed to be the same as that which produces anti-inflammatory effects.

The events leading to cleft lip and palate formation in mice given the antiepileptic drug dilantin have been clarified. Dilantin causes a severe retardation of growth in the embyonic lateral nasal processes, which are then too small to fuse with the median nasal processes, thus forming a cleft lip. This growth reduction is associated with inhibition of the mesenchymal cell process network which normally develops.

In studies of craniofacial anomalies in hamsters caused by H-1 parovirus, it was shown that the morbidity and mortality of the newborns varied directly with the mother's level of the hormone progesterone. When the progesterone level was high, the morbidity and mortality levels were high, but when progesterone levels dropped, no virus effect was seen. High progesterone levels suppress "T" lymphocytes.

Studies in cats of biochemical mechanisms in orthodontic tooth movement showed an increase of prostaglandin E_2 in the bone during treatment. These increases were evident in areas of tension and compression.

Clinical scientists confirmed the belief that respiratory allergies alter the pattern of growth of the face and jaws; allergic children had longer and narrower faces than normal.

Research on restorative materials included a variety of studies. In one project, investigators devised an automated system of minicomputers that enables them to compare dental filling materials clinically. They have used the system to develop composite plastic filling materials for posterior teeth, and to evaluate copper-containing alloys. The investigators have found that a composite material containing strontium particles served up to 5 years as a successful posterior filling in over 90% of the teeth tested. As a result, a filling material of this type has become available on the dental market. Two of the copper-containing alloys they tested showed resistance to corrosion and maintained a superior marginal adaptation without fracture, but a third one did not.

Scientists at the American Dental Association Laboratories at the National Bureau of Standards are developing data on binary combinations of 20 transition elements which could prove useful for dental appli-

cation. These elements include silver, copper, mercury, gold and other metals. This data source should facilitate the development of better alloys for dental appliances, and may be of benefit for a wide range of applications.

Scientists concerned with the biocompatibility and safety of endodontic filling materials have devised a reliable screening approach for use by manufacturers in developing new products and in maintaining quality control. In a study to compare the controversial Sargenti procedure with conventional endodontic treatment, scientists have so far shown that the early phase of healing was poor after the Sargenti method had been used.

Recent tooth implant research on titanium implants in Rhesus monkeys suggests that implant success is dependent upon ankylosis. Surface treatment of cast metal subperiosteal implants by "glow discharge cleaning" improved tissue acceptability because it completely removed the waxy residue left by the casting process.

In studies on the head stabilizer for patients with cerebral palsy, scientists confirmed previous findings that restorative procedures can be carried out using the stabilizer rather than general anesthesia, and also showed that it can be used to train patients to control their movements voluntarily.

Nutrition. Monkeys with subacute Vitamin C deficiency had more severe experimental periodontal disease than nondeficient animals, and had leukocytes with reduced ability to phagocytize and kill microorganisms. The effect of high protein diet on human calcium excretion was found to vary according to the protein source, the length of the study and the amount of phosphorus intake. Calcium absorption from the gut was unaffected by the high protein diet. Vitamin A-deficient rat pups raised on milk from Vitamin A-deficient mothers had higher caries rates than controls. Presumably the deficiency affected tooth development. Folate deficiency in cultures of mouse palates caused an epithelial union between palatal shelves which prevented mesenchymal fusion. In preliminary studies zinc deficient STR/N mice had more severe periodontal bone loss than STR/N mice on adequate zinc intake. This experimental model will be useful in future studies.

Since cryosurgery for treating inflammatory and neoplastic diseases of oral-facial tissues shows advantages over radiation and other procedures, investigators are conducting microscopic studies of cryolesions in subhuman primates. They have found that some components of the salivary glands are more resistant than others to destruction from freezing. Recently they used these findings to advantage in treating an aggressive acinar cell carcinoma of the parotid gland. Cryosurgery destroyed the malignant tissue but caused only transient damage to the facial nerves. The investigators have shown that cryosurgery is followed by optimum repair and regeneration, with minimal scarring; thus it faciliates surgical reconstruction.

A new anti-herpes virus vaccine protective against infections in animals, and not likely to promote carcinomas, has been developed. The new vaccine, containing nucleic acid-free viral subunits, gave 100% protection against localized lip infections and fatal encephalitis, and 70% protection against latent ganglionic infections. To prove that the protection resulted from activating the body's immune system, the investigators suppressed the immune system with drugs and showed that the protective effect was reversed.

Salivary Secretions. The salivary glands of rats decreased in size when soft or liquid diets were fed but they enlarged when increased mastication was required. The liquid diets also caused the disappearance of a major salivary protein fraction, which was restored when solid diets were resumed. Human subjects on liquid diets also show reduced salivary protein.

Mineralization. Calcium-phospholipid-phosphate complexes, which are closely linked with nearly all mammalian mineralization, have recently been isolated from the matrix vesicles in epiphyseal cartilage. In addition, scientists are using a synthetic protein-acidic-phospholipid to study mineralization in vitro.

Studies on the effect of fluoride on the ameloblast in vitro indicated that small doses cause disturbances in the ameloblast function. This finding may explain the occurrence of tooth mottling in areas where there is excessive fluoride in the water.

Calcium-binding proteins dependent upon Vitamin K have been shown to be associated with bone mineralizing systems. In one study when Vitamin K was blocked with Warfarin and then supplements were given shortly thereafter, a zone of dentine with decreased calcium was formed. This zone was presumably associated with the failure of the calcium-binding proteins to be synthesized.

Pain research. Scientists showed that the dental nerve endings form treelike extensions at the sides of the odontoblasts with some processes extending into the predentine and dentine layers. These findings suggest that the odontoblast layer serves as the chief pain receptor within the tooth.

Neuromuscular Control of Jaw Movements. Studies indicate that highly organized groups of nerve cells in the brain function as pattern generators to activate the rhythmic jaw movements involved in drinking and chewing. In these studies, scientists discovered a specific pattern generator that inhibits the powerful jaw-closure muscles so that the jaw can open without resistance.

Arthroscopy, a widely used technique for the diagnosis of knee-joint pathology, is now being applied to the temporomandibular joint (TMJ). Recent studies have established its usefulness for the diagnosis of pathology in the TMJ of animals, and current research is assessing its applicability to the TMJ of humans.

Fear of Dental Treatment. Whether individuals with an exaggerated fear of dental treatment also have a low pain tolerance was investigated. The findings indicated that fearful people do not have a lower pain tolerance than nonfearful people. The authors concluded that the emotional and cognitive aspects of dental pain are more significant in determining the individual's reaction than the actual pain involved.

Studies of children's behavior during dental treatment indicate that parental presence does not produce greater anxiety or noncompliant behavior, and dentists using neutral or nonevaluative responses to the child are more effective in minimizing disruptive behavior than those using positive or negative responses.

Prepared by A. A. Rizzo

ANNUAL REPORT FY 1979

PERIODONTAL DISEASES PROGRAM

INTRODUCTION

Periodontal diseases constitute a major health problem throughout the world. These diseases have already caused millions of adult Americans to lose their teeth and will cause many more individuals to become edentulous unless effective preventive measures become available. To develop such measures and ultimately to eradicate these diseases is the mission of the Periodontal Diseases Program. To achieve this mission the Program supports research on the cause, nature, diagnosis, treatment, and prevention of these diseases. Since periodontal diseases have a multifactorial etiology and complex manifestations, the research encompasses a wide variety of subject matter, which includes the identity and nature of the oral microorganisms suspected of causing disease and the complex immune responses activated in the host by the disease. The microbiologic studies emphasize anaerobic bacteria, and the host response research emphasizes the cellular and biochemical mechanisms involved in inflammation and tissue destruction. Clinical and laboratory activities are coordinated in an effort to accelerate the development of preventive measures for public health application.

ADMINISTRATION

During FY 1979, the Program awarded a total of $6.5 million for 53 regular research grants, 3 clinical research centers, 1 program project, and 12 new investigator awards. In addition, 14 regular research grants, 2 special dental research grants, and 1 program project were active but did not receive FY 1979 funds. A total of $721,967 was awarded for 6 institutional training grants supporting about 37 trainees and fellows and an additional $185,025 was awarded for 16 fellowships. A total of $265,583 was expended for 7 career development awards.

Awards for the 3 specialized clinical research centers funded this year totaled $1,612,831. These centers were created to develop coordinated programs which include both basic and clinical research on periodontal diseases. Emphasis is placed on efforts to identify the causative organisms, to determine the host responses to these organisms, and to develop improved therapeutic and preventive measures.

Table I shows the distribution of research and training funds during FY 1979.

Table I: DISTRIBUTION OF FUNDS DURING FY 1979

A. RESEARCH GRANTS

	ACTIVE	FUNDED IN 1979	FUNDS
Microbiology	18	15	$1,501,648
Inflammation and Immune Response	33	24	1,409,141
Bone Metabolism	13	11	618,287
Tissue Structure and Metabolism	16	13	806,696
Prevention, Diagnosis, and Treatment	3	2	157,399
Clinical Research Centers	3	3	1,635,752
Career Development Awards	7	7	265,583
Conference - IADR Meeting Osaka, Japan	1	1	75,104
Total	94	76	$6,469,610

B. TRAINING

Institutional Grants	6	6	$ 721,967
Individual Fellowships	16	16	185,025
Total	22	22	906,992
Program Total			$7,376,602

STAFF ACTIVITIES

Staff made visits to 11 institutions to program, evaluate, or monitor research projects, participated in several scientific meetings, and sponsored a scientific workshop on current treatment methods in periodontics. These activities are listed below.

A. Site Visits: Initial Review, Monitoring, and Programming

University of Michigan, Ann Arbor	October 1978
Harvard School of Dental Medicine, Boston	October 1978
University of California, Los Angeles	October 1978
Harvard School of Dental Medicine, Boston	October 1978
Forsyth Dental Center, Boston	January 1979
University of Southern California, Los Angeles	January 1979
University of Pennsylvania, Philadelphia	February 1979
Forsyth Dental Center, Boston	June 1979
Virginia Commonwealth University, Richmond	June 1979
University of Washington, Seattle	June 1979
State University of New York, Buffalo	July 1979

B. Meetings

Annual Periodontal Symposium, Los Angeles	February 1979
International Association for Dental Research, New Orleans	March 1979
District of Columbia Dental Society, Meeting, Washington, DC	April 1979
Greater Washington Periodontal Society	May 1979
International Symposium: Phenytoin Induced Teratology & Gingival Pathology, Chapel Hill	May 1979

C. Periodontal Diseases Advisory Committee Meeting — December 1978

D. Sponsored Workshop

Periodontal Therapy, Bethesda	June 1979

RESEARCH HIGHLIGHTS

Microbiology

Periodontal tissues are believed to harbor hundreds of different species of microorganisms in health and disease. Over the past several years, investigations of patients with periodontal diseases of varying severity, indicate that some of these microorganisms are pathogenic. Four types of evidence support this contention: the suspected pathogens are predominant members of the plaque; they are found at the sites of greatest tissue destruction; they cause periodontal disease in experimental animals; and elimination of the organisms arrests the disease. In more recent microbiological studies investigators have intensified their efforts to isolate, identify, and characterize the organisms associated with particular types of periodontal disease. Because this task is such an enormous undertaking, it is being carried out at several institutions, including the three Periodontal Disease Centers. Although all of these teams of investigators have the same general aim in mind, each group has developed its own approach to the microbiology of periodontal disease.

Since previous studies have convinced the investigators at Forsyth Dental Center that the predominantly anaerobic subgingival flora plays a more crucial role in causing periodontal disease than the supragingival flora, they have concentrated on the predominant organisms in the deepest portions of periodontal pockets. To obtain samples from these regions they insert a barbed broach through a 19 gauge needle which is flushed with oxygen-free gas to maximize the yield of oxygen-sensitive anaerobes.

Investigators at the SUNY (Buffalo) Center, on the other hand attempt to obtain a representative sample of the predominant organisms throughout the pocket by sampling the full length of the pocket with a paper point also under anaerobic conditions. Both the Forsyth and Buffalo scientists sample soft plaque material and tend to avoid hard calculus adherent to

the tooth roots. Although these workers culture for all viable organisms, they only isolate and identify the predominant species.

At Virginia Commonwealth University Center, Richmond, investigators use a curette to specifically sample the soft plaque material attached to the tooth root in the deep portion of the pocket. These investigators attempt to isolate and identify every individual organism in the sample. Thus, although their samples are probably more localized than those taken at Buffalo and Forsyth, their identification scheme is more comprehensive. As one might expect, the results from these three approaches have shown differences.

Preliminary results obtained by investigators at Virginia Commonwealth University and Virginia Polytechnic Institute attest to the enormity of identifying the total flora in a sample which may be limited. Thus far 1105 isolates have been characterized and identified to the species level. These include 141 different species, 87 (61%) of which are previously undescribed or unreported species. Three species of Eubacterium -- E. bracy, E. timidum, and E. nodatum -- were often found in large numbers in these samples. Spirochetes were plentiful; out of 8 species of treponomes recovered, 6 had not been reported previously.

The pathogenic potential of Bacteroides asaccharolyticus and Actinobacillus actinomycetem-comitans in causing certain types of periodontal disease is indicated by clinical and laboratory evidence from Forsyth Dental Center, SUNY Buffalo, the University of Michigan, in Ann Arbor and the University of Washington, in Seattle. Investigators at Forsyth had shown that numerous B. asaccharolyticus were present in inflamed and suppurating periodontal pockets, and that the organism would cause periodontal disease in gnotobiotic rats. Moreover, it has been shown that many adult patients suffering from periodontal disease have an elevated serum antibody to these organisms. In more recent studies at Buffalo, scientists have recovered proportionately large numbers of B. asaccharolyticus from periodontal lesions induced by ligatures placed subgingivally around the teeth of monkeys. Similar results were found in the periodontal lesions of beagle dogs by researchers at the University of Michigan. Investigators at the University of Washington, Forsyth and SUNY, Buffalo have shown that B. asaccharolyticus from subgingival sites in human subjects can be eradicated or reduced by surgery or by systemic tetracycline therapy for six months and during this period, disease activity declines.

In order to determine the nature of the immune response to B. asaccharolyticus, the investigators at Forsyth immunized rats with this organism. Although the rats responded antigenically to whole cell sonicates, purified lipopolysaccharides (LPS) from the organism elicited neither a specific immune response, nor a nonspecific mitogenic response. Thus, it seems unlikely that LPS from this organism could be a significant factor in disease.

Laboratory evidence indicates that B. asaccharolyticus could cause disease by a number of additional mechanisms. The organism produces

enzymes including collagenase, fibrinolysin, DNase, and RNase, and such noxious agents as ammonia, hydrogen sulfide, and methyl mercaptan, all of which can cause tissue destruction. Moreover, the capsule of the organism inhibits phagocytosis by polymorphonuclear leukocytes (PMNs). Thus, a variety of evidence implicates B. asaccharolyticus in the etiology of the inflammatory, suppurative, and destructive periodontal diseases of adults.

The second organism implicated in periodontal disease is the gram-negative, short, slightly curved, round-ended, capnophilic rod, Actinobacillus actinomycetem-comitans (Y4). The Forsyth group has recovered large numbers of this organism from the periodontal pockets of young adults with destructive periodontal disease and patients with localized juvenile periodontitis (LJP). These investigators have also shown that young patients with localized juvenile periodontitis have an elevated antibody to Y4. The investigators at Pennsylvania determined that the Y4 isolated from LJP patients is identical to the American type culture strains designated A. actinomycetem-comitans using DNA base composition, fermentation properties, morphological and antigenic profiles.

Investigators at SUNY, Buffalo measured precipitating antibodies to A. actinomycetem-comitans in sera from patients with severe periodontal diseases, including LJP, generalized juvenile periodontitis, acute necrotizing ulcerative gingivitis, and severe adult periodontitis; normal and edentulous patients served as controls. Sera from 60% of the patients with LJP contained antibodies that precipitated with antigens of the Actinobacillus, whereas sera of the other patient groups rarely showed precipitating antibodies to this organism. Investigators at the University of Pennsylvania and SUNY, Buffalo have demonstrated that sera from patients with LJP inhibits the Actinobacillus leucotoxin that lyses polymorphonuclear leukocytes. Apparently the specific antibody produced by these patients not only neutralizes the Actinobacillus leucotoxin but also prepares the organism for opsonization and subsequent phagocytosis by neutrophils. The investigators at SUNY have used these findings to implicate A. actinomycetem-comitans as the main pathogen in LJP and to hypothesize that this disease remains confined for a time to molars and incisors, because specific antibody mechanisms prevent the spreading of the initial infection.

Long suspected as a periodontal pathogen, Bacteroides melaninogenicus has been recently implicated in pregnancy gingivitis, where these organisms seem to be dependent upon host hormone levels for sufficient growth to cause disease. Investigators found a threefold increase in B. melaninogenicus subspecies intermedius during pregnancy when the amount of circulating estradiol and progesterone was increased, and also showed in vitro that this microorganism actually utilizes more estradiol and progesterone than control organisms. In future studies they will seek definitive evidence to show whether the pregnancy gingivitis is actually caused by the increase of B. melaninogenicus ss intermedius and whether the hormones estradiol and progesterone enhance their growth in vivo.

Until recently A. viscosus and A. naeslundii were distinguished by their reactions to catalase: A. viscosus is catalase positive and A. naeslundii catalase negative. Now investigators at the University of Pennsylvania are able to further differentiate between the two organisms by means of ultrastructural and immunofluorescent techniques. Cells incubated with homologous rabbit antiserum and then with fluorescein isothiocyanate (FITC) conjugated anti-rabbit IgG showed a completely smooth outline for A. naeslundii and an interrupted, irregular outline for A. viscosus.

