

ANNUAL REPORT  
OF  
PROGRAM ACTIVITIES  
NATIONAL INSTITUTES OF HEALTH

1961

NATIONAL INSTITUTE OF DENTAL RESEARCH

NATIONAL INSTITUTES OF HEALTH  
PUBLIC HEALTH SERVICE  
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE



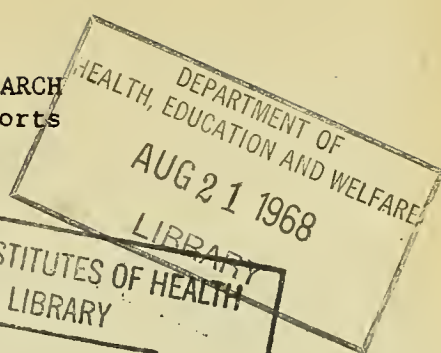






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NATIONAL INSTITUTE OF DENTAL RESEARCH  
Annual Narrative and Project Reports  
Calendar Year 1961



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# National Institute of Dental Research

## Annual Report of Administration

Calendar Year 1961

The National Institute of Dental Research is conducting research in the broad areas of dental caries, periodontal disease, various oral-systemic relationships, and abnormalities of growth and development affecting the oral cavity, face, and head. Related to these studies is a wide range of basic research and clinical investigations covering such areas as genetic influences on patterns of oral health and disease, biochemical and histological evaluations of function as related to both normal and abnormal tissues; epidemiological aspects of disease in the aged and chronically ill; and the utilization of various research tools including germ-free techniques and electron microscopy and X-ray diffraction. The Office of the Director is responsible for the over-all planning and coordinating of these research programs and for the administration of the dental research grants program.

Due to program expansion, the following organizational changes have been made: (1) establishment of the position of Assistant Director; and (2) establishment of the following new sections: (a) Section on Protein Chemistry; (b) Section on Oral Pharyngeal Development and Function; (c) Training Section; and (d) Research Grants Section. During the coming year another important addition to the Institute will be the establishment of a program analysis operation in the Office of the Director.

During the summer of 1961, the Office of the Director, assumed responsibility for coordinating the dedication plans with the Director, NIH and officials in the Public Health Service and the Department.

To accommodate the extended research activities in the new building, a Research Services Section was established to provide assistance to the research staff through its glassware preparation and cage washing facilities and coordination of the animal experimental program. Another central service, established in the Laboratory of Histology and Pathology, is related to the operation of a maintenance and developmental instrument shop. This service is available to all NIDR laboratories for the repair of existing physical equipment and the construction and testing of new laboratory instruments.



# The National Institute of Dental Research

## Annual Report on Informational Activities

Calendar Year 1961

The Dental Institute Information Office carries out a program of professional and public information that focuses on the improvement of general health through the conduct of research on oral and related diseases. This is a continuing operation with its general function specified in the Dental Research Act. Thus, program efforts during the year continued toward the development of more effective means of communication between the general public, professional and lay groups, and individuals both within and outside the Government upon whose interest and support much of the success of the NIDR program depends.

### Trends in Dental Information

The National Institute of Dental Research has become in the space of 13 years a symbol and a means for further enhancing the dental profession. Its appropriations and programs, although small in relation to other Institutes, have had, and will continue to exert, a notable influence on the course of dental education, research and practice. Evidence of this is seen in the history of grant supported research papers presented at the annual program of the International Association for Dental Research, a principal scientific meeting. In 1957, seventeen percent of the papers were supported by NIDR grants. By 1961 the percentage had increased to 81 percent.

A fundamental change is also occurring in the scientific orientation and attitude of investigators directly and indirectly associated with the Intramural Program. Evidence of this change is seen in the knowledge that NIDR scientists are now contributing to the scientific community primarily through publications in the basic science journals, where formerly research reporting was mainly confined to a relatively few dental journals. Current records show, for example, that the research papers submitted from NIDR last year were published in 33 different journals representing almost all scientific disciplines and specialties. While this trend is indicative of progress, many dentists practicing today were trained in the absence of a research environment, and are not accustomed, nor do they have the opportunity to read extensively in the basic science literature.

The resolution of this basic communications problem, not wholly unique to dentistry, continued in 1961 to be a priority target of the NIDR information program. Examples of progress made included the publication, in selected dental journals, of specially-tailored articles reporting on intramural research work at the Institute. Also in the coming year, the Research Highlights Booklet will be stylized to make it more readable to a wider audience. Additional experience has shown that these articles and booklets are attracting many science writers from the national press and from subprofessional journals and newspapers





(widely distributed among dentists), whereas, classical research reports in professional journals have failed to do so.

### Public and Professional Relation

Noteworthy among accomplishments in 1961 was dedication of the new dental research building with primary responsibility for planning and conduct of the ceremony assigned to the Information Office.

The dedication took place on May 26, and many distinguished persons were present including the Secretary of HEW, the Surgeon General, the Director of the W.K. Kellogg Foundation, Congressmen, the President of the American Dental Association, and many others. Also attending were some 500 guests. Considerable publicity was associated with the dedication and subsequent occupancy of the research building; and, the past eight months have witnessed a sharp increase in the number of visitors to the Institute.

Other opportunities for public and professional relation activities are offered on a continuous basis in reply to the many requests for information from the general public and individuals in dental and related health professions. In addition to mail, telephone, and in-person inquiries received directly by the Information Office, inquiries were also referred for appropriate handling from other areas of the Institute, the Service, the Department, other Government agencies, and members of Congress. Also, the past year saw an increase in the coverage of dental intramural research by many facets of the professional and lay media with emphasis on press, special announcements, exhibits, etc. Particular attention was directed to servicing science writers, and staff members of national publications, and house organs.

### Internal Reports

Internal reports prepared in whole or in part by the Information Office constituted another broad area of activity in Calendar 1961. Such documents, required by all Institutes, include weekly reports of selected dental research advances and program developments prepared for the Director, NIH, the Surgeon General, Congressional personnel and others; the annual report of the Dental Institute for publication in the over-all annual report of the DHEW; and, an annual report on activities of the Dental Information Office. Special documents for budgetary and appropriation hearings are also prepared. These include the Director's Opening Statement and supporting material, a compilation of highlights of research progress and program developments, and a special report on recent progress in the field of genetics for the House Appropriations Committee. Also included among internal documents prepared or contributed to during the year are speeches and articles for key personnel at various echelons of Government.



THE ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

The National Institute of Dental Research

Calendar Year 1961

by

Dr. Seymour J. Kreshover

Summary statements by the Dental Institute's program leaders, introductory to the project reports of their principal investigators, provide in varying degrees of adequacy, a highlight account of research activities by organizational categories. While such reports understandably emphasize an integrated project effort within the respective segments of the Institute and make prideful reference to the more noteworthy evidences of productivity, the actual daily experience of research operations reflects an optimum orientation of the laboratory and branch chiefs toward programs of research rather than scientific disciplines.

Appreciably influencing the daily conduct of research activities throughout the Institute is a continuing series of regularly scheduled scientific sessions where program leaders share responsibility for making critical appraisals of programs and discuss implications for future planning. At these weekly luncheon meetings, investigators occupying positions of team leadership present detailed accounts of their programs. Thus, all echelons of research administration are kept informed of the total Institute effort; a forum is provided for the discussion and assessment of new and continuing programs; ways and means are sought to further program productivity; and relationships of the various programs to each other are considered in terms of actual and potentially fruitful collaborations with other NIH scientists and outside groups.

It has been said that groups conducting research are confronted with two tasks: to give relative weight to the goals of its individual members, and to establish priorities for the utilization of its total resources. Inasmuch as research programs are ideally characterized by forward movement with, however, an always existing prospect of reversal or stasis, some yardstick of measurement is desirable to assess the differential of productivity or diminishing returns. Recognizing that a judgment of accomplishment must frequently await the test of time, and that a presumably important contribution today may become the insignificant event of tomorrow, any inventory of research accomplishment should be retrospective.

In attempting to evaluate our current program, a valuable take-off point is the analysis prepared five years ago for the HEW Secretary's Consultant Group on Medical Research and Education (Bayne-Jones Committee). This report, covering calendar year 1957, described the Institute's over-all



program under three general categories: areas receiving major emphasis and warranting future expansion; areas presenting promising leads for future expansion; and minor project areas receiving careful evaluation for evidences of productivity and justification for continuation. The following comparative program descriptions, covering a five year span, hopefully will provide an objective view of the patterns of change, the successes and failures in terms of the foreseen and the unforeseen, and implications for the future.

Program Activities for  
Calendar Year 1957

Course of Program and  
Current Status

I. Program Areas of Major Emphasis

1. Human Genetics:

Studies during this year were limited largely to an inbred population group in Southern Maryland (Brandywine Program). Among the more significant findings was an incidence of hereditary dental defects 300 times greater than previously reported for any other population group in the U.S. A correspondingly high incidence of other genetically determined conditions (sickle cell anemia, albinism, etc.) attracted the interest of other categorical Institutes of NIH as well as a number of investigators from outside institutions. While productivity was reflected in the publication of three papers, the major effort during the year was devoted to problems of program organization and planning for a considerably broadened base of operations to include other isolate population groups in the Eastern U.S. as well as an emphasis on the biochemical aspects of hereditary disease.

A considerable expansion during the past four years included (a) cytological studies of a newly described oral disease in an isolate population in North Carolina; (b) a study of hereditary patterns of dental development in the offspring of consanguinous marriages in Japan; (c) a study of human chromosomal aberrations in relation to growth defects such as clefts of the lip and palate, micrognathia and other oral and facial deformities; (d) a study of the genetics of secretor factor in saliva and its relationship to other genetic markers; and (e) the identification and study of additional isolate population groups in Eastern U.S. These activities led to the publication of fourteen reports during this period. Among the more significant contributions were the description of an heretofore unrecognized hereditary oral disease (benign intraepithelial dyskeratosis); the clarification of mechanisms of abnormal oral development based on longitudinal twin studies and analysis of family data; the identification of a rare gene mutation defect resembling Albers-Schönberg disease; and the collection of histochemical, genetic, social,



physical, dental, and laboratory data from the Brandywine group. Major emphasis in the future will be given to the programming of this data for machine analysis.

## 2. Periodontal Disease:

a. Epidemiological studies. Following the development and testing of a field method for the mensuration of periodontal disease, efforts were directed toward the compilation of a descriptive epidemiology. In the first of these studies, conducted in India (in cooperation with the WHO, the Indian Council on Medical Research, and the University of Michigan), data were assembled which showed a prevalence of advanced periodontal disease at earlier ages than ever previously recorded for comparable population groups in the U.S. While oral hygiene status undoubtedly contributed to this incidence, nutritional factors were an additional consideration. Another study in Alabama called attention to the importance of socio-economic factors in explaining the marked racial (negro vs. white) differences in prevalence. A later attempt to implicate various chronic systemic illnesses was unsuccessful. The total year's effort led to the publication of five papers. Plans for the future were to collaborate with the ICNND in its international surveys.

b. Laboratory studies. Of particular interest during this year was a study to compare the biochemical pattern of inflammatory response in normal periodontal tissues with comparable structures in animals subjected to various systemic disturbances such as alloxan diabetes. The observed alterations in glycogen, DNA, and soluble organic phosphorous led to

A considerable expansion of epidemiological studies occurred during the past four years. Particular emphasis was given to surveys conducted in cooperation with the Interdepartmental Committee on Nutrition for the National Defense. Findings of significance were reported in a series of twelve publications. While it may be concluded from the narrative report of the Epidemiology and Biometry Branch that considerable progress was made, a major problem was encountered in the recruitment of professional staff. Because of the critical shortage of qualified dental epidemiologists in this country, much of the total program effort in epidemiology was directed toward preceptorship training of promising young investigators. Plans for the future include an increased attention to this inservice training program and a parallel effort to attract recent graduates of dentistry to the postgraduate programs in epidemiology recently initiated at the Universities of North Carolina and Michigan.

Broad expansion of laboratory activities included analytical studies of collagen and other proteins in relation to function and disease, and the utilization of germ-free technics to clarify the role of bacteria and calculus formation in periodontitis. Inasmuch as collagen is a major structural protein and appears to play a specific role in





speculation regarding the factors that control resistance and susceptibility to inflammation. Other areas of laboratory research were concerned with the identification of microorganisms associated with periodontal disease, and the histochemistry of the attacked connective tissue. These studies were moderately productive and were reported in eight publications. Plans for the following years were to broaden the base of activity to include germfree studies and an emphasis on a better understanding of the composition and structure of collagen.

certain metabolic processes, an understanding of its molecular structure was considered essential to an understanding of pathologic processes in periodontal disease. The recent elucidation of the cross-linking mechanism between subunits of the collagen molecule may provide one explanation of the changes that occur in connective tissue with age and thus account for the greater prevalence of periodontal disease in elderly persons. However, the continuing complexity of the problem of periodontal disease may be illustrated by the further finding that calculus, a provocant of periodontitis, can form in the germfree mouse, whereas, in the rat, there is evidence to suggest that it may be induced by inoculation of pure cultures of microorganisms recovered from diseased animals. The contributions from these and other laboratory studies of periodontal disease have been reported in over sixty papers during the past four years. While plans for the future are to continue an emphasis on the laboratory approach to this complex problem, a considerable effort will be made to develop a more meaningful and productive clinical program.

### 3. Nutritional Aspects of Oral Disease:

Major attention during 1957 was directed to the further demonstration of the role of nutrition in dental caries. Following the evidence that lysine supplementation in lysine deficient rats appreciably reduced the incidence and severity of caries, studies were initiated to evaluate the possible anti-caries effect of phosphate compounds. A substantial body of evidence was accumulated to show that a significant relationship exists and that the effect is presumably mediated through some local

Particular attention was directed during the past two years to the initiation of a caries study in American Indian children who were given  $\text{CaHPO}_4$  in the form of a two per cent additive to the daily bread consumption. This study was planned in collaboration with the NIAMD, the Division of Indian Health, and the Bureau of Indian Affairs. Data accumulated after a one year period does not support the early laboratory evidence of a relationship between dibasic calcium phosphate and caries. However, a longer period of



activity on the tooth surface. This hypothesis was supported, to some extent, by the evidence that mineral ash, calcium, and phosphorous content of enamel and dentin were not altered following extreme deficiencies or variations in the Ca and P of the diet.

The investigations pursued during this year were reported in three publications. Plans for the future included a clinical trial of the phosphate compound.

observation will be required before a reliable judgment can be made. In another recently initiated study on the same population group, an effort is being made to evaluate the feasibility of using a dietary calcium additive as a means of reducing the skeletal burden of strontium-90. This project, in collaboration with the NIAMD, is formulated on the premise that the dilution of strontium-90 (ubiquitously present in dairy, grain, and other foodstuffs) with dietary calcium free of this nucleide, will bring about a reduction in strontium deposition in skeletal tissue.

The degree of activity in the nutritional program during the past four years is reflected by the publication of approximately twenty papers. While the dollar support has substantially declined since FY 1958, this field will continue to receive attention. Plans for the future include a detailed strontium and calcium balance study on Indian children brought to the Clinical Center from the caries testing program in South Dakota.

#### 4. Bacteriological Aspects of Oral Disease:

##### a. Germfree studies.

This program emphasized two major problems; technical operations and diet preparations. In the latter category, although the process of autoclaving was found to cause a considerable reduction in nutritional value of caries test diets, success was achieved in the maintenance of a caries-potentiating effect. In other studies designed to test microbial relationships, an unavoidable delay was experienced because of the limited capacity of the two available tanks. Although progress during the year was slow, a considerable expansion of activities was envisioned for the early future.

Due to the considerable requirements for space, significant expansion of the germfree program continued to be delayed until facilities became available in the new laboratories of the Dental Institute in July 1961. Nevertheless, the previous years of limited activity were quite productive. The several contributions of note were related principally to studies of periodontal disease, dental caries, calculus formation, and the interaction of microbial and nutritional factors as they affect the oral and systemic welfare. Most significant, perhaps, was the finding that caries in the rat and hamster is a transmissible, infectious disease and



that a single, host-specific micro-organism can be implicated. Although the causative organism in each animal species is of similar type (anaerobic streptococcus), neither strain is cariogenic for the heterologous species. A further observation of significance is that since both organisms are potent producers of lactic acid, and neither produces extracellular proteolytic enzymes, acidogenesis is obviously not the only factor involved in caries initiation. An important direction of research during the next year will be the application of these findings to studies in man.

b. Other microbiological studies.

Major attention during this year was given to a study of bacteremia following dental treatment. Conducted in cooperation with the NHI, this investigation provided the important information that bacterial invasion of the blood stream, following routine operative and oral surgical procedures, was considerably higher (82-88 per cent) than previously reported. The obvious implications were for more effective prophylactic procedures to remove risk to patients with rheumatic heart disease and other cardiac lesions. No plans were made to extend this study into succeeding years. The remaining activities in the area of microbiological research were devoted in large part to studies of the nutritional requirements and metabolism of oral bacteria, and the isolation, cultivation, and identification of oral spirochetes.

Personnel previously occupied with the bacteremia studies were able in the past several years to direct their attention to other program areas. In addition to the expansion of the gnotobiotic program, areas of emphasis during the past year have included viral infections of oral tissues; relationships between tissue enzymes and experimental infections; and the nutrition and metabolism of oral bacteria. Productivity of this over-all, expanded operation in microbiology is reflected by the publication of over forty papers (exclusive of those previously mentioned in connection with periodontal disease research) in the course of the past four years as compared with two in 1957. Plans for the future are to establish an Immunology Section for the study of cellular and humoral mechanisms of resistance to the oral microbiota; and a further exploration of tissue hypersensitivity to antigens of the oral microbiota in relation to periodontal inflammation.



## 5. Electron and X-ray Microscopy:

For the greater part, this program was directed toward (a) the determination of the crystal structure of calcified tissues by electron diffraction; (b) the investigation of the reaction of various chemical agents, such as fluoride compounds, on tooth surfaces, with emphasis on the mechanisms by which acid-solubility of enamel is altered; and (c) the development of technics for the application of contact and projection x-ray microscopy to studies of hard and soft tissues. While these objectives were, in great part, successfully met, considerably more was learned about the sub-microscopic structure of organic matrix of calcified tissues than about the mineral content. These and other findings, were reported in four publications during the year. Plans for the future were to increase the scope of studies related to mechanisms of calcification, crystallographic approaches to a better understanding of the structure and composition of mineralized tissue, and further development of technics of x-ray microscopy for application to problems of interest in dental pathology.

While earlier efforts emphasized the development of new and improved biophysical methods for studying calcified dental tissues, the current program has been characterized by a considerably broader area of activity. Receiving major attention have been studies to (a) characterize, in detail, the inorganic components of mature and developmental calcified tissues (including composition, atomic structure and dimensions of the fundamental apatite crystals in tooth and bone), and (b) describe the crystallization processes involved in normal and abnormal calcification (using both vital and model systems), and the chemical reactivity of the mineral phases.

Among the more challenging concepts that emerged from these studies were (a) that the major phase of all biological apatites is some calcium deficient hydroxyapatite with compensatory hydrogen bonding, and (b) that the higher chemical reactivity of young calcified tissue compared to older tissue may be a result of the gradual perfection of this calcium deficiency. The degree of program productivity during the past four years is demonstrated by the publication of over thirty papers. Plans for the immediate future will be to continue an emphasis on crystallographic studies (in parallel with studies of the organic matrix) in order to provide a better understanding of the structural alterations involved in malformation and caries.





## II. Program Areas Presenting Promising Leads for Future Expansion

A number of activities, although receiving modest levels of support during the year, gave promise of significant accomplishments in the early future. These included:

1. The development of new histochemical procedures for the demonstration and precise localization of enzyme activity in teeth and supporting connective tissues.
2. Studies of the physiological response of ambulatory patients to various general anesthetic agents.
3. Studies of prosthetic reconstruction procedures for maxillo-facial defects and the formulation of principles of design that could contribute to improved functions of mastication.
4. Studies of the chemical composition of saliva in caries-free and caries-active individuals.
5. The development of caries-susceptible and caries-resistant strains of animals as an approach to the further study of genetic factors involved in the caries process.

The productivity of this heterogeneous group of project activities was evidenced by the collective publication of ten papers.

While some portions of the program effort considered promising five years ago did not later justify significant increases in dollar support, their degree of productivity was sufficient to warrant continuation. These included the assembling of baseline physiological data for evaluating patient response to newer drugs used in general dental anesthesia, and the design and construction of obturators and other prosthetic devices used in cleft palate and post-surgical cases involving extensive loss of tissue.

Areas of current major emphasis that evolved from the earlier, modestly supported projects were: (a) an expanded program effort in histopathology and histochemistry that led to the development of new histochemical staining technics and ultimately to the discovery of a new connective tissue fiber (oxytalan), and (b) an expanded animal caries program that led to the discovery that caries resistance and caries-susceptibility in different strains of hamsters is governed by the absence or presence of a specific microorganism and not by genetic constitution alone. This major contribution to our knowledge of dental caries is now being further augmented by large scale studies to identify and classify the causative microorganisms in other animal species. It is hoped that the early future will see an application of these studies to a better understanding of the disease process in man.

The degree of productivity represented in the three continuing projects and the two major program areas is reflected by the publication of almost forty papers during the past four years (exclusive of those previously mentioned in the microbiology program).



### III. Minor Program Areas

A number of research projects were pursued during the year with minimal support. Although designated as minor, each was evaluated carefully as a prospective area for future emphasis. Included among the studies were:

1. Biological effects of certain instrumentation procedures on dental pulps and surrounding tissues.
2. The biological effects of fluorides.
3. The effectiveness of chemical agents in the control of dental caries.
4. Factors which influence the pathogenicity of oral spirochetes.
5. Diseases produced by fungi in the oral cavity.
6. Differentiation of strains of oral viruses.

Although varying degrees of progress were made, all the listed studies were considered sufficiently promising to warrant continued support in the next year. Accomplishments were reflected by the publication of seven papers in three of the study areas; pulp response to operative dental procedures, fluorides, and oral spirochetes.

While moderately increased levels of support were provided during the past four years for most of the project areas, particular attention was given to studies of the biological effects of operative dental procedures on pulpal tissue, and the effectiveness of certain chemical agents in the control of dental caries, and the pathogenicity of oral spirochetes. The following are some of the more significant accomplishments which have been reported during the past year in a series of eight publications.

1. Pulpal response to the newly introduced high rotary speed instruments, and to restorative filling materials was shown to vary directly with the speed and pressure used in instrumentation, the thickness of dentin overlying the pulp, the type of coolant used, and the size of the rotary cutting tool.