Host Response

The host response to periodontal disease includes many cellular and chemical reactions which protect the host, but may also cause soft tissue destruction and bone resorption. In current work plaque-induced lysosome release by PMNs, polyclonal activators, and PMN impairment have been studied; recent findings are outlined below.

Previous investigations have shown that when PMNs are incubated with dental plaque, they release their lysosomal constituents. Since the microbial composition of dental plaque is extremely complex and variable, investigators at the University of Pennsylvania tested the response of PMNs to plaque collected at various intervals during the development of experimental gingivitis. Although plaque taken at 21 days did not differ from that taken at 3 days in stimulating the release of lysozyme and myeloperoxidase by PMNs, the 21-day plaque caused the PMNs to release more lactoferrin than the 3-day plaque. Since lactoferrin is segregated in specific granules of PMNs along with cationic proteins, collagenase and neutral proteinases, this result suggested that potentially destructive enzymes are also released. Since serum was required, the investigators postulated that release of lysosomes was due to complement activation. In more recent investigations the University of Pennsylvania scientists showed that PMN extracts, or factors released by PMNs incubated with A. viscosus, stimulated a 10-fold increase in mononuclear proliferation. They were able to detect lysosomal material in the PMN extracts and in the factors released by PMNs. Thus the investigators concluded that the release of lysosmal constituents by the PMNs could account for periodontal inflammation by more than one mechanism.

Investigators at Virginia Commonwealth University and University of Washington postulated that the proliferation of B-lymphocytes and plasma cells in advanced periodontal lesions is the result of polyclonal activators. Polyclonal activators are molecules which stimulate B-lymphocytes to divide and differentiate to produce nonspecific antibodies. These investigators demonstrated that peripheral B-lymphocytes obtained from young adults with severe periodontitis showed enhanced proliferation both to extracts of oral organisms and to staphylococcal protein, a well known polyclonal B-cell activator. Moreover, they showed that extracts of many plaque organisms such as B. melaninogenicus, A. viscosus, A. naeslundii and A. isralei contain potent polyclonal activators. According to the Virginia investigators the B-lymphocytes present in gingival tissue divide and differentiate following activation by the

polyclonal activators of the oral flora. The activated B-lymphocytes can then participate in cell mediated immunity by producing lymphokines, including osteoclast activating factor (OAF). The release of OAF and other factors by B-lymphocytes could be partly responsible for the bone resorption observed in periodontal lesions. Therefore the development of severe periodontal disease among some young adults with severe periodontitis may be related to hyperactive B-cells stimulated by these nonspecific bacterial activators.

The investigators at SUNY, Buffalo and the University of Washington, Seattle have previously reported that peripheral PMN chemotaxis and phagocytosis are impaired in patients with localized juvenile periodontitis (LJP). These research groups have now extended their findings on the chemotactic function of PMNs to a larger number of LJP patients and to other types of periodontal disease. The group in Buffalo found cellular chemotactic defects in the PMNs from 26 of 32 young LJP patients and 7 of 10 adult patients with a history of LJP. Serum from 75% of the patients with LJP also contained an inhibitor for chemotaxis stimulated by the standard chemotactic factor from E. coli. In contrast, PMNs from patients with adult periodontitis showed normal or elevated chemotaxis. Random migration and chemokinesis were normal in the PMNs of LJP patients.

A series of experiments were designed to test whether the chemotactic impairment is due to an intrinsic defect of the PMNs. The investigators at Buffalo found that the impairment of chemotaxis in PMNs persisted in all of the 10 patients who were treated for localized juvenile periodontitis. They also showed that the PMNs of adult patients with a history of localized juvenile periodontitis had defective neutrophils. Previously the same investigators had found that some of the non-diseased siblings of patients with LJP also had defective neutrophils. These three lines of evidence suggest that the neutrophil defect is an intrinsic defect and is not caused by the disease localized juvenile periodontitis.

Both the Buffalo and Seattle groups have shown that a serum factor from patients with LJP specifically inhibits the bacterially derived chemotactic factor from E. coli but does not inhibit the complement-derived chemotactic factor. It appears that both the intrinsic defect of the PMNs and the factor in the serum which inhibits the bacterially derived chemotactic factor may account for the susceptibility of certain patients to periodontal infection.

Treatment

Many investigations have implicated dento-bacterial plaque as a primary cause of periodontal disease. Workers in Sweden showed that the frequent removal of plaque by professionals coupled with strict personal oral hygiene can prevent periodontal disease. After 324 test and 156 control patients ranging from 20 to 71 years had been examined, they were given prophylaxis and instruction in oral hygiene. The test patients were recalled for prophylaxis and oral hygiene instruction every 2 months for

the first 2 years and every 3 months during the last year of the study. The control patients were recalled only once a year and did not receive further instructions in oral hygiene. At the end of 3 years, the test groups had only negligible signs of gingivitis and no further loss of periodontal attachment, but the control group showed significant loss of attachment.

Investigators at the University of Pennsylvania working with investigators of the University of Gothenburg have tested whether quantitative darkfield microscopy is a feasible technique for monitoring the periodontal flora of patients being treated for gingivitis and periodontitis. Before treatment coccoid forms predominated in normal sites, whereas other types such as motile rods, curved rods, and small, medium-sized, and large spirochetes predominated in diseased sites. The investigators then monitored the flora of periodontal pockets in patients who had been treated by 3 methods: scaling, tetracycline therapy, and a combination of both. After treatment, the tissues of all three groups were considerably improved clinically and the flora in the periodontal pockets resembled that of healthy pockets; the proportion of coccoid cells had increased and motile rods and spirochetes had decreased. Thus active treatment caused substantial changes in the microbial flora. The results indicate that monitoring the flora is helpful in determining the progress of the disease.

Since tetracycline is commonly used as an aid in periodontal therapy, investigators at Forsyth have developed an assay for measuring this antibiotic in small samples of crevicular fluid. Early results show that after a systemic administration of a single 250 or 500 mg dose, the tetracycline levels in crevicular fluid peaked at 6 to 20 mg/ml (5 to 7 hours), whereas the highest concentration of tetracycline in the blood was only 1.5 to 2.5 mg/ml (3 hours). The higher levels of tetracycline in the gingival crevice may explain the efficacy of tetracycline in periodontal therapy.

Collagen and Bone Metabolism

The NIDR continues to support numerous basic studies on the structure and metabolism of the connective tissues involved in periodontal disease. Emphasis has been placed on the two major extracellular components of the soft connective tissues, collagen and proteoglycans, and on bone resorption. This year's highlights include studies on the roles of mononuclear cell proteins, proteoglycans, and epithelial cells in the formation of collagen and studies on osteoclasts and macrophages in bone resorption.

Although a great deal is known about the synthesis of collagen, scientists are still discovering new factors which influence the formation of this important structural protein. Recently, investigators at the University of Pennsylvania found that mononuclear cells synthesize and secrete a protein factor which can selectively inhibit collagen synthesis by fibroblasts.

Collagen in the form of fibrils endows the tissue with tensile strength and the proteoglycans provide resistance to compressive forces. According to investigators at the University of Michigan the proteoglycans also influence the fibrillogenesis of collagen and the organization of the fibrils into fibers. In vitro studies showed that the fibrillogenesis of collagen can be retarded or accelerated by placing the collagen in solution with different kinds of proteoglycans. Thus, the size of collagen fibrils, and hence the function of a tissue, may be a specific expression of the kind and amount of proteoglycans in that tissue.

Investigators at the University of Alabama had previously shown that the basement membrane, which controls the passage of material between the epithelium and the connective tissue, contains a family of related but distinct collagenous components. Since collagen synthesis is traditionally associated with fibroblasts, scientists expected to find that these cells provided the collagenous components of this intermediate structure. However, recent tissue culture experiments at the University of Michigan clearly demonstrated that epithelial cells produced basement membranes which were composed mainly of collagenous material and were antigenically identical with the native basal lamina produced in vivo.

Investigators at the University of Massachusetts have used the ia rat (incisor absent) as a model to study the origin of osteoclasts and the role of these cells in the defective bone resorption seen in this abnormality. Microscopically, they observed osteoclasts with no ruffled border associated with osteopetrotic bone. This condition could be cured by temporary parabiosis or by transfusing spleen, thymus, or liver cells from normal litter mates. After either parabiosis or transfusion, the bone contained functional osteoclasts with ruffled borders which resorbed bone normally and osteopetrosis was no longer evident. Future studies will show whether the bone was repopulated by transplanted cells or whether the mutant osteoclasts of the recipient were stimulated to function normally by some agent produced by the donor cells.

Investigators from Washington State University at Pullman and Washington University at St. Louis have shown that peritoneal macrophages can resorb bone in vitro. The Pullman group showed that when peritoneal macrophages were cultured with calvaria that had been frozen and thawed to destroy viable calvarial cells, the calcium within the calvaria decreased by 23%. Calvaria cultured without macrophages showed no decrease. Their evidence indicated that the bone resorption was due to the release of hydrolytic enzymes from the macrophages, rather than to the transformation of the macrophages into osteoclasts. The St. Louis group demonstrated that mouse peritoneal macrophages cultured with isotopically labeled bone matrix began to resorb bone matrix after only 3 hours of culture, and degraded particles at a nearly linear rate for 2 days. Contact between the macrophages and matrix was essential for optimal resorption.

MEETINGS SPONSORED

The Periodontal Diseases Advisory Committee (PDAC) held one meeting during FY 1979. Subsequently its charter expired and a new committee called the NIDR Programs Advisory Committee was formed. The new committee, chartered in May 1979, is composed of the Periodontal Diseases Advisory Subcommittee and the Dental Caries Advisory Subcommittee.

At the 17th and final meeting of the Periodontal Diseases Advisory Committee, Dr. Daniel Fine reviewed the various measures used for treatment and prevention in periodontal therapy. After discussing these measures, the committee concluded that more data are needed to determine the comparative efficacy of surgery, curettage, root planing, and antibiotics in the treatment and control of periodontal disease. The committee agreed that the natural history of periodontal disease is still unknown and should be studied. In another presentation, Dr. William Butler, described the structure of collagen and elaborated on the characterization techniques he uses in his laboratory.

The committee discussed the reasons why there are so few successful grant applications for clinical periodontal research, and urged the Institute to strengthen its efforts to develop more clinical research and training.

During the workshop on "Periodontal Therapy" held June 11 and 12, 1979, Dr. Jan Lindhe of the University of Gothenburg, Dr. Harald Loe of the University of Connecticut, and Dr. Sigurd Ramjford of the University of Michigan reviewed in detail their research on periodontal therapy. Participants agreed that the most important aspect of periodontal therapy is the removal of plaque and calculus from tooth surfaces; the selection of one surgical technique over another is not critical as long as access to the tooth surfaces is adequate for cleaning. The workshop members also agreed that periodontal therapy would be an appropriate topic for a consensus development conference at NIH.

Publications

The proceedings of the grant-supported International Conference on Research in the Biology of Periodontal Disease, held at the University of Illinois in June 1977, are now available to the public from the American Academy of Periodontology, Chicago, Illinois. A summary of the staff-organized workshop, "Leukocyte Function in Bacterial Diseases with an Emphasis on Periodontal Diseases", held in Bethesda in 1978, was published in the Journal of Infectious Diseases Vol 139: 604-612, 1979; reprints are available.

FUTURE PLANS

In order to develop adequate research on the multiple factors involved in periodontal disease the Program will expand both its grant and contract programs, and will continue to support basic research on oral biology in

health and disease.

Microbiological research over the past several years has identified about a dozen species of bacteria as causative organisms for different types of periodontal diseases. In recent investigations at the Periodontal Clinical Research Centers a systematic approach to the isolation, identification and characterization of periodontal microorganisms has begun. Researchers have shown that both Bacteroides melaninogenicus and Actinobacillus actinomycetem-comitans predominate in human periodontal lesions. They have also shown that these two organisms cause periodontal disease in animal models. Future studies will continue the systematic identification of suspected pathogens and will also focus on the mechanisms by which they cause disease.

A wide variety of local inflammatory reactions are mounted in response to antigenic challenges by periodontal microorganisms. Because of the technical difficulties of obtaining large samples of oral tissues, studies of these reactions have been carried out in vitro with peripheral blood leucocytes serving as the test tissue. Last year new techniques were developed to obtain inflammatory cells directly from the local gingival sites and studies were initiated to determine the validity of observations made previously on peripheral blood cells. Special efforts will be made to expand this promising area of research.

Preliminary results show that tetracycline is concentrated in the sulcular fluid after systemic therapy. Since the efficacy of tetracycline in periodontal therapy may be due to the concentration of this antibiotic in the sulcular fluid, program staff will support studies to elucidate this mechanism of tetracycline concentration, and will initiate studies to screen other antibiotics and antimicrobial agents for such properties. Continued efforts will also be made to develop slow release systems to deliver antimicrobials to periodontal lesions in the oral cavity.

In a recent workshop the participants discussed periodontal treatment and prevention, and recommended that staff plan a consensus development conference to evaluate different approaches to periodontal therapy.

SUMMARY

The Program awarded $6.5 million for 53 regular grants, 3 clinical centers, 1 program project, 12 new investigator grants and 7 career development awards. A total of $0.9 million was awarded for 6 institutional training grants supporting 37 trainees, and for 16 individual fellowships.

Microbiology. Investigators have intensified efforts to identify and characterize organisms associated with periodontal disease. At one center, they identify the predominant organisms only in the deepest portions of the periodontal pockets, whereas at another center, scientists seek to identify predominant organisms throughout the pocket. At a third center, investigators attempt to identify all organisms in samples taken from the soft plaque attached to deep portions of the tooth root.

These scientists have identified and characterized 1105 isolates from 25 sites in 19 patients and found 141 different species, 61% of which had not been described before. Eubacterium and spirochetes were abundant.

The evidence against B. asaccharolyticus, A. actinomycetem-comitans (Y4), and B. melaninogenicus has been strengthened by new findings. Patients with disease have numerous B. asaccharolyticus in periodontal pockets and show specific antibody to this organism. Moreover, the organism produces collagenase, fibrinolysin, and other destructive agents, and its capsule prevents leukocytes from engulfing it. Patients with localized juvenile periodontitis (LJP) have large numbers of the Y4 organism, and many such patients show specific antibody against the organism and the leucotoxin it makes.

In pregnancy gingivitis B. melaninogenicus ss intermedius increased when the levels of estradiol and progesterone increased. This organism also utilized more estradiol and progesterone in vitro than controls.

Host response research included studies on plaque-induced PMN lysosome release, polyclonal activators, and PMN malfunction. Plaque taken from human experimental gingivitis sites after 21 days caused the PMNs to release more lactoferrin than the 3-day plaque. The results suggest that destructive enzymes are also released. Factors released by PMNs incubated with A. viscosus also stimulated a 10-fold increase in mononuclear cell proliferation. Thus, lysosomal constituents could account for inflammation by more than one mechanism.

The abundance of lymphocytes and plasma cells in advanced periodontal lesions may be due to polyclonal activators, which stimulate B-cells to divide and differentiate, and to produce nonspecific antibodies and destructive lymphokines, such as osteoclast activating factor (OAF). Potent polyclonal activators have been found in many plaque organisms including B. melaninogenicus and A viscosus.

Investigators have extended previous findings that PMN chemotaxis and phagocytosis are impaired in LJP. Since this impairment persisted in all 10 patients who were treated for LJP, it is now considered to be an intrinsic trait.

Treatment. Frequent removal of plaque by professionals coupled with strict oral hygiene prevented periodontal disease. Patients given prophylaxis and hygiene instructions every 2 or 3 months for 3 years had healthier tissues than control patients given prophylaxis only once a year with no oral hygiene instructions.

Monitoring the flora by quantitative darkfield microscopy was helpful in determining the status of patients being treated for periodontitis. Before treatment coccoid forms predominated in normal sites, whereas motile rods, curved rods, and spirochetes predominated in diseased sites. After treatment, the tissues were improved and the flora of diseased sites showed a decrease in motile rods and spirochetes, and an

increase in coccoid cells.

Using a new assay for tetracycline in crevicular fluid, scientists showed that after systemic administration, crevicular levels peaked at 6-20 mg/ml whereas blood levels only reached 1.5-2.5 mg/ml.

Collagen and bone research. Mononuclear cells secrete a protein factor which inhibits collagen synthesis by fibroblasts. Proteoglycans influence the rate of collagen fibrillogenesis; some accelerate the process, whereas others retard it. The basement membrane was shown to contain collagen secreted by the epithelial cells rather than by fibroblasts, the expected cellular source.

In the ia rat (incisor absent) osteoclasts have no ruffled border and the bone is osteopetrotic because resorption is inadequate. When this condition was cured by parabiosis or transfusion, functional osteoclasts with ruffled borders were seen and osteopetrosis was absent. The functional osteoclasts were either donor cells, or recipient cells which had been cured by substances from the donor.

Prepared by P. F. Parakkal and A. A. Rizzo

ANNUAL REPORT 1979

CRANIOFACIAL ANOMALIES PROGRAM BRANCH

INTRODUCTION

The Craniofacial Anomalies Program Branch supports research and research training related to the prevention, diagnosis, etiology, and treatment of craniofacial malformations. The areas of research include cleft lip/palate, other congenital anomalies involving oral or craniofacial structures, disfigurements resulting from surgery or accidents, malocclusion of teeth and jaws, and the effects of anatomic malformation on oral function.