2. A pronounced caries-inhibiting effect was demonstrated in rats when the carbonyl-binding compound, sodium metabisulfite, was added to the diet. The degree of effectiveness approached 83 per cent reduction in caries when the chemical was made available on an uninterrupted basis for 90 days. Intermittent feeding of the compound for lesser periods continued to show a beneficial effect, although to a lesser degree. Parallel studies have been in progress to evaluate the effectiveness of other carbonyl-binding compounds.

Efforts in the next year will be directed to a continuation of these programs with the primary objective of evaluating newer dental restorative materials and rotary instruments; and testing a variety of chemicals for their anti-caries properties. Continued attention also will be given



to the study of viral infections of the oral tissues, the role of bacteria in the formation of dental calculus, and methods of cultivating oral spirochetes.

While this review of intramural activities is necessarily deficient in its coverage of the Institute's over-all program activity, its purpose has been to deal in generalities so as to provide a readily assimilated digest of the relative productivity of different program efforts; the manner in which changes and trends in the recent past have brought us to the present; and the implications of these events for the future.



# THE NATIONAL INSTITUTE OF DENTAL RESEARCH

## Annual Report on Extramural Research

Calendar Year 1961

### Research Grants Program

During the year effort has been expended in most of the major areas of interest to dental research. Currently these areas are: caries, periodontal disorders, congenital anomalies (cleft palate), calcification (bone and enamel), growth and development, and salivary studies. In addition, efforts will be continued in those other aspects of dental research such as basic science studies related to oral health, dental public health investigations, and studies to improve dental materials.

Research progress achieved by the Institutes' grantees is an indication of the importance and development of the Institute's extramural program. All of the 49 dental schools in the United States have now participated in the Institute research grants program. Approximately 80 percent of the funds allocated for research projects are devoted to research support at these institutions. During 1961, the number of active research grants totalled 477 at 139 institutions in 39 states and territories and 13 foreign countries compared with 372 grants at 101 institutions in 36 states and 7 foreign countries last year.

New findings on the etiology and mechanism of experimental caries were announced. Grantee investigators at the Harvard School of Dental Medicine have reported recently on studies of inherited characteristics that influence susceptibility and resistance in selected strains of rats. These studies were designed to provide varying degrees of contact between representatives of the Harvard susceptible and resistant strains of rats at varying periods in their life history to determine if they could influence each other's rate of caries activity. All animals were reared under identical environmental conditions and maintained on the same cariogenic diet. Results showed that rats of the susceptible strain developed high rates of caries activity when placed in contact with animals of the resistant strain. However, three-quarters of the members of the resistant strain that were maintained in direct contact with caries active members of the susceptible strain remained free of decay throughout the experimental period of 250 days. The remaining quarter developed a small number of slowly progressive decay lesions. Thus, under this highly specific set of experimental conditions, the investigators were able to classify animals susceptible or resistant to caries, presumably on the basis of hereditary factors.

A coordinated in vivo test of SnF<sub>2</sub> prophylaxis paste and 8 percent topical SnF<sub>2</sub> treatment has continued to receive support from the Institute. The effectiveness of the use of a stannous fluoride tooth paste in preventing caries in children is being compared with the standard topical technique. This joint survey is being conducted by the Minnesota and North Dakota Department of Health, Division of Dental Health. The investigators are





studying the dental health of approximately 750 children 11-13 years of age following various prophylactic treatments. The data obtained will be evaluated statistically to determine the value of the paste treatment and the amount of discoloration derived from the various treatments.

Moreover, during the year a new grant was approved which will support a study on fluorine: a new method for determining fluoride in drinking water. The proposed new method of fluoride analysis employing an organic compound as the reagent is completely different from other methods because it eliminates the use of the zirconium-alizarin lake entirely. This grant, at the University of Pittsburgh, will support studies designed to produce new data on the accurate determination of fluoride in water supplies. With the growth of the practice of fluoridation of water supplies as a Public Health measure, a simplification of these methods is of increasing importance.

Support to grantees at the California State Department of Public Health investigating hypoplasias in varying fluoride and temperature zones has continued. The ultimate objective of this proposal is the determination of optimum levels of fluoridation for communities with differing climates, as indicated by variations in patterns of dental caries and fluorosis.

During the current year, special emphasis is being directed to augment the multidisciplinary approach to the cleft palate problem. One such study is designed to evaluate the success of cleft palate management, and to predict the value of the various diagnostic procedures in terms of speech adequacy. Working as a team in this effort are speech pathologists, physicians, and oral surgeons.

Another study involves the dynamics of speech in cleft palate patients who wear a dental prosthetic "speech aid." It is expected that information, relative to the optimal placement of a speech appliance, will contribute to our knowledge and treatment in the areas of prosthetics, plastic surgery, and speech pathology.

Indicative of the activity in the cleft palate field currently is the receipt of four applications for cleft palate centers. Ultimately our efforts will be directed to recognize the magnitude of the problem of a child with congenital malformation including an examination of the malformation throughout its biologic continuum, from genesis through post-natal development. Basic research utilizing a team approach in the medical, dental, and social disciplines seeks information to augment our understanding of this anomaly and to provide a better basis for optimal rehabilitation.

Prominent this year in the field of congenital anomalies was the award of a grant in support of a research program to explore a method of effecting dental-orthopedic adjustments of the premaxillary and maxillary elements in the infant with wide complete unilateral or bilateral clefts of the lip and palate. This technique, i.e., early orthodontic adjustments, coupled with ideal palatal surgery and orthodontic guidance during the development periods, may aid materially in minimizing or perhaps eliminating in many instances one of the major problems of growth and development in this segment of the cleft palate population. This is an important area of the cleft palate



study which needs further research from the standpoint of orthodontic intervention beginning at age of 3 weeks, and the institution or repositioning of the premaxilla prior to lip surgery.

This program, conducted at the United Hospitals of Newark, Newark, New Jersey, will emphasize an integrated approach to the cleft palate problem in the orthodontic, surgical, and rehabilitation phases. Since the program involves a broad spectrum of investigations ranging from an evaluation of psycho-social factors to orthodontic and surgical treatment procedures, provisions have been made to use the services at the Barkhorn Memorial Speech and Hearing Center for psychological, otolaryngeal, and social services consultation in keeping with the total child concept.

The National Advisory Dental Research Council, through its Program Planning Committee, initiated the establishment of a grant to plan a comprehensive team approach to the study of periodontal disease, exploiting the long-term basic science approach rather than a disease oriented emphasis. It is expected that the information resulting from this planning grant will provide a realistic basis for further work in the study of periodontal disease.

Indicative of the broadening base of dental research today is a proposal to study germ-free animals in its approach to dental diseases and the environmental relationship of organisms. The requirements for growth and pathogenicity of bacteria involved in the etiology of experimental caries and periodontal diseases will be studied.

A considerable body of evidence suggests that microorganisms are involved in the pathogenesis of periodontal disease in man and in some experimental animals. Organisms from periodontal disease in man have been shown to be capable of eliciting mixed anaerobic infection in guinea pigs. The essential minimum components of one such infection have been shown to be four in number. These organisms or others associated with periodontal disease in man have been demonstrated to elaborate enzymes and other metabolites capable of destroying the principal chemical components of connective tissue. Among these products are collagenase, fibrinolysin, desoxyribonuclease, ribonuclease, hyaluronidase, beta glucuronidase, chondroitin sulfatase and ammonia. It is possible that the capacity of these organisms to elaborate tissue-destroying metabolites is essential to the pathogenesis of periodontal disease. The theory can be put to a direct test by trying to produce periodontal disease in hamsters exposed only to the four key organisms which are presumed to be involved.

Disorders of the supporting structures of the teeth constitute one of the major problems in oral medicine. Fundamental to the control of this disease is an understanding of bone formation and destruction. These are complex processes, having many factors which require and facilitate the fabrication of organic matrices, enzyme systems, hormones, vitamins, etc., as well as the transport and precipitation of complex minerals upon a matrix to form a functional structure. Thus, a determined and vigorous effort must be made in the basic sciences related to dentistry, if a major breakthrough in periodontal diseases and the calcification complex are to be achieved.



Five more years of support have been awarded to grantees at the New York University School of Dentistry for studies on the basic nature of the mineralization process in all hard tissues. Both vertebrate and invertebrate animals will be studied, to elucidate such factors as the chemical mechanism which "triggers" mineralization and the "critical" period in the embryological mineralization process. Humans also will be studied to explore further the placental transfer and uptake by the fetal hard tissues of the tetracycline antibiotics. It is expected that this research project will contribute to our basic knowledge of mineralization; and also, that some of these studies may be of therapeutic interest and add to the clinical armamentarium.

The prediction of the amount and direction of facial growth is crucial for the diagnosis and treatment planning of specialties concerned with child growth and development. The hereditary factor, although acknowledged as being a major consideration, has not been thoroughly investigated. Thus, the role of heredity in the determination of facial growth will be initiated and the investigation of nutritional and endocrinological factors in the etiology of postnatal dento-facial anomalies will be expanded. Special significance will be placed on research concerned with the embryologic development of the face and jaws.

A grant resulting from the concerted efforts of the Dental Study Section and the Council to stimulate research in a basic area essential to a better understanding of oral health is supporting an International Conference on Salivary Glands and their Secretions. The Conference proceedings will be published, and it will include a comprehensive review of the current knowledge in the field which will be used as a point of departure to stimulate research in this area and broaden our understanding of the biology of salivary glands and their secretions.

Grantees at the Eastman Dental Dispensary during the course of an investigation into the fermentation of foodstuffs by saliva and the development of a new method of measuring acid production in these mixtures, directed their attention toward the relative fermentative capabilities of whole saliva, salivary sediment, and supernatant.

The relative fermentative capabilities of whole saliva, salivary sediment, and salivary supernatant on foods was examined, using the periodic neutralization technique. In no case did sediment or supernatant produce as much acid as did whole saliva. The intrinsic glycolytic activity of saliva was shown to rest in the sediment, but the supernatant appeared to contain some adjuvant which increased the glycolytic potential of the organisms. In an effort to identify this adjuvant, artificial amylase and buffer systems were added to salivary sediment. While boosting acid production, these artificial additions were not so effective as natural supernatant in restoring sediment to the full glycolytic activity of whole saliva.

The Dental Study Section in its survey of the status of research in dentistry, has determined that research on adhesive restorative materials is one of the areas in which research should be expanded. The need for accelerated investigation in this area has furthermore been emphasized by the National Advisory Dental Research Council. Subsequently, a grant to support a workshop on



adhesive restorative materials at the University of Indiana was approved.

The Institute this year is supporting a new study on the development of dental adhesives at the Eastman Dental Dispensary,\* Rochester, New York. The aim of this research is the development of adhesive, restorative, lining and cementing materials, either organic or inorganic, that will form a strong lasting bond, under oral conditions, to enamel, dentin, and cementum. .

The extensive development of commercial adhesives offers a broad field for investigation. Generally, most commercial adhesives are not suitable for dental use; they fail to meet requirements for dental materials such as moisture resistance, adherence upon immersion in saliva, non-toxicity to pulp and tissue, short curling times, low temperature setting, ease of application, etc. There is, however, a large reservoir of materials developed in the course of commercial research of possible use in dentistry which have been shelved because they lack properties desired for industrial needs. For instance, such materials may set too rapidly so that working life is short or they may be too expensive to manufacture. Such characteristics render the materials undesirable for most industrial application. Consequently, they are not publicized and must be sought out. It is these materials which may have dental application.

The National Advisory Dental Research Council sponsored a conference in January to devise methods for stimulating sound behavioral science research programs in the field of dentistry. Principal topics under discussion were those dealing with sociological factors that influence public opinion and programs of oral hygiene; studies in doctor-patient relationship; design of research involving anxiety producing conditions; and experimental programs in dental education. Problems associated with the development of dental-behavioral science research and training were accorded particular attention during the two day session. Forthcoming from these discussions were specific recommendations for consideration by the National Advisory Dental Research Council at their March meeting. These recommendations included the provision of program support to selected dental schools and the extension of public health research programs concerned with sociological aspects of dentistry.

To further the study of human genetics, and particularly its role in oral malformations and disease, the Council on Dental Research of the American Dental Association in cooperation with the National Institute of Dental Research conducted a special symposium, held at the National Institutes of Health, April 3-5, that brought together, for the first time, outstanding geneticists and dental investigators to exchange information on present and proposed studies.

Indicative of the broadening base of dental research today was the presentation of fifteen scientific papers covering recent developments in medical genetics, heritable diseases of dental interest, and genetic methods

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\* D-1404





applicable to the study of intrinsic dental disorders.

An example of collaborative work between the Intramural and Extramural Branches of the National Institute of Dental Research was the approval of a total of \$126,902 for a five-year period of time to the National Children's Cardiac Hospital, Miami, Florida, for the studies of "Cariogenic Streptococci." The research proposed in this application is primarily concerned with serological and chemical studies of cariogenic and non-cariogenic streptococci furnished by NIDR, and an epidemiological study of caries exposure in children with rheumatic fever who receive long-term penicillin treatment. The work contemplated represents an expansion of the NIDR's current program, since it will cover important areas in which NIDR is not maximally engaged but which is essential to the full development of the program.

The Council, realizing the importance of research abroad agreed to use up to 2 percent of the research grant budget to scientists in foreign countries in support of especially worthwhile research. Consonant with this viewpoint, 16 foreign grants amounting to approximately \$119,000 were activated during the calendar year.

During the Calendar Year 1961, the Extramural Programs Branch, NIDR, received 387 research grant applications requesting \$7,304,221 for the first year of support; of these, 188 applications were approved for a sum of \$3,092,906.

Council also approved the payment of 243 grants in the amount of \$3,322,855 which were previously committed.

### Training Grant Program

The primary objective of the NIDR graduate training program is to increase the number of competent researchers in dental schools and other dental research institutions throughout the United States. The program is designed to meet the critical shortage of clinical and basic science personnel presently available to these institutions. It has been established that substantial numbers of additional personnel for research will be required in the near future.

Two million, four hundred and forty-one thousand dollars has been apportioned for training grants from fiscal year 1962 funds. It is estimated that these funds will support approximately 50 training centers and 250 trainees. Currently, there are 45 active training grants supporting approximately 200 trainees at 38 training centers. These are located in 22 states and the District of Columbia.

During the 1961 calendar year, 7 new grants were activated. Six of these were for training centers and one supported a conference dealing with training. In addition, eight training centers received supplemental grants.

This calendar year has seen the bringing of the fiscal year of training grants into phase with the normal academic year of training institutions. All training grants have been prorated and financed according to adjusted



project period dates corresponding to the government fiscal year. For most grants this was accomplished in two steps, the first of which established a common beginning date of January 1. The second adjustment, to a beginning date of July 1, was completed this year. This includes forward financing of the next year's grant period from current fiscal year funds.

## Fellowships Program

### A. Regular Research Fellowships

The National Institute of Dental Research fellowship program has been offering three types of awards this year, (1) Predoctoral; (2) Postdoctoral; and (3) special research fellowships.

The primary purpose of the research fellowship program is directed toward graduate training in the basic sciences. Special emphasis has been placed on increasing the number of trained individuals in the basic sciences related to the study of periodontal diseases, cleft palate, and dental caries. The Institute has recognized the dental research manpower shortage and the need of competently trained personnel and has sought to support sound research training.

During 1961, a total of \$521,299 was awarded for 71 regular fellowships at 37 institutions located in 17 states, the District of Columbia, and 2 foreign countries. In comparison, last year 52 regular fellowships were awarded for a total of \$330,292.

### B. Research Career Awards

The Research Career Award Programs merges and modifies the Career Research Professorship, Senior Fellowship Grant, and Special Fellowship Grant Programs.

Research Career Awards are available to institutions in the United States on behalf of qualified candidates to provide increased numbers of stable career opportunities for scientists of superior potential and capability in the sciences related to health. Awards are intended to provide individuals with some measure of assured support reasonably early in their careers. Two groups of awards are available as follows:

1. Research Career Award - Awards in this group are intended to finance additional stable positions for experienced investigators who are continuing to develop in productive careers of independent research and teaching. Awards are made on the basis of nationwide competition, and the research standards expected of candidates are high.

2. Research Career Development Awards - Awards in this group are intended to finance positions for able scientists who plan to pursue careers in independent research and training. These awards are for young investigators who desire experience and further training in a productive research environment as well as for scientists undertaking independent research who need further experience to qualify for senior positions.



In 1961 a total of \$137,016 was awarded for 12 Research Career Development Awards. These are located in 11 institutions in 8 states. No Research Career Awards have been made in 1961.



Annual Report of the Laboratory of Microbiology  
National Institute of Dental Research  
Summary Statement

Occupancy of the new dental research building in 1961 made possible for the first time geographical consolidation of the laboratory's programs, concomitant intensification of research in microbial physiology, and initiation of concerted effort in immunology. For convenience, the year's advances are summarized in the categories of dental caries, periodontal disease, microbial physiology, immunology, and virus research, although the research of most of the staff overlaps into several of these areas.

Our gnotobiotic studies of the etiology and pathogenesis of experimental dental caries in rats and hamsters are now extensive enough to permit some generalizations and to demonstrate the great ecological subtlety of this problem. To date, only acidogenic, nonproteolytic streptococci, of a kind differing from all recognized species, have been found to serve as the specific transmissible microbial factor in rodent caries. Well-known acidogenic, nonproteolytic and proteolytic streptococci, and a number of species from other genera are not cariogenic. Furthermore, cariogenic streptococci from rats are not cariogenic in hamsters, and vice versa. Explanation of these specific host-parasite associations should be very significant for human dental caries. This should be greatly facilitated by the recent development of a much more nearly defined cariogenic diet than hitherto available, which enables better control of this important determinant of caries. A logical sequel to the discovery of a specific etiological agent of caries would be development of a protective vaccine. To date vaccines of the cariogenic streptococci have engendered no detectable circulating antibody in tests with hamsters. To be sure, the absence of such antibody does not preclude active resistance to infection. However, vaccinated hamsters have as yet evidenced no increased resistance to caries. In another kind of attack on the caries problem, continuing studies of the inhibition of experimental caries by sodium metabisulfide have shown that certain addition complexes of this salt with aldehydes and ketones were also effective caries preventives; these derivatives might offer practical advantages. Also, even intermittent administration of the anticaries agent, e.g., for one day each week, reduced the caries scores by nearly half. It is felt that these experimental studies offer a sound basis for serious consideration of clinical trials of these anticaries agents, as in a dentifrice.

Formation of dental calculus in the gingival area is recognized as the most important immediate provocation of periodontal disease. Systematic studies of the bacterial deposits that initiate calculus deposition are therefore essential. Contrary to the general impression that filamentous forms predominate, our studies show that various species of streptococci outnumber all other types at all stages of calculus formation, and in both supra- and subgingival locations. Large numbers of Neisseria species, gram-negative bacilli, diphtheroids, and fusobacteria are present during the first two weeks (starting with clean teeth), but then decrease. Filamentous organisms such as Actinomyces and Nocardia





species, Leptotrichia buccalis, and Bacterionema matruchotii were rare during the first two weeks but increased steadily thereafter. The over-all predominance of streptococci alters sharply the focus of study of the etiology and prevention of dental calculus. Accordingly, the calcification of various oral bacteria has been studied in a model system in vivo, namely, within cellophane sacs implanted intra-abdominally in rats. Under these conditions the bacteria receive a constantly renewed supply of nutrients and calcifying fluid without interference of body cells, mucus, and the like. There seems to be little specificity at the calcification stage, for all of a variety of oral bacteria tested have developed calcifications, both extra- and intracellularly, strikingly similar to those seen in human dental calculus, as judged by optical and electron microscopy, and X-ray diffraction.

In the field of microbial physiology, continued study of the enzymatic synthesis of folic acid by lactobacilli elucidated the role of phosphorylation of a reduced pteridine as an intermediate step. In another study, metabolism of galactosamine by a lactobacillus was shown to proceed via galactosamine-6-phosphate, which is deaminated to yield ammonia and tagatose-6-phosphate. A third study concerned the role of lipoic acid in the dissimilation of lactic acid. The evidence indicates that lipoic acid functions as an electron acceptor to convert lactic acid to pyruvic acid as the first step in dissimilation. A fourth project concerned the biochemistry of growth and development, as investigated through the biochemical factors initiating and controlling morphogenesis of Dictyostelium discoideum. Stimulation of differentiation is ion-specific (potassium, magnesium, and phosphate stimulate, but sodium chloride and sulfate do not) but not critically dependent on ionic strength. Hydrogen-ion concentration likewise is not critical throughout the range pH 5 to 7. There is no correlation between ability of a substance to chelate and its ability to stimulate differentiation. Histidine accelerates morphogenesis during the early stages, but apparently not by stimulating protein synthesis. Rather surprisingly, highly effective stimulators of differentiation do not increase respiration of D. discoideum.

Continuing studies of the pathogenic potentialities of gingival bacteria in relation to periodontal disease developed on the immunological side. It was shown, contrary to all expectation from the literature, that vaccination of mice with killed or living veillonellae engendered significant resistance specifically against the lethal effect of glucolipid endotoxin from the homologous bacteria. On the other hand, guinea pigs and rabbits vaccinated with oral viridans streptococci developed typical allergic inflammatory reactions to intracutaneous injections of streptococcal protein. In a separate immunological study, use was made of the ability of endotoxins to coat erythrocytes and make them agglutinable by homologous anti-endotoxin serum (indirect hemagglutination); in this way, five well-distinguished serotypes of Veillonella species were discerned, although chemical analyses revealed no significant difference in chemical composition of the respective endotoxins. Another aspect of endotoxic activity relates to nonspecific changes in host resistance. It was found that certain doses of glucolipid produce in mice a marked elevation of the plasma level of lactic dehydrogenase. However, this phenomenon is not a useable or specific measure of endotoxic action but (as in the generality of cases) an indication of massive trauma, for it develops only with nearly lethal doses of endotoxin.



As an outgrowth of the foregoing experiments, collaborative studies with the National Cancer Institute revealed a contaminating virus in several supposedly purified virus strains, namely, a hitherto unsuspected virus whose only known manifestation is a many-fold increase in plasma lactic dehydrogenase. The specificity of this manifestation, in contrast to the increase of plasma lactic dehydrogenase owing to trauma, is indicated by the lack of a concomitant increase of plasma glutamic-oxalacetic transaminase. Two other virus studies were brought to fruition during the year. Five serogroups of herpes simplex virus have been defined by kinetic analysis of the neutralization reaction between the respective strains of virus and homologous and heterologous antisera. Finally, definitive evidence has established, by serial transmission in tissue culture, the occurrence of a presumably new virus in the salivary glands of germfree rats but not in any of the many other organs tested. It is believed that this is the first viral agent isolated from germfree animals. It has been found also in nearly all ordinary laboratory rats of the same strain.