To achieve the program objectives of reducing the morbidity and mortality associated with craniofacial anomalies, more knowledge is needed at both the laboratory and clinical levels. Consequently, the Program provides research funds for studies ranging from basic investigations in developmental biology to epidemiological surveys. Clinical studies in growth and development and treatment are also supported. In preparation for a formal program evaluation to be completed next year, a major effort was made during FY 1979 to refine and bring into sharper focus the objectives of this program area. Staff effort was supplemented by three workshops covering the three major areas of research activities.

ADMINISTRATION

In FY 1979 grant support totaling $8.6 million was distributed among 88 research grants, which included 6 program projects, 70 regular research grants, and 12 new investigator awards. Five research contracts were awarded for $352,300. The Program also supported 47 trainees in research. Of the total support for trainees, $548,584 was awarded to support 37 fellows on 5 Institutional training grants, and $124,499 was awarded for 10 individual postdoctoral fellowships. Eight research career development awards were made at a cost of $292,446.

Table 1 shows the distribution of grant support according to funding mechanism. Approximately 25% of the grant funds were used for program projects, and 60% for regular grants. Table 2 shows the distribution of research grants by disease category. The specific categories of malocclusion, oral-facial clefts, and other craniofacial anomalies each received an increased funding compared to FY 1978. The increase overall was approximately 15%. This increase is also registered in Table 3 with respect to program classification. All show increased dollar support compared to FY 1978 except the area of epidemiology in which no projects were supported.

TABLE 1. FY 1979 RESEARCH SUPPORT BY FUNDING MECHANISM
(in thousands of dollars)

Type of Grants		No. of Grants Active	Funded	Cost	Percent
Program Projects	(P01)	6	6	$ 2,186	25.3
Regular Research	(R01)	83	70	5,090	59.4
Special Research	(R23)	12	12	325	3.8
Career Development	(K04)	8	8	292	3.5
Institutional Training	(T32)	5	5	549	6.4
Individual Training	(F32)	10	10	124	1.5
Conference	(R13)	1	1	2	0.1
		125	112	$ 8,568	100.0

TABLE 2. FY 1979 RESEARCH SUPPORT BY CATEGORY

		No. of Grants	Cost	Percent
I.	Craniofacial Anomalies-General	33	$ 2,383	31.5
II.	Cleft Lip/Palate	20	1,774	23.4
III.	Other Congenital Anomalies	8	1,381	18.2
IV.	Malocclusion	27	2,034	26.9
		88*	$ 7,572	100.0

*Does not include K04, T32, F32, and R13 Awards.

TABLE 3. FY 1979 RESEARCH SUPPORT BY CLASSIFICATION

		No. of Grants	Cost	Percent
I.	Etiology	46	$ 3,165	41.8
II.	Diagnosis and Treatment	24	2,688	35.5
III.	Pathology	18	1,719	22.7
IV.	Epidemiology	--	---	-- -
		88*	$ 7,572	100.0

*Does not include K04, T32, F32, and R13 Awards.

STAFF ACTIVITIES

During FY 1979, staff activities included visiting institutions, monitoring grants and contracts, communicating with researchers, participating in scientific meetings, and publications. Through these professional activities, staff was able to maintain close communication with scientists working in the field of craniofacial anomalies. These activities included:

A. Site Visits: Initial Review, Monitoring, Programming & Communication

University of Illinois, Chicago	Oct 1978
University of North Carolina, Chapel Hill	Oct 1978
Harvard School of Dental Medicine, Boston	Nov 1978
Eastman Dental Center, Rochester, New York	Nov 1978
University of Michigan, Ann Arbor	Nov 1978
New York University Medical Center, New York City	Dec 1978
University of California, San Francisco	Jan 1979
University of Washington, Seattle	Jan 1979
University of Maryland, Baltimore	Feb 1979
Eunice Kennedy Shriver Center for Mental Retardation, Inc., Waltham, Massachusetts	Apr 1979
University of Connecticut, Farmington	May 1979
University of Illinois, Chicago	Jun 1979
University of Minnesota, Minneapolis	Jun 1979
Eastman Dental Clinic, Rochester, New York	Jul 1979
Tufts University, Boston	Jul 1979
New York University Medical Center, New York City	Aug 1979
University of Texas, Houston	Aug 1979
University of North Carolina, Chapel Hill	Sep 1979
University of Michigan, Ann Arbor	Sep 1979

B. Meetings

1978 Annual Scientific Meeting of the American Congress of Rehabilitation Medicine and the American Academy of Physical Medicine and Rehabilitation, New Orleans; Presented Exhibit on "Acquired Craniofacial Defects."	Nov 1978
"Workshop on the Etiology of Facial Clefting," Grant No. 1 R13 DE 05213-01, Indiana University, held in Warrenton, Virginia. (Participant)	Dec 1978
36th Annual Meeting of the American Cleft Palate Association, San Diego, California	Feb 1979
Seminar for Establishing a Uniform Method to Assess Hypernasality in Cleft Palate Patients in North America, New Orleans. (Participant)	Mar 1979

American and International Association for Dental Research, and served as Moderator of the Symposium, "State of the Art Technology, New Approaches to Craniofacial Morphology," New Orleans. (Dr. Christiansen) Mar 1979

American Association of Orthodontists, Washington, D. C. May 1979

Craniofacial Workshop on Cleft Lip/Palate, sponsored by the Medical College of Georgia, Augusta. (Keynote Address by Dr. Christiansen) May 1979

The Teratology Society Meeting, Cedar, Michigan Jun 1979

New England Regional Conference on Standards for Service to Handicapped Children, Providence, Rhode Island. (Participant) Sep 1979

C. Sponsored Meetings and Seminars:

Workshop to refine the Research Objectives and Approaches for Acquired Craniofacial Disfigurement, Bethesda. Dec 1978

Planning Sessions for the "NIH Consensus Development Conference for Removal of Third Molars," to be held November 28-30, 1979, Bethesda. Apr 1979
Jun 1979

D. Staff Development

Biomedical Instrumentation Course, Princeton, New Jersey. Oct 1978

Training Course for Project Officers, Manassas, Virginia Oct 1978

The Federal Budget Process, Bethesda. Jun 1979

Effective Planning and Decision Making, Bethesda. Jul 1979

E. Publications

"The Future of Human Genetics Research and Craniofacial Anomalies in Federal Programs," in the book The Teaching of Human Genetics in Dental Education, The Proceedings of a Workshop Conference, (Ed. Kopel, H.) pp. 29-30, Dental Concepts. Inc., Los Angeles. (Dr. Christiansen) 1979

"Nomenclature and Classification of Craniofacial Anomalies," in the book The Teaching of Human Genetics in Dental Education, The Proceedings of a Workshop Conference, (Ed. Kopel, H.) pp. 77-82, Dental Concepts, Inc., Los Angeles. (Dr. Christiansen) 1979

"Lateral Pressures of the Relaxed Tongue," Angle Orthodontist, Vol. 49(1):44-46. (Dr. Christiansen) Jan 1979

"Facial Growth and Associated Cranial Base Defect," Angle Orthodontist, Vol. 49(2):92-97. (Dr. Christiansen) Apr 1979

RESEARCH HIGHLIGHTS

A. Developmental Biology

Studies at the Molecular and Cellular Level. Previous research has shown that the neural crest is involved in craniofacial morphogenesis. This embryonic structure gives rise to the skeletal and connective tissue structures of the craniofacial region as well as to certain muscular, neural, and endocrine elements. The mechanisms which control the migration and differentiation of the pluripotent neural crest cells have been studied by scientists at the Universite De Nantes in France. To explain the patterns of migration, these scientists suggest two possibilities: that preferential pathways or spaces within the tissue enable the cells to move to their destination without obstruction, or that neural crest cells are selectively attracted by chemical signals originating in specific regions of the embryo.

One group of neural crest cells, the autonomic neuroblasts, become localized in either sympathetic or parasympathetic ganglia and accordingly become committed to one of two possible metabolic pathways of neurotransmitter synthesis: they become either adrenergic or cholinergic. When transplanted to the vagal level (somites 1-7), neural crest cells from the adrenomedullary region give rise to cholinergic enteric ganglia. When transplanted into the adrenomedullary region, cells from any level of the mesencephalic or rhombencephalic crest colonize the suprarenal gland and give rise to adrenomedullary cells which synthesize adrenalin. These researchers also showed that the choice of neurotransmitter metabolism in the developing cholinergic ganglia could be reversed by transplanting cells from cholinergic ganglia back to an adrenomedullary level of the neural crest in a younger embryo. These transplanted cells reverted to neuroblasts and remigrated to an adrenergic site at which they became adrenergic synthesizers. Thus the neural crest is equipotential at all levels in its ability to provide the whole spectrum of autonomic ganglion and adrenomedullary cells. This being so, the cells in any given region are not preprogrammed for their ultimate function. These studies also suggest that the migration pattern is imposed on the neural crest by embryonic morphogenesis which establishes preferential migration routes. One pathway leads the cells toward the gut in the vagal region, another guides them to the suprarenal gland. Whether the neuroblast ultimately differentiates into an adrenergic or cholinergic producer is determined by extrinsic signals from the environment into which it migrates.

Scientists at the University of Southern California are studying the cellular aspects of vascularization. When endothelial cells from the aortas of 1- to 2-day old calves were cloned at a high efficiency in fibrin-coated dishes, both the primary cultures and the clones produced high fibrinolytic activity which was 90% dependent upon the presence of plasminogen. This high level of specific proteolytic activity was found in both the cell lysate and the culture medium. Both the synthesis and secretion of this protease increased during the log phase of cell growth, reached a maximum at cell confluency, and remained at high levels thereafter. The morphological growth patterns of these endothelial cells was comparable to those of transformed neoplastic cells when they were grown in acid-treated fetal calf, dog, or human serum. Furthermore, the cells demonstrated anchorage-independent growth, forming large colonies in semisolid media. Spontaneous neoplastic transformation of these cells was excluded by chromosomal analysis and lack of tumorigenicity in nude athymic mice.

High fibrinolytic activity, morphological changes in the appropriate serum, and growth in semisolid media are characteristics of transformed cells. Since their results had shown that endothelial cells share these characteristics with transformed cells, the investigators hypothesized that vascular endothelial cells are basically invasive cells. However, in vivo they are regulated in some way, possibly by tissue protease inhibitors or specific inhibitors of endothelial cell proliferation. Subsequent experiments in which cartilage was used as a nonvascularized "model" tissue supported this hypothesis. These experiments also isolated a factor from the cartilage which selectively inhibits endothelial cell proliferation but does not affect the proliferation of smooth muscle cells or fibroblasts. Preliminary studies indicate that this factor arrests the endothelial cell cycle in the G phase.

Studies at the Tissue Level. Researchers at the University of North Carolina have continued their studies on the formation and enlargement of tissue masses in the embryonic face. To follow the migration of cells in the face, they developed implant labeling procedures, using sable hair probes as carriers to introduce tritiated thymidine marker in precise areas of the neural crest. Probes, labeled with tritiated thymidine, were inserted into the facial processes and adjacent structures of chick embryos in ovo. Embryonic tissues were processed and the resulting autoradiographs showed that localized labeling could be achieved with this technique which could also be used to analyze morphogenetic movement. Using this technique to examine older specimens, investigators observed that lateral nasal process mesenchyme remained in a nearly constant position relative to the optic chiasma. By contrast, the maxillary process grew forward, inferior and medial to the lateral nasal process, as a distinct and separate population of mesenchymal and epithelial cells. Furthermore, the maxillary process grew medially

over the roof of the stomodeum to contribute most of the mesenchymal and epithelial components of the developing nasal septum. In addition, a major translocation of mesenchyme occurred between the medial and lateral nasal process. These precise data on the morphogenetic movements involved in early facial development are basic to an exact knowledge of the pathogenesis of cleft lip and other facial anomalies.

At the University of Maine experimental studies were made on the regions of membrane-bone formation in the upper and lower jaw and palate of vertebrate embryos. Results showed that the bone-forming tissue needed the overlying epithelium to induce bone formation. Recombination studies indicated that this need was not absolute. Epithelium from other body regions (e.g., the limb-forming region) as well as from an older embryonic stage were adequate to initiate osteogenesis in the bone-forming tissue. The need for epithelium exists only during a specific time period early in the development of the facial region.

B. Cleft Lip and Palate

Etiology and Pathogenesis. In the mouse the complex genetic locus, (H-2), first discovered because it affects graft rejection, has been associated with other cellular and developmental processes including cleft palate. The frequency of cleft palate in offspring of pregnant mice given steroids depends on H-2 types. The percentage of cleft palates in offspring of these animals ranges from about 20% to over 80% depending upon the major histocompatibility (H-2) type of the mother. Research workers at Johns Hopkins University believe that the relation between the incidence of cleft palate and H-2 is based on the production by the H-2 gene complex of substances which inhibit cell adhesion. According to this hypothesis the lack of initial adhesion of the cells overlying the palatal shelves of the embryo prevents the shelves from fusing and a cleft results. Thus far, the scientists have found that the adhesiveness of single adult liver cells varies with their H-2 type. These experiments are being continued to compare the adhesion rates of cells of different types and to attempt to interfere with adhesion by means of antibodies to H-2 products.

Scientists at the University of Pennsylvania are taking a different approach to determine how steroids produce cleft palate in mice. They examined the inhibitory effects of corticosteroids and other drugs on an important developmental change, the programmed cell death of the epithelium of the medial edge in the fetal palate. Failure of normal epithelial breakdown could result in palatal clefting because intact epithelium prevents the underlying connective tissue from penetrating across the midline. Recent evidence has shown that the basic biochemical action of anti-inflammatory

corticosteroids is to interfere with phospholipid metabolism and to depress arachidonic acid release and thereby to reduce prostaglandin synthesis from this precursor. Investigations at Pennsylvania are aimed at testing whether certain teratogens function through anti-inflammatory mechanisms by inhibiting the normal release of arachidonic acid from the phospholipids of palatal membranes. Previous work by this group showed that the administration of arachidonic acid reduces the high incidence of steroid-induced clefting. Additional evidence shows that the anti-inflammatory drug, indomethacin, reverses the effects of arachidonic acid and thereby promotes increased clefting. Indomethacin presumably inhibits prostaglandin cyclo-oxygenase and thus blocks the transformation of arachidonic acid to prostaglandin G_2, the precursor of all other prostaglandins. These findings seem to implicate the anti-inflammatory pathway in clefting and to emphasize the need to study more precisely the effects of arachidonic acid and prostaglandins on palatal programmed cell death.

Each year in the United States, an estimated 5,000 to 14,000 children are born to epileptic women on anticonvulsant drug therapy. At least 11% of these children are believed to be affected by a variable combination of abnormalities referred to as the Fetal Hydantoin Syndrome, so named because it is usually associated with the commonly used therapeutic drug, diphenyl hydantoin, or phenytoin (also known by the brand name Dilantin). Although cleft lip was the first pathologic condition to be linked to teratogenic anti-epileptic drugs, mental retardation is now believed to be the most common and most serious consequence.

Investigators at the University of North Carolina have extended their earlier studies on the mechanism of action of anti-epileptic drugs in the formation of cleft lip. In those studies on the genetically susceptible A/J mouse, they established that a single oral dose of Dilantin given to a pregnant mouse at a precise time and in an amount sufficient to raise the blood levels to the human therapeutic range caused a 4 to 5 times greater incidence of cleft lip in the offspring than in those of untreated animals.

During the past year, single (intraperitoneal) injections of phenytoin resulted in an almost 100% incidence of cleft lip without increasing embryonic deaths (resorptions) or other external malformations. Using this approach, the scientists examined in detail the developmental alterations leading to cleft lip. Growth of the lateral nasal processes was severely retarded; as a result, these processes failed to achieve sufficient size for contact and subsequent fusion with the medial nasal process to form a normal lip. This growth reduction was associated with the absence or severe restriction of a cell process meshwork of the mesenchymal cells which is usually seen in the normal growth process.

A similar mechanism may be involved in other developmental abnormalities that resulted from phenytoin administration. This interpretation is strongly supported by recent observations at Georgetown University, where investigators administered phenytoin to A/J mice bearing fetuses one day younger than those at North Carolina. This treatment produced changes in the cell process meshwork of developing limb buds which were almost identical with those seen in cleft lip formation. These changes were associated with gross finger abnormalities (digital reductions) similar to those seen in the human Fetal Hydantoin Syndrome.

Patient Management. Clinical investigators at the University of Iowa are using beagle dogs as models to improve the treatment of human cleft lip and palate. Current surgical procedures for repairing cleft palate include the precaution of placing the incision as far from the dentition as possible. Cleft palate surgeons have observed this difficult precautionary measure for many years, even on the small palates of human infants. Recent data from the beagle dog experiments, however, indicate that this precaution is not only not necessary but may even be undesirable. If confirmed by cross-validation, these findings will facilitate improvements in surgery and may lead to long-range benefits for patients. Normal speech development and maintenance require a certain degree of palatal length and mobility, which may be easier to achieve if the surgeon can use wider palatal flaps without damage to the dentition.

When cleft palate surgery is performed early in life, maxillofacial defects may develop during subsequent growth and development. Before 1974, little attention was paid to surgery for cleft lip as a potential etiologic factor in such defects. Since then, however, the research team at the University of Iowa Cleft Palate Center has developed a more balanced program which now considers how surgery for cleft lip affects the development of the maxillofacial complex.

Midfacial retrusion with incisor crossbite is a common sequel to surgical repair of clefts of the lip and palate. Since the vomer and other septal structures are often involved in such surgery, research directed at the effects of vomer surgery on subsequent growth is of considerable clinical importance. In a study of this problem, Iowa investigators removed a major part of the vomer bone in one group of beagle dogs, subjected a second group to sham operations, and used a third group as controls. Resection of the vomer caused decreased antero-posterior growth of the maxilla immediately after surgery and severe maxillofacial deformity later on. The data seem to support the contention that surgery for cleft palate may adversely affect maxillofacial development.

C. Other Craniofacial Anomalies

A limited but definite number of human birth defects are caused by viruses and other infectious agents; a notable example is the constellation of abnormalities caused by prenatal infection with rubella virus. Therefore, the NIDR supports research to investigate the effect of maternal virus infections on newborns.

At the Institute for Medical Research in Bennington, Vermont, scientists have studied the craniofacial anomaly and tooth loss seen in embryonic and newborn hamsters whose mothers had been infected with H-1 parovirus. Two main conclusions have been drawn from their studies. First, the morbidity and/or mortality of the hamsters varies according to the day of pregnancy that the virus is given; second, the virus locates in mesenchymal tissues only, especially in those that are actively proliferating, such as the base of developing cartilage and in tissues within the tooth pulp. In the latter the odontoblasts and dentine degenerate preferentially while the connective tissue supporting the teeth deteriorates at the same time. Thus, the loss of teeth is an unavoidable sequel to this virus infection. The H-1 parovirus also attacks the osteoblasts of the skull flatbones, destroying them and leaving bone remnants and defects that cause the flattened foreface characteristic of the H-1 parovirus-treated hamster. Sections of skin from infected embryos showed virus in the nuclei of all areas of connective tissue, including the connective tissue sheath and papillae of the hair follicles, but not in the rest of the developing hair and skin epidermis. Why the epidermal cells are resistant and the mesenchymal cells susceptible to H-1 parovirus is not yet known. The investigators hypothesize that surface receptors for the virus may differ in the two classes of cells.

In additional studies, different hamster mothers were injected with the same amount of virus on different days of the 16-day gestation period. The resulting incidence of morbidity/mortality among the babies formed a tri-modal curve with a small peak at 3 days, a wider and higher peak at 8 to 10 days and a sharp peak at 13+ days. A curve of progesterone levels in the average pregnant hamster during gestation showed the same tri-modal pattern. When the progesterone level was high, the morbidity/mortality effect of the virus was also great. When progesterone levels plummeted on the 15th day, no virus effect on the embryos was seen except when large amounts of virus were given. The mothers often did not produce antibodies even when given fairly high doses, but instead became tolerant to the specific parovirus for the rest of their lives, even though they could produce antibodies to other paroviruses. Scientists believe that these results may represent the suppression of thymus lymphoid "T" cells by progesterone. Future experiments are designed to show a direct relation between proges-

terone and maternal and embryonic lymphoid proliferation and the degree of craniofacial deformity in embryos and newborn infants.

D. Malocclusion

Bone Growth and Remodeling. How bone is remodeled by the physical forces exerted on teeth is of interest to basic and clinical scientists. Investigators at Case Western Reserve University are conducting research to determine how the collagenous fibers of the periodontal membrane and the periosteum maintain their attachment to resorbing bone surfaces that are undergoing remodeling. So far, they have found at least two mechanisms for fibrous attachment on such resorbing surfaces. One involves a direct conversion of bone matrix fibers into periodontal and periosteal fibers while the rest of the bone surface undergoes resorption. Documentation of this process at the EM level has added to data from their previous light microscope studies. The second mechanism by which fibers anchor onto resorptive bone surfaces has never before been described. Under the EM, scientists have discovered a layer or zone of glue-like substance at the membrane-bone interface. The composition of this zone has not been determined, but it is homogenous in structure, merges directly with the collagenous fibers on the membrane side, and appears to be cemented onto the bone surface on the other side. The substance of this layer may reattach the connective tissue of the periodontal or periosteal membrane onto bone surfaces in which the connective tissue has become detached either by normal remodeling resorption or by surgical dysjunction.

In continuing studies on the biochemical mechanisms involved in orthodontic tooth movement, scientists at the University of Pennsylvania are now studying the role of prostaglandins, biologically active substances that mediate a wide array of physiological effects. Indirect evidence indicates that prostaglandins of the E and F groups participate in bone remodeling. The Pennsylvania workers have developed a method for extracting and measuring prostaglandins from bone. They have also adapted a micro method for detecting DNA in bone, so that quantities of bone prostaglandins can be related to bone cell numbers. Using these methods, they sampled the bone around orthodontically treated canine teeth in cats and found that prostaglandin E_2, the most potent bone-resorbing prostaglandin, increased after 1, 7, and 14 days of treatment. In areas of tissue tension, the increase was most pronounced at the earliest period, whereas at areas of tissue compression, the increase was more pronounced at the later period. These results suggest that certain prostaglandins are produced locally and exert their effect on bone during orthodontic tooth movement. Thus, such drugs as aspirin, which inhibit an enzyme responsible for producing prostaglandins, could affect the course of orthodontic treatment in human patients.

During the last three years, investigators at the University of Connecticut have demonstrated that under controlled culture conditions, mechanical forces of physiological magnitude and electrical perturbations of the "capacitor" type affect the proliferation of bone and cartilage cells. By monitoring several biochemical changes, they have obtained evidence that the external perturbation is transduced into a message at the cell membrane via changes in cyclic nucleotide levels and in cation distribution. For example, their recent studies on the effect of mechanical pressure on the induction of ornithine decarboxylase, an enzyme which rises early in cell division, point to K^+ (potassium) as an important regulatory ion. Sensitivity to K^+ can be altered pharmacologically by local anesthetics or K^+ permeators (ionophores). Current research may show how such drugs may be used to modulate the effects of mechanical forces on the growth of skeletal tissue.

For the last several years, orthopedic researchers have treated nonunion (nonhealing) fractures with electrical stimuli. One of the methods uses oscillating external electromagnetic fields (EMF) to produce a therapeutic electrical perturbation. Using these clinical devices, basic scientists at Connecticut found that low-intensity EMF pulses caused a 50 to 100% increase in DNA synthesis in cultured bone cells. This effect depended on many aspects of the signal itself, such as pulse frequency and shape, as well as on cell culture conditions. The demonstration of the EMF effect on bone cells in vitro strengthens the scientific rationale for this form of clinical treatment and offers an experimental tool for screening various signal modalities. Further investigation is designed to determine the relation between the EMF effect and cell specificity (cell type and cell cycle stage), response specificity (cell division vs. matrix production), and the biochemistry of signal transduction.

Clinical Research. Clinical investigators at the University of Michigan have examined the inheritance of the morphology of the craniofacial complex in the nuclear family. The maxilla, mandible, cranial base, and cranium of members of the family were compared for similarity or dissimilarity. Computer graphics were used to make a quantitative analysis of shape differences.

The siblings were found to be similar to the average of their parents (termed midparent) or some similar value 85% of the time, but the frequency of similarity to mother or father alone was only 10%. In analyses of the number of bones found to be similar between siblings and parents, the midparent produced the maximum number of similar scores. The fact of the close similarity between children and the midparent, despite discernible difference between parents, led these investigators to conclude that craniofacial variability is the result of a polygenic effect and not a major single gene effect. These results have prompted these workers to

stress the importance of polygenic inheritance in craniofacial variability and to conclude that the nuclear family offers the optimal unit against which to compare the individual patient in diagnosis and treatment planning.

Respiratory allergies create breathing difficulties and frequently lead to mouth breathing. Since clinical observation indicates that these conditions may affect the growth of the jaws, scientists at the University of Pittsburgh are attempting to determine whether early correction of allergies will prevent or cure dentofacial deformity. In the first phase of the study, they confirmed the belief that respiratory allergies alter the pattern of growth of the face and jaws; allergic children had longer and narrower faces than normal. In the second phase, they will treat the allergies of these patients to determine whether their facial proportions will return to normal as a result.

FUTURE PLANS

The studies identified in this report will be continued in FY 1980. Developmental biology studies will emphasize the role of cell membranes in growth and development, with particular attention to transmembrane communication and the morphogenetic signals involved. These signals are thought to be important in the development of the craniofacial structures from primordial tissues derived from neural crest cells. Studies of the migratory pathways of labelled neural crest cells in the craniofacial region will also be continued.

Studies to address the functional problems associated with trauma to facial musculature will be initiated in 1980. For example, surgical procedures for improved treatment of facial paralysis, which may result from injuries, will be studied in animals. These procedures will involve skeletal muscle transplantation and translocation of portions of muscles. The projected studies will examine the impact of loss of innervation of muscles and test the feasibility of reinnervation from a different nerve source. Thus, our understanding of the biology of adaptation of skeletal muscle will be increased.

Since orthognathic surgery has become an increasingly common method of treating dentofacial disfigurement, the postoperative sequelae associated with this approach must be thoroughly studied. Therefore, projects to examine the factors which may produce instability of the treated jaws will be initiated. These factors include biting forces, swallowing patterns, respiration, tongue position, and overall posture of the head and neck structures. Identifying the critical factors which produce instability will enable clinicians to alter the treatment and improve prognosis.

A formal NIH Consensus Development meeting will be held in FY 1980 to assess the indications and contraindications for removal of third molars. This conference was organized because there is limited scientific evidence and major differences of opinion about the influence of third molars on occlusion, on the growth potential of the jaws, on periodontal health, and on future prosthetic needs. Discussions will be held on the varied pathologies associated with third molars, and on the optimal time and techniques for removal. The conferees will also identify the areas where further research is needed.

A formal evaluation of the Craniofacial Anomalies Program will be conducted in FY 1980. This evaluation will examine the adequacy of the NIDR programs in the three major areas of congenital defects, dentofacial malrelations, and acquired defects. In preparation for the evaluation, staff and consultants have recently completed a refinement of the goals, objectives, and approaches of each of these three subcategories. It is anticipated that the evaluation will be a valuable tool for program planning.

SUMMARY

During FY 1979 the Craniofacial Anomalies Program Branch made grant awards totaling $8.6 million, which included $5.4 million in individual research grants and $2.2 million in program projects. Eight career development awards totaled $292,446, and 37 fellows were supported on 5 institutional training grants for a cost of $548,584. Ten postdoctoral fellows were supported on individual grants, totaling $124,499. Research contracts totaled $352,300.

Research highlights in this report emphasize studies on the mechanisms which control the migration and differentiation of neural crest cells. Since these cells form the skeletal and connective tissue framework of the face, studies of neural crest cells are important for understanding both normal and abnormal development of the craniofacial region.

Studies of vascular tissue formation have shown that during normal development, vascular endothelial cells are highly invasive, but are carefully regulated. Knowledge of these regulating mechanisms are important not only for understanding development but also for understanding neoplasia.

Research on the formation and enlargement of tissue masses in the embryonic face has advanced significantly as a result of studies using sable hair probes to precisely label neural crest cells with radioactive markers. The detailed knowledge of cell migration patterns gained by this technique is providing an understanding of the pathogenesis of cleft lip and other facial anomalies.

Studies of the relationship between histocompatibility types and cell adhesiveness have shown how specific genes may affect the initial fusion of palatal shelves, a development process important in cleft formation. Other basic research is directed at the question of how steroids produce cleft palate in mice. Findings suggest that steroids interfere with programmed cell death in the medial edge of the palatal shelves and thereby prevent fusion. Some evidence indicates that the mechanism of drug action here is the same as that which produces anti-inflammatory effects.

Follow up studies of the role of anti-epileptic drugs in the formation of cleft lip have shown that in the Ajax mouse fetus, Dilantin causes a severe retardation of growth in the lateral nasal process. Formation of a cleft lip occurs because the size of this process is insufficient for normal contact and fusion with the medial nasal process. This growth reduction is associated with inhibition of the mesenchymal cell process network which normally develops. This cell process network is also absent in limb abnormalities produced by administering Dilantin to Ajax mice earlier in pregnancy than in the cleft lip experiments.

Studies of craniofacial anomalies in hamsters caused by H-1 parovirus infection have shown that the organism locates only in mesenchymal tissue and primarily in that which is actively proliferating. The incidence of morbidity/mortality among these hamster babies varied directly with the progesterone levels of the mothers at the time of virus inoculation. Since high progesterone levels of the mother tend to suppress "T" lymphocytes, these findings suggest a relationship between this anomaly and embryonic lymphoid proliferation.

Studies of biochemical mechanisms involved in orthodontic tooth movement suggest that locally produced prostaglandins exert effects on bone during orthodontic tooth movement. These studies as well as others relating enzyme induction and the application of force to teeth give promise of future applicability in clinical treatment. It seems possible that certain drugs may be useful in modulating the effects of mechanical forces on the growth of skeletal tissues.

Family studies of the inheritance of craniofacial variability have failed to demonstrate a simple genetic basis for most of the variation encountered. Research in this area points to a polygenic or multi-factorial basis for the common forms of malocclusion encountered by the orthodontist.

Human and animal studies indicate that respiratory function affects facial development. Respiratory allergies may prove to be important in the causation of a significant number of malocclusions.

Prepared by Richard L. Christiansen and Jerry D. Niswander

ANNUAL REPORT FY 1979

RESTORATIVE MATERIALS PROGRAM BRANCH

INTRODUCTION

The Restorative Materials Program serves as a primary focus at the NIDR for supporting research and development in dental biomaterials and instrumentation. Since the sequelae of oral diseases are damaged tissues, there is a continuing need for materials to repair these tissues and restore function and appearance.

Through grants, contracts and interagency agreements, the Program funds research in the development of new and improved materials. These research areas fall into eight categories: 1) restorative filling materials for repairing teeth; 2) bonding agents, adhesive coatings and cements to prevent decay on the chewing surfaces of teeth and at the margins of fillings; 3) intraoral prostheses for replacing missing teeth and other oral tissues; maxillofacial prostheses to replace defects resulting from congenital abnormalities, surgery, or accidents; 4) artifical tooth implants to replace missing teeth and to serve as anchors for bridges and dentures; 5) materials and techniques for improved root canal therapy; 6) transplants and replants of natural teeth; 7) diagnostic equipment and devices to improve dental care; 8) improved restorative materials for prevention. Special emphasis is given to clinical studies of implants and maxillofacial materials.

ADMINISTRATIVE

Table I shows the distribution of funds for research and research training in FY 1979. The Program area supported 46 research grants (including 6 research career development awards) totaling approximately $2.5 million; 1 contract and 2 interagency agreements totaling $562 thousand. The Program also awarded $397 thousand for 6 research training grants, and $45 thousand for 3 individual postdoctoral fellowships.

FY 1979 DISTRIBUTION OF FUNDS

RESTORATIVE MATERIALS PROGRAM BRANCH

Program Area	No. of Projects	Obligated Funds (thousands)	% of Funds
GRANTS[1]			
Filling Materials	18	$ 855	35
Bonding Agents	2	88	4
Prostheses (Oral)	6	394	16
Prostheses (Facial)	3	226	9
Implants	9	541	22
Transplants & Replants	2	64	3
General Studies	4	124	5
Prevention	1	54	2
Endodontics	1	68	3
Total	46	$ 2,414	99
CONTRACTS AND INTERAGENCY AGREEMENTS			
Filling Materials	1	$ 317	56
Bonding Agents	1/4	68	12
Prostheses (Oral)	1 (approx.)	177	31
Prostheses (Facial)	1	0	
Total	3	$ 562	99
TRAINING AWARDS			
Institutional Grants	6	$ 397	90
Fellowships[2]	3	45	10
Total	9	$ 442	100
GRAND TOTALS	58	$ 3,049	

1. Includes 6 Research Career Development Awards for $222 thousand

2. One of these did not receive FY 1979 funds

STAFF ACTIVITIES

During FY 1979 Program staff made numerous visits to research institutions to program and monitor grants and contracts. Through these professional activities and participation in scientific meetings they were able to stay abreast of scientific developments and to maintain close liaison with scientists working in the area of restorative materials. These professional activities are listed below.

A. Site Visits: Initial Review, Monitoring, and Programming

University of North Carolina	Oct 1978
Gulf South Research Institute, New Orleans	Apr 1979
Tulane University, New Orleans	Apr 1979
Forsyth Dental Center, Boston	May 1979
Harvard School of Dental Medicine, Boston	May 1979
University of Arizona, Tucson	Jun 1979
University of Colorado, Denver	Jun 1979
PHS Hospital, San Francisco	Aug 1979
VA Hospital, Sepulveda	Aug 1979
University of the Pacific, San Francisco	Aug 1979
Harvard School of Dental Medicine, Boston	Sep 1979
National Bureau of Standards, Gaithersburg	Sep 1979

B. Scientific Meetings

Annual Meeting of the Amer. Acad. of Implant Dentistry, Las Vegas	Oct 1978
Second International Prosthodontic Congress, Las Vegas	Oct 1978
Invited Lecture by Dr. Valega at the Conf. for Teachers of Dental Materials, W. Va. University, Morgantown	Nov 1978
Amer. Assoc. of Dent. Schools, New Orleans	Mar 1979
Inter. Assoc. of Dent. Res., New Orleans	Mar 1979
Amer. Nat. Standards Committee, New Orleans	Mar 1979
Amer. Acad. of Oral Path., San Diego	Apr 1979
Society for Biomaterials, Clemson, S.C.	Apr 1979
D.C. Dent. Soc. Annual Mtg., Wash.,D.C.	Apr 1979
NASA and Case-Western Dent. Implant Assess. and Recommend. Seminar, Cleveland	May 1979
Sixth Annual Symp. on Coordinating Clin. Trials, Boston	May 1979
American Chem. Soc. Nat. Org. Chem. Symp., Tucson	Jun 1979
Gordon Conf. Science & Techn. of Biomaterials, Tilton, N.H.	Jul 1979
Invited Lecture by Dr. Valega at the Fifth Congr. of the Hellenic Soc. of Oral Implantology, Thessoloniki, Greece	Aug 1979
American Chem. Soc. Nat. Mtg., Wash., D.C.	Sep 1979
Amer. Dent. Assoc. Annual mtg., Dallas	Oct 1979

C. Staff Development

Grants and Contracts: "The Mystique of Management"	Feb 1979
Performance Appraisal Training-Phase I	Sep 1979

D. Additional Staff Activities

Dr. Valega was appointed by the NIH Associate Director of Extramural Research and Training to a committee to revise the instructions for the "Summary Progress Report" section for Type 5 research grant applications (PHS form 2590-1).