1. Microbiology
2. Gnotobiotics
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease.

Principal Investigator: Dr. R. J. Fitzgerald

Other Investigators: Dr. H. V. Jordan  
Dr. P. H. Keyes  
Dr. R. H. Larson

Cooperating Units: Germfree Services Section, Sanitary Engineering Branch (DRS-84).

Man Years (calendar year 1961):

Total:	4-1/2
Professional:	1
Other:	3 1/2

Project Description:

Objectives:

A continuation of Project NIDR-2 (1960) in which germfree and gnotobiotic animals are used in the study of dental caries, calculus formation and periodontal disease.

Methods Employed:

1. Standard technics are employed for the breeding and rearing of germfree animals, principally rats of the Lobund or Fischer inbred strains. The germfree animals are then subjected to different experimental conditions including inoculation with pure cultures of specific microorganisms in order to determine the relative importance of such factors in the etiology of oral diseases.
2. The term gnotobiotic refers not only to germfree animals but is also extensible to animals in which the microbial ecology is subject to control. For this reason previously germfree animals which have been purposely infected are gnotobiotics and, by extension, "conventional" animals known to be lacking one or more components of the normal microflora may also be used as gnotobiotics in specific studies.



Part A (continued)Major Findings:

1. Earlier studies had shown that dental caries was induced by inoculation of germfree rats with a specific acidogenic but non-proteolytic streptococcus. Germfree rats have<sup>now</sup> been infected with two other rat oral streptococci, (1) a Streptococcus lactis which is acidogenic and non-proteolytic and (2) Streptococcus faecalis var. zymogenes which is both acidogenic and proteolytic. With neither of these strains has any caries activity been noted although both became established in the gnotobiotic rats. In addition a streptococcus previously shown to be cariogenic in hamsters was established in germfree rats but was devoid of caries activity in this host.

2. Observations reported in previous years from this Institute noting that dental caries in rats and hamsters had a number of the attributes of a specific infection have led to attempts to protect hamsters against caries by immunologic means. We have sought to immunize hamsters against a cariogenic streptococcus, either passively by injections of immune rabbit serum specific for this organism, or actively by injections of a phenol killed vaccine of the same organism. These experiments performed in collaboration with Dr. P. H. Keyes (Histology and Pathology Laboratory, NIDR) have been uniformly negative. Not only has no protective effect against caries been noted but also serological tests have revealed no evidence of circulating antibodies (agglutinins) against the cariogenic streptococcus in the serum of the vaccinated animals.

Significance to Dental Research:

The evidence from studies of caries in experimental animals justifies the conclusion that, in rodents at least, dental caries is a transmissible infectious disease. In addition, our studies in the gnotobiotic rat and in the hamster are suggestive that caries may be a highly specific disease within each species. To date we have found only a single organism that is cariogenic for the rat and a single organism that is cariogenic for the hamster. Although both are similar types of anaerobic streptococci neither strain is cariogenic for the heterologous species of animal. In addition, both the caries producing streptococci are potent producers of lactic acid from sugars and neither produces extracellular proteolytic enzymes to any detectable extent. This might suggest that the primary mechanism of tooth cavitation is via acid destruction of the mineral phase. However, other equally acidogenic microorganisms have had no effect on the teeth of the test animals indicating that acidogenesis is not the only factor involved. These experimental findings indicate that, as a disease, dental caries is far more specific than hitherto realized and depends on a critical





Part A (continued)

interaction between host, diet and microflora.

The negative results of attempts to immunize hamsters with the cariogenic streptococcus are consistent with other observations in humans and animals that one episode of caries does not confer immunity against succeeding attacks. This in turn suggests that even if it is possible to determine the etiology of caries in humans, measures other than immunologic may have to be sought to control the disease.

Proposed Course of Project:

In basic outline this project will continue along the present lines. Now that it is possible to induce caries at will and with specific microorganisms, the intention is to explore in more intimate detail the ultimate mechanisms by which specific microorganisms are able to produce this disease. Other studies are expected to enlarge upon earlier observations concerning calculus deposition in the germfree rat. Germfree and gnotobiotic animals will also be utilized in collaborative studies with Dr. Mergenhagen concerning the role of microbial endotoxins in gingival pathology as related to periodontal disease.

Part B included:           Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Keyes, Paul H. and Fitzgerald, Robert J. Dental Caries in the Syrian Hamster IX. Studies on the Fate of "Labelled" Cariogenic Streptococci. Arch. Oral Biol. 1961, in press.

Honors and Awards relating to this project:

None



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Manifest and Inapparent Viral Infections of Oral Tissues

Principal Investigator: Dr. H. W. Scherp

Other Investigator: Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1961):

Total: 3-1/2

Professional: 1

Other: 2-1/2

Project Description:

Objectives:

1. To test for the presence of inapparent viruses in the tissues of "germfree" and conventional animals.
2. To isolate, identify, and determine the etiological significance of viruses from various lesions of the oral mucosa of undetermined etiology.

This project is a continuation of NIDR-11 (1960).

Methods Employed:

Samples containing tissue cells are frozen and thawed to liberate intracellular viruses. To test for viruses, the samples are inoculated into standard tissue cultures of rabbit kidney cells, monkey cells, HeLa cells, mouse L cells, chicken embryo cells, and human skin cells. After incubation, cultures are frozen, thawed and transferred to a fresh tissue culture; serial transfers are made. In addition, specimens of biopsy tissue are maintained in tissue culture medium for protracted periods to allow development of latent viruses. Evidence for a virus is sought by microscopic examination for cytopathologic changes in the cultured cells and by hemagglutination and hemadsorption tests



Part A (continued)

of the cultured fluids with guinea pig and chicken erythrocytes. Isolates of viruses are identified by neutralization tests with specific antisera, when applicable. Refined antigenic analysis of related viruses is accomplished by comparison of the kinetics of their neutralization by homologous and heterologous antisera using a plaque-count method for quantitation of virus. Results are expressed as a pseudomonomolecular rate constant,  $K$ ; homology is evidenced by the highest  $K$  values.

Major Findings:

1. Serologic differentiation of strains of herpes simplex virus: Preliminary results reported in NIDR-23 (1959) showed the feasibility of distinguishing between strains of herpes simplex virus by determination of their  $K$  values with homologous and heterologous antisera. Accordingly, during 1960 effort was concentrated on working toward an antigenic schema based on the necessary reciprocal neutralization tests with 15 strains of herpes simplex virus (4 stock strains and 11 strains freshly isolated from patients; each test repeated at least once) and rabbit antisera to 5 of them. It was reported in NIDR-11 (1960) that the 15 strains of virus studied in detail fell quite distinctly into 4 antigenic groups (A-D): 5 in group A; 2 in group B; 3 in group C; and 5 in group D. The homologous and heterologous  $K$  values of group D strains, however, indicated antigenic overlapping with all of the strains.

Consequently, during 1961 the experimental plan was extended to include antisera against 6 more of the original 15 strains, and this phase of the project was completed. The results of reciprocal neutralization experiments, in which all 11 antisera were tested against all 15 strains of virus, have shown that the strains in group D can be further differentiated and a fifth group E can be proposed. Groups A, B, and C remain unchanged. However, the number of strains in group D is reduced to 2 and group E has 3 strains that were previously assigned to group D. The strains in group E are serologically heterogeneous and demonstrate the same degree of antigenic overlapping as did the strains in group D prior to the incorporation of the 6 additional antisera into the study.

Two other points of significance have developed as a result of extending the number of antisera to 11. First, strain HF (the classical Rockefeller Institute strain, isolated in 1922) remains





Part A (continued)

immunologically distinct from the other 14 strains of virus even though it has been passed a total of 12 times in rabbit kidney cell cultures. It had been thought that this number of passages in this system might alter its antigenic structure and render it immunologically less distinct from the other 14 strains of virus. Secondly, two strains isolated from the same individual at an interval of two years have been found to be immunologically distinguishable from each other although they are not distinguishable from some of the other strains studied. One of these strains has been found to be immunologically similar to strains in group A while the other is similar to strains in group D. Since the last report, 12 new strains of herpes simplex virus have been isolated in this laboratory for future study. Among these strains are 9 which were isolated from recurrent episodes of herpetic lesions in three individuals.

2. A cytopathic agent from the salivary glands of rats:

Previously we reported (NIDR-23, 1959 and NIDR-11, 1960) some of the properties of a possibly viral agent found in the salivary glands, but not in any of a variety of other organs and tissues, of both conventional and germfree rats. During 1961 these studies have been extended, with particular attention to the critically important question of maintenance of the agent in serial transfers in tissue cultures. Previously, using rabbit kidney cell cultures in roller tubes and a tenfold dilution with each transfer, only a maximum of 4 passages had been possible--barely sufficient to satisfy the criterion for a virus, as contrasted to a nonreproducing toxic factor. By increasing the scale of operations sixtyfold, and using stationary cultures in large vessels (Blake bottles), it was found possible to slow down the development of the cytopathic effect so that discrete foci developed gradually. In this way, serial transfer through 8 passages in 2 separate series had been accomplished. Accordingly, we conclude that this agent is indeed a virus, so far as we know the first one isolated from germfree animals. This virus is apparently stable indefinitely at  $-55^{\circ}\text{C}$ ., since isolates stored at this temperature for more than 2 years retain their cytopathic activity. However, activity is diminished significantly by exposure to  $56^{\circ}\text{C}$ . for 60 minutes. On the other hand, the virus is ether-resistant.

A cold hemagglutinin has been found to be associated with this virus in rat salivary gland extracts and in the first few transfers in culture. Fresh rabbit erythrocytes are used and hemagglutination occurs in 12 to 18 hours at  $4^{\circ}\text{C}$ . in a pH range of



Part A (continued)

7.3 - 7.6. Hemagglutination titers as great as 1:20,480 have been obtained. No hemagglutination is demonstrable when guinea pig, rat, chick, or mouse erythrocytes are used. Hemagglutination occurs only at 4°C. and not at 23°C. or at 37°C. Differential ultracentrifugation studies indicate that this hemagglutinin can be separated from the virus. Also, since it disappears in later passages of the virus in tissue culture, it is presumably not integral with the virus.

Rabbits have been immunized with this virus in order to determine the extent of its antigenicity based upon conventional neutralization tests and hemagglutination inhibition studies. The virus has now been recovered from 43 of the 57 germfree glands tested.

Significance to Dental Research:

Oral soft tissue lesions of unestablished but possibly viral etiology are frequently encountered by the dentist. The still incompletely understood phenomena of latency and activation of herpes virus are accordingly of direct dental interest; they also provide prototypes of fundamental viral relationships. The availability of a method to distinguish between strains of herpes virus will make possible elucidation of previously inaccessible phases of the ecology of this ubiquitous agent. Study of inapparent viral infection in otherwise germfree animals affords another approach to the study of the consequences of such infections on "normal" physiology.

Proposed Course of Project:

1. Since a serological distinction was demonstrated for the first time between the strains of herpes simplex virus isolated from the same person during successive recurrences of herpes labialis after a considerable interval, it is clearly important to investigate the generality of this phenomenon and its significance in the pathogenesis of recurrent herpetic infections.
2. Having demonstrated the viral (i.e., transmissible) nature of the cytopathic agent found in salivary glands of germfree animals, attention will be turned to further characterization of the virus, e.g., physical and chemical properties, immunological relationships, host-cell range, possible pathogenicity for embryonic and newborn animals.

Part B included

Yes



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Ship, I. I., Ashe, W. K., and Scherp, H. W. Recurrent  
"Fever Blister" and "Canker Sore" - Tests for Herpes Simplex  
and Other Viruses with Mammalian Cell Cultures. Arch. Oral  
Biol. 3: 117-124, 1961.

Honors and Awards Relating to this Project:

None



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Part A

Project Title: The Relationship Between Tissue Enzymes and  
Experimental Infection

Principal Investigator: Dr. Abner Louis Notkins

Other Investigators: None

Cooperating Units: National Cancer Institute  
(Dr. Robert E. Greenfield)

Man Years (calendar year 1961):

Total: 2

Professional: 1

Other: 1

Project Description:

Objectives:

Immunologists have done a vast amount of work on the relationship between antibody titer and infection. However, relatively little work has been done on the relationship between tissue enzymes and experimental infection. Alteration in enzyme activity following the administration of various foreign and toxic materials in normal and sensitized animals has also been a much neglected area of study. These enzyme changes offer a method for recognizing and evaluating tissue damage and studying biochemical changes within the cell. Areas to be investigated include experimental infections, antigen-antibody reactions, hypersensitivity, natural resistance, toxic states, and tumor-host relationship.

Methods Employed:

The glycolytic enzyme lactic dehydrogenase was studied following the administration of endotoxin in the normal and pertussis sensitized mice. A correlation was found between the amount of endotoxin given and the subsequent level of plasma lactic dehydrogenase. Approximately ten times more endotoxin was required to





Part A (continued)

produce an increase in LDH activity in the normal animal as in the pertussis sensitized animal. The enzyme elevation occurred much earlier in the sensitized animal. Further studies seemed to indicate that the apparent difference in enzyme response between the normal and sensitized animal was related to the known enhanced susceptibility to endotoxin of the pertussis sensitized animal. High LDH activity following the administration of endotoxin was shown to be related to lethality.

Recent work reported by Riley and Wroblewski (Science, 132, 151, 1960) called attention to the correlation between the plasma level of the enzyme LDH and the growth of several transplanted mouse tumors. A transmissible agent capable of producing a 5 to 10 fold rise of plasma lactic dehydrogenase in normal animals within 72 hours after inoculation was found associated with all 26 mouse tumors examined (Riley, et al., Science, 132, 545, 1960). Experiments conducted in our laboratories and in those of the NCI revealed that the Moloney leukemia virus (of mouse origin) produced a 4 to 6 fold increase in plasma LDH activity in the mouse. This same virus, however, failed to produce an increase in plasma LDH in the rat. Further experiments showed that if the Moloney leukemia virus was reisolated from the rat and then inoculated into the mouse, no increase in mouse plasma LDH occurred. From this and other experiments it was concluded that the factor responsible for the increase in plasma LDH was not directly related to the neoplastic process, but was probably a virus of mouse origin which was being carried as a contaminant by certain serially transplanted mouse tumors. Thus, the activity of plasma LDH in a mouse with a particular tumor depends on whether or not the LDH factor has become associated with that tumor.

Further studies were undertaken to investigate enzyme activity in normal mice, normal mice inoculated with the LDH factor, tumor-bearing mice without the LDH factor, and tumor-bearing mice inoculated with the LDH factor. These studies are near completion. The major findings include: (1) Any 8 to 10 fold increase in plasma LDH activity and plasma glutamic-oxalacetic transaminase (SGOT) activity in tumor bearing animals which did not contain the LDH factor. (2) An 80 fold increase in plasma LDH activity in the tumor-bearing animal (plasma cell tumor) which had been experimentally infected with the LDH factor as compared to the normal control (without tumor or LDH factor). (3) SGOT is not elevated in the normal animal following the inoculation of the LDH factor in contrast to the enzyme lactic dehydrogenase.



Part A (continued)

In light of the ability of the LDH factor to produce this marked and rapid rise in plasma LDH, it became of interest to see if other known virus agents would produce a similar change. The relationship of these known viruses to tissue damage and enzyme elevation is still under investigation. Preliminary findings reveal a relationship between plasma enzyme activity and direct tissue damage by the infecting virus. Contamination of virus preparations with the LDH factor has been noted.

The LDH agent is of great interest and provides an intriguing area of study in addition to offering an excellent tool for the investigation of virus-host-enzyme interaction. Pathology studies and metabolic experiments are in progress in hopes of further characterizing this agent.

Significance to Dental Research:

The areas of experimental infection, hypersensitivity, non-specified resistance, toxic states, tumor-host relationship, and tissue damage all deal with basic biological phenomena which are of great concern to both physician and dentist. The studies undertaken in this laboratory have been concerned with the recognition of these phenomena, the mechanisms involved, and eventual application to clinical problems.

Proposed Course of Project:

Further investigation into the relationship between experimental infection and enzyme alteration. Further characterization of the LDH factor. Examination of some of the mechanisms of sensitization and nonspecific resistance.

Part B included

Yes



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Notkins, A. L. Berry, R. J., Moloney, J. B., Greenfield, R. E.  
Relationship of the Lactic D hydrogenase Factor to Certain  
Murine Tumors. Nature (in press).

Honors and Awards Relating to this Project:

None



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Part A

Project Title: Nutrition and Metabolism of Lactic Acid Bacteria

Principal Investigator: Dr. T. Shiota

Other Investigators: Dr. H. B. Blumenthal

Cooperating Units: None

Man Years (calendar year 1961)

Total: 3-3/4

Professional: 1-3/4

Other: 2

Project Description:

Objectives:

To study amino acid, peptide, vitamin and hexosamine metabolism of lactic acid bacteria with specific attention to the enzymatic synthesis of folic acid-like compounds by purified extracts of Lactobacillus plantarum and galactosamine metabolism by Lactobacillus casei.

Methods Employed:

1. The determination of folic acid-like compounds was carried out by microbiological assay, chromatography and spectrophotometry.
2. The preparation and isolation of phosphorylated pteridine was carried out by chemical phosphorylation followed by various treatments including ion exchange and cellulose chromatography.
3. The metabolism of galactosamine was measured by ammonia and ketohexose formation.
4. Chemical analyses for phosphorus, nitrogen, hydrazones, etc., were performed by usual methods.





Part A (continued)Major Findings

1. Folic Acid: The enzymatic coupling of 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine with p-aminobenzoyleglutamate yielding dihydrofolate by extracts of Lactobacillus plantarum requires adenosinetriphosphate. Hence, a phosphorylated reduced pteridine was suspected as an intermediate in dihydrofolate synthesis. The pteridine was chemically phosphorylated and the resulting crude mixture was found to be active in dihydrofolate synthesis in the absence of adenosinetriphosphate. The problem of preparation and identification of the chemically phosphorylated pteridine was undertaken. By ion-exchange and cellulose chromatography three factions, each containing a phosphorylated pteridine, were obtained. By ultraviolet light absorption spectroscopy and nitrogen and phosphorous analyses, it was found that fraction one contained a pteridine with one phosphate, fraction two contained a pteridine with two phosphate, and fraction three a pteridine with three phosphate, per mole of pteridine. When each of the factions was reduced chemically to the dihydro-derivatives and tested for enzymatic coupling with p-aminobenzoyleglutamate, only fraction two, the diphosphate pteridine derivative, was active. We conclude that this compound is 2-amino-4-hydroxy-6-pteridinylmethylpyrophosphate which, when reduced to the dihydro level, is the active phosphorylated intermediate.

2. Galactosamine Metabolism: Cell free extracts were prepared from Lactobacillus casei, A.T.C.C. 7469, grown on a casitone-yeast extract-salts medium supplemented with 1% galactose. These extracts deaminated galactosamine-6-phosphate to tagatose-6-phosphate and ammonia. Tagatose-6-phosphate was isolated by ion-exchange chromatography and identified as a phosphorylated ketohexose by paper chromatography. Furthermore, when the isolated material was treated with periodate, the product was identical with glycolaldehyde phosphate. This indicated that the sugar was phosphorylated in the terminal position. When the isolated material was treated with phosphatase and phenylhydrazine, the melting point of the resultant hydrozone compared favorably with authentic tagatosazone. The melting point of the mixed hydrozones showed no depression.



Part A (continued)Significance to Dental Research:

The occurrence of lactic acid bacteria in the oral cavity and their capacity to produce acid have incriminated them as being etiologically associated with dental caries. Hence, the study of their nutrition and metabolism will not only help to broaden knowledge in the general field of microbiology and biochemistry, but may contribute to a better understanding of the complex microbic processes in the oral cavity.

Proposed Course of Project:

It is proposed that the project in folic acid be continued with broadening scope. Although we have demonstrated that a chemically prepared phosphorylated pteridine can serve as an intermediate in dihydrofolate synthesis, we have yet to show that the pteridine is enzymatically phosphorylated by adenosinetriphosphate. We anticipate the demonstration of this reaction and isolation of enzymatically phosphorylated pteridine.

Reduced pteridines, such as tetrahydrofolic acid, and tetrahydropteridines have been found by other investigators to act as coenzymes in the hydroxylation of phenylalanine and tryptophane. We hope to make a survey of similar hydroxylation systems for amino acids such as proline, lysine, etc.

Part B included

Yes



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Part B: Honors, Awards and Publication

Publications other than abstracts from this project:

L'inhibition de la croissance bacterienne par la S-( $\beta$ -aminoethyl)-cysteine. Role privilegie des dipeptides ayant la lysine ou son analogue en position N-terminale. Mauron, J., Folk, J. E., et Shiota, T. *Chimie* 15: 426-428 (1961).

The enzymic synthesis of dihydrofolate from 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine and p-aminobenzoylglutamate by extracts of Lactobacillus plantarum. Shiota, T. and Disraely, M. N. *Biochem. Biophys. Acta* 52: 467-473 (1961)

Inhibition of lysine and lysine peptide utilization in bacteria by peptides of S-( $\beta$ -aminoethyl)-cysteine. Shiota, T., Mauron, J. and Folk, J. E. *Biochem. Biophys. Acta* 53: 360-365 (1961).

The formation of tagatose-6-phosphate from galactosamine-6-phosphate by extracts of Lactobacillus casei. Shiota, T., Blumenthal, H. B., Disraely, M. N. and McCann, M. P. *Arch. Biochem. Biophys.* (in press).

The cultivation of D-galactosamine-metabolizing lactic acid bacteria. Blumenthal, H., Shiota, T., McCann, Mary p. and Disraely, M. N. *J. Gen Microbiol.* 24: 81-85 (1961).

Honors and Awards Relating to this Project:

None



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Part A:

Project Title: A study of Oral Filamentous Organisms and Related Bacteria and Their Relationship to Disease of the Oral Cavity

Principal Investigator: Dr. A. Howell, Jr.

Other Investigators: Dr. A. A. Rizzo

Cooperating Units: None

Man Years (calendar year 1961):

Total: 4  
Professional: 1-1/4  
Other: 2-3/4

Project Description:

Objectives:

1. To study the incidence and identity of filamentous micro-organisms and related bacteria in the oral cavity.
2. To determine the relationship of these organisms to calculus formation.