Dr. Reese will serve as a member of the Review Panel for the Extramural Associates Program.

A program exhibit entitled, "Clinical Evaluation of Restorative Dental Materials" has been developed in collaboration with Dr. Joseph Moffa, U.S. Public Health Service Hospital, San Francisco, California.

The exhibit was initially displayed at the American Dental Association 120th Annual Session, October 21-25, 1979, in Dallas, Texas, where it received a Certificate of Honor, Second Award-category.

RESEARCH HIGHLIGHTS

Restorative Materials

To hasten the acceptance of new and improved dental restorative materials, investigators must determine clinical efficacy and safety, promptly and efficiently. Such a task requires rapid acquisition of information from many human subjects and intelligent interpretation of the data. Dental investigators at the Public Health Service Hospital in San Francisco recently devised an automated system of minicomputers which can develop, maintain, and analyze comprehensive records of dental treatment and related clinical observations. This system enables scientists to rapidly compare the clinical performance of any combination of materials and thus facilitates research leading to improved care and longer lasting restorations. The new system currently contains clinical information on more than 3,000 restorations in 900 patients and can provide longevity data on as many as 50 different filling materials. The Public Health Service investigators are using the system in two current projects. In one, a tooth-colored, composite plastic filling for restorations in posterior teeth is being tested, and in the other, silver amalgam formulations which promise increased longevity are being evaluated. The findings to date are described in the following paragraphs.

Efforts to develop composite plastic filling materials suitable for posterior teeth continue because these materials are similar to natural tooth structure in appearance, electrical conductivity, and thermal behavior and do not corrode or fracture easily. Scientists are, however attempting to counteract their tendency to wear down too quickly by adding other components. In one study, they added minute particles of strontium glass filler and later evaluated the resulting filling material in controlled clinical studies of 198 restorations in 56 patients. The new filling material did not show the excessive wear and disintegration seen in earlier plastic materials. After 5 years, more than 90% of the restorations showed only minimal wear and excellent marginal adaptation to the adjacent natural tooth structure. Moreover, the new material proved to be highly stable in color. As a result, a proprietary prototype material similar in composition and properties to the material tested has been introduced into the dental market and should provide a model for developing other esthetically acceptable, wear-resistant filling materials with broad dental applications.

The restoration of teeth damaged by dental caries accounts for much of the total cost of dental care in this country. The limited longevity of the restorative materials now in use has led investigators to search for a means to improve longevity and thereby to reduce the cost of dental care. About 85 per cent of the restorations of posterior teeth consist of silver amalgam which often fails at the margins even when the cavity is properly prepared and the material is well compacted and carefully carved to the margins.

Some laboratory studies indicate that the edges fracture only after corrosion has occurred in the gamma-2 phase, a structural component of the alloy. Earlier laboratory studies had showed that the weak gamma-2 phase could be eliminated by adding specific proportions of either gold or copper to the alloy. These new alloys appeared to perform better clinically than those without gold or copper, but long-term human clinical studies have not yet been completed. Currently, Public Health Service investigators are using the new automated minicomputer system to evaluate the performance of several of the new copper-containing alloys. After three years, two high copper alloys, Dispersalloy and Tytin, showed resistance to corrosion and maintained a superior marginal adaptation without fracture, whereas a third high copper amalgam alloy, Sybraloy, showed high rates of tarnish, corrosion, and marginal breakdown. Apparently, adding copper to dental amalgams does not guarantee resistance to corrosion and marginal breakdown; other factors are also involved.

Investigators at Indiana University and at Louisiana State University are studying the relation of "creep" and corrosion to the marginal fracture of dental amalgam fillings. Creep is the deformation that occurs when silver amalgam fillings are subjected to pressure. When scientists have measured the rate of creep of different amalgams under specified laboratory conditions and have later tested these amalgams clinically over long periods of time, they have found that amalgams with high creep rates generally undergo more marginal fracture and do not

last as long as amalgams with low creep rates. Nevertheless, since their results are not absolutely clear cut, there is growing controversy among materials scientists about whether creep is directly related to the breakdown of the dental amalgams. Some investigators believe that corrosion is more important and that creep is incidental. To clarify the issue, investigators at Louisiana State studied the various microstructural phases which make up the composite structure of amalgam and related them to creep and corrosion behavior. Tests indicated that the creep rate of the experimental amalgam rose with an increase in either the gamma-1 or gamma-2 phase or both, whereas corrosion increased only with an increase in the gamma-2 content. Thus, these studies have clarified to some extent the relation between creep and corrosion but not between these phenomena and marginal fracture.

The scientists at Indiana University have taken a different approach to the problem of amalgam creep and marginal fracture. Observing that previous studies had used different alloy systems, they argued that the exact effect of creep on marginal breakdown could not be isolated from other variables, such as alloy composition and the nature of the particle. Hence, they tested a single commercial alloy and found that the creep could be markedly altered by varying the trituration time from 5 to 18 seconds. The creep of the 5-second mix was 45 per cent less than that of the 18-second mix while other properties remained the same. Encouraged by these findings, the investigators then conducted a clinical study on alloys with different rates of creep which resulted from different trituration times. After 3 years, evaluation of 39 paired restorations showed significantly less marginal breakdown in amalgams with low creep (mixed for 5 seconds) than in those with high creep (triturated for 18 seconds). Examination of microstructure of the amalgams triturated for different times showed that the two mixes produced different microstructures.

The LSU investigations indicate that predicting the clinical efficacy of a dental amalgam involves more than its creep value in vitro; on the other hand, the Indiana research indicates that the creep values are indicative of the relative propensity for marginal breakdown. The correlation between the different microstructures, marginal breakdown, and creep values is being investigated further.

Prostheses

Since metallic elements in the pure state seldom provide optimum properties for a given purpose, alloys must be developed to achieve the desired characteristics. To do so, scientists first conduct basic laboratory studies to determine how individual metals interact and what structures result from combining different proportions of each metal at different temperatures. Next they select promising compositions from these data for further research and development. If all of the possible combinations of the 50 metallic elements and the 10 nonmetallic elements that can form alloys were known, the task of developing precise alloys for specific purposes in dentistry or in other fields would be greatly

facilitated. Since, however, the systematic testing of different alloy combinations had yielded only incomplete data, scientists at the American Dental Association Laboratories at the National Bureau of Standards began more than a decade ago to develop experimental data on binary combinations of about 20 transition elements which could prove useful for dental application. These elements include, besides silver, copper, mercury, and gold, other metals with exceptional resistance to corrosion. From their systematic exploration of the properties that result from the binary combination of transition elements with noble metals, the investigators have amassed a great deal of useful information which they have expressed as "phase diagrams." A phase diagram is a graphic representation of the changing properties of an alloy when the temperature and composition are varied. These new data, added to existing information, have enabled these scientists to develop a comprehensive, easily interpretable arrangement of over one hundred color-coded phase diagrams on a large wall chart. Metallurgists can now easily identify new relations and immediately apply this information to the development of improved alloys. This important data source should greatly facilitate the development of better alloys not only for dental appliances but also for a wide range of industrial applications. In fact, important studies on the potential use of titanium in casting alloys for dental crown and bridge work have already begun.

Endodontics

To achieve successful endodontic therapy it is necessary to clean and ream the root canal thoroughly and to fill it with inert biocompatible material. Investigators at the University of Connecticut Health Center, School of Dental Medicine, are developing new methods to evaluate the efficacy of endodontic materials. Not only are they devising methods to test the biocompatibility of endodontic filling materials, but they are also attempting to establish better biologic standards and criteria for performance and safety and to establish tests to determine whether new products meet these criteria.

In their current research three endodontic materials, AH 26, Kerr Sealer, and Kloroperka N.O. were evaluated for biocompatibility in both *in vitro* and *in vivo* models. In the *in vitro* experiments, all three materials demonstrated high toxicity. The *in vivo* research, carried out in rats, guinea pigs, and baboons, also showed that the three materials are toxic. The methods of evaluation included spectrophotometric, autoradiographic, SEM and electron probe, and histological techniques. To arrive at a final assessment of a given material, the investigators correlated the results of these techniques. The protocol they have developed provides a reliable screening approach for use by manufacturers in developing new products and for maintaining quality control. As a result of the research, specifications for the biologic evaluation of endodontic materials are being developed for the Federation Dentaire Internationale.

During recent years some endodontists have claimed that the Sargenti endodontic treatment procedure is more economical than the methods used by most American dentists. Developed in Europe and commonly referred to as the N_2 method, this controversial technique has been used widely in America during the past few years, even though it has never been adequately tested.

At the College of Medicine and Dentistry of New Jersey, investigators have undertaken a study to compare the one-visit Sargenti procedure with the conventional treatment described by American authorities. One hundred anterior and posterior teeth of eight Maccaca rhesus monkeys were infected with a bacterial strain commonly isolated from endodontically involved human teeth. After a waiting period of 30-50 days to establish a pulpal and periapical infection, the infected teeth were isolated and direct access obtained to the pulp canal. Subsequently instrumentation and debridement of the canal were carried out either according to the Sargenti method or the conventional method. Sargenti-treated teeth were instrumented and filled during the same session, whereas conventionally treated teeth were cultured, medicated with camphorated chlorophenol and filled at the next treatment visit. All the teeth were sealed with amalgam restorations and the animals sacrificed after periods of six, twelve and twenty-four months.

Serial sections were prepared from 35 teeth treated with the conventional American technique, 16 teeth treated with the N_2 technique, and 14 teeth treated with the N_2 technique but filled with a zinc oxide-eugenol paste (ZOE). Chronic inflammatory cells were present in all specimens. This response was mild to moderate in conventionally treated teeth with significant bone resorption occurring in only eight of the 35 specimens (23%). In the N_2 treated animals the response was usually moderate to severe with significant bone resorption occurring in 12/16 teeth examined (75%). Teeth prepared by the N_2 method but filled with ZOE showed a mild to moderate response with bone resorption evident in 5/14 teeth (35%). After six months the conventionally treated teeth showed only a mild inflammatory response which remained at the apical foramen and did not result in a significant periapical change in 77% of the cases. In contrast, the N_2 treatment evoked at least a moderate inflammation in all cases and caused periapical bone resorption in 75% of the cases.

So far, these studies have shown that periapical healing is poor for the first six months when the N_2 method is utilized. Thus, these results suggest that the N_2 method of treatment for teeth might not be an acceptable clinical procedure. However, before firm conclusions can be drawn, the scientists must await the results of the 12- and 24-month evaluations.

Implants

In investigations at the Medical University of South Carolina porous titanium dental implants have served as free standing individual teeth

in Rhesus monkeys for periods exceeding two years. Nine of these implants, in 5 animals, have functioned for periods ranging from 104 to 146 weeks with little evidence of mobility.

Of the various factors monitored at the monthly clincal evaluations, early mobility was the least sensitive indicator of success or failure. This is not surprising, since the porous roots become ankylosed and would not be expected to show mobility until immediately before the loss of all bony attachment. Since the mode of failure involved the formation of intrabony defects and a lowering of the crestal bone height, radiography and pocket depth measurements were useful indicators of the status of the implants. However, they alone were not always sure indicators of success or failure. Intrabony defects and bone loss occurring buccally were often obscured in the periapical radiographs.

In some instances, the South Carolina investigators were able to determine the specific cause of implant failure. One implant failed quickly after 140 weeks of satisfactory clinical performance because of a hemolytic streptococcal infection; several other implants failed after long periods of satisfactory service. They were able to associate these failures with periodontal disease and anatomical factors. Their calculations of stresses on implants of their design indicated that mechanical trauma due to overstressing of the implant could have caused aggravation of the periodontal problems. The gradual increase in mobility which usually occurs in failing implants which are non-porous and not ankylosed, did not occur in their specimens. Even when five of their failing implants were removed surgically, they still had no mobility.

These findings have brought new attention to the significance of ankylosis in the artificial dental implant field. Some scientists now believe that the success of implants is dependent upon ankylosis.

In the research described above, the nature and texture of the surfaces presented to the tissues by the titanium implants encouraged new bone to grow in close apposition to the implants and become attached by ankylosis. As a result there was less mobility than in natural human teeth and presumably little periodontal space for disease to develop. Thus ankylosis may be advantageous for artificial dental implants, even though it is considered pathological in human dentition. In certain animals such as sharks, ankylosis is the natural mode of tooth attachment.

Researchers at SUNY (Buffalo) have discovered that the surface treatment of cast metal subperiosteal implants is a critical step in preparing implants for insertion. First they found that the accepted procedures for casting and finishing metallic implant devices do not provide a microscopically clean surface with appropriate distribution of surface free energy; subsequently, they learned how to improve the tissue acceptability of such dental implants by applying surface chemical and physical methods.

Measures of the actual surface free energy of the prepared implants revealed that standard polishing and cleaning techniques left on the metallic surfaces extremely thin, but still crucial, waxy coatings which prevented the tissue bonding necessary to immobilize the implant. Instead, a fibrous capsule formed around the implant, walling it off from the surrounding oral tissues. These observations were made by inspecting the surfaces of both the implants and the adjacent tissues with the scanning electron microscope, and analyzing the elemental composition of these phases by an energy-dispersive x-ray technique. Such unfavorable implant tissue relations could result in bacterial infection and implant loss.

As a result of these findings, the scientists applied a rapid and nondestructive surface treatment technique called "glow discharge cleaning" to the implants to restore the surface energies to their desired maximum values. This process also sterilized the devices. Preparing specimens in this manner resulted in considerable improvement in tissue bonding and in new bone growth adjacent to the subperiosteal implants. According to scanning electron microscope observations, when mechanical disruption of the bone between the implant and tissue was attempted in an excised sample, rupture occurred in the adjacent tissue mass rather than at the tissue/implant boundary. This result indicated that a strong, infection-free bone had been formed between the implant and the tissues.

Although investigators are optimistic about the possiblity of achieving satisfactory implant fixation and function, many problems must be solved before dental implants become routinely successful. For example, some investigators have now concluded that ankylosis of the implant to the alveolar bone is necessary, whereas others believe that a compliant, elastomeric cushion between hard metallic implants and the soft biological tissues of the host is needed to minimize mechanizal forces that could dislodge the device.

To develop such a cushion, scientists are applying surface chemical and physical techniques including the glow discharge treatment mentioned earlier. Preliminary findings indicate that this technique is not only improving the bonding of the biomedical elastomers to the metal devices, but is also improving the tissue receptivity of the new elastomeric surfaces.

Device for Handicapped Patients

Investigators at the University of Washington have continued their research on the head stabilizer for dental patients with cerebral palsy. As indicated in previous annual reports, the device provides active external stabilization of the head for diagnostic, hygienic and restorative procedures. During the past year the investigators have confirmed previous results and have found the stabilizer useful in the training of patients to control their movements voluntarily. These studies have demonstrated that the prototype stabilizer can provide

externally imposed head control sufficient for fairly simple procedures, such as composite restorations on readily accessible teeth; such procedures can be carried out with ease using the head stabilizer rather than general anesthesia to eliminate undesirable head motion. Feasibility testing is continuing with the prototype in use for more demanding tasks, such as amalgam restorations in posterior teeth. Before developing methods to duplicate the present electronic prototype of the stabilizer for technological transfer to other dental clinics, the Washington University investigators have decided to build a second generation apparatus which is entirely pneumatic. The pneumatic system will be simpler in design, smaller, more reliable and less expensive than the present prototype.

Meanwhile, clinical studies with the original prototype have shown that some patients can learn to control their head movements voluntarily by using the stabilizer as an exercise machine. In this study, five patients, 7 to 31 years of age, with a serious inability to control head movements, exercised with the stabilizer for one hour each week or more. After eight months or less, all five subjects had gained the ability to hold their heads almost completely on center without any mechanical help. The investigators believe that the apparatus not only helps strengthen the muscles, but also provides enhanced proprioceptive feedback, so that the patients develop an increased awareness of muscular movements. These results indicate that it is possible to compensate in part for the brain damage involved in cerebral palsy.

MEETINGS SPONSORED

The favorable impact of the consensus development conference, "Dental Implants: Benefit and Risk," held at Harvard June 13-14, 1978, continues to be evident. The high level of interest stimulated by this conference among the profession ensures that the consensus development effort will achieve its long-range goal of establishing a uniform, interpretable body of knowledge in the controversial field of dental implants.