Methods Employed:

Standard bacteriological methods for the isolation and identification of oral bacteria (including filamentous forms) have been employed. In order to study the bacteriological changes occurring during calculus formation specimens were taken at various time intervals after complete scaling and polishing of the areas to be subsequently sampled. During the past year studies have been completed on 20 such samples from 5 patients bringing the total in this study to 62 samples from 9 patients. In addition 14 samples of supra- and subgingival calculus from 8 patients have been compared to determine whether or not these two types of calculus contain the same bacteriological flora.





Major Findings:

1. It was reported previously (1960) that a variety of bacteria were possibly involved in the development of calculus. Further studies support this conclusion. By the technic employed, various species of streptococci were found to be the predominant organism in all samples studied regardless of the age of the specimen. In the earlier samples (i.e., specimens up to 14 days old) species of Neisseria, several varieties of gram negative rods, diphtheroids, and fusobacteria were found to be present in very large numbers, while filamentous organisms such as species of Actinomyces, Nocardia-like organisms, Leptotrichia buccalis, and Bacterionema matruchotii were seldom found before the 7th to 14th day. Large numbers of this latter group of organisms, with a consequent decrease in the relative numbers of gram-negative and gram-positive cocci (other than streptococci), were not found until after 4-6 weeks. In nearly all specimens, regardless of age, other bacteria such as species of Veillonella and Lactobacillus, other gram-positive rods and unidentified gram-positive cocci were usually present in variable numbers.
2. No significant qualitative differences were found in the bacterial flora of samples of supra- and subgingival calculus from a given individual except that Bacterionema matruchotii was isolated only from supragingival calculus (5 of 8 individuals). As noted above, streptococci were the predominant organisms isolated from all specimens, with species of Actinomyces, gram-negative and gram-positive rods, and fusobacteria next in abundance. A variety of other organisms were recovered in smaller numbers from nearly all specimens.

Significance to Dental Research:

The finding that streptococci are the predominant organisms in samples of calculus at all stages of development is at variance with most reports in the literature. Most previous studies, however, have been on a morphological basis rather than on a cultural basis. It is therefore probable that the studies reported here represent a more accurate determination of the bacterial flora of calculus than those reported previously. These findings should be of value in interpreting the role of particular organisms in periodontal disease.

Proposed Course of Project:

1. To continue the study of the filamentous organisms (and related forms) from the oral cavity.
2. To determine the incidence and significance of such forms in patients with rampant caries.

Part B included Yes



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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Gilmour, M. N., Howell, A., Jr., and Bibby, B. G. The Classification of Organisms Termed Leptotrichia (Leptothrix) buccalis. I. Review of the Literature and Proposed Separation into Leptotrichia buccalis Trevisan, 1879, and Bacterionema Gen. Nov., B. matruchotii (Mendel, 1919) Comb. Nov. Bacteriol. Rev. 25: 131-141, 1961

Howell, A., Jr. and Pine, L. The Classification of Organisms Termed Leptotrichia (Leptothrix) buccalis. IV. Physiological and Biochemical Characteristics of Bacterionema matruchotii. Bacteriol. Rev. 25: 162-171, 1961.

Gilmour, M. N., Howell, A. Jr., and Bibby, B. G. Proposal for Designation of Neotype Strains of Leptotrichia buccalis and Bacterionema matruchotii. Intern. Bull. Bacteriol. Nomen. Taxon. 11: 161-163, 1961.

Honors and Awards relating to this project:

None



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Part A

Project Title: Immunological, Biochemical, and Pathological Factors  
in the Taxonomy and Ecology of Oral Bacteria

Principal Investigator: Mr. M. Rogosa

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)

Total: 3-1/2

Professional: 1

Other: 2-1/2

Project Description:

Objectives:

To determine the immunological, biochemical, and pathological characteristics of the veillonellae, lactobacilli, diphtheroids, and other oral microorganisms. This project is a continuation of NIDR-22 (1959).

Methods Employed:

1. Antisera, prepared in rabbits against bacterial cells, are tested against the organisms or antigens extracted from them by the techniques of agglutination, precipitation, and selective antibody absorption.
2. Biochemical characteristics are determined by standard and newly developed procedures.
3. Pathogenicity studies are conducted in appropriate animal hosts, inoculated by various routes.



Major Findings:

1. The major antigenic groupings of Veillonella species have been defined. However, isolations from the mouse seem to be antigenically distinct. This finding may be significant in relation to the extremely large doses of endotoxin required in challenge experiments with mice.

2. Biochemical Studies. Glucose analyses of spent media from Veillonella cultures showed that, contrary to published descriptions, these organisms cannot utilize glucose for energy.

Continuing studies of H<sub>2</sub>S production are showing that H<sub>2</sub>S may be produced by all Veillonella under appropriate conditions. Two possible pathways of H<sub>2</sub>S production may be involved, one by means of a cysteine desulfhydrase, and one by other thio compounds such as thiocyanates or thiosulfates.

Significance to Dental Research:

Since systematic immunological and pathogenicity studies are lacking, these studies are important in differentiating organisms; in increasing knowledge of the structure of the cell; and in determining the pathogenic potential of the oral microbiota.

Proposed Course of Project:

The immediate future will be devoted to further biochemical and serological studies of the Veillonella. Attempts are being made to develop a more selective medium for diphtheroids. In cooperation with Drs. Stephan and Howell, material from rampant caries is to be sampled for lactobacilli and Veillonella.

Part B included:        Yes





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Part B: Honors, Awards, and Publications

Publications other than Abstracts from this Project:

Rogosa, M. Experimental Conditions for Nitrate Reduction by Certain Strains of the Genus Lactobacillus. J. Gen. Microbiol. 24: 401-408, 1961.

Rogosa, M., Franklin, J. G., and Perry, K. D. Correlation of the Vitamin Requirements with Cultural and Biochemical Characters of Lactobacillus. J. Gen. Microbiol. 25: 473-482, 1961.

Ford, J. E. and Rogosa, M. The Nutrition of a Lactobacillus acidophilus Variant Isolated from the Duodenum of a Chick. J. Gen. Microbiol. 25: 249-252, 1961.

Honors and Awards relating to this Project:

Selected as member of the subcommittee on lactobacilli of the International Committee on Bacteriological Nomenclature.



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Part A

Project Title: Studies on the Oral Spirochetes

Principal Investigator: Dr. E. G. Hampp

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (calendar year 1961):

Total: 1  
Professional: 1  
Other: None

Project Description:

Objectives:

1. To investigate methods for surface cultivation of the small oral treponemes, Borrelia vincentii and Borrelia buccalis.
2. To study and develop methods for better mass cultivation of the various pure strains of oral spirochetes to be utilized for preparing endotoxins for further studies on their chemical properties and biologic activities.
3. Studies on Borrelia buccalis: a) Survival time of these organisms in their basic agar medium; b) Growth of these spirochetes in a broth medium, free of agar, which heretofore has not been possible.

Methods Employed:

1. Pure cultures of the small oral treponemes, B. vincentii and B. buccalis were streaked on the surface of Huntoon's "hormone" medium containing agar concentrations of 2, 3, 4 and 5%. The media were enriched at the time of use with 0.1% reduced glutathione and either ascitic fluid, defibrinated rabbit or sheep blood. All cultures were incubated at 35°C. in anaerobic jars using hydrogen or hydrogen containing 5% carbon dioxide.
2. a) It was previously reported that 40 liters of Huntoon's medium, with its ascitic fluid and glutathione enrichment, was required to



obtain 1.75 gms. of dry cells of the small oral treponeme. Due to the many technical difficulties in preparing the basic broth medium in large quantities, attempts were made to prepare the broth in two steps. The veal heart base and egg was infused and made double strength and sterilized by fractional procedures. The Thiopeptone (BBL) salt, and water mixture was prepared separately and also double strength. It was sterilized in the autoclave. By such procedures the latter portion of the medium could be prepared in large containers and the veal heart base and enrichment, i.e. ascitic fluid and glutathione, added before inoculation with spirochetes. In addition veal heart base was used to prepare media of various compositions, many of which revolved around the thioglycollate type medium. b) The use of TEM4T (Diacetyl tartaric acid ester of tallow monoglycerides) as advocated by Power and Pelczar for the growth of the Reiter strain of Treponema pallidum was utilized in various concentrations in an attempt to increase cell crops of the oral spirochetes both in Huntoon's "hormone" broth and in spiroplate broth (BBL).

3. a) Stock strains of B. buccalis were maintained in anaerobic jars on their enriched agar media up to 9 months at 35°C. Subcultures of the various strains of organisms were removed at designated intervals for subculturing procedures to determine viability of the spirochetes. Other groups of cultures that had been stored and incubated in air at 35°C. in rubber stoppered tubes for up to three years were also studied. b) In the past, B. buccalis has not been grown in broth, with its usual enrichment, without at least 0.2% agar in the medium. Four strains of these organisms were maintained in 0.2% agar containing complete broth and transferred at weekly intervals over an extended period. The agar content of the medium was gradually reduced at various transfer intervals until this material was deleted from the medium.

#### Major Findings:

1. Huntoon's "hormone" medium with 3% agar was found to be the most effective concentration of this material for surface cultivation of the small oral treponeme, B. vincentii and B. buccalis. Defibrinated rabbit or sheep blood was equally effective as a serum type enrichment. Since such colonies were water clear, ascitic fluid containing medium presented difficulties in visualization of the minute colonies due to lack of a contrast background. Hydrogen with 5% Co<sub>2</sub> was better than hydrogen alone for producing anaerobic conditions in jars. The number of colonies obtained were not commensurate with the number of organisms plated. A mixture of the three pure strains of spirochetes could be separated by surface culture procedures; however, plating of oral scrapings for isolation of spirochetes was unsuccessful. The method could possibly be used to separate mixtures of spirochetes obtained from well plates but does not offer much advantage over serial dilutions in shake tubes for the same purpose.



2. a) The two-step method of preparing Huntoon's "hormone" broth was effective only from the ease in preparing large batches of medium and by reducing precipitates. The recombined broth did not permit as large amounts of spirochetal growth as did the medium prepared in the usual manner. However, the veal heart base when enriched with ascitic fluid and glutathione would support moderate growth of spirochetes as did the peptone, egg, salt water mixture. Apparently certain growth promoting substances are lost during the two step method of preparing this medium. Further, attempts at producing a better and easily reproducible clear broth medium by using veal heart base in conjunction with other media ingredients were not successful.

b) The most encouraging results have been recently obtained in the incorporation of TEM4T (Diacetyl tartaric acid ester of tallow monoglycerides) both in Huntoon's "hormone" broth and in Spirolate broth (BBL). The TEM4T markedly increases the growth of the small oral treponeme, B. vincentii and B. buccalis in both types of media; however, rough estimates of growth do not parallel the results reported by Power and Pelczar even when the Reiter strain of spirochetes was used as a control. Of significance in this study is that the easily prepared precipitate free Spirolate broth may be used for mass cultivation of oral spirochetes for other studies concerned with endotoxin production. This work is being continued.

3. a) It was found that subcultures of B. buccalis remained viable and could be routinely transferred up to two weeks without loss of strains. After this period results were erratic and unpredictable. A group of 28 cultures maintained in rubber stoppered tubes at 35°C., stored from 9-36 months and transferred at various intervals, showed that 7 cultures of the group could be recovered on subsequent transfer to fresh media. Three of the seven cultures had been stored for 31, 32 and 36 months respectively. All cultures stored in anaerobic jars remained viable for a period of 9 months. This problem is concerned with the possibility of the spirochetal granules being germinative units.

b) One out of four strains of B. buccalis was successfully adapted to grow in Huntoon's "hormone" broth free of a small percentage of agar. It has also been grown in spirolate broth containing ascitic fluid (BBL) in the presence of TEM4T. It is now possible to prepare mass cultures of this organism for other studies. Attempts are being made to adapt the other three strains of B. buccalis to grow in the same manner.





Significance to Dental Research:

1. It is hoped that the development of routine and reproducible methods for surface cultivation of the various strains of oral spirochetes may be obtained to facilitate isolation procedures for these organisms from highly contaminated oral inocula.
2. The development of a broth medium for mass cultivation of oral spirochetes is needed for preparing endotoxins for further studies on their chemical composition and biologic activities.
3. The growth of B. buccalis in an agar free broth will be helpful for mass cultivation of these organisms for subsequent animal and laboratory studies directed toward the illucidation of these organisms, if any, in oral diseases.
4. The studies herein reported are necessary in order to continue certain facets of animal infection experiments to determine the significance of fusospirochetal organisms in human oral diseases.

Proposed Course of Project:

These studies will be continued and expanded and used in subsequent work designed to determine the etiology of ulcerative gingivostomatitis.

Part B included            Yes



Part B: Honors, Awards and Publications

## Publications other than abstracts from this project:

Omata, R. R. and Hampp, E. G. Proteolytic Activities of Some Oral Spirochetes. J. Dent. Res. 40: 171-176, 1961.

Mergenhagen, S. E., Hampp, E. G. and Scherp, H. W. Preparation and Biological Activities of Endotoxins from Oral Bacteria. J. Infect. Dis. 108: 304-310, 1961.

Hampp, E. G. and Mergenhagen, S. E. Experimental Infections with Oral Spirochetes. J. Infect. Dis. 109: 43-61, 1961.

Rizzo, A. A., Hampp, E. G. and Mergenhagen, S. E. Spirochetal Abscesses in Hamster Cheek Pouch. Arch. Oral. Biol. 5: 63-64, 1961.

## Honors and awards relating to this project:

1961 Award of Merit from Georgetown Dental School for contribution to the Profession of Dentistry. A plaque and citation was presented.



PHS-NIH  
Individual Project Report  
Calendar Year 1961Part A

Project Title: Studies on Lactate Fermentation by Butyribacterium rettgeri.

Principal Investigator: Dr. C. L. Wittenberger

Other Investigators: Dr. Martin Flavin, Section on Enzymes, National Heart Institute, National Institutes of Health

Cooperating Units: American Dental Association

Man Years (calendar year 1961)

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

The anaerobic bacterium Butyribacterium rettgeri carries out a modified butyric acid type fermentation of lactate. The major products of the fermentation are butyrate, acetate and carbon dioxide. An unusual feature of this metabolic process emerged when Barker and co-workers discovered that the organism required lipoic acid for growth when lactate served as the main energy source but not when pyruvate or glucose was substituted for lactate in the growth medium. These results suggested that lipoic acid was not involved in the classical  $\alpha$ -keto acid oxidation sequences but rather was functioning in a new and perhaps unique metabolic capacity. Other studies by Barker's group on the metabolism of B. rettgeri revealed that the acetate formed by this organism is partially derived from the condensation of two moles of carbon dioxide. The nature of the pathway and the intermediates involved in this fixation of CO<sub>2</sub> have not been resolved.

This investigation is oriented toward resolving the role of lipoic acid in lactate dissimilation by B. rettgeri and toward determining the pathway of acetate formation from carbon dioxide. It is hoped that the information derived from this study will not only provide a basis for a better understanding of the metabolism of the organism but might also, in a broader sense, contribute to the general knowledge of carbon dioxide fixation processes and the metabolism of



Part A (continued)

one-carbon units.

Methods Employed:

All are standard techniques routine to the types of studies herein described.

Major Findings:

Results from this laboratory have confirmed an earlier observation by Barker and co-workers that lactate but not pyruvate decomposition is sensitive to arsenite in resting cells of B. rettgeri. These findings suggest that either lipoate is involved in the conversion of lactate to pyruvate or that pyruvate is not an obligatory intermediate in lactate dissimilation. The former alternative was considered the most likely when it was demonstrated that qualitatively identical fermentation products are formed from both lactate and pyruvate. Furthermore, by means of radioactive tracer techniques, it has been possible to show a complete equilibration of the carbons of lactate and pyruvate during the fermentation of lactate. Although these results indicate that a pathway for lactate dissimilation does exist in which pyruvate may function as an intermediate, they do not exclude the possibility that alternate pathways for lactate metabolism may also exist.

When B. rettgeri cells are dried by lyophilization, they are subsequently unable to decompose lactate although their ability to ferment pyruvate is unaltered. The addition of either ferricyanide or lipoic acid to these preparations fully restores their capacity to dissimilate lactate. Both ferricyanide and lipoate are required as net electron acceptors and balance studies have shown that both acceptors are reduced stoichiometrically by lactate. The ferricyanide reaction is insensitive to arsenite, which seems to eliminate the possibility that lipoate is involved in the transport of lactate into the cell. The reduction of added lipoate by lactate, on the other hand, is completely inhibited when cells are preincubated with arsenite. This observation suggests that the reduction of exogenous lipoate is mediated by endogenous, possibly enzyme-bound lipoate, and supports the hypothesis that, in B. rettgeri, lipoic acid functions as an integral component of the electron transport system involved in the disposition of lactate hydrogens.

In an effort to gain more specific information regarding the metabolic role of lipoic acid, attempts have been made to study the lactate fermentation in cell-free extracts. Cell-free extracts prepared by various means have been found to be incapable of





Part A (continued)

catalyzing the anaerobic decomposition of lactate unless ferricyanide or another suitable dye is added as a net electron acceptor. Under these conditions lactate dissimilation is not arsenite sensitive. The ferricyanide reaction is thus apparently not mediated by endogenous lipoate. Lipoic acid will not substitute for ferricyanide as an electron acceptor in extracts, which may indicate that the lesion induced by cellular disruption is in the transfer of lactate hydrogens to enzyme-bound lipoic acid. Although unsupplemented cell-free extracts are devoid of lactate decomposing activity, they do catalyze the reversible reduction of lipoic acid by DPNH. This reaction is mediated by an endogenous dithiol which may be bound lipoic acid. This possibility is supported by the observation that lipoic acid amide is much more active as an acceptor for DPNH hydrogens than is the free acid.

Significance to Dental Research:

Many of the organisms comprising the oral flora, such as Veillonella, require rather high partial pressures of carbon dioxide for growth. Indeed, so far as known, virtually all living organisms require a certain level of carbon dioxide for growth and reproduction. The nature of this carbon dioxide requirement is incompletely understood and it is anticipated that studies on the mechanisms of carbon dioxide fixation in B. rettgeri will provide basic information which will contribute to the better understanding of this fundamental phenomenon in all organisms.

Proposed Course of Project:

Studies now in progress are directed toward obtaining cell-free preparations of B. rettgeri which are capable of effecting an arsenite sensitive dissimilation of lactate. Various means of disrupting cells, such as treatment with lysozyme, will be employed in an effort to obtain active preparations. If successful, a purification of the lactate decomposing system will be undertaken for the purpose of localizing the specific biochemical site of lipoate action.

Further work on the pathway of CO<sub>2</sub> fixation in B. rettgeri and the intermediates involved in this process is also anticipated. Initial studies will be conducted with intact cells to establish the optimum physiological conditions for this metabolic process. More detailed work will be carried out with sub-cellular fractions. Special consideration will be given to the possible involvement of certain vitamins, such as biotin and B<sub>12</sub> in the CO<sub>2</sub> fixation pathway.

Part B included:

No



1. Microbiology
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Role of Bacteria in the Formation of Dental Calculus.  
I. The Capacity of Dental Plaque Organisms to Serve as  
a Matrix for Calcification.

Principal Investigator: Dr. A. A. Rizzo

Other Investigators: Dr. D. B. Scott  
Dr. G. R. Martin  
Dr. S. E. Mergenhagen

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1-1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

1. To discover the role of bacteria in the development of mineralized deposits on teeth.
2. To investigate, specifically, the capacity of individual oral organism to serve as matrix for calcification.

Methods Employed:

Washed pure cultures of organisms isolated from calcifying human dental plaques were transferred into sealed dialysis bags and implanted in the peritoneal cavities of rats. Non-viable organisms and several other substances were also implanted. Animals were sacrificed after varying intervals and the contents of the bags were subjected to standard bacteriological procedures, X-ray diffraction analysis, and electron microscopy.

Implanting test material enclosed in a dialysis bag brings a refinement and simplification to the intraperitoneal implant method of studying calcification (mentioned in a previous annual report).



Part A (continued)

The advantages of this method are that the test specimens are continuously exposed to a supersaturated solution of calcium and phosphorus similar to serum and saliva; and yet the material under study remains isolated from host cells and high molecular weight compounds which might influence calcification.

Major Findings:

Ten viable organisms, representing gram-positive, gram-negative, filamentous, bacillary, and coccoid types, have been tested so far and all of them appear capable of inducing calcification. Hydroxyapatite crystals were detectable by X-ray diffraction between 14 and 26 days after implantation, whereas control specimens of sterile saline (0.85% and 4.0%) and of distilled water implanted for 51 days did not calcify. Killed organisms developed similar calcifications which were detectable somewhat earlier than those of the viable bacteria. Under both the optical and electron microscope, these calcifications were strikingly similar to those seen in human calculus. In smears stained by the von Kassa method, early calcifications were seen as rough spheroids from 2 to 6 microns in diameter. Electron micrographs showed needle-like crystals of hydroxyapatite both within and between bacterial cells.

Significance to Dental Research:

These studies have shown that oral organisms of several types may serve as a readily calcifiable matrix material. Although previous workers have emphasized the importance of gram-positive filamentous bacteria in the mineralization of dental deposits, these recent findings indicate that other types of organisms are equally important.

Considering the high prevalence of dental calculus and the fact that very few studies of this type have been carried out, the new data brought forth constitutes a much needed addition to our knowledge of the oral bacteria involved in calculus formation. The successful use of the in vivo model system described suggests that the same experimental system might be profitably applied to test other substances implicated in calculus formation, such as salivary secretions, leucocytes, and epithelial cells.

Proposed Course of Project:

1. Using similar methods, additional organisms will be tested both individually and in combination.
2. Other substances present in dental plaques such as saliva, leucocytes, and epithelial cells will also be tested.



Part A (continued)

3. The influence of different organisms on the attachment of calcifications to teeth will be studied by implanting teeth with bacteria.

Part B included:                      Yes





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Rizzo, A. A., Martin, G. R., Scott, D. B., and Mergenhagen, S. E.:  
Mineralization of Bacteria. Science, 1961, in press.

Honors and Awards relating to this project:

None



1. Microbiology
2. Gnotobiotics
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Role of Microbiological, Nutritional and Host Factors on Experimental Dental Caries.

Principal Investigator: Dr. R. H. Larson

Other Investigator: Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (Calendar Year 1961):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

The development of experimental procedures to facilitate evaluation of the separate roles of host animal, diet and oral flora in the development of experimental dental caries.

Methods Employed:

The project is aimed at developing or modifying conventional methods for studying effects of specific variations of microbiology, nutrition and host on the carious process.

Major Findings:

1. A semi-purified caries-test diet has been formulated which appears to be nutritionally adequate for the Sprague-Dawley and Osborne & Mendel rats. Caries activity developed on all tooth surfaces. A useful variation of the diet, on which only slight activity developed, was made by substituting corn starch for confectionary sugar. The starch diet was essentially isocaloric with the caries-test diet and was equally adequate for growth and well being.
2. There is some evidence in the literature that as animals mature the teeth become less susceptible to caries development. These earlier studies gave no consideration to possible differences in



oral flora when caries studies were initiated at difference ages. We have placed animals on a caries-test diet at different ages (18 to 40 days). In an attempt to equalize the flora, the animals of each group were treated with a broad spectrum antibiotic prior to the caries test period. They were then "reinfected" at the beginning of the test period by being housed with an older caries active animal. The bucco-lingual caries activity of the animals started at 40 days of age was only 13% of that of the animals started at 18 days of age. Proximal caries was 59% and sulcal caries 69% of that in the animals started at 18 days of age.

3. A streptococcus made resistant to streptomycin and shown to be caries-active in the hamster was inoculated into the rat by various methods including plaque transfer from an infected hamster. The organism was recovered from the rats at intervals during the experiment but no increase in caries activity was demonstrated. These results agree with the findings in a comparable study with gnotobiotic rats.

Significance to Dental Research:

1. The availability of a relatively simple, nutritionally adequate, semi-purified diet, should facilitate the study of the role of individual dietary components in the caries process. The high protein content of the diet plus the excellent nutritional status of the animals receiving it are in direct contrast to the usual experience with some of the more commonly used caries-test diets in which nutritional stresses of varying degrees are associated with the development of caries.

2. The results of the caries studies initiated on animals of different ages showed a decrease in activity with increase in age in spite of efforts to equalize the flora. It has not been shown whether this decrease was due to tooth maturation or to some other physiological change in the host.

Proposed Course of Project:

The NIH Black rat shows low levels of caries activity compared with the highly active Osborne & Mendel rat, thus providing two strains of animals in which various host factors may be compared. Studies are now under way to compare caries activity in both first and second generation hybrid animals with those of the original strains, giving special consideration to possible differences in the microbial ecology of the two strains. Plans are also being made to conduct parallel studies of other constitutional factors in the two strains which may be relatable to caries activity, such as electrophoretic patterns of saliva and tooth solubility in acids or other products of bacterial metabolism.

Part B included: Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Larson, R. H., Zipkin, I., and Rubin, M. Effect of Administration of EDTA by Various Routes on Dental Caries in the Rat - Possible Role of Coprophagy. Archives of Oral Biology, 5, 49-54, 1961

Larson, R. H., and Zipkin, I. Effect of Tetracycline in the Transmission of Dental Caries in Rats. J. Dent. Res. 40, 264-267, 1961.

Honors and Awards relating to this project:

None





1. Microbiology
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Mechanisms of Action of Oral Bacteria and their Products in Periodontal Disease.

1. Biological, Physical, and Immunochemical Studies on Endotoxins
2. Allergenic Properties of Oral Bacteria

Principal Investigator: Dr. S. E. Mergenhagen

Other Investigators: Dr. S. B. Jensen  
Dr. A. A. Rizzo

Cooperating Units: None

Man Years (calendar year 1961):

Total:	4-1/2
Professional:	2
Other:	2-1/2

Project Description:

Objectives:

1. To determine which members of the oral microbiota possess endotoxins or other products capable of inducing hypersensitivity in the animal host.
2. To analyze, by physical and immunochemical techniques, the nature of these bacterial products.
3. To analyze the mechanism of action of endotoxins and other bacterial products on host tissue and to study the role they may play in the pathogenesis of experimental infections with oral bacteria.

Methods Employed:

The two-phase phenol-water extraction of Westphal has been applied to oral bacteria with a view of releasing soluble endotoxins from the cells. Toxicity of isolated bacterial fractions was tested by intracutaneous and intravenous injection in rabbits and intra-



peritoneal injection in mice. Pyrogenic activities of endotoxic preparations were tested in rabbits. Development of lesions from primary injections in rabbits was followed grossly and histologically. Similar observations were made on rabbits given a second injection intravenously to elicit the localized Shwartzman reaction. Toxicity for mice was calculated as the 50 per cent lethal dose. In addition, the effects of endotoxin on oral tissues was investigated by injecting these preparations intramucosally in the gingival-palatal area of the rabbit. Both direct toxicity to oral mucosa and Shwartzman reactions in oral mucosa were followed histologically. The effects of intramucosal injection were further studied by measuring systemic temperatures and examining regional lymph nodes draining the injected oral sites. Chemical and physical analysis of these lypopolysaccharide endotoxins utilizes paper chromatography and specific colorimetric tests for polysaccharide elements, ultracentrifugation for purification of endotoxins and to determine the molecular properties of the material, and chemical fractionation of the endotoxin to determine whether the toxic moiety is really only a part of the whole complex isolated by the phenol-water method.

Immunological analyses of lypopolysaccharide endotoxins are being carried out by using immunoelectrophoresis, agar diffusion techniques, agglutination, hemagglutination, and hemagglutination inhibition techniques. Biological specificity of veillonella endotoxins has been performed by testing the effect of prior experience with living or dead oral bacteria on the response of mice and rabbits to endotoxins. This study has been extended to using germfree rats monoinfected with oral veillonella.

Sensitization of various experimental animals (mice, guinea pigs, rabbits) has been effected by prior vaccination with oral viridans streptococci, i.e., viable streptococci or heat-killed streptococci injected in an adjuvant. Animals were challenged after a suitable period intracutaneously and intramucosally with soluble nucleoprotein complexes isolated from the streptococci. Toxicity in sensitized and control animals' dermal and mucosal tissues was followed histologically.

#### Major Findings:

1. Endotoxins, isolated by the phenol-water extraction procedure, have been extracted from oral strains of Veillonella, Selenomonas sputigena, Bacteroides melaninogenicus, Fusobacterium nucleatum, small oral treponemes, Borrelia vincentii and Borrelia buccalis, but not from viridans streptococci. These endotoxins produced intracutaneous inflammatory necrotic lesions, prepared skin sites for the local Shwartzman reaction, were pyrogenic in rabbits, and were lethal for mice. In addition, the endotoxin from Veillonella strains (others not tested) produced inflammatory necrotic lesions



Part A (continued)

in the oral mucosa of the rabbit and prepared this tissue for the local Shwartzman reaction. These effects were obtained with as little as 1 microgram of endotoxin. A comparison of the response of oral mucosa and of skin to similar local injections of endotoxin revealed that the gross and histologic manifestations following this specific type of insult were basically similar in both tissues. It was observed, however, that an intramucosal injection of 1 microgram produced temperature elevation, whereas 25 times this dose given intracutaneously was not pyrogenic.

2. The lipopolysaccharide-containing endotoxin fraction, isolated by the phenol-water procedure from veillonella cells, has been characterized chemically as follows: it contained 10 per cent Kjeldahl nitrogen, 4 per cent protein, 4.2 per cent phosphorus, 60 per cent chloroform-soluble lipid after acid hydrolysis, 48 per cent nucleic acid.

Nucleic acid contamination of the above endotoxins could be eliminated by preliminary treatment of the bacterial cells with 0.5 per cent formol or by ultracentrifugation of the dialyzed water phase after phenol-water extraction. This procedure proved useful for getting highly toxic preparations free of nucleic acid. Veillonella endotoxin so prepared had low contents of nitrogen (0.4 per cent), phosphorus (0.5 per cent) and protein (1.8 per cent). Concomitant with the reduction in these chemical components, the minimum lethal dose (LD<sub>50</sub>) for mice and the minimum effective preparatory dose for the local Shwartzman reaction were reduced. Analytical ultracentrifuge data indicate that this endotoxin is polydisperse and has several components of varying molecular weight. However, products separated in a Sephadex column indicate that the fractions with the greatest molecular weight (>50,000) are responsible for the toxicity of the whole fraction tested. Polysaccharide components thus far identified in veillonella endotoxin have been glucosamine, galactosamine, sialic-acid like components, and a fast moving hexose with chromatographic properties similar to 3-methylglucose. The hexosamine components were identified colorimetrically and by ion-exchange chromatography. Sialic acid was identified by the thiobarbituric acid assay method and also by paper chromatography with known references of N-acetylneuraminic acid and N-glycolyneuraminic acid. Sialic acid-like materials have been found also in the endotoxin of Selenomonas sputigena but not in the others.

3. Utilizing hemagglutination techniques, an immunological study of veillonella lipopolysaccharide endotoxin demonstrated that the endotoxic moiety is in part responsible for the specific agglutinating properties of the veillonella organism. Although the lipopolysaccharide proved to be a poor antibody-producer in vivo,



Part A (continued)

it could be used as a hapten in hemagglutination tests in which rabbit antiserum to whole cells agglutinated endotoxin sensitized sheep erythrocytes. Biologically active lipopolysaccharides (i.e.; Shwartzman reactive, pyrogenic) were prepared by phenol-water extraction from several other strains of oral veillonellae. Quantitative analyses of the lipopolysaccharides for lipid, protein, polysaccharide, N, and P contents revealed a striking similarity in all endotoxin preparations. Certain lipopolysaccharides, however, appeared to be serologically distinct as determined by hemagglutination inhibition tests which established five serotypes of veillonellae. Preliminary observations, utilizing paper chromatography, may indicate the basis for this immunological specificity. Paper chromatography of certain hydrolyzed lipopolysaccharides revealed fast moving hexoses, tentatively identified as a methylglucose, which may be responsible for the specificity of the endotoxin from which they were isolated on chromatograms.

Toxicity tests in veillonella vaccinated and nonvaccinated mice with serologically specific endotoxins have revealed some interesting preliminary findings. In a number of separate experiments, intraperitoneal injection of heat-killed veillonellae or living veillonellae conferred resistance on mice to the lethal effects of veillonella endotoxin and to the lethal effects of other gram negative pathogens. The protection afforded the mouse seemed to be best conferred on those animals with endotoxin prepared from the strain of veillonella used for vaccination. Utilizing weight loss to sub-lethal injections of endotoxin as a parameter of toxic physiological effects on the mouse, there was observed some overlapping of protection among homologous and heterologous vaccinated mice.

Sensitization of the guinea pig and the rabbit to oral viridans streptococci was demonstrated in that dermal and mucosal tissues reacted to soluble nucleoprotein complexes in a manner which is consistent with the hypersensitive response. Tissues of such animals after injection of challenging protein showed marked edema, necrosis, monocytic invasion, and massive plasma cell accumulation.

Significance to Dental Research:

The demonstration by chemical and biological means of endotoxins in certain strains of oral bacteria, especially those which are found in greater abundance in the oral cavity in pathologic processes of oral soft tissues, supports the frequently advanced speculation implicating toxic components of the oral microbiota in the inflammatory, necrotic phases of periodontal disease. The demonstration of specific antigens (lipopolysaccharides) in gram negative oral bacteria by immunological and biological





Part A (continued)

techniques may contribute to a more complete understanding of the host-parasite relationship in periodontal disease. Evidence for the susceptibility of gingival tissue to endotoxins and to antigens other than endotoxins in hypersensitized animals may implicate the allergenic properties of oral bacteria in oral disease.

Proposed Course of Project:

To continue our investigations as outlined above with particular reference to mechanisms of action of endotoxin and other bacterial products on host tissue, availability of oral tissues to such bacterial products, and a physical, biological, and immunochemical study of endotoxins from gram negative bacteria.

Part B included:            Yes



PHS-NIH  
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E. Endotoxic Properties of Oral Bacteria as Revealed by the Local Shwartzman Reaction. J. Dental Res. 39: 267-272, 1960.

Rizzo, A. A. and Mergenhagen, S. E. Local Shwartzman Reaction in Rabbit Oral Mucosa with Endotoxin from Oral Bacteria. Proc. Soc. Exp. Biol. and Med. 104: 579-582, 1960.

Mergenhagen, S. E., Hampp, E. G., and Scherp, H. W. Preparation and Biological Activities of Endotoxins from Oral Bacteria. J. Infectious Diseases 108: 304-311, 1961.

Mergenhagen, S. E., Zipkin, I. and Varah, E. Immunochemical Studies on an Oral Veillonella Endotoxin. J. Immunology, In press.

Honors and Awards relating to this project:

None



1. Microbiology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Biochemistry of the Differentiating Cellular Slime Mold, Dictyostelium discoideum.

Principal Investigator: Dr. M. I. Krichevsky

Other Investigators: None

Cooperating Units: Department of Chemistry, Howard University, Washington, D.C., Dr. William Hills.

Man Years (calendar year 1961)

Total: 2-1/4

Professional: 1

Other: 1-1/4

Project Description:

Objectives:

To investigate the biochemical factors initiating and controlling morphogenesis of Dictyostelium discoideum. Presently the main effort is concerned with the factors affecting the rate of differentiation but not the quality. Also, a study has been started concerning the nature of the pigments of D. discoideum and D. purpureum. The pigments are apparently contained only in the mature spores. The stalk cells remain colorless. Thus we have a qualitative rather than quantitative difference between the two cell types facilitating study of factors controlling the appearance of cellular differences.

Methods Employed:

All are standard techniques routine to the types of studies herein described.

Major Findings:

The effect of a variety of compounds on the efficiency and rate of morphogenesis in D. Discoideum was determined. It was found that many unrelated materials would stimulate differentiation indicating that no one simple metabolite or type of metabolite is rate-limiting. Since some ions ( $K^+$ ,  $PO_4^{-3}$ ,  $Mg^{+2}$ , etc.) stimulated while others did not ( $Na^+$ ,  $SO_4^{-2}$ , etc.) it was concluded that ionic strength is not a limiting factor but that the species of ion is important. The



Part A (continued)

pH of the medium (from pH5 through 7) had no marked effect on spore formation. The phenomenon of chelation does not play a central role in the differentiation since there is no correlation between ability to chelate and ability to stimulate. Histidine did not stimulate protein synthesis during early stages of morphogenesis yet this amino acid stimulates only at these same stages. The effect of energy generation is complex. All those compounds stimulating differentiation theoretically could increase adenosine triphosphate levels. However, a number of highly stimulatory materials do not lead to an increase in oxygen consumption. The converse is also true.

Some preliminary progress has been made towards purification of various pigments of the organisms under study. The yellow pigments of D. discoideum have been partially purified. At the moment four distinct pigment fractions have been obtained with apparently similar spectral properties. They have been found to be light sensitive and acidic in nature.

The purple pigment of D. purpureum has been successfully extracted from the spores after a mild acid hydrolysis. The pigment fraction is considerably more polar than that of D. discoideum.

Significance to Dental Research:

The phenomena of non-genetic functional changes in cell types, such as embryological differentiation, microbial spore formation, adaptive enzyme formation, cancerous de-differentiation, aging, etc., are an integral and fundamental part of the life history of all organisms. It is desirable to have information regarding the biochemical factors influencing the initiation and course of such changes since many aspects of medical phenomena are intimately affected by these functional differences among cells.

The amoeboid slime molds have several advantages as a model system for such studies:

1. The amoebae, which are a uniform population in the vegetative stage, form two and only two differentiated cell types (i.e., spore cells and stalk cells).
2. They are simple to grow in quantity under routine laboratory conditions.
3. The formation of the two cell types takes place under starvation conditions, thereby allowing close nutritional control of the process. In addition, the cellular differentiation is unconfounded by associated phenomena due to growth.





Part A (continued)

4. The spore mass is attached to the stalk only by surface tension, thus the separation of the two is facilitated.

Proposed Course of Project:

Future experiments will be designed to test the effect of a variety of compounds on the intracellular adenosine triphosphate levels of differentiating cells to ascertain whether energy generation is rate limiting.

A study of the nutritional requirements of the vegetative amoebae will be undertaken. A defined medium is required so that the initiation of differentiation can be closely controlled and studied.

The study of pigmentation of the slime mold spores will involve determination of: (a) purification procedures, (b) chemical structures, (c) nutritional factors in biosynthesis, (d) pathways of biosynthesis and (e) factors controlling the formation of biosynthetic enzymes.

Part B included:

No



Serial No. NIDR-13  
1. Microbiology  
2. Gnotobiotics  
3. Bethesda 14, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Inhibitors and Experimental Caries

Principal Investigators: Dr. H. V. Jordan

Other Investigators: Dr. I. Zipkin  
Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1961)

Total: 2-1/4

Professional: 1

Other: 1-1/4

Project Description:

Objectives:

1. To study dental caries in experimental animals by the use of chemical agents which inhibit the caries process.
2. To observe the action of these inhibitors on the growth and metabolic activities of oral bacteria as a means of understanding their mode of action.
3. To detect nutritional and metabolic differences between cariogenic and noncariogenic streptococci which will aid in their detection and may point to the basis for their pathogenicity. This is a continuation of NIDR-6 (1960).

Methods Employed:

The methods employed are essentially those described in the previous report (NIDR-6, 1960). The inhibitors selected for test are incorporated into a coarse corn particle, high sugar diet (585) to observe their in vivo effect on experimental caries. Animal experiments are accompanied by in vitro bacteriological tests on pure cultures of caries-active streptococci from rats and hamsters.



Part A (continued)Major Findings:

1. Morphological and histological changes of the teeth and other oral tissues are known to occur in lathyritic experimental animals. The lathyrogenic agents semicarbazide (SCH) and  $\beta$ -aminopropionitrile (BAPN) were tested for their effect on experimental caries in rats. At concentrations above 0.01% BAPN and 0.005% SCH in diet 585, significant inhibition of sulcal caries was observed. Incorporation of the same levels of SCH into diet 636 had no effect on the smooth surface caries normally produced by this diet. BAPN potentiated caries on diet 636. Neither compound was markedly inhibitory to the in vitro growth of caries active streptococci. The nonlathyrogenic nitriles cyanoacetic acid,  $\alpha$  cyanoacetamide, propionitrile,  $\beta$  dimethylaminopropionitrile, ethylene cyanohydrin, and acetonitrile at a level of 0.4% in diet 585 had no effect on sulcal caries.

2. In continuing studies on the inhibition of experimental caries by sodium metabisulfite, the effect of this compound in the form of its addition complex with certain carbonyl-containing compounds was tested. Acetone sodium bisulfite and acetaldehyde sodium bisulfite were compared with sodium metabisulfite at equivalent bisulfite concentrations (0.02 gm M per kilo of diet 585). Caries inhibitions were 66, 57, and 83% respectively. Four addition complexes are currently under test: menadione sodium bisulfite, benzaldehyde sodium bisulfite, formaldehyde sodium bisulfite, formaldehyde sodium bisulfite and sodium formaldehyde sulfoxalate.

The specific ketone-binding reagent p-hydrazinobenzene-sulfonic acid, tested at a level of 0.026 gm M per kilo of diet 585 was found to have no effect on sulcal caries. Previous studies (NIDR-6, 1960) had reported that carbonyl-binding agents which bind both aldehydes and ketones were very effective as caries inhibitors and that a specific aldehyde-binding agent was only moderately effective.

As a means of reducing total intake of metabisulfite, the effect of varied and intermittent exposure to the agent was tested by adding the agent to the diet for 1 day of each week, 1 week of each month, and for only the first 2 weeks of the experiment. Caries scores were reduced 42, 45, and 37%, respectively. This is compared to an 80% inhibition of caries obtained by uninterrupted exposure for the full 90 days.



Part A (continued)

Bacteriological sampling of the cecal contents of rats fed metabisulfite revealed a 200-fold reduction in the lactobacillus population compared to control rats on diet 585. The coliform count was doubled and the streptococcus count was halved in the experimental group. The average free thiamin level of the cecal contents of rats fed metabisulfite was reduced to one-half that of the control group. There was no apparent difference in the average cecal pH between the control and experimental groups.

3. In studies on the fatty acid metabolism of caries active streptococci, the observation was made that these strains were more sensitive to oleic acid than were certain caries-inactive strains. Experiments with other fatty acids, both saturated and unsaturated as well as substituted fatty acid analogues indicated that inhibition was related to unsaturation at the 9-10 position. Linoleic acid and linolenic acid were also active.

Sodium oleate was tested against experimental caries in rats. The saturated analogue sodium stearate was also tested for comparison. Both compounds were without effect on caries. This may be related to the observation that in vitro oleate inhibition was reversed by CO<sub>2</sub>.

Increased sensitivity of caries active strains to ethyl alcohol was also observed. Ethanol inhibition was also reversed by CO<sub>2</sub>.

Significance to Dental Research:

The studies described in this report are believed to constitute a rational approach to the discovery of chemical agents which may have a potential value in the problem of caries control. Study of compounds with a known chemical effect, i.e., selective affinity for carbonyl groups, may lead to a closer delimitation of the chain of events leading to caries. Compounds which are more specific in their action could be ultimately more useful than agents which act in a general way to suppress the whole oral flora.

The in vitro bacteriological studies on pure strains of caries producing streptococci are of both practical as well as theoretical value. Any biochemical differences between these organisms and the noncariogenic strains may be exploited for the selective detection and cultivation of these active strains and also by the use of selective inhibitors to block the particular enzymatic step in question. More importantly, a knowledge of the differences between these two kinds of streptococci may provide important clues for understanding the basis for their pathogenicity.





Part A (continued)Proposed Course of Project:

Studies on other carbonyl-binding compounds and their effect on experimental caries are planned. A more detailed investigation of the effect of the selective carbonyl-binding agents on the metabolism of caries active streptococci is also indicated. Preliminary results on the bisulfite effect on glucose metabolism of streptococci given in a previous report (NIDR-15, 1960) will be extended to include a complete carbon balance.

Continuing nutritional and metabolic studies on caries-active streptococci will include experiments to obtain a semidefined or a defined medium in order to detect vitamin, amino acid, purine and pyrimidine requirements. More extensive studies on the oleate and ethanol sensitivity will continue to determine the metabolic significance of these observations.

A cooperative study has been initiated with Dr. Keyes to investigate the bacteriology of a transmissible plaque which produces periodontal inflammation in hamsters.

Part B included

Yes



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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Jordan, H. V., Fitzgerald, R. J., and Berger, N. D.  
Carbonyl-binding Compounds as Inhibitors of Experimental  
Caries in Rats. J. Dental Res. 40: 199-203, 1961.