Perhaps the most important acknowledgement of the value of the conference was the commentary published by the Council on Dental Materials and Devices of the American Dental Association (ADA) in the March, 1979, issue of the Journal of the American Dental Association, which also contained a summary of the consensus conference. The Council will soon publish its own updated position paper on endosseous implants. The complete proceedings of the conference are being prepared for publication and will be published by the Government Printing Office for distribution to the profession.

FUTURE PLANS

The Program will initiate a clinical research program on endosseous tooth implants. With the aid of consultants, staff developed a protocol and announced its availability in the NIH Guide to Grants and Contracts. The purposes of this announcement were to apprise the scientific community

of NIDR's intent to sponsor this clinical trial, to determine the degree of interest in it, and to offer interested persons an opportunity to participate in its development. We have asked for comments on the choice of implants to be studied, the detailed methodology of the protocol, the proposed plan for the study, the magnitude of the project, and on human subjects-ethical issues.

The program plans to begin this study during FY 1980 by awarding an initial contract to a Lead Clinical Center and its collaborating Data Coordinating Center for a one-year field trial of the study. This initial project will include finalizing all data collection forms, patient screening and evaluation, pre- and post-surgery data collection, and patient follow-up. By the end of the first year, additional clinical centers may be added to the study.

The program will also begin a study of the mathematical technique of finite element analysis in several areas of dental research. Requests for research on the use of this approach for tooth implant research will be initiated by RFA or RFP. In addition, we intend to initiate a project which will use finite element analysis to study fixed and removable protheses. The following subjects may be pursued individually or as a package: 1) construction of a finite element model to depict natural teeth as abutments for both fixed and removable protheses; 2) stress, strain, and displacement analysis of the protheses; comparisons of the stress distribution of various designs; 3) fatigue analysis of different clasps and framework designs; 4) stresses induced upon abutments by various prosthetic designs.

Future plans also call for a project to establish objective criteria for success and failure of prostheses. Since a literature survey by staff indicated that no such criteria exist, an RFA on this subject will be published in the NIH Guide.

SUMMARY

During FY 78 the Restorative Materials Program awarded $2.5 million to support 46 research grants, including 6 research career development awards, one contract and two interagency agreements. The Program also awarded a total of $397 thousand to support 6 training grants, and $45 thousand for 3 individual postdoctoral fellowships.

Investigators at U.S. Public Health Service Hospital in San Francisco have devised an automated system of minicomputers that enables them to rapidly compare the clinical performance of any combination of dental filling materials. They are using the system in studies to develop composite plastic filling materials for posterior teeth, and to evaluate the performance of copper-containing alloys. The investigators have found that an experimental composite containing strontium particles served for as long as 5 years as a successful posterior filling in over 90% of the teeth tested. As a result, a filling material of this type has become available on the dental market. Two of the copper-containing

alloys they tested showed resistance to corrosion and maintained a superior marginal adaptation without fracture, but a third one did not. Researchers at Indiana University and at Louisiana State University studied the relation of creep, corrosion, amalgam microstructure and trituration time to marginal fracture of dental amalgam fillings, but their results have not yet provided firm conclusions.

Scientists at the American Dental Association Laboratories at the National Bureau of Standards are developing experimental data on binary combinations of approximately 20 transition elements which could prove useful for dental application. These elements include silver, copper, mercury, gold and other metals with exceptional resistance to corrosion. This data source should facilitate the development of better alloys for dental appliances, and may be of benefit for a wide range of industrial applications.

At the University of Connecticut Health Center, School of Dental Medicine, researchers are developing new methods to test the biocompatibility of endodontic filling materials and attempting to establish better biologic standards and criteria for performance and safety of these materials. The protocol they have developed provides a reliable screening approach for use by manufacturers in developing new products and for maintaining quality control. Researchers at the College of Medicine and Dentistry of New Jersey are comparing the controversial Sargenti procedure with the conventional endodontic treatment. Early results showed that healing was poor after the Sargenti method was used.

Progress in tooth implant research by scientists at the Medical University of South Carolina have added new dimensions to the significance of ankylosis. On the basis of studies of titanium dental implants in Rhesus monkeys some scientists now believe that implant success is dependent upon ankylosis. Scientists at SUNY (Buffalo) are finding that the surface treatment of cast metal subperiosteal implants by a technique called "glow discharge cleaning" improves the tissue acceptability of these implants. This non destructive technique removes the microscopic waxy residue left by less effective methods and optimizes the surface energy of the implants.

Investigators at the University of Washington continued their studies on the head stabilizer for dental patients with cerebral palsy. They confirmed previous findings that simple restorative procedures can be carried out using the head stabilizer rather than general anesthesia, and showed that the stabilizer can be useful in training patients to control their movements voluntarily.

The consensus development conference, "Dental Implants:Benefit and Risk", held at Harvard in 1978, apparently had a favorably impact on the profession. A summary of this conference was published in the March, 1979 issue of the Journal of the American Dental Association, and the complete proceedings of the conference are being prepared for publication

by the Government Printing Office. Plans are being developed to initiate a program of clinical trials on endosseous tooth implants. The program plans to award the initial contract during FY 1980.

Prepared by Thomas M. Valega and Joyce A. Reese

ANNUAL REPORT FY 1979

SOFT TISSUE STOMATOLOGY AND NUTRITION PROGRAM BRANCH

INTRODUCTION

The Soft Tissue Stomatology and Nutrition Program Branch supports research in four major areas: soft tissue diseases, nutrition, salivary glands and their secretions, and mineralization. The program's main objectives are to generate knowledge of a) the etiology, diagnosis, treatment, and prevention of oral soft tissue diseases and disorders, b) the role of nutrition in the growth, maintenance, function and health of hard and soft tissues of the craniofacial complex, c) the anatomy, biochemistry, physiology and pharmacology of normal and abnormal salivary glands and their secretions, and d) the mechanism of mineralization, with special emphasis on the role of cells and other regulatory mechanisms.

ADMINISTRATIVE

During FY 1979, 83 research grants were funded at a cost of $4,917,153. In addition, 7 contracts were funded at a level of $459,872, and one interagency agreement received $77,040. Table 1 illustrates the distribution of grant and contract funds by subject category.

During this fiscal year, 6 training grants received $591,561. These grants supported a total of 5 predoctoral and 21 postdoctoral trainees. Funds were also provided for 9 individual postdoctoral fellowships ($104,557), 8 career development awards ($250,618), and 1 career award ($32,454).

Program staff devoted considerable effort to the NIH Nutrition Coordinating Committee during FY 1979. This effort involved reporting NIDR nutrition activities and preparing reports on current nutrition research projects. Program staff also represented NIDR on the NIH Digestive Diseases Committee and on the NIH Cystic Fibrosis Coordinating Committee.

During this fiscal year two contracts to determine the clinical efficacy of the antiviral compound 9-hydroxy-ethoxymethyl guanine were jointly sponsored with the National Institute of Allergy and Infectious Diseases. This collaborative effort will continue for at least one additional year. The interagency agreement with the Veterans Administration in East Orange, New Jersey to study the detection of early cancerous lesions in the oral cavity was renewed for an additional year.

TABLE I. DISTRIBUTION OF ACTIVE GRANTS & CONTRACTS FY 79

	Projects Funded FY 1979	FY 1979 Funds Expended
RESEARCH GRANTS		
Nutrition	10	$ 426,851
Salivary Secretions	20	832,020
Soft Tissue	21	1,305,931
Mineralization	32	2,352,351
TOTALS	83	$4,917,153
CONTRACTS		
Nutrition	1	$ 68,020
Herpes Infections*	2	78,680
Oral Cancer	1	77,040
Aphthous Stomatitis	3	293,372
Fluoride	1	19,800
TOTALS	8	$ 536,912
TRAINING		
Individual Fellows	9	$ 104,557
Institutional Training Grants (Trainees - 26)	6	591,561
TOTALS	15	$ 696,118

* Jointly supported with NIAID

STAFF ACTIVITIES

Monitoring, Evaluation, and Programming Visits

1. Temple University, Philadelphia
   October 18, 1978 (Programming)

2. Ad Hoc Oral Biology & Medicine Study Section Meeting,
   Long Beach, October 21-22, 1978

3. University of California, Los Angeles,
   October 23-24, 1978 (Project site visit)

4. University of North Carolina, Durham,
   October 29-30, 1978 (Project site visit)

5. Harvard University, Boston,
   November 2, 1978 (Project site visit)

6. University of Washington, Seattle,
   November 30-December 1, 1978 (Project site visit)

7. AADR Chapter Meeting, Morgantown
   May 26, 1979 (Programming)

8. University of Washington, Seattle
   June 25-27, 1979 (Project site visit)

9. University of Washington, Seattle
   June 28, 1979 (Project Officer's visit)

10. Veterans Administration Medical Center, Takoma
    June 29, 1979 (Programming)

Scientific Meetings

1. Annual Sponsor's Scientific Meeting,
   Monell Chemical Senses Center,
   University of Pennsylvania, Philadelphia,
   October 19-20, 1978

2. American Dental Association Meeting,
   Anaheim, California, October 22-25, 1978

3. AADS Meeting, New Orleans
   March 26-28, 1979

4. IADR Meeting, New Orleans
   March 29-April 1, 1979

RESEARCH HIGHLIGHTS

Nutrition

In FY 1977 we reported in vitro studies which showed that horseradish peroxidase introduced into the gingival sulcus passed through the epithelial barrier in subhuman primates with an acute Vitamin C deficiency, but not in those with adequate Vitamin C. In follow-up studies, subacute Vitamin C deficiency was established in some of these same animals and the molar teeth of both deficient and nondeficient animals were ligated with silk ligatures to develop an experimental periodontal pocket, according to the procedure developed at the Eastman Dental Center. The resulting localized periodontal disease was measured by gingival index, calculus and debris index, and pocket depth. Disease was noticeably worse in the deficient animals than in the pair-fed controls, although the deficient group showed no clinical signs of deficiency. All of the periodontal indices were significantly elevated. Another important finding was that the polymorphonuclear leukocytes in the circulating blood of the monkeys with subacute ascorbic acid deficiency had a reduced ability to phagocytize and to kill engulfed Candida albicans in a standardized in vitro assay system.

These studies indicate that marginal ascorbic acid intake increases susceptibility to periodontal disease and that two mechanisms may account for the increase in susceptibility: slow repair and impaired leukocyte function.

Studies previously reported by investigators at the University of Connecticut indicated that high-protein diets caused an increase in calcium excretion in the urine of rats, guinea pigs, and man. The diet of the human subjects consisted of low-fat cottage cheese with the calories and minerals provided by a liquid supplement. Calcium excretion showed an average increase of 93% among the nine subjects studied, but varied widely from no increase to more than a 200% increase. Females had a mean increase of 124% compared with 41% for males. This finding may be related to the higher prevalence of osteoporosis among females.

Subsequent studies however, have not confirmed that high protein causes an increase in calcium excretion. These studies indicate that calcium excretion in the urine depends upon several interrelated variables including the exact amount of protein ingested, the source of the protein, the amount of available phosphorus in the diet, and the length of the study. In one of these studies, by a grantee at the Veterans Administration Hospital in Hines, Illinois, the effects of high meat diets on calcium metabolism were studied in adult male subjects. To determine the influence of calcium intake levels on excretion, the calcium intake in this experiment was carefully regulated at 200, 800, 1100, and 2000 mg per day (800 mg per day is considered normal). At low or normal calcium intake, there was no significant increase in calcium in the urine either in the group as a whole or in individuals

that received the high protein (meat) diet. It was noted, however, that two subjects on a high calcium intake did show an increase in urinary calcium excretion, which then declined to base line levels within 2 months even though both the high calcium and high protein intake were continued. In this study the phosphate content of the high protein meat diet was 230-290 mg per day higher than in the low protein diet. As a result, there was a slight positive increase in phosphate balance. Calcium absorption from the intestine did not increase during high-protein intake, and according to radioisotope measurements, no significant change in the specific activity of urinary calcium was observed in the control or high protein studies. Apparently the high phosphorus content of the high-meat diet limited calcium excretion either through decreased bone resorption or through an akaline-buffering effect of the phosphorus on kidney function. Thus, although high-protein intake may increase urinary calcium under certain conditions, other variables such as dietary phosphorus may modify this effect.

Investigators at the University of Alabama have been studying the effect of Vitamin A deficiency on tooth and bone formation. A severe nutritional problem in many parts of the world, Vitamin A deficiency is also a problem among young children in the United States (Ten State Study, 1970; HANES, 1972). Previous studies have shown that Vitamin A deficiency interferes with normal sulfur metabolism in the matrix of developing bone. Current studies in rats indicate that this deficiency increases the susceptibility to dental caries. Rat pups, born to dams which consumed a Vitamin A-deficient diet near the time of conception, were normal at birth if the diet was adequate for 14 days before mating, but were distinctly smaller if the mother's diet was adequate for only 10 days before mating. Vitamin A concentrations in the serum of dams on a Vitamin A-deficient diet decreased within one week after parturition; thus the pups were raised to weanling age on Vitamin A-deficient milk. At weaning they were removed from the dams and placed on an adequate Vitamin A but mildly cariogenic diet and inoculated with Strep mutans 6715. Within three weeks, the rate of caries among these pups was significantly higher than that in normal animals. These findings clearly indicate that Vitamin A deficiency adversely affects the normal development of teeth and makes them more susceptible to caries.

Folate deficiency in women has been associated with spontaneous abortion in early pregnancy, toxemia of pregnancy, premature deliveries, and fetal abnormalities and thus is related to the early nutritional requirements of the fetus. In an in vitro study at Fairleigh Dickinson University, rabbit palatal shelves were subjected to folic acid deficiency before fusion. After 72 hours in culture, the palates grown on media deficient in folic acid tend to maintain an epithelial union which prevented mesenchymal fusion. Preliminary data from explants taken earlier than 72 hours indicate even more serious effects of folate deficiency.

In seeking to identify a suitable animal model for nutritional studies

of periodontal disease, an investigator at the University of Minnesota has examined the STR/N strain of mice. He has confirmed earlier NIDR intramural studies which showed that this animal develops spontaneous periodontal disease including loss of alveolar bone preceded by progressive gingivitis and periodontal pocket formation. These confirmatory studies showed clearly that at 12 months of age STR/N mice had sustained significantly more alveolar bone loss than Swiss Webster controls. Histologic examination indicated that the bone loss was due at least in part to osteoclastic resorption. In addition, the investigators have ruled out the possibility that the spontaneous STR/N periodontal disease might be related to underlying predisposing conditions such as diabetes mellitus, disturbed mineral metabolism, food or hair impaction, or gross anomalies of the teeth. Having characterized the experimental model, the investigators have now begun disease-related nutritional studies.

In their first study on STR/N mice, preliminary data indicated that adult animals on a zinc-deficient diet suffered more severe periodontal bone loss than those on adequate or high dietary supplements of zinc. How zinc deficiency contributes to increased periodontal bone loss is unknown. According to some studies, zinc deficiency causes disturbances in epithelial linings, and may also depress normal cellular immune responses.

Soft Tissue

The last 8 to 10 years have seen an increase in the number of reports on the use of cryosurgery freezing for treating inflammatory and neoplastic diseases of oral-facial tissue. However, little has been done to characterize, in animal model systems, the tissue reaction(s) to the time and temperature variations used in this approach and to different methods of instrumentation, such as direct spray or probe application. Investigators at the State University of New York in Buffalo have obtained favorable clinical results with cryosurgical methods on various structures and organs of the oral-facial complex. Currently they are characterizing microscopically experimental cryolesions on mucosa, skin and glands in subhuman primates. Results so far indicate that the various components of salivary glands responded differently to low temperature. The duct tissue of the salivary glands was resistant to cryosurgery unless a two-cycle cold application was used. They have also found that the submandibular gland in the rhesus monkey could be completely destroyed by two-cycle freezing.

The results of these animal studies have been transferred rapidly to human application. Recently the investigators used their experimental findings in treating an aggressive recurrent acinar cell carcinoma of the parotid gland of a patient in whom conventional surgery was contraindicted. The fact that cryosurgery on the parotid gland resulted in only transient damage to the facial nerves suggests that cryosurgery may be preferable to conventional surgery for parotid neoplasms.

Two-cycle freezing of the hard palate also uniformly destroys the minor salivary glands. Of major importance is the finding that the palatal bone which is devitalized by this procedure responds like a devitalized bone graft; it undergoes optimum repair and eventually total regeneration of the bone takes place. Preliminary studies of the buccal mucosa and tongue suggest that either the probe or spray technique is useful in treating diffuse epithelial lesions or deep-seated nodular or dissecting vascular neoplasms in these regions. The technique used on buccal mucosa freezes the whole area from the intraoral mucosa to the skin and thus destroys the entire diseased tissue including epithelium, connective tissue, and glands, and yet leaves sufficient tissue intact for repair. The intraoral mucosa resurfaces with minimal scarring. These results suggest that cryosurgery can also be used to treat erosive lichen planus, diffuse epithelial dysplasia, or hyperkeratosis. A potentially important advantage of cryosurgery over radiation therapy is that the tissues subjected to this treatment can be reconstructed surgically at a later date, whereas irradiated tissues usually cannot.