Nevin, T. A., and Jordan, H. V. Natural or Derived Materials  
which Replace Ascitic Fluid for the Growth of an Oral Spirochete.  
J. Dental Res. 40: 622, 1961.

Jordan, H. V., and Berger, N. D. Testing of Antioxidants  
against Experimental Caries in Rats. J. Dental Res. 40: 878-883,  
1961.

Jordan, H. V., Fitzgerald, R. J., and Berger, N. D. A Comparison  
of the Caries Inhibitory Potential of Sodium Metabisulfite and  
Related Compounds. J. Dental Res., in press.



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

ENZYME CHEMISTRY: The major accomplishments of the Section on Enzyme Chemistry concern the increased understanding of the chemical nature of two enzymes: carboxypeptidase B and aldolase. At this time the mechanism of action of no enzyme can be explained in terms of the chemical structure of the protein; therefore, the progress made in describing the catalytic properties of these enzymes is of significance in leading to an understanding of the mechanism of enzyme catalysis in general.

The activity of carboxypeptidase B as studied by Dr. Folk and Dr. Wolff was found to depend upon the single atom of divalent metal incorporated in each molecule of enzyme. A metal (cadmium) that produces a very active catalyst for one type of substrate (an ester) is completely inactive with another type of substrate (a peptide) that is split rapidly by enzyme containing another metal (zinc or cobalt). Further analysis of the reactions catalyzed by these enzyme variants with several substrates and inhibitors has permitted the estimation of rates of the partial reactions that compose the over-all reaction. The determination of the effect of each metal on the separate processes of substrate binding, product binding, and substrate splitting is becoming possible through these kinetic analyses, and there is now a prospect for defining the role of the metal in this type of enzyme.

The principal developments in the studies of aldolase conducted by Dr. Mehler are: the evidence supporting the identity of the enzyme-substrate combination that is measured spectrophotometrically with the catalytically active Michaelis compound, the appreciation of the role of electrostatic forces in binding the enzyme and substrate through analysis of the effect of ionic strength on the kinetic and spectrophotometric phenomena, and the alteration of the enzyme by proteolytic degradation to give a new enzyme species with altered catalytic properties. These findings have permitted the elaboration of a model of aldolase that is being tested and refined through current studies.

PRENATAL AND DIETARY FACTORS IN EXPERIMENTAL DENTAL CARIES: In continuation studies by Dr. C. T. G. King the influence of prenatal factors on dental caries and development of oral structures was investigated. This year's results confirmed previous evidence that acidosis increases the susceptibility to dental caries in the progeny of white rats. Since non-specific stressor agents, corticosterone, tremorine, electroshock, and induced anemia, did not increase caries susceptibility, it appears that acidosis is acting as a rather specific agent. In additional studies on the relation of variations in amniotic pressure to the development of the foetus, agar was introduced into the amniotic sac to produce 50 percent resorption. Of the remaining viable embryos, 37 percent had cleft palate, 29 percent had limb deformities and 6 percent had a variety of deformities including umbilical hernia. Upwards



of 50 percent of the viable embryos which appeared grossly normal demonstrated skeletal malformations.

In other studies by Dr. F. J. McClure on the effect of phosphates on experimental rat caries, results demonstrated interesting variability in the cariostatic effect of different phosphate minerals. Added to phosphorus deficient cariogenic diets the soluble phosphates, i.e., ammonium phosphate, and sodium metaphosphate were very cariostatic. A complex commercial insoluble phosphate, victamide was not cariostatic. With respect to organic phosphorus compounds, sodium phytate was again demonstrated to be highly cariostatic, and in addition phytin, a calcium and magnesium phytate, as well as beta-glycerol phosphate proved to be highly cariostatic. The results with the organic phosphorus compounds are most provocative particularly as regards the availability of their phosphorus within the oral cavity. The results pose interesting speculation as regards a significant role of phosphorus in the control of dental caries.

SALIVA CHEMISTRY: Under the direction of Dr. I. Zipkin research has continued to perfect analytical procedures for the spectrophotometric determination of tyrosine and tryptophan in parotid saliva. Correlation with chemical procedures is satisfactory particularly with respect to tyrosine. A good correlation was found also for tryptophan but the presence of a constant non-tryptophan ultra-violet absorbing material was also indicated.

In collaboration with Dr. L. Avioli (NCI), a study was made of the transport of calcium by the parotid gland. Following injection of  $\text{Ca}^{47}$ , the salivary  $\text{Ca}^{47}$  showed a maximum specific activity between one fourth and one half hours.  $\text{Ca}^{47}$  was always lower in the saliva than in the blood and paralleled the blood  $\text{Ca}^{47}$  after the first 2-4 hours. The difference in specific activity between blood and saliva may reflect a delay mechanism from blood to saliva, or may represent a relatively non-measurable pool of salivary calcium.

PROTEIN CHEMISTRY: The major problem under investigation by Dr. K. A. Piez was the structure of collagen and its role in metabolic processes. Several important advances were made during the past year. Specifically it was shown that the collagen molecule contains subunits which probably correspond to single chains. There appear to be three such chains in the molecule of which one is different in composition and properties from the other two. The two types can be divided and studied separately. Further studies are in progress to more completely characterize these subunits. Dr. Piez has also shown, in collaboration with Dr. J. Gross of Massachusetts General Hospital, that as collagen matures in vivo, intramolecular crosslinks form. This results in the formation of chain pairs which can be separated from the single chain subunits. The process of crosslinking continues intra- and intermolecularly and appears to be intimately involved in the maturation of the connective tissue and the development of the properties essential to the normal function of the tissue. For example in lathyrism, a toxic condition in which connective tissue has a drastically lowered tensile strength and the collagen is easily soluble in neutral salt solution, crosslinking between chains occurs at a greatly diminished rate. This maturation process is amenable to further study since it also occurs in vitro in purified collagen stored at 37°.





Dr. George Martin studied mineralization in the aorta as isolated from rats. Incubated in serum, the aortas fix calcium and phosphate as hydroxy-apatite. Since elastase, but not collagenase or other proteases, prevents the mineralization, it appears that in this system elastin rather than collagen may be active in the initiation of a process related to calcification. This recent observation indicates that this system may provide a new approach to the complex problem of calcification.

In a collaborative study with Dr. Davie of Western Reserve University, Dr. Folk and Dr. Gladner (NIAMD) have shown that  $\beta$ -lactoglobulins A and B, which are genetic variants, have different amino acid residues in two positions in the polypeptide chain. The amino acid differences are sufficient to explain the different properties. These results have important genetic implications, with regard to the molecular events responsible for the expression of a mutation.

An important part of the program in protein chemistry is the learning and development of new procedures. In this area, Dr. Lewis has become proficient in the use of the ultracentrifuge and associated equipment and devised new methods for the determination of molecular weights and other properties of macromolecules. In parallel technical studies, Dr. George Martin has put into operation a gas chromatogram which can be used for the analysis of many classes of compounds. This relatively new tool will permit many studies not previously possible. Equipment for monitoring radioactivity in a flowing stream has also been acquired and tested. Methods have been devised which will permit simultaneous determination of concentration and radioactivity in the effluent from columns used to separate amino acids and proteins.

#### CALCIFICATION AND STRONTIUM STUDIES:

In cooperation with Dr. S. Natelson, The Roosevelt Hospital, New York, Dr. R. C. Likins is exploring the feasibility of using a dietary calcium additive as a means of reducing the skeletal burden of strontium-90 in children. The study was made possible through the current study by the NIDR and the NIAMD to determine the cariostatic effect of a calcium phosphate additive to bread.

The special study group consists of 500 children equally divided into a test group receiving bread supplemented with  $\text{CaHPO}_4$  and a control group receiving unsupplemented bread. Children in both groups were given 5.0 mg. of strontium as the chloride twice daily for ten days. At the end of this period, 20 ml. of blood were obtained from each of the subjects. Studies are currently in progress to ascertain whether or not the calcium supplement has significantly reduced the serum Strontium:Calcium ratio. Strontium:Calcium ratios in urine samples are also being determined in order to provide information on the absorption and excretion of strontium.

In an additional project, Dr. Likins in cooperation with Dr. Jacob Menczel (NIH Foreign Fellow from Hadassah University Medical School), has initiated studies on osteoporosis as induced in white rats. Specifically,



one objective is to evaluate the relation of fluoride to bone resorption. Thus far the technique for the total immobilization of the rat for periods up to two weeks by means of a whole body cast has been perfected. During this period of induced immobilization, bone resorption occurs, and may be studied by means of the calcium balance and subsequent tissue analysis. In due time the investigation will be extended to include a study of the effect of orally administered strontium, as well as fluoride, on osteoporosis.



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Part A

Project Title: Analytical and Structural Aspects of Collagen and Other Proteins in Relation to Function.

Principal Investigator: Dr. K. A. Piez

Other Investigators: Dr. G. Martin, Dr. M. S. Lewis, Dr. J. E. Folk

Cooperating Units: Massachusetts General Hospital  
Section of Physical Biology, NIAMD

Man Years:

Total:	2 1/4
Professional:	3/4
Other:	1 1/2

Project Description:

Objectives:

It is the long-range purpose of this project to examine collagens and proteins of related interest with respect to their chemical and physical nature and to correlate function and structure in normal and pathological conditions. The immediate problems relate to (1) the separation and characterization of the subunits of collagen, (2) the characterization of noncollagenous, calcified proteins, and (3) a comparison of two genetically different  $\beta$ -lactoglobulins.

Methods Employed:

Usual laboratory procedures.

Major Findings:

(1) When collagen is denatured by heat or urea, two fractions representing different weight classes are observed in the ultracentrifuge. By chromatography on CM-cellulose it has been shown that each of these fractions can be separated into two subfractions. Examination in the ultracentrifuge and by amino acid analysis has demonstrated that these subfractions represent two distinct types of subunits of the collagen molecule which are probably related as follows: The molecule as originally synthesized contains three



subunits, which are probably single chains, of two different kinds. These are designated  $\alpha 1$  and  $\alpha 2$ , each molecule containing two  $\alpha 1$  chains and one  $\alpha 2$  chain. As a function of time and environment, crosslinks form between chains (intramolecularly) to give chain pairs. These are designated  $\beta 1$  ( $\alpha 1$ - $\alpha 2$ ) and  $\beta 2$  ( $\alpha 1$ - $\alpha 1$ ). Three-chain ( $\alpha 1$ - $\alpha 1$ - $\alpha 2$ ) units also appear to form and crosslinks may eventually be created between molecules.

(2) Enamel protein and protein from many calcified invertebrates are not collagens as shown by the usual structural and chemical criteria. There are however some similarities to collagen. For example, enamel protein and at least some invertebrate calcified tissues (the shell of the snail and a partially calcified cartilage-like material from the crab) contain hydroxylysine, an amino acid once thought to be unique to collagen. Complete amino acid analyses of these materials have been obtained.

(3)  $\beta$ -Lactoglobulins A and B, genetically different proteins from milk, have been analyzed. It was shown that each molecule contains two identical subunits of 18,300 molecular weight and that A and B are identical except for two amino acids out of 162 in each subunit. A valine and an aspartic acid in A are replaced by an alanine and a glycine in B.

#### Significance to Dental Research:

(1) Collagen is a major structural protein and appears to play a specific role in certain metabolic processes. An understanding of its molecular structure is therefore basic to an understanding of many normal and pathological conditions. For example, the elucidation of the cross-linking mechanism between subunits of the collagen molecule appears to explain some of the changes that occur in connective tissue with age.

(2) In determining the role which a protein matrix plays in calcification, a knowledge of the properties of the matrix is a necessity. In particular, a comparison of calcified collagens and noncollagenous proteins may help to elucidate the mechanism by which they exert an effect on the initiation and progression of calcification. The presence of hydroxylysine in these otherwise diverse proteins may be of importance in this respect.

(3) A knowledge of the means of genetic control of protein structure and function has become an important part of all biochemical research.  $\beta$ -Lactoglobulins A and B represent a pair of easily studied genetic variants. The principles elucidated may apply to many genetic variations, some of which are expressed as pathological conditions. The  $\beta$ -lactoglobulins have particular theoretical interest because two amino acid residues are involved unlike the hemoglobins where only one residue appears to be affected. As in the case of the





hemoglobins, the differences in the  $\beta$ -lactoglobulins demonstrate the profound effect produced by a mutation operating through minor alterations in the amino acid structure of a protein.

Proposed Course of Project:

- (1) Studies are continuing to further elucidate the structure and function of collagen subunits.
- (2) Additional samples of calcified tissues will be collected and analyzed to provide further evidence concerning the role of the protein matrix in calcification.
- (3) The studies on  $\beta$ -lactoglobulin are completed insofar as this project is concerned.

Part B included

Yes



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Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Piez, K. A. and Saroff, H. The Chromatography and Electrochromatography of Amino Acids and Peptides in "Chromatography and Electrochromatography" ed. by E. Heftmann, Interscience. In press.

Piez, K. A. Amino Acid Composition of Some Calcified Proteins. Science 134:841-842, Sept. 1961.

Piez, K. A. The Chemistry of the Protein Matrix of Enamel in "Keratinization" ed. by E. O. Butcher, American Association for the Advancement of Science. In press.

Piez, K. A., Lewis, M. S., Martin, G. R., and Gross, J. Subunits of the Collagen Molecule. Biochim. et Biophys. Acta 53:596-598, Nov. 11, 1961.

Martin, G. R., Gross, J., Piez, K. A. and Lewis, M. S. On the Intramolecular Crosslinking of Collagen in Lathyrotic Rats. Biochim. et Biophys. Acta 53:599-601, Nov. 11, 1961.

Piez, K. A., Davie, E. W., Folk, J. E., and Gladner, J. A.  $\beta$ -Lactoglobulins A and B. I. Chromatographic Separation and Amino Acid Composition. J. Biol. Chem. 236:2912-2915, 1961, Nov.

Honors and Awards Relating to this Project:

None



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Part A

Project Title: Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

Principal Investigator: Dr. K. A. Piez

Other Investigators: None

Cooperating Units: Laboratory of Cell Biology, NIAID

Man Years:

Total:	3/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

It is the broad purpose of this project to obtain basic information relative to protein and amino acid metabolism in mammalian cells in culture. The current studies are specifically concerned with (1) the intracellular concentration of amino acids required for protein synthesis and (2) the biosynthesis of cystine.

Methods Employed:

Usual laboratory procedures.

Major Findings:

(1) Cultured human cells concentrated several of the essential amino acids 3- to 30-fold. The minimal effective intracellular concentration required for protein synthesis was 0.01 to 0.05 mM. Nearly maximal rates were obtained at 0.03 to 0.15 mM.

(2) It was discovered that the mammalian cell lines tested, synthesized cystine by the classical pathway from serine and methionine with homocystine and cystathionine as intermediates. In spite of this operative pathway cells required cystine under the usual culture conditions. This was the result of loss of intermediates to the medium at a rate faster than they could be



synthesized. At very high cell population levels (20 to 40 x 10<sup>4</sup> per ml) the loss was not limiting and cystine was not required.

Significance to Dental Research:

Cell culture is a relatively new tool suitable for the study of many metabolic processes. The present findings contribute to the understanding and use of this tool by all branches of the medical sciences.

Proposed Course of Project:

This project has been terminated.

Part B included      Yes





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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Eagle, H., Piez, K. A., and Levy, M. The Intracellular Amino Acid Concentrations Required for Protein Synthesis in Human Cell Cultures. J. Biol. Chem. 236:2039-2042, July 1961.

Eagle, H., Piez, K. A., and Oyama, V. I. The Biosynthesis of Cystine in Human Cell Cultures. J. Biol. Chem. 236:1425-1428, May 1961.

Honors and Awards Relating to This Project:

None



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Part A

Project Title: The Molecular Basis of Connective Tissue Metabolism and Function.

Principal Investigator: Dr. G. R. Martin

Other Investigators: Dr. K. A. Piez, Dr. M. S. Lewis, Dr. D. B. Scott, Dr. S. Mergenhagen, Dr. A. A. Rizzo, Dr. H. Fullmer

Cooperating Units: Massachusetts General Hospital  
National Heart Institute

Man Years (calendar year 1961):

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

I. To elucidate the molecular basis of connective tissue disorders. In particular, to investigate the level, metabolism and structure of various connective tissue components in normal animals and in such experimental conditions as lathyrism, scurvy and in inflamed tissue.

II. To study the role of the organic matrix in calcification in order to gain insight into the factors initiating and regulating the deposition of mineral in hard tissues.

Methods Employed:

Usual laboratory procedures.

Major Findings:

I. We have examined the subunits of rat skin collagen isolated by two different extraction procedures. The results indicate that newly formed collagen (salt-extracted) is composed of three subunits of similar size, one of which (C 2) differs in composition



from the other two ( $\alpha 1$ ). Older collagen (acid extracted) was found to contain in addition two larger components apparently formed by cross-linking of  $\alpha 1$  and  $\alpha 2$ . Further maturation gives rise to a collagen in which all three subunits are joined. Finally, intermolecular crosslinks join adjacent molecules and produce a very insoluble and stable matrix.

Since marked changes, including a loss of tensile strength and a pronounced increase in the extractability of collagen are produced in connective tissue by the administration of lathyrogenic agents such as  $\beta$ -aminopropionitrile (BAPN), we have studied the structure of acid extracted collagen from normal and BAPN-treated animals and the incorporation of glycine-C<sup>14</sup> into the subunits.

Collagen extracted by acetic acid from the skins of rats administered BAPN for 34 days was found to have a greatly lowered content of chain pairs. Collagen derived from acutely toxic animals contained normal quantities of chain pairs, but radioisotope incorporation studies indicated that intramolecular crosslinking of newly formed collagen was greatly reduced.

These findings indicate that there is a defect at the molecular level in the normal intramolecular crosslinking of collagen in lathyritic animals. While it is uncertain at present as to the degree to which the symptoms of lathyrism observed grossly and at the histological level can be ascribed to this defect, a direct relationship is not unreasonable.

II. Following a twenty-four hour lag period significant quantities of calcium and phosphate are removed from serum and fixed to aortas incubated in vitro. Standard X-ray diffraction techniques indicated that the aortas had mineralized with hydroxyapatite. Histological examination revealed that the mineral was deposited on elastin fibers. Treatment with enzymes which specifically remove various components of the aorta prior to incubation indicated that the presence of elastin was essential if mineralization was to occur. The twenty four hour lag period appeared to be caused by labile inhibitors present in fresh serum. Alkaline phosphatase was found to accelerate mineralization indicating that the inhibitors are destroyed by this enzyme. These findings suggest that the elastin component of aorta may be important in the initiation of mineralization under these conditions and that the deposition and localization of mineral may be regulated by inhibitors.

#### Significance to Dental Research:

Since collagen is an important constituent of the hard and soft tissues of the oral cavity, a clarification of the events involved in its formation, calcification and destruction may contribute to our understanding of normal and pathological conditions in which



collagen has a role.

Proposed Course of Project:

I. It is planned to study the structure of collagen isolated from subcellular fractions in order to obtain evidence concerning the mechanism of collagen synthesis.

II. Further studies on the biochemical events in calcification are in progress.

Part B included            Yes





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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Martin, G. R., Mergenhagen, S. E. and Scott, D. B. Relation of Ionizing Groups to the Structure of the Collagen Polymer. *Biochim. Biophys. Acta* 49:245-250, 1961.

Martin, G. R. and Mecca, Christyna E. Studies on the Distribution of L-ascorbic Acid in the Rat. *Arch. Biochem. Biophys.* 93:110-114, 1961.

Martin, G. R. Studies on the Tissue Distribution of Ascorbic Acid. *Annals New York Acad. Science* 92:121-127, 1961.

Martin, G. R., Fullmer, H. M. and Burns, J. J. Observations on the Effect of 3-methylcholanthrene in Scorbutic Guinea Pigs. *Proc. Soc. Exptl. Biol. Med.* 106:157-160, 1961.

Fullmer, H. M., Martin, G. R. and Burns, J. J. Role of Ascorbic Acid in the Formation and Maintenance of Dental Structures. *Annals New York Acad. Science* 92:286-294, 1961.

Martin, G. R., Mergenhagen, S. E. and Prockop, D. Influence of Scurvy and Lathyrism (Odoratism) on Hydroxyproline Excretion. *Nature* 191:1008, 1961.

Martin, G. R., Gross, J., Piez, K. A., and Lewis, M. S. On the Intramolecular Crosslinking of Collagen in Lathyritic Rats. *Biochim. Biophys. Acta* 53:599-601, 1961.

Piez, K. A., Lewis, M. S., Martin, G. R. and Gross, J. Subunits of the Collagen Molecule. *Biochim. Biophys. Acta* 53:596-598, 1961.

Honors and Awards Relating to this Project:

None



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Part A

Project Title: Physical Chemistry of Proteins.

Principal Investigator: Dr. M. S. Lewis

Other Investigators: Dr. K. A. Piez, Dr. G. R. Martin

Cooperating Units: NIAMD

Man Years (calendar year 1961):

Total:	1 1/4
Professional:	1
Other:	1/4

Project Description:

Objectives:

The long-range purpose of this project is to study the physical chemistry of collagens and other proteins of related interest, particularly with respect to the functional relationship of molecular weight, dimensions, and structure.

Methods Employed:

Analytical ultracentrifugation was used for the determination of sedimentation coefficients and molecular weights. Optical rotation was used for studies on helical content of native and denatured collagens. Viscometry was used for studies on molecular dimensions.

Major Findings:

(1) Various chromatographic fractions of denatured collagens have been examined by sedimentation velocity ultracentrifugation. The data obtained have been used with chromatographic and amino acid analytical data to postulate the relationship of the subunits to the structure of the intact molecule.

(2) Tentative values have been assigned to the molecular weights of salt soluble collagen and its subunits on the basis of equilibrium and approach to equilibrium ultracentrifuge studies. The relationship



of these subunits to the subunits of acid extracted collagen has been studied.

(3) The molecular weight of the 3S component of myosin has been determined in 8 M urea. The weight was found to be one-third that found for this component in ordinary solvents, supporting the concept that myosin has a three stranded structure similar in some respect to collagen.

(4) A new method of performing approach-to-equilibrium-molecular-weight-determinations, has been devised. This method gives significantly better accuracy and greatly decreases the time involved in the necessary measurements and calculations.

Significance to Dental Research:

Collagen is of major significance as a structural protein, and an understanding of its molecular structure is basic to understanding the structure and function of connective tissue. Myosin is of importance as a contractile structural protein. The comparison of the molecular structure of this protein with that of collagen is useful for relating their similarities and differences to their respective functions.

Proposed Course of Project:

(1) Studies are continuing on the structure and function of the various collagens and their subunits.

(2) Additional studies will be carried out on other proteins that may give information concerning some particular aspect of the studies on collagen.

Part B included            Yes



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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Piez, K. A., Lewis, M. S., Martin, G. R., and Gross, J. Subunits of the Collagen Molecule. *Biochim. et Biophys. Acta* 53:596-598, Nov. 11, 1961.

Martin, G. R., Gross, J., Piez, K. A. and Lewis, M. S. On the Intramolecular Crosslinking of Collagen in Lathyrctic Rats. *Biochim. et Biophys. Acta* 53:599-601, Nov. 11, 1961.

Honors and Awards Relating to this Project:

None





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Part A

Project Title: Correlation of Clinical Oral Health Findings to Biochemical Stress-related Factors in Personnel Wintering in Antarctica.

Principal Investigator: Dr. C. T. G. King

Other Investigators: Dr. I. Zipkin

Cooperating Units: U. S. Naval Medical Research Laboratories, U. S. Naval Submarine Base, New London, Conn.

Man Years (calendar year 1961):

Total:	3/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

During a period of stress, stimulated adrenal gland activity increases epinephrine, nor-epinephrine and adrenal-corticoid steroid levels in the blood. Personnel who winter in Antarctica are subject to emotional and physical stress due to isolation and exposure to extreme cold. A biochemical study of stress-related factors in blood, urine, and saliva should lead to some understanding of systemic manifestations of this stress environment. The objective of this study is to correlate clinical oral health findings with various biochemical-stress related factors.

Methods Employed:

A number of samples of blood, urine, and saliva have been obtained from Navy personnel prior to their departure to Antarctica and more samples are to be obtained during their stay there. The following determinations will be made:

Blood Serum: Adrenal corticoid level, calcium, sodium and potassium will be determined.

Urine: Catechol amines: Epinephrine and nor-epinephrine.



Saliva: (Specifically, parotid saliva) Steroids, Na, K, proteins, tyrosine, tryptophan, uric acid and total carbohydrate.

Clinical Phase: (To be carried out by Dr. T. Allensworth, U.S.N., in the Antarctica)

1. Evaluation of gingival tissue: (a) P M A index; (b) intra-oral colored photographs.
2. Evaluation of supporting structure of teeth: (a) Full mouth radiographic survey at six months intervals; (b) Russell's index; (c) Tooth Movilometer.
3. Evaluation of Nutritional Status: (a) Intradermal dye-oxidation test for tissue levels of Vitamin C; (b) Wood's light illumination of tongue for fluorescent patterns and possible photographic record.

Significance to Dental Research:

As previously mentioned, it is the objective of this study to correlate clinical oral health findings with various biochemical or physiological stress related factors.

Present Status of Project:

The base line determinations on blood serum and saliva have been completed. The catechol amine determinations in the urine will be carried out during January and February of 1962.

All blood, saliva and urine samples have been collected in the Antarctica and are on their way to the U.S.A. in a deep freeze. The clinical phase of the project has been completed and an evaluation from the U. S. Naval Submarine Base in New London, Conn. will follow.



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Part A

Project Title: The Effect of Prenatal Factors on Fetal Development and Dental Caries in the Progeny.

Principal Investigator: Dr. C. T. G. King

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	2 1/4
Professional:	3/4
Other:	1 1/2

Project Description:

Subproject A:

Dental caries in the progeny of acidotic and non-specifically stressed pregnant rats.

Objectives:

To study the effect of a respiratory acidosis and non-specific stressor agents during pregnancy on the caries susceptibility of the offspring.

Methods Employed:

The methods employed were the usual procedures outlined in last year's progress report.

Major Findings:

The results confirm the ones obtained in the first experiment, in that acidosis increases the susceptibility to dental caries in the progeny (control score 10.00; experimental 15.00). The differences are statistically significant but they are not as large as in the first experiment.

Non-specific stressor agents (i.e. corticosterone, Tremorine, electroshock, anemia) did not increase the caries susceptibility of



the young like CO<sub>2</sub> did which points to the fact that this gas is acting as a rather specific agent.

This experiment is now terminated and the material is being organized for publication.

Significance to Dental Research:

This project attempts to evaluate the effect of pre-natal influences on the development of dental caries.

Proposed Course of Project:

Long bones and teeth obtained from the animals on this experiment will be analyzed for calcium and phosphorous content in an effort to evaluate the effect of acidosis during pregnancy on calcium deposition in the developing embryo.

Subproject B:

The effect of manganese deficiency in the diet of the rat during development and gestation, on the caries susceptibility of the progeny.

Objectives:

To study the effect of manganese deficiency in the diet of the rat from weaning and during gestation on the caries susceptibility of their progeny.

Methods Employed:

Normal 21 day old female rats from normal mothers on a stock diet were put on a manganese deficient diet for 60 days. At this time the young rats (approximately 81 days of age) were mated to normal, sexually mature male rats. After mating, and throughout the gestation period the animals were fed the deficient diet. The offspring of these animals were weaned at 21 days of age and put on a cariogenic diet. They will remain on this diet for a period of 60 days. At this time the animals will be killed and their molar teeth scored for caries as against another group of animals on the same cariogenic diet but whose mothers were raised on stock laboratory chow.

Significance to Dental Research:

This study will attempt to evaluate the effect of prenatal influences (trace element deficiency) on the development of dental caries.





Proposed Course of Project:

At the present time this experiment is in progress. It should be terminated early in 1962 at which time an evaluation of the results will be made.

Subproject C:

Prenatal influences on fetal development.

Objectives:

To study the relation of variations in amniotic pressure on the development of the rat foetus.

Methods Employed:

The methods employed were the usual procedures outlined in last year's progress report. In addition, the following experimental procedure was devised: a 3 percent agar solution was made at 80° C. The solution was allowed to cool to 40-39° C with constant stirring. When it reached this temperature it was injected (via a warm syringe .10 cc) directly into the exposed uterine horn. As the agar came in contact with intrauterine or amniotic fluid at 37.5° C, it immediately froze and plugged the hole made by the needle. This procedure allowed us to use more foetuses per pregnant rat and to determine the exact area where the pressure was induced.

Major Findings:

Agar was introduced into 185 amniotic sacs. This procedure produced 50 percent resorptions (93 foetuses) and 92 foetuses were viable; of these 92, 37 percent had cleft palate, 29 percent had limb deformities, 6 percent had a variety of deformities such as umbilical hernias, etc. The remaining 28 percent of the viable young appeared normal by gross examination; however when they were cleared with KOH and stained with alizarin red S, half of the 28 percent demonstrated skeletal malformation, i.e. ribs, skulls, etc.).

In summary, increased amniotic pressure within the sac leads to foetal malformation or resorption. Intra or outer uterine increased pressure does not necessarily lead to the same results.

These results were presented at the 5th Pan American Congress of Endocrinology in Lima, Peru on October 20, 1961.

Significance to Dental Research:

This study was carried out to evaluate some of the factors which may contribute to congenital abnormalities in the oral structures.



Subproject D:

Effect of exogenous adrenal cortical hormones during gestation on congenital malformations.

Methods Employed:

Pregnant strain A mice were administered 5 mg of corticosterone acetate daily during the 11th and 12th day of gestation.

Pregnant Sprague Dawley rats were treated with corticosterone and 17 hydroxycorticosterone acetate at different stages of gestation.

The animals were killed one day previous to the expected delivery date and the fetuses examined grossly for malformations.

Major Findings:

Corticosterone or 17 hydroxycorticosterone produces 95 percent cleft palates in the strain A mice when injected during the 11th and 12th day of gestation (this confirms other investigators' findings). However, neither of these adrenal steroids were able to induce malformations in the rat regardless of the stage of gestation that they were administered.

Significance to Dental Research:

This study was carried out to determine the effect of adrenocortical steroids, administered during gestation, on the development of the foetus.

Proposed Course of Project:

Together with Dr. F. Kendrick we will attempt to determine if the effect of these hormones in the mice is via an increase in amniotic pressure.

Part B included            No



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Enzyme Structure and Mechanism of Action

Principal Investigator: Dr. A. H. Mehler

Other Investigators: None

Cooperating Units: NIAMD

Man Years (calendar year 1961):

Total:	2 1/2
Professional:	1
Other:	1 1/2

Project Description:

Objectives:

To determine the primary structure of the enzyme aldolase; to determine the nature of linkages between enzyme and substrate; to determine the factors that influence catalytic activity; and to ascertain the nature of the catalytic process.

Methods Employed:

N-Terminal amino acids are identified and determined quantitatively by specific chemical methods. C-Terminal amino acids are determined after liberation by enzymatic digestion. Aldolase is degraded by specific proteolytic enzymes and by chemical reagents that attack certain amino acids, and the end groups formed as the result of these treatments are determined. The altered enzyme and fragments produced during various treatments are separated by chromatography, gel filtration, and other physical methods, and their properties determined through ultracentrifugal, amino acid, and end group analyses. Kinetic assays of enzymic activity are carried out on native and altered enzyme preparations with various substrates and inhibitors in the spectrophotometer. The spectrophotometric changes produced by combination of enzyme and substrates are measured.



Major Findings:

The kinetics of aldolase have been examined as a function of ionic strength and the competitive inhibition of the binding of substrate by salt has been established. The direct spectrophotometric determination of the formation of an enzyme-substrate complex between aldolase and dehydroxyacetone phosphate has been used to calculate a dissociation constant for this substrate without the use of kinetic data. The effect of salt on this dissociation supports the conclusion that the observed complex is indeed the catalytically active complex. Degradation of aldolase by chymotrypsin in the presence of substrate has been found to produce an enzyme with altered catalytic properties, similar to the enzyme produced by carboxypeptidase, but differing in chemical composition from the carboxypeptidase-altered enzyme.

Significance to Dental Research:

These studies are part of a program to elucidate the basic nature of enzymes. Since all biological processes are fundamentally enzymatic, it is necessary to understand the nature of enzymes in order to obtain an understanding of normal and pathological biological processes.

Proposed Course of Project:

All of the approaches mentioned above will be continued to define additional structural features and catalytic properties of aldolase. Similar enzymes from other sources will be investigated in order to determine which properties may be correlated.

Part B included      No





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies on Soluble Ribonucleic Acid.

Principal Investigator: Dr. Fred H. Bergmann

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/2
Professional:	1/2
Other:	none

Project Description:

Objectives:

Enzymatic reactions forming amino-acyl ribonucleic acid compounds from low molecular weight ribonucleic acid fractions (RNA) and amino acids have been described. Long-term objectives of this project are: to characterize this type of reaction with respect to the detailed chemical nature of specific ribonucleic acids, and the nature of this catalytic process. A more immediate objective is the development of methods for the isolation of specific biologically active RNA molecules.

Methods Employed:

Amino-acyl RNA synthetases and soluble RNA are prepared from Escherichia coli. Conventional radiochemical methods of assay are used to measure partial reactions of the enzymes, and the rate and extent of amino-acyl RNA formation.

Major Findings:

None

Significance to Dental Research:

The reactions described are currently believed to be early steps in the synthesis of proteins. A more thorough understanding of this



process is a desirable objective in understanding the reactions of all biological material at the molecular level.

Proposed Course of Project:

Investigation of the feasibility of separation of specific soluble RNA species by reactions of  $\alpha$ -amino groups of amino-acyl RNA with N-carboxy anhydrides.

Part B included            No



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes: Chemical and Molecular Studies. II. Blood Clotting: Molecular Mechanism and Enzymology. III. Protein Structure.

Principal Investigator: Dr. J. E. Folk

Other Investigators: Dr. E. C. Wolff, Dr. K. A. Piez

Cooperating Units: Section of Physical Biology, NIAMD  
Section of Histochemistry, NIAMD

Man Years (calendar year 1961):

Total: 3  
Professional: 1  
Other: 2

Project Description:

Objectives:

- I. (a) Specificity and mode of action of the carboxypeptidases.  
(b) Purification and activation studies on the zymogen of carboxypeptidase B.  
(c) Biochemical and histochemical studies on enzymes which hydrolyze or modify L-glutamyl peptides.
- II. (a) Mechanism of the thrombin-induced conversion of fibrinogen to fibrin.  
(b) Physiological properties of the peptides formed during this conversion.
- III. Amino acid composition and arrangements in the genetic species of  $\beta$ -lactoglobulin.

Methods Employed:

- I. (a) Detailed kinetic studies have been carried out and are being continued with carboxypeptidase B. Methods employed in these studies have been modified and improved to cover most conditions of substrate concentration, pH, salt concentration, etc.  
(b) Steps in the purification of carboxypeptidase B zymogen have



been followed by the methods in (a) as have the rates and means of activation to the enzyme.

(c) Substrates which are potential chromogenic agents have been synthesized for study of enzymes which act upon glutamyl peptides.

II. Chemical and enzymatic methods of sequence analysis have been applied to peptide fragments of Peptide B from fibrinogen. Portions of certain sequences of this peptide are in the process of synthesis. A physiological testing system has been established for assaying the activity of fragments (natural and synthetic) from this peptide.

III. The peptides from two genetic species of  $\beta$ -lactoglobulin have been separated and amino acid composition and sequences studied by high voltage electrophoresis and automatic amino acid analysis.

#### Major Findings:

I. (a) The  $K_m$  (Michaelis constant) and  $k_o$  (rate constant for breakdown of enzyme-substrate complex to products) have been determined with a number of substrates for native carboxypeptidase B and the modified enzyme under varying conditions of pH, temperature, ionic strength, etc. The  $K_I$  (enzyme-inhibitor dissociation constant) values have been determined for a large number of competitive inhibitors of carboxypeptidase B.

(b) A several-fold purification of precarboxypeptidase B has been accomplished by means of Sephadex chromatography. However, studies are hindered by the very labile nature of this material.

(c)  $\gamma$ -glutamyl- $\beta$ -naphthylamide has been prepared and demonstrated to be a specific and extremely useful histochemical substrate for  $\gamma$ -glutamyl transpeptidase. The histochemical localization of enzyme(s) which affect the  $\alpha$ -glutamyl- $\beta$ -naphthylamide is underway.

II. Peptide B and certain trypsin-formed fragments are extremely active in potentiating the physiological activity of bradykinin.

III. Two amino acid interchanges in the 2 genetic species of  $\beta$ -lactoglobulin have been observed. The peptides of tryptic digests in which these amino acid interchanges are located have been identified (see project report of Dr. Piez).

#### Significance to Dental Research:

I. Understanding of the mechanism of action and specificity of proteolytic enzymes, the use of these enzymes for protein and enzyme modification and structure studies, and the knowledge of the particular functions and interrelationships of these enzymes contribute to the organization and understanding of basic biochemical processes.





II. A knowledge of the primary structures, physiological changes and effects, and the basic mechanisms involved in the fibrinogen-fibrin transition may contribute to a systematic pharmacological and clinical approach to the correction of certain blood clotting abnormalities.

Proposed Course of Project:

I. Further studies are under way which have been designed to gain insight into the mode of action of carboxypeptidases. The particular function of metal ion in catalysis and the effects of enzyme modification on specificity are under study.

Attempts to identify the factors involved in the intercellular release of naphthylamine from the  $\alpha$ -glutamyl peptide are being made.

II. Studies designed to determine the sequence of amino acids necessary for the physiological effects noted with Peptide B are underway. Synthesis of further peptide fragments with possible activity is proposed.

Part B included      Yes



PHS-NIH  
Individual Project Report  
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Folk, J. E., and Gladner, J. A. Influence of Cobalt and Cadmium on the Peptidase and Esterase Activities of Carboxypeptidase B. *Biochim. et Biophys. Acta* 48:139-147, March 18, 1961.

Folk, J. E., Braunberg, R. C. and Gladner, J. A. Carboxypeptidase B. V. Amino- and Carboxyl Terminal Sequences. *Biochim. et Biophys. Acta* 47:595-596, March 4, 1961.

Mauron, J., Folk, J. E. and Shiota, T. L'inhibition de la croissance bacterienne par la S-( $\beta$ -aminoethyl)-cysteine. Role privilegie des dipeptides ayant la lysine on son analogue en position N-terminale. *Chimia* 15:426-428, Aug. 1961.

Glenner, G. G. and Folk, J. E. Glutamyl Peptidases in Rat and Guinea Pig Kidney Slices. *Nature* 192:338, 1961.

Piez, K. A., Davie, E. W., Folk, J. E. and Gladner, J. A.  $\beta$ -Lactoglobulins A and B. I. Chromatographic Separation and Amino Acid Composition. *J. Biol. Chem.* 236:2912-2915, Nov. 1961.

Shiota, T., Mauron, J., and Folk, J. E. Inhibition of Lysine and Lysine Peptide Utilization in Bacteria by Peptides of Thiosine (S-( $\beta$ -aminoethyl)cysteine). *Biochim. et Biophys. Acta* 53:360-365, Oct. 28, 1961.

Honors and Awards Relating to this Project:

None



PHS-NIH  
Individual Project Report  
Calendar Year 1961Part A

Project Title: The Role of Citrate in Calcifying Tissues.

Principal Investigator: Dr. I. Zipkin

Other Investigators: Dr. Jacob Menczel, Hadassh University Medical  
School, Jerusalem, Israel  
Dr. Aaron Posner, Laboratory of Histology and  
Pathology, NIDR

Cooperating Units: Pennsylvania State University

Man Years (calendar year 1961):

Total: 2

Professional: 1/2

Other: 1 1/2

Project Description:

Objectives:

To elucidate the role of citrate in calcified tissues.

Subproject A:

Relation of fluoride to citrate content of bone.

Methods Employed:

It had been shown previously that the citrate concentration in human bones was inversely proportional to the fluoride content. This finding has been confirmed in tibia-fibula preparations in the rat. During the past year additional confirmation has been provided from analysis of mandibles and vertebrae. Fluoride has been determined by methods previously utilized in this laboratory and citrate by a modification of the method of Hess et al.

Major Findings:

The concentration of citrate in the mandibles of rats receiving 0, 10, 50 and 100 ppm F in the drinking water at 22 days of age was 0.52%, 0.44%, 0.44% and 0.31%; at 102 days of age it was 0.46%, 0.42%, 0.37% and 0.33%; and at 161 days it was 0.47%,



0.40%, 0.41% and 0.34%. The citrate concentration in the vertebrae at 72 days of age was 0.55%, 0.53%, 0.46% and 0.38%; at 102 days, 0.57%, 0.49%, 0.46% and 0.37%; and at 161 days the concentration of citrate was 0.51%, 0.42%, 0.40% and 0.33%. It has also been shown that the decrease in citrate concentration is real and not a dilution effect due to bone growth since the increase in total citrate of the bones was depressed.

Proposed Course of Project:

The effect of level of calcium on the fluoride-citrate relation will be studied. In addition other calcified structures such as teeth will be examined to see if the same relation obtains.

Subproject B:

Effect of fluoride in human bone on the crystallinity of bone apatite.

Methods Employed:

Using standard X-ray diffraction technics, Aaron S. Posner of the Laboratory of Histology and Pathology, will examine by X-ray diffraction technics human bone specimens to determine crystallinity and specific surface of the bone crystal.

Major Findings:

Preliminary work with three human bones has shown that fluoride makes the bone crystal more crystalline and thus decreases the specific surface and/or the strain within the crystal. With a decrease of surface area in relation to mass, the concentration of surface oriented ions must decrease. This mechanism may explain the inverse relation between citrate and fluoride in bone.

Significance to Dental Research:

The effective role of fluoride in human nutrition has been limited to its capacity to inhibit caries. Studies are in progress to determine the efficacy of fluoride in combating bone loss in such diseases as osteoporosis. If fluoride reduces the specific surface of the enamel crystal, a new idea will be introduced to help elucidate the inhibition of caries by fluoride.

Proposed Course of Project:

Rat bones of various ages will also be examined by X-ray diffraction patterns to delineate the effect of time as well as fluoride concentration. Collaborative studies are in progress with Dr. Schraer of Pennsylvania State University and Dr. Menczel on the





effect of fluoride on radio strontium discrimination in bone. Studies have begun in collaboration with R. Mencil, on the excretion of citrate in osteoporotic states. To date, studies have been in progress on the production of osteoporosis in rats with hydrocortisone.

Part B included      No



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Biochemistry and Physiology of the Salivary Glands  
and Their Secretions.

Principal Investigator: Dr. I. Zipkin

Other Investigators: None

Cooperating Units: National Cancer Institute  
U.S. Naval Training Station, Great Lakes, Illinois

Man Years (calendar year 1961):

Total: 1 1/2

Professional: 1/2

Other: 1

Project Description:

Objectives:

To study basic components of parotid and submaxillary saliva and  
their relation to oral and systemic disease.

Subproject A:

Development of a simplified procedure for the determination of  
uric acid, protein, tyrosine, and tryptophan in whole human parotid  
saliva by ultraviolet absorption spectrophotometry.

Methods Employed:

The ultraviolet absorption spectrum of whole human parotid saliva  
was taken before and after uricase treatment. The difference in  
density of 292 m $\mu$  represented the uric acid content. Three drops  
of 6N NaOH were then added and the absorption spectrum taken again.  
The tyrosine and tryptophan contents were calculated using dif-  
ferential spectrophotometric equations for a two component system.  
Protein was calculated from the Kalckar modification of the  
Warburg-Christian equation. Tyrosine, tryptophan and protein  
were determined by the Udenfriend-Cooper, Steers-Sevag and the  
Lowry procedures, respectively.



Major Findings:

Parotid saliva contained  $2.4 \pm 0.2$  mg % uric acid. Excellent agreement was found between the chemical and spectrophotometric determination of tyrosine in the whole parotid saliva. The mean  $\pm$  SE was  $6.7 \pm 0.5$  mg %, of which 78.7% was present in the TCA precipitable fraction. The regression line for tryptophan was  $Y = 0.87 X + 1.35$  where X and Y are the concentrations of tryptophan by chemical and spectrophotometric procedures respectively. A good correlation was found, but the presence of a constant non-tryptophan ultraviolet absorbing material was indicated. The mean  $\pm$  SE was  $3.1 \pm 0.3$  mg % for tryptophan with over 90% present in the TCA precipitable fraction. Only 45.0% of the Lowry positive material in the whole parotid saliva absorbed in the ultraviolet and approximated the TCA precipitable fraction.

Subproject B:

The transport of calcium by the parotid gland in the human.