Studies on pigmented skin showed that melanocytes are specifically sensitive to certain temperature ranges; thus, cryosurgery may be useful in treating melanomas. The periosteum and cortex of the jaw bone appear to resist low temperatures. If the cortex is devitalized in focal segments, the periosteum will use the remaining structure as a lattice to regenerate the bone. When gingival tissue was frozen at -40 to -60 degrees Centigrade it became resurfaced with normal epithelium. These findings encourage the use of cryosurgery in the treatment of periodontal disease and other inflammatory lesions of the jaws. Pulp tissue was also sensitive to cryosurgery. When pulp tissue was devitalized by cryosurgery neither periapical rarefaction nor increased susceptibility to cariogenic attack or periodontal disease resulted. Because of the susceptibility of odontogenic epithelium to freezing, scientists were able to treat an aggressive ameloblastoma by a combination of conventional surgery and cryosurgery rather than by a disfiguring block resection.

Herpes simplex virus type I is an extremely common human virus which causes painful recurrent lesions of the lips and mouth, occasionally causes blindness or death, and is believed to be involved in the development of epidermal carcinomas. Investigators at the Medical College of Virginia and Duke University have made highly significant progress in 3 areas of herpes research. The Virginia workers have developed a new vaccine that is protective in animals and is not likely to promote carcinoma development. They have also produced positive evidence that chronic infection with herpes can aid in inducing carcinomas. The Duke University scientist has made basic discoveries on the body's cellular defense mechanisms against this common virus.

In developing the new herpes type I virus vaccine the investigators first studied primary infections. In mice infected on an abraded portion of the upper lip, primary virus replicated and a vesicular epithelial lesion resulted in five days. Within 48 hours the virus had

spread from the lip to the ipsilateral trigeminal ganglia and thereafter to the cerebellum, the contralateral trigeminal ganglion and the cerebrum. The vaccine tested was a nucleic acid-free viral subunit which was intended to protect the animals against primary trigeminal and recurrent oral lesions, and against latent infection and fatal encephalitis. Studies with single vaccine doses of between 4 and 215 ug resulted in a linear reduction in the severity and duration of the lip lesion. Two doses of 40 ug each given 10 days apart protected all the test animals from fatal infection and 630 ug protected 70 percent of the mice from latent ganglion infection. Animals protected at this 70 percent level had only five plaque-forming units (PFU) of virus per ganglion whereas those receiving 40 ug or less had over 60 PFU virus per ganglion.

A companion study showed that with a standardized vaccine dose, protection from lethal encephalitis was 100 percent when the animals were challenged with virus 4 weeks after vaccination, but declined to 78 percent protection when the challenge was made 24 weeks after vaccination. The vaccine was less effective against latent ganglion infections. Only 50 percent protection was obtained after the four-week interval, and no protection was evident after the 24-week interval. The addition of Freund's adjuvant to the vaccine improved protection against lethal encephalitis, and was absolutely necessary to protect against latency.

These investigators also successfully reactivated the virus in the latently infected trigeminal ganglia and in the lips of the latently infected animals by immunosuppressing the animals with cyclophosphamide every three or four days. The reactivation was proven by tissue culture methods. Although previous workers had observed herpes simplex antigens after immunosuppression, none had previously succeeded in isolating the infectious virus.

In the studies linking HSV Type I and epidermal cancer, the Virginia investigators have induced malignancy of the lip in mice infected with the herpes virus. In this animal model they exposed the lip to short intervals of UV light during the vesicular stage of the labial herpes lesion and then applied a tumor-promoting agent (TPA) three times per week for about six months. So far, 34 of the 156 animals have developed tumors and of these, 6 have progressed to squamous cell carcinoma. When the three agents were used separately, no tumors were produced, but UV light plus TPA produced three benign papillomas, and the herpes simplex virus plus TPA produced two.

The investigator at Duke University has studied the interaction of herpes simplex virus and human leukocytes. He observed that herpes simplex virus does not infect peripheral blood leukocytes from normal human subjects and from most patients with leukemia; the virus cannot replicate in these cells. However, the virus does replicate in cells from patients with hairy cell leukemia and in cells from about half the normal newborns tested. These findings suggest that newborn infants and

some leukemic patients may be more susceptible to severe or lethal herpes infections, because viruses can replicate in these protective cells which normally inactivate the virus.

Since exposure to the stresses (sunburn, trauma, fever) associated with herpes infections cause the elaboration of increased amounts of prostaglandins, and since steroids aggravate herpetic infections, the investigator tested these substances to determine whether they stimulated or increased virus replication in leukocytes. The experiments showed that prostaglandins and steriods had no effect on virus replication, but both substances completely inhibited another defense mechanism known as antibody-dependent, cell-mediated cytolysis. This mechanism is active against human fibroblasts infected with the herpes simplex virus. Another finding made by these investigators was that exposure to high concentrations of virus did not affect the phagocytic activity of either polymorphonuclear leukocytes or monocytes.

Salivary Secretions

Proteins and glycoproteins found in salivary secretions fall into two general classes: serum proteins and intrinsic secretory materials manufactured in the salivary gland. How these components are secreted is not clearly understood; only a few studies have explored how the secretory stimulus affects the secretion of proteins in saliva. For example, the secretion of intrinsic proteins like amylase is known to depend on the autonomic nervous system, but it is not known whether this system regulates the secretion of serum proteins. Recent studies at the University of Alabama have helped to clarify the role of the autonomic nervous system in regulating protein secretion in the saliva of adult rats. In these studies, the effects of sympathetic and parasympathetic stimulation were compared. The effects of adrenergic glandular receptors were distinguished from those of cholinergic receptors by comparing pilocarpine-induced secretions in the neurally intact gland on one side with pilocarpine-induced secretions in the acutely sympathectomized gland on the contralateral side. The concentrations of amylase, IgG, IgA, and total protein were measured. Flow rate was also recorded. In the secretions from the intact glands, both the total protein and the amylase concentrations were much higher (20X) than the secretions from the sympathectomized glands. Following the pattern of amylase, IgA levels in the intact gland secretions were also higher (9X) than the IgA levels in the secretions from the sympathectomized glands. The IgA concentrations in saliva from the intact glands averaged 46 mg% immediately after stimulation and gradually decreased to 14 mg% after 45 minutes. Pilocarpine-induced saliva from the sympathectomized glands contained 5 mg% of IgA and did not change appreciably with time or the flow rate. These data support the hypothesis that the acinar cell of the parotid plays a role in secretion of IgA. However, since IgA secretion was not identical in all respects with amylase secretion, probably not all subunits of secretory IgA are derived from the acinar cell. This concurs with the belief that part of the immunoglobulin moiety of IgA is serum-derived and becomes conjugated with the secretory piece during

passage through the glandular epithelium. The secretory piece of the IgA molecule may be under the control of the autonomic nervous system. In contrast, IgG levels in the parotid saliva of rats were independent of flow rate or autonomic stimulation. Virtually identical levels of IgG were found in secretion from both sympathectomized and neurally intact glands stimulated by pilocarpine. This evidence indicates that these molecules are passively transported from the serum to the saliva.

Saliva is an important component of the oral milieu: changes in its composition can alter the oral environment and oral health. Thus, modifications induced by the lack of mastication could result in conditions detrimental to oral health. At the University of Washington scientists have studied the effects of mastication on the size and function of salivary glands. In rats fed a standard laboratory chow blended with water (but not in those fed the same chow in pellet form), workers observed atrophy of the parotid gland. Gland weight, RNA content, and amylase decreased by 40-50%; DNA decreased by 25%; and cell size and cell number also decreased. When 30% non-nutritive bulk was added to standard chow and pelletized to increase mastication, the parotid glands of the rats enlarged, their RNA, and amylase content increased by 50% to 60% above controls on standard pelletized chow, and cell size and number increased. The pelletized bulk diet also caused a 25% to 35% increase in protein concentration, amylase, RNase, and DNase in the gland. Electrophoretic patterns of saliva from rats fed the bulk diet were the same as those of the saliva from controls. The liquid diet, however, caused striking changes in saliva composition. Amylase and RNase decreased 70%, DNase decreased 44%, and protein concentration decreased 54%. Electrophoresis in anionic slab gel showed that three slow-moving bands were missing. In cationic gel, three major bands were missing and a fourth band was reduced. These bands reappeared when the same rats were returned to the control diet for one week. Other experiments showed that the missing bands were in fractions associated with the proline-rich protein. These findings may be relevant to human oral health since the protein content of human saliva is severely reduced in individuals on liquid diets such as Metrecal.

Mineralization

Calcium-phospholipid-phosphate (Ca-PL-PO_4) complexes have been isolated from mineralized rabbit tissue by an investigator at the Hospital for Special Surgery, Cornell University Medical College. These complexes were found in all the mineralized tissues including bone, calcified cartilage, and tooth but not in non-mineralized tissues such as muscle, ear cartilage, bone marrow, or adipose tissue. The amount of Ca-PL-PO_4 was highest in those tissues involved in active mineralization. All isolated complexes induced in vitro hydroxyapatite formation from metastable calcium-phosphate solutions. At the Medical College of South Carolina Ca-PL-PO_4 complexas were recently isolated from the matrix vesicles of epiphyseal cartilage. Since the extracellular matrix vesicles seem to be associated with the onset of mineralization, it is believed that these complexes may perform an important function in the

mineralization process.

In other studies involving investigators at the Hospital for Special Surgery and the Dental Science Institute, University of Texas Health Science Center, Ca-PL-PO_4 complexes were isolated from the calcifying bacteria Bacterionema matruchotii and its proteolipid-containing fractions but not from non-calcifying Actinomyces naeslundii. The fact that both calcified and uncalcified B. matruchotii contained these complexes suggests that the organisms were primed for calcification even when grown in a maintenance medium. When different fractions of B. matruchotii were tested for the ability to initiate hydroxyapatite formation from metastable calcium phosphate solutions, the Ca-PL-PO_4 fraction yielded the greatest amount of hydroxyapatite. Since most of the Ca-PL-PO_4 complexes are part of the proteolipid of the organism, they are probably membrane components. Although phospholipids occur in a wide variety of plant and animal tissues, not all tissues calcify. Studies with a non-calcifying microorganism such as A. naeslundii suggest that the total membrane environment is involved in the regulation of proteolipid-initiated calcification. Thus, the microbiological model may turn out to be an ideal system for examining how calcification is regulated by the cell.

In complementary studies, investigators at the University of Texas Health Science Center in Houston have developed a synthetic protein-acidic-phospholipid analogue which forms hydroxyapatite from metastable solutions as do proteolipid and protein acidic-phospholipid complexes from calcifying microorganisms. Kinetic studies of calcium uptake showed that after a gradual uptake of both calcium and phosphate, hydroxyapatite was formed by 72 hours. This system should enable scientists to find anti-calculus agents as well as to define the mode of mineral inhibition. The sympathetic complexes make it possible to define and control such factors as the type of protein and phospholipid, the protein-to-phospholipid ratios, and the ionic concentrations in the test system.

An investigator at the University of Pennsylvania has developed an in vitro system for studying the effect of fluoride on ameloblasts. First molar tooth germs were removed from one-day-old mice and grown in culture on a millipore filter for as long as 6 days. Specific quantities of fluoride (0.1, 1, 2, 5, 10, and 50 ppm) were added to the medium at 24 hours and at later stages during this period. Light and electron microscopic findings confirmed those of earlier studies which had shown that doses of fluoride 5 ppm or greater totally arrested enamel matrix formation and caused large vesicles to be formed in the distal cytoplasm of the ameloblast. The vesicles were filled with a material which appeared identical with that normally formed in the matrix by the in vitro controls. These vesicles accumulated only during the secretory stage. They were not seen in immature ameloblasts. Control specimens formed a wide zone of enamel matrix during the same culture period. With 2 ppm fluoride in the medium, enamel formation was arrested but the

vesicles were smaller and less numerous. With 1 ppm fluoride, enamel appeared to form but was only about one-half as thick as that of controls and differed structurally. The effects of lower doses are still being evaluated. Current experiments will determine the exact time course of the effects at various doses and the reversibility of the fluoride effect. If successful, these studies will provide a better understanding of the mechanism of tooth mottling caused by excessive amounts of fluoride in some well water.

Investigators at the Georgia Institute of Technology are the first to refine the crystal structure of human tooth enamel. This was accomplished by means of the Rietveld analysis, which employs whole pattern-fitting-structure analysis of diffraction data from powdered enamel. Although the data are regarded as preliminary, they do locate the principal atoms and give some information about the location of OH, F, Cl, and some CO_3, and of structurally incorporated H_2O. These structural refinements use the least-squares fit between observed and calculated intensities obtained by both x-ray and neutron diffraction techniques. Since x-rays and neutrons are diffracted differently by certain atoms or ionic groups, each technique complements the other. For example, in order to confirm the suggested location of water, samples must be heated to drive off the water; this appears to result in a movement of CO_3 into the water channel. Since x-rays cannot detect the difference between H_2O and CO_3 but neutrons can, it is expected that these data will be easily obtained in the next project period. Since the location of such constituents as H_2O, CO_3, HPO_4, and Cl significantly affect not only fluoride uptake and release, but also enamel reactivity in the oral environment (including its resistance to bacterial acid), these structural data may help to explain why individuals differ in their susceptibility to decay. Such data may also lead to methods of protecting teeth.

Previous reports described a group of unique calcium-binding proteins dependent on Vitamin K. These proteins have been isolated from the bones of several animals including man, where they amount to about 1 percent of the total bone protein. Studies at Children's Hospital Medical Center in Boston showed that the calcium-binding protein, gamma-carboxyglutamic acid (GLA), increased in chick bone just before mineralization and was higher in the dense fraction of rachitic bone. The less dense bone fraction in rachitic animals which did not contain GLA was composed principally of unmineralized osteoid. Thus GLA appears to coincide with mineralization.

In recent studies at Northwestern University, a limited period of acute Vitamin K deficiency was created in rats by blocking Vitamin K with Warfarin and following this by Vitamin K supplementation shortly thereafter. This treatment caused a disturbance in the dentine of the rat incisors that was identified by electron probe analysis as a zone of decreased calcium concentration presumably associated with a failure to synthesize GLA. The bones of the Vitamin K-deficient animals also

showed a decreased calcium concentration. Although the exact role of GLA in mineralizing tissues is not yet known, these interesting proteins seem to be associated with the accumulation of mineral in a variety of normal and pathologic conditions.

FUTURE PLANS

Expanded research activities on the etiology and prevention of oral viral and ulcerative diseases are planned. Since new findings on immunization against latent viral infections suggest excellent opportunities for progress, the program will encourage additional studies in this area. NIDR has committed partial support for an international conference on human herpes virus set for March of 1980. It is hoped that this meeting will lead to expanded activity and progress in this important area of research.

The program will also continue to emphasize salivary gland studies, nutrition studies and mineral and fluoride metabolism studies. The program recently published an announcement seeking grants in the area of fluoride research and has initiated a research resources contract to make animals available to interested investigators from two colonies of mice which have been on defined high and low fluoride diets for over five years. Continued emphasis will be placed on training of individuals for the solution of soft tissue and nutrition research problems associated with the oral facial complex.

SUMMARY

During FY 1979 the Soft Tissue Stomatology and Nutrition Program Branch expended nearly $5 million for 82 research grants and $0.5 million for 7 contracts and one interagency agreement. Funds were also provided for 6 institutional training grants supporting 26 trainees ($591,561), 9 individual fellows ($104,557), 8 career development awards ($250,618) and one career award ($32,454).

Nutrition

Monkeys suffering from subacute Vitamin C deficiency exhibited worse periodontal health around molar teeth ligated with silk ligatures than nondeficient animals. Leukocytes from the same deficient animals showed a reduced ability to phagocytize and kill organisms in vitro. Studies of the effect of calcium excretion due to high protein diet in humans indicate that the protein source, length of time of study and the amount of phosphorus intake influence calcium loss from the kidney. Calcium absorption from the gut was unaffected. Vitamin A deficient rat pups raised on milk from Vitamin A deficient mothers experience higher caries rates than control animals. These findings clearly indicate that Vitamin A deficiency adversely affects tooth development. Folate deficiency in the media of cultures containing growing mouse palates caused an epithelial union between palatal shelves which prevented

mesenchymal fusion. The STR/N strain of mice has been shown to develop spontaneous periodontal disease by twelve months of age. Local and systemic predisposing conditions have been ruled out and preliminary studies indicate that zinc deficient animals suffer more severe periodontal bone loss than those on adequate or high zinc supplements. This experimental model will be useful in future nutrition-periodontal disease studies.

Soft Tissues

Cryosurgery has been studied in subhuman primates to determine its potential for treating pathological conditions of the oral facial area. Significant results have been obtained on salivary glands, lesions involving bone and epithelial lesions such as lichen planus. Advantages of cryosurgery are its minimal effect on nerves and the fact that the treatment area can be reconstructed surgically later whereas irradiated tissue usually can not be subjected to extensive surgical procedures. Scientists have developed a vaccine which provides complete protection against lethal herpes simplex virus type I infections, and provides partial protection against latent ganglion infections in laboratory mice. In related studies it was found that the herpes virus does not infect peripheral blood leukocytes from most normal adult human subjects but does infect leukocytes in about 50 percent of the newborns tested. It was also found that prostaglandins and steroids inhibited antibody-dependent cell-mediated cytolysis, a mechanism which is normally active against virus-infected fibroblasts.

Salivary Secretions

It has been confirmed that the secretion of salivary IgA is under the influence of the acinar cell of the parotid gland, whereas salivary IgG is independent of gland function and reflects serum levels. In studies on the effects of mastication the salivary glands of rats decreased in size when soft or liquid diets were fed but they increased in size when increased mastication was required. Liquid diets also caused striking changes in salivary composition. The main alteration was the disappearance of a major protein fraction which was restored when solid diets were resumed. Human subjects on liquid diets also show reduced salivary protein.

Mineralization

Calcium-phospholipid-phosphate complexes have been found in all mineralizing tissues and are highest in tissues involved in active mineralization. These complexes have also recently been isolated from matrix vesicles of epiphyseal cartilage which are believed to be associated with the onset of mineralization. A synthetic protein-acidic-phospholipid has been developed which makes it possible to study mineralization mechanisms in vitro. Such studies may lead to the development of anticalculus agents. Studies of the effect of fluoride

on the ameloblast in vitro indicated that relatively small doses cause disturbances in the ameloblast function. These findings may lead to a better understanding of the mechanism of tooth mottling caused by excessive amounts of fluoride in water. Studies on the structure of enamel using x-ray and neutron diffraction have been advanced by a whole pattern-fitting-structure analysis known as the Rietveld analysis. Information about the localization of important ions such as OH, F, Cl and CO3 and the structurally incorporated water have been obtained. Calcium-binding proteins dependent upon Vitamin K have been shown to be associated with several bone mineralizing systems. In one study when Vitamin K was blocked with Warfarin and then Vitamin K was supplemented shortly thereafter, a zone of dentine with decreased calcium was formed. This zone was presumably associated with the failure of the calcium binding proteins to be synthesized.

Prepared by P. D. Frazier

ANNUAL REPORT FY 1979

PAIN CONTROL AND BEHAVIORAL STUDIES PROGRAM BRANCH

INTRODUCTION

The Pain Control and Behavioral Studies Program Branch supports research and research training on the etiology, prevention, diagnosis, and treatment of orofacial pain and on the sociopsychological factors that affect oral health. These factors include preventive attitudes and behaviors, fear and anxiety about dental therapy, psychosocial concomitants of malocclusion and facial disfigurement, and such stress-related oral disorders as bruxism and temporomandibular joint pain. The Program also supports studies on other oral sensory and motor functions including gustation, olfaction, mastication, and deglutition.

In its simplest form, pain is a warning signal to the organism alerting it to the existence of a functional or organic problem. Once it has served this purpose, however, pain becomes a major problem in itself with sometimes severe social and economic consequences. Although man has always attempted to understand pain and to ameliorate its ravages, we are still far from succeeding in either. In recent years, however, it has become clear that human pain is not just a simple stimulus-response phenomenon but a combination of perceptual and emotional experiences resulting in various autonomic and behavioral responses determined by a complex interaction of individual, cultural, and societal factors. Much current research focuses on learning what and how much these individual components contribute to the total pain experience. Since orofacial and dental pain constitute a major part of the human pain experience, research support along the above lines are important activities at NIDR. This program supports not only the usual research studies but also international scientific meetings and workshops.

Oral diseases are clearly affected by such individual and societal behaviors as dietary choices, the use or neglect of good oral hygiene practices and professional services, cooperation with dental care providers, and community adoption of preventive agents such as water fluoridation. The Pain Control and Behavioral Studies Program strives to develop a strong research base in behavioral and social sciences so that health-relevant behaviors can be understood and improved. Vigorous programming during the past year has helped to expand the range of topics supported; further program development will be emphasized in FY 1980.

ADMINISTRATIVE REPORT

From October 1, 1978, to September 30, 1979, the Program funded research grants totaling $2.15 million. These awards included five new investi-

gator awards and two Research Career Development awards. In research training, the program funded seven individual fellowships totaling $76,000 and two institutional training grants totaling $184,000.

Table I shows the distribution of funding by major research areas.

Table I

FY 1979 DISTRIBUTION OF RESEARCH FUNDS

Research Area	Grants Funded	Amounts ($000s)	Percent of Total
Neurophysiological	19	$ 977	45
Behavioral	10	606	28
Clinical	5	336	16
Combined and Miscellaneous	1	232	11
Totals	35	$2151	100

STAFF ACTIVITIES

In FY 1979, staff began preparations for a formal evaluation of its program activities. To expedite this project, Dr. Donald Kruper, Chairman of the Department of Behavioral Science, University of Pittsburgh School of Dental Medicine has accepted a five-month NIDR assignment under the Intergovernmental Personnel Act to work with program staff in formulating and refining program objectives and in developing evaluation criteria.

During the past year, program staff participated in site visits, monitored grants, communicated with researchers and professional organizations, and participated in scientific meetings, thereby maintaining close communication with scientists working both in orofacial pain research and social-behavioral research relevant to oral health.

Scientific Meetings Attended

*American Dental Association	October 1978
*Association for the Advancement of the Behavior Therapies	November 1978
*Behavior Science Group, American Dental Association Research Institute, Chicago	November 1978
*American Dental Association Council	February 1979
American Association of Dental Schools and American Association for Dental Research, New Orleans	March 1979

*Staff made formal presentation(s).

*Conference on "Oral Motor Behaviors" co-sponsored by NIDR, NICHHD, and NIA Bethesda	May 1979
*First Annual Meeting of the Academy of Behavioral Medicine Research Snowbird, Utah	June 1979
American Psychologcial Association New York	September 1979
*First General Scientific Meeting American Pain Society, San Diego	September 1979

Monitoring, Evaluation, and/or Programming Visits

University of Florida Gainesville	June 1979
State University of New York Buffalo	August 1979
University of Washington Seattle	August 1979

RESEARCH HIGHLIGHTS

Visualization of Nerve Endings in the Tooth Structure

Investigators at the University of Iowa and at the University of California at San Diego are trying to define within the tooth structure the nerve endings which transmit pain from caries and from other injuries to the dentine. Combining highly specialized electron microscopic techniques with a special computer program, they have demonstrated that the nerve endings interface with the odontoblasts at the periphery of the pulp. Three-dimensional representations of this interface indicate that the nerve endings form tree-like extensions at the sides of the odontoblasts and that a few extend with the odontoblast process into the predentine and the dentine. Thus, the odontoblast layer may serve as the chief receptor site of pain within the tooth.

Neuromuscular Control of Jaw Movements

Investigators at the University of California in Los Angeles are using guinea pigs to study the neuromuscular mechanisms that control chewing and swallowing. Their latest findings showed that, without any input of external information, the brain can produce rhythmic jaw movements similar to those of drinking and chewing. This finding indicates the existence of intrinsic pattern generators, consisting of highly organized groups of nerve cells. Presumably, these generators are organized before birth so that infants can suckle and perform other vital func-

tions. While examining the behavior of single cells within the areas of the brain that control jaw muscle contractions during jaw movement, these scientists discovered a pattern generator which inhibits jaw closing, so that the jaw can open without resistance from the powerful jaw-closure muscles. This simple mechanism is apparently necessary for such activities as normal chewing, yawning, and panting, but may be compromised by pathologic conditions which cause trismus or restrict jaw opening.

Arthroscopy of the Temporomandibular Joint

Diagnostic arthroscopy has been widely used in knee-joint pathology but has only recently been applied to temporomandibular joint (TMJ) disorders. These problems often require a nondestructive diagnostic technique like arthroscopy since open biopsy of the TMJ is contraindicated. To develop this diagnostic adjunct, scientists at the University of Illinois devised a procedure enabling them to perform arthroscopic examinations and biopsies of the bone and soft tissues of the TMJ with no untoward results.

After establishing the feasibility of the procedure, these investigators set out to determine if pathologic changes in the TMJ could be recognized. Surgical lesions, steroid arthropathy, adjuvant (rheumatoid-like) arthritis, and inflammatory changes were induced in rabbits. The TMJs were then examined arthroscopically, and confirmatory biopsies of the involved areas were performed. Correlation of the gross and microscopic observations indicated that condylar and meniscal damage, early degenerative changes, and inflammation of the synovial tissues are easily visualized and diagnosed by the arthroscope. This technique, therefore, appears to provide a relatively safe and effective instrument for examining the TMJ. It is now being used in patients with clinical and radiographic evidence of organic TMJ pathology to establish its usefulness in human patients. This procedure should help to reduce the amount of diagnostic surgery performed on the TMJ and should provide a more rational basis for treatment planning.

Fear of Dental Treatment

Whether individuals who have an exaggerated fear of dental treatment and go to great lengths to avoid it also have a low threshold of pain tolerance had previously been unclear. To settle this question, investigators at North Dakota State University measured the reaction to pain of a group of college students who were fearful of dental work and a control group who were not fearful.

Using well-controlled procedures, these investigators tested the students for tolerance to electric shock of the incisor and forearm. A constant-current DC stimulator was adapted to administer extremely low levels of current in fine increments. As the levels were increased, subjects reported "very faint sensation," "very faint pain," and "tolerance." The

levels for each response were recorded in microamps and the means for each group calculated.

The results showed that the high-anxiety group did not differ from the low-anxiety group in their pain tolerance levels for either forearm or incisor shock. In other words, fearful people were not more sensitive to objectively measured pain stimulation than nonfearful people. The groups did differ, however, in their emotional responses to the same stimulation. High-fear subjects scored higher than low-fear subjects on measures of anxiety taken during the pain tolerance tests. This difference was evident in tests for both arm and tooth pain.

The groups also differed in their pain ratings. After each tolerance test, subjects rated the intensity of the most severe pain they experienced during stimulation. Although there were no differences in the actual physical intensity of the stimulation received by the two groups, the high-fear people rated dental stimulation considerably more painful than did their low-fear counterparts.

Since these findings indicate that the emotional and cognitive aspects of the dental pain experience are more important than pain tolerance, they may provide an important lead for treatment studies designed to reduce adverse reactions and the concomitant avoidance of dental treatment.

Children's Response to Dental Treatment

Since childhood experiences with dentistry appear to be powerful determinants of later attitudes and behaviors toward dental care, investigators at the University of Connecticut, the University of Florida, and the University of Washington are studying children's behaviors during dental treatment. They hope to discover management techniques which will lead to improved attitudes and greater cooperation during treatment.

Scientists at the University of Connecticut are observing children 2 to 5 years old during sequential dental visits. The same pedodontist treats all patients; detailed behavioral and psychophysiological data are collected at each visit. These investigators have found that contrary to prevailing opinion, the presence of the child's parent in the dental operatory does not increase anxiety and noncompliant behavior. The scientists are also trying to find out whether the age of a child's initial exposure to dental treatment affects his/her emotional response and compliance at an older age.

Investigators at the University of Florida are studying the effect of the dentist's management strategy on disruptive and anxious behaviors. Fourth-year dental students are trained to use alternative behavioral "scripts." All of the "scripts" include clear information and directions for the child, but they vary in management strategy. One

script suggests positive comments after cooperative behaviors; the second script suggests negative comments after disruptive behaviors, and a third script, neutral (nonevaluative) comments only. Preliminary results indicate that fewer disruptive behaviors followed the use of the "neutral" comments.

Similar results were reported by investigators at the University of Washington who videotaped dentist-patient interactions of both "normal" and "disruptive" children and used a specific behavior-coding scheme designed for this purpose to score them. Their data show not only how the dentist's behaviors affected the child, but also how the child's behaviors affected the dentist.

Studies like these should expand our knowledge of the psychological impact of dental treatment on children and should lead to practical recommendations for minimizing or preventing the development of deleterious attitudes and behaviors.

MEETINGS SPONSORED

Second World Congress on Pain - During FY 1978, NIDR provided major support for the Second World Congress on Pain held in Montreal under the auspices of the International Association for the Study of Pain. This meeting drew scientists, physicians, dentists, and other health professionals from various parts of the world to discuss research findings and to reappraise current knowledge of and practices in pain therapy. About 25% of the scientific program was devoted to papers related to oral-facial pain. A four-hour plenary session on oral-facial pain, which consisted of 50 scientific presentations, was especially important to the success of the entire congress.

Extensive press coverage of the Montreal meeting resulted in immediate dissemination of information on the Congress in over 100 newspapers throughout the world. Proceedings of the meeting will be published as Vol. 3 of Advances in Pain Research and Therapy by Raven Press (released in October, 1979).

A separate satellite meeting, which was held in Toronto to focus on the scientific aspects of the dental and orofacial pain, consisted of three major sessions. The first dealt with peripheral mechanisms of dental sensitivity. The second dealt with central projections of afferent dental nerve fibers, particularly at the level of the brain stem. These afferents project to both the rostral and caudal components of the trigeminal brain stem complex; their functional role in mediating tooth pain and jaw and facial motor reflexes is of great interest to researchers. Chemical mediators at these sites were also discussed. In the third session, new methods of assessing clinical pain both in vivo and in vitro were described. Chronic orofacial pain syndromes, including the TMJ pain dysfunction syndrome, were also discussed.

Interdisciplinary Conference on Oral Motor Behaviors

An interdisciplinary conference, "Oral Motor Behaviors: Impact of Oral Conditions and Dental Treatment," was held in May 1979, under the joint sponsorship of NIDR, NIA, and NICHHD. Developed by the staffs of the three institutes, this conference synthesized our current knowledge of oral motor behaviors and their dental effects. The participants agreed that treatment failures and pathologic conditions caused by maladaptive oral motor behaviors constitute a significant health problem which exacts a heavy toll in human and financial resources.

The conferees discussed the special oral health problems of children, adults, and the elderly, and identified areas of research need. They concluded that the relation between oral habits and the development of malocclusion remains unclear, despite claims to the contrary by some clinicians. They also pointed out inconsistencies in the results arrived at by different measurement techniques which have been used in research on bruxism and temporomandibular joint pain and specifically recommended research on the etiology and treatment of these conditions. They also observed that nearly all animal and human studies of oral function and behaviors have excluded older individuals despite the fact that they may be particularly vulnerable to oral motor disorders and to difficulties in changing motor behaviors as natural dentition is lost and replaced by dentures. These problems affect nutrition, speech, and social interactions. Proceedings of the conference have been published as a book entitled Oral Motor Behaviors; Impact on Conditions and Dental Treatment which will be available for distribution in December, 1979.

FUTURE PLANS

During FY 1980 attention will be principally directed toward broadening the scope and strengthening the quality of the research and research training supported by the program. Program balance will also be emphasized. An important related activity will be to establish evaluation criteria to determine how effectively program objectives are being met. Dr. Donald Kruper, a visiting dental scientist and educator, will coordinate this evaluation activity.

Pain Research

Using the new insights provided by recent discoveries of the brain's own pain suppressive mechanisms, mediated by endorphins and other neuropeptides, scientists may soon be able to develop techniques that will help individuals to control their own pain. To better attain this goal, staff will encourage research in these areas, especially that which is relevant to both acute and chronic dental and orofacial pain. Staff will also encourage projects that use acute dental pain, such as that associated with third molar extractions, as a model for clinical trials of new pharmacological and nonpharmacological pain control methods.

Plans for the major conference on dental anesthesia and sedation, to be held in the spring of 1981, will be finalized. Despite efforts by NIDR to increase research and research training in this field, its scientific base remains seriously deficient. The aims of the proposed conference are to identify the serious knowledge gaps and to develop strategies for bridging them.

Social and Behavioral Research

During FY 1980, a major effort will be made to expand the scope and strengthen the quality of social/behavioral research in dentistry. A specific focus will be on research related to the social and behavioral concomitants of orthodontic treatment and orthognathic surgery and to the factors that are most likely to induce patients to adopt and maintain preventive practices. The program planned for the First National Research Conference on Oral Health Behavior, to be held in April, 1980, should provide a much-needed "state-of-the-art" review of research on the determinants of oral health behaviors since it stresses the impact of health practitioners, institutions, and communities on the use of preventive measures in combating oral disease. The conference should thereby advance program goals by stimulating additional research on the social and behavioral factors that relate to prevention. Papers prepared for the conference will be distributed to the behavioral/social science and dental research communities through journal articles planned for publication in late FY 1980.

SUMMARY

During FY 1979, the Pain Control and Behavioral Studies Program made 35 research grant awards totaling $2.1 million. The program funded seven fellowships in research training at a cost of $76,000 and two institutional training programs totaling $184,000. Highlights of the research supported are summarized below.

Visualization of Nerve Endings in the Tooth Structure

Using highly specialized techniques and analyses, scientists showed that the dental nerve endings form tree-like extensions at the sides of the odontoblasts with some processes extending into the predentine and dentine layers. These findings suggest that the odontoblast layer serves as the chief pain receptor within the tooth.

Neuromuscular Control of Jaw Movements

Studies indicate that within the brain highly organized groups of nerve cells function as intrinsic pattern generators to activate the rhythmic jaw movements involved in drinking and chewing. Specifically, a pattern generator was found that inhibits the jaw from closing, so that the jaw can open without resistance from the powerful jaw-closure muscles. This finding may provide insights into the etiology and treatment of clinical

problems associated with restricted jaw opening.

Arthroscopy of the Temporomandibular Joint

Arthroscopy, a widely used technique for the diagnosis of kneejoint pathology, is now being applied for the first time to the temporomandibular joint (TMJ). Recent studies have established its usefulness for the diagnosis of pathology in the TMJ of animals, and current research is assessing its applicability to the TMJ of humans.

Fear of Dental Treatment

The question of whether individuals with an exaggerated fear of dental treatment also have a low pain tolerance was investigated. The findings indicate that fearful people do not have a lower pain tolerance than nonfearful people. The authors concluded that the emotional and cognitive aspects of dental pain are more significant in determining the individual's reaction than the actual pain involved.

Children's Response to Dental Treatment

The results of several studies of children's behavior during dental treatment indicate that:

1. Contrary to prevailing clinical opinion, parental presence in the room does not produce greater anxiety or noncompliant behavior in the child;

2. Dentists using neutral or nonevaluative responses to the child are more effective in minimizing disruptive behavior than those using positive or negative responses.

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