Methods Employed:

In collaboration with Dr. L. Avioli, formerly of the National Cancer Institute, blood and parotid saliva samples were collected at 0, 1/4, 1/2, 3/4, 1, 2, 4, 8, 12 and 24 hours following intravenous injection of 15  $\mu$ curies of  $Ca^{47}$ . Stable calcium was determined by an EDTA chelatometric method, and the isotope was measured in a well-type scintillation counter.

Major Findings:

The salivary  $Ca^{47}$  showed a maximum specific activity between 1/4 and 1/2 hours following injection of the isotope. The specific activity of salivary  $Ca^{47}$  was always lower in the saliva than in the blood and paralleled it after the first 2-4 hours. During the linear portion of the curves the difference in specific activity between blood and saliva was inversely related to the concentration of blood calcium. The differences in specific activity between blood and saliva may reflect a delay mechanism from blood to saliva, or may represent a relatively non-measurable pool of salivary calcium. The former explanation seems more attractive.

Significance to Dental Research:

Since saliva is in intimate contact with the teeth and other oral structures, it is important to study its composition in both normal and pathological states. Since the parotid secretion is a discrete body fluid, it is important to determine to what extent it may reflect oral and systemic disease.



Proposed Course of Project:

- (1) In collaboration with the U.S. Naval Training Station at Great Lakes, Illinois, studies are being completed on the uric acid, tryptophan, tyrosine and protein content of the parotid saliva of caries-normal, caries-rampant, and caries-immune naval trainees.
- (2) The third collection (May 1961) of saliva specimens from personnel of the South Dakota phosphated-bread dental study, has been analyzed for Ca and P and the results await statistical treatment.
- (3) In collaboration with Dr. King, parotid salivas from trainees prior to departure to the Antarctica have been analyzed for protein, tyrosine, tryptophan and uric acid by ultraviolet absorption spectrophotometry. Parotid saliva will be collected from these trainees under "Antarctica Stress" for analysis of the above constituents.

Part B included      Yes





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Zipkin, I., Mergenhagen, S. E., and Kass, R.: The Sialic Acid Content of Human Saliva. *Biochem. & Biophys. Res. Comm.* 4:76, 1961.

Larson, R., Zipkin, I., and Rubin, M.: Effect of Administration of EDTA by Various Routes on Dental Caries in the Rat. Possible Role of Coprophagy. *Arch. Oral Biol.* 5:491, 1961.

Zipkin, I.: Chemical Agents Affecting Experimental Caries. *J. Amer. Med. Assoc.* 177:210, 1961.

Larson, R. H., and Zipkin, I.: Effect of tetracycline on the Transmission of Dental Caries. *J. Dent. Res.* 40:264, 1961.

Mergenhagen, S. E., Zipkin, I., and Varah, E.: Immunochemical Studies on an Oral Veillonella Endotoxin. *J. Immunol.* In press.

Honors and Awards relating to this project:

Invited to chair session on Biochemistry of the Salivary Glands and Their Secretion at the International Conference on Salivary Glands and Their Secretions, August 26-28, 1962, Seattle, Washington.

Guest Lecturer 1961 U.S. Naval Dental School, Bethesda, Maryland.



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Biochemical and Biophysical Studies of Calcification.

Principal Investigator: Dr. R. C. Likins

Other Investigators: See subprojects.

Cooperating Units: The Roosevelt Hospital, New York

Man Years (calendar year 1961):

Total:	2
Professional:	1
Other:	1

Project Description:

Subproject A: (With Dr. S. Natelson, The Roosevelt Hospital, New York)

Objectives:

To explore the feasibility of using a dietary calcium additive as a means of reducing the skeletal burden of strontium-90 in children.

Methods Employed:

Subjects were selected from school children residing in two Indian Mission boarding schools located in South Dakota. The children of one of these schools are now receiving a dietary calcium additive which has increased the average daily calcium intake to a value approximately twice that of their unsupplemented controls. The study group is composed of five hundred children equally divided between the control and test groups. The subjects were matched according to age and sex, and apportioned so as to obtain an equal distribution among the age groups of six through thirteen years inclusive.

To minimize the variability in strontium intake between test and control groups (mean daily intake probably ranges from 1-2 mg.) two hundred children in each school were given 5.0 mg. of strontium as the chloride twice daily for ten days. At the end of this period, 20 ml. of blood were obtained from each of these subjects by venipuncture; blood was also obtained from an additional fifty



children in each school who had not received the strontium supplement. Rising morning-urine samples were obtained from fifty children in each school prior to strontium supplementation, and at the end of 1, 2, 5, and 10 days thereafter.

Serum obtained from the subjects will be analyzed for strontium and calcium by X-ray spectroscopy to ascertain whether or not the calcium supplement has significantly reduced the serum Sr 1:Ca ratio. The Sr 1:Ca ratios in the urine samples will also be determined to provide information on the absorption and excretion of strontium following supplementation with this ion.

Nine control and nine calcium-supplemented children were admitted to the Clinical Center for detailed calcium balance studies during the summer of 1961. These same children have also served as subjects in strontium balance experiments designed to provide additional information on the metabolism of this ion relative to that of calcium. Total strontium analysis of food, blood, and excreta are being carried out in parallel with the calcium study which is under the direction of Dr. G. D. Whedon, NIAMD.

#### Major Findings:

The clinical and field studies are complete and the collected samples are now being analyzed for strontium and calcium.

#### Significance to Dental Research:

Concern over possible hazards associated with the ingestion of strontium-90 has focused attention on strontium metabolism and, particularly, on means of effecting a reduction in present and future skeletal burdens. There is reason to believe that the dilution of strontium-90, ubiquitously present in dairy, grain, and other foodstuffs with sources of dietary calcium free of this nucleide would bring about such a reduction. The attendant lowering of the dietary Sr<sup>90</sup>/Ca ratio would be expected to increase the competitive disadvantage of strontium-90, relative to calcium, and result in a diminished absorption and retention of the former. Further, since the bulk of strontium-90 is deposited during periods of rapid skeletal accretion, it follows that efforts directed toward prevention must be centered primarily in the child and adolescent.

#### Proposed Course of Project:

The initiation of additional research on this problem must await the results of the clinical and field studies.

Subproject B: (With Dr. Jacob Menczel, NIH Foreign Fellow, Hadassah University Medical School)



Objectives:

To investigate the effect of fluoride ingestion on experimentally induced osteoporosis.

Methods Employed:

Rats were injected daily for two months with calcium-45 and given either distilled water or a 50 parts per million fluoride solution as a source of drinking water. At various time intervals, animals from both groups have been placed in whole body plaster of Paris casts to induce osteoporosis. During this period of immobilization they are fed a soluble, chemically defined diet. The state of calcium balance is being determined by the daily analysis of the excreta for total calcium. Parallel analysis of the excreta and selected bones for Ca<sup>45</sup> provides a measure of the comparative release of skeletally bound calcium in the control and fluoride treated animals. The excreta are also being analyzed for phosphorus and for citrate to provide additional information on the metabolism of these ions in osteoporosis.

Major Findings:

The technique for the total immobilization of the rat for periods up to two weeks by means of a whole body cast has been perfected.

Significance to Dental Research:

The incidence and severity of osteoporosis is reportedly less among population groups ingesting high levels of fluoride. It has also been shown that the administration of large doses of fluoride can reverse the negative calcium balance in osteoporosis. The present investigation is designed to investigate the effect of fluoride on bone resorption.

Proposed Course of Project:

It is anticipated that the investigation will be extended to include a study of the effect of orally administered strontium on osteoporosis.

Part B included

Yes





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Individual Project Report  
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Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Likins, R. C., Posner, A. S., Paretzkin, Boris, and Frost, Ann P. Effect of Crystal Growth on the Comparative Fixation of Sr<sup>89</sup> and Ca<sup>45</sup> by Calcified Tissues. J. Biol. Chem. 236:2804-2806, October 1961.

Rubin, Meyer, Likins, Robert C., and Berry, Elmer G. On the Validity of Radiocarbon Dates from Snail Shells. Accepted for publication in J. Geol.

Honors and Awards relating to this Project:

None



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Relation of Dietary Supplements of Phosphorus and Protein to Dental Caries.

Principal Investigator: Dr. F. J. McClure

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total: 1 1/2  
Professional: 1  
Other: 1/2

Project Description:

Objectives:

To obtain information on the effect of phosphorus supplements and cereal protein on experimental dental caries.

Major Findings:

The effect of different phosphorus supplements in experimental cariogenic diets was as follows: added to phosphorus deficient diets consisting essentially of whole wheat flour 75% and cerelese 18%, the soluble phosphates, i.e., ammonium phosphate and sodium meta phosphates, were very cariostatic; a complex commercial insoluble phosphate, victamide, was not cariostatic. Added to diets consisting essentially of dry ground bread and cerelese, sodium phytate (phosphorylated inositol) and ammonium phosphate again proved to be very cariostatic. Insoluble tri-calcium phosphate in the presence of salt in the diet was also cariostatic.

Significance to Dental Research:

Results continue to support previous evidence of the pronounced inhibitory effect of phosphorus supplements on dental caries. Although the mechanism of this phosphorus effect remains unresolved, the possibility of utilizing phosphate for the control of human dental caries is of continued interest.



Proposed Course of Project:

Continuing experimental studies will attempt to resolve the mechanism of the cariostatic effect of phosphorus supplements. The effect of a dentifrice containing 10% of soluble sodium phosphate vs. a nonphosphate dentifrice will be evaluated in a clinical study in cooperation with the Epidemiology and Biometry Branch, NIDR.

Part B included      Yes



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Individual Project Report  
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

McClure, F. J.: Effect of Diet on Alveolar Bone Resorption.  
J. Dent. Res. 40:380, 1961 (March-April).

McClure, F. J.: Diet and Dental Caries. J. Am. Dent. Assoc.  
62:511-515, 1961 (May).

Honors and Awards relating to this project:

Honorary Member, American Dental Association.





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Prenatal Influences on Fetal Development

Principle Investigator: Dr. F. J. Kendrick

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To study the effect of a variety of maternal factors upon the development of the rat fetus with special reference to oral structures.

Methods Employed:

- A. Alteration of amniotic fluid hydrostatic pressure
1. Determination and recording of amniotic fluid hydrostatic pressures by means of physiological pressure transducer, amplifier, and recorder.
  2. Tying off a renal artery or ureter, or clamping a renal artery and vein for various measured periods of time.
  3. Bilateral nephrectomy with and without parabiosis and dialysis.
  4. Water loading by means of (a) intraperitoneal saline, (b) pitressin with and without tap water by stomach tube, (c) aldosterone with and without saline by stomach tube, (d) desoxycorticosterone with and without ACTH and saline by stomach tube, the mineralocorticoid activity being monitored by serial plasma sodium determinations.
  5. Dehydration by water deprivation with and without a



sodium free diet and ammonium chloride.

B. Immunologic study of rat palate

1. Production of anti fetal rat palate rabbit serum and testing for antibodies by (a) precipitation in gel diffusion plates, (b) intravenous injection of antiserum into pregnant rats, and (c) injection of antiserum into the uterine lumen of pregnant rats.
2. Establishment of fetal rat palate in tissue culture to test for cytotoxic factors in antiserum.
3. Repeated breeding and injection of rats with homogenized palate of their own young in an effort to elicit an auto-immune reaction.

Major Findings:

- A. 1. It was found early that a U tube manometer does not possess the sensitivity and the rapidity of response required for measurement of amniotic fluid hydrostatic pressure. A physiological pressure transducer used in conjunction with an amplifier and a recorder seems to meet these requirements. Myometrial contractions complicate the determination of basal pressures. Autonomic drugs are being employed to minimize this factor. Preliminary pressure determinations indicate that there is a probable increased amniotic fluid pressure on the 16th gestational day if the amniotic sac is punctured on the 15th gestational day (a procedure which results in a high incidence of cleft palates). Elevated pressures have also been observed three days following bilateral nephrectomy on the 14th day of gestation. These studies are being continued.
2. Clamping of the renal artery and vein unilaterally for two hours on the 11th gestational day has produced some skeletal deformities. This phenomenon is being studied further.
3. Bilateral nephrectomy on the 14th and 15th gestational days has resulted in the finding of fetuses with open palates on the 17th and 18th gestational days. The palate closes normally on the 16th day when the fetal weight is approximately 500 mgms. All of the experimental fetuses were smaller in size and weight than the control fetuses, and parabiosis with normal rats at the time of nephrectomy or within two days resulted in closure of the palates if the fetal weight reached approximately 500 mgms. Whether the



open palates beyond the 16th day of gestation represents a true malformation or simply a total retardation of growth has not been determined.

4. As a result of various methods of water loading pregnant rats, aldosterone alone has produced a few skeletal malformations.

5. Water deprivation plus a sodium free diet and ammonium chloride diuresis during the 13th through 17th gestational days produced some skeletal deformities.

B. 1. Anti fetal rat palate rabbit serum produced some precipitation with fetal rat palate extract in gel diffusion plates which was not eliminated by absorbing the antiserum with lyophilized rat plasma. Intravenous injection of antiserum into pregnant rats and into the lumen of the pregnant uterus produced no malformations.

2. Cultures of fetal rat palate have been established.

3. Injection of rats with homogenized palate of their own young from each litter for as many as eight litters per rat has produced no malformations.

Significance to Dental Research:

An understanding of some of the factors concerned with the etiology and pathogenesis of oral anomalies may lead to effective means for their prevention.

Proposed Course of Project:

Further determinations of the precise effects and the mechanisms of action of teratogens.

Part B included Yes



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Individual Project Report  
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Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Feild, L. E., Kreshover, S. J., and Lieberman, J. E.:  
Temporary Uterine Circulatory Arrest as a Cause of Abnormal  
Fetal Development. J. Dent. Res. 39:1240-1247, Nov.-Dec. 1960.





Annual Report of the Laboratory of Histology and Pathology  
National Institute of Dental Research  
Summary Statement

The program of the Laboratory of Histology and Pathology is broadly subdivided into three categories: biophysical studies of the mineralized tissues, histochemical investigations of connective tissues, and experimental pathological studies of dental caries.

The present year has seen both a marked improvement in the armamentarium of equipment available for the biophysical studies, and the addition of a number of essential personnel. Although it is not generally realized, enamel and dentin were by several years the first of the organized tissues to be studied by electron microscopy and diffraction, and it was in this laboratory that the earliest beginnings were made. Over the ensuing years, rapid technical advances occurred in this area, and in general it was possible to keep abreast of the times. However, much of the equipment was rapidly becoming obsolescent, particularly the available electron microscopes, and the acquisition of a modern high resolution instrument this year was of vital importance. In addition, other newly-developed related instruments, such as those involved in contact and projection microradiography, have recently come to the forefront as valuable tools for both structural and analytical studies of the mineralized tissues. The laboratory is now well equipped for these applications of X-ray techniques. Further, it is hardly possible to operate a well-rounded program in biophysics without the utilization of X-ray diffraction and spectrographic methods. This year we have added to our staff two physical chemists, experienced in crystallography, and have equipped a modern crystallographic laboratory.

The basic ultrastructural studies of the crystalline component of dental enamel have been continued, with an eye toward defining more accurately the dimensions of the crystals and their orientation within the prismatic units of the enamel. This work has been carried on mainly by electron microscopy and diffraction of mature and developing material, and it presently appears as if the crystals are considerably longer than previously thought. Determination of crystal arrangement, which is quite intricate, has been largely a matter of morphological definition. A clear understanding of enamel crystallinity is essential for interpretation of the structural alterations involved in malformation and caries.

During the latter half of the year, with the opening of the crystallographic laboratory, investigations of the crystal chemistry of the calcium phosphates (apatites) involved in mineralized tissues were initiated. These parallel the ultrastructural studies, and are concerned with such matters as the atomic arrangements within crystals, crystal texture and perfection,



and the chemical properties of the mineral. The concept of "defect apatite," characterized by missing calcium atoms in the crystal lattice, as the crystalline substance really present in calcified tissues, is being pursued, both by X-ray diffraction and infrared spectrophotometry. The latter technique provides a means of determining the hydrogen bond content of the apatite, which is thought to compensate for the missing calcium atoms. There have already been numerous ramifications of the work on crystal chemistry, the principal one being a study of the effect of fluoride on crystallinity in bone. The results indicate the presence of improved, or more perfect, somewhat larger crystals in the bone of animals which have been exposed to fluoride-containing drinking water. It has also been shown both in vitro and in vivo that Sr<sup>89</sup> is rejected to a greater extent by larger apatite crystals than by smaller. This has led to the initiation of a study of the reaction to Sr<sup>89</sup> of bone apatite in animals exposed to fluoride for periods of up to a year, on the assumption that larger, more perfect crystals should be present than in non-fluoride animals. All of this latter work has a bearing both on the basic biological mechanisms in water fluoridation, and on the relation of fluoride content of hard tissues to retention of ingested strontium (fallout).

The problem of mineralization itself has for some time been of prime interest in this laboratory. Inasmuch as numerous workers in biochemistry and microbiology have similar interests, it is natural that in this area our personnel should be involved in considerable collaborative, as well as individual effort. The primary objective, from the biophysical and histological standpoints, is to determine the relation between inorganic crystals and organic matrix, the mechanisms and sites of crystal nucleation, the sequence in which mineralization occurs, and the cytological basis for calcification. All of these facets are being approached by electron microscopy, microradiography and related techniques. Several projects within the laboratory concern normal mineralization of various substances. One, a microradiographic study of the mineralization sequence in rodent enamel, is of particular interest, because it has been found that the pattern is not the same as in other species, including the human. This is especially important, because the rodent is the most commonly used of all experimental animals. Another study deals with the development and mineralization of cementum, a tissue which has been largely neglected, and yet is of great importance as a dental constituent involved in periodontal disease. Mineralization is also being studied in ectopic calcifications such as calculus; inasmuch as this substance forms externally on teeth, it can be handled experimentally in the form of deposits laid down on plastic strips fastened to the teeth of human subjects. A series of studies has been completed in which a first, membranous structure has been identified, on which a microbial and fibrillar organic layer is subsequently laid down. Deposition of mineral (apatite) within this matrix has been followed, as well as the crystal growth which takes place. Various collaborative studies on the mechanisms of calcification have also been continued; these include such systems as intraperitoneal implants of collagen, bacteria, and other organic matrices. It is of interest that both native and certain reconstituted collagens, as well as living or dead cultures of microorganisms will mineralize when implanted in dialysis bags. These findings



suggest that the factors heretofore thought to be involved in promoting or inhibiting calcification need further investigation. A beginning has been made over the last two years in the study of abnormal mineralization. For this first work at the ultrastructural level it was felt that a test system should be employed which was fairly well defined physiologically as well as histologically at the optical level. The pathological condition selected was avitaminosis D, established by a rachitogenic diet. Attention was this year directed toward a comparison between dentin formation in normal and in rachitic rats. The electron microscopic observations indicate that in tooth formation in rachitic animals dentin development seems to begin normally, and then suddenly becomes atypical. The odontoblasts associated with formation of the tissue become distorted, and there is a marked delay in the production of collagenous matrix fibrils, and an even greater lag in mineralization. Once under way, matrix formation and calcification seem to progress normally, so far as microstructure is concerned. Beyond the direct data obtained, studies such as this are of some importance in several other respects. First, they provide a basis in experience for forming some impressions on the degree to which ultrastructures in the developing tooth germ may reflect very acute generalized pathosis in bone formation and calcification. These ideas will serve as a guide in planning future experiments in pathological mineralization. Second, they give new information on the possible interrelationships between cells and the extracellular production of organic matrices and their mineralization. This in turn bears on the common present-day problem in cytology, the relation between structure and function.

Turning to the histochemical program, emphasis has for the past few years been placed upon determination of the chemical composition and reactive groups in normal and diseased connective tissues. These investigations are not only contributing to our general knowledge of the connective tissues, but also have a direct bearing on the structure and function of the supporting tissues about the teeth. A new type of fibers, named oxytalan, was discovered in this laboratory several years ago. These fibers have now been shown as a normal constituent in various specialized connective tissues, such as periodontal membranes and ligaments. In addition they have been found to develop in the reparative tissue following damage in periodontal disease, radicular cysts and granulomas. Current comparative embryologic and histochemical studies have indicated that the fibers probably should be classified in the elastic group, most likely being pre-elastic in nature.

Further histochemical investigation of a large series of human specimens obtained at autopsy has led to the finding that bone and periodontal tissues also undergo degradation at sites remote from the inflammatory foci in periodontal disease, and without concomitant localized inflammation. Observation of this phenomenon leads to some questions regarding the role of inflammation in periodontal disease, and future work will be directed toward clarification of this point.

Various other collaborative histochemical studies have been continued, including work on the mechanisms of vitamin C action, the dermal connective tissue changes associated with amyotrophic lateral sclerosis, and the development of selective stains for nucleoprotein and collagen.



The remaining group of studies have been concerned with dental caries, a long range project on the etiology and characteristics of the disease as investigated in carefully controlled animal experiments. Various factors in the host-parasite-diet complex have been under study, and this year emphasis has been placed on the microbial and dietary factors affecting the course of the carious process. The use of tracer strains of microorganisms, primarily pure cultures of streptococci rendered identifiable through induced antibiotic resistance, has been continued. Such labelled organisms have been recovered from carious lesions or plaques both in animals inoculated directly and in animals which have received the organisms by natural transmission from a previously inoculated mother. This technique has made it possible to study both the transmission of caries activity into animals previously uninfected, and to make some estimations of the degree of caries activity. A new strain of streptomycin-resistant streptococci has been found which seems to establish itself more effectively than the strains used earlier. As an extension of this work, an attempt was made to produce an active immunity in animals by injection of a vaccine prepared from known cariogenic streptococci. Results were negative. Another promising technique for the induction of caries has been transfer of plaque material from animals with the disease. An interesting sidelight in development of the method is the preliminary observation that periodontal disease also seems to arise in animals subjected to transfer of suitable plaque material. The discovery of the infectious nature of plaque substance recovered from the periodontium may well lead to a profitable series of investigations on experimental periodontal disease. The microbial characteristics of these plaques are now under investigation in collaborative efforts with the Laboratory of Microbiology, NIDR, and the possibility of inducing periodontal disease with pure cultures of organisms is being studied.





1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

Principal Investigator: Dr. D. B. Scott

Other Investigators: Dr. M. U. Nylen

Cooperating Units: None

Man Years (calendar year 1961):

Total:	2 3/4
Professional:	3/4
Other:	2

Project Description:

Objectives:

1. Determination of the crystal structure of enamel, with special emphasis on crystal orientation and size.
2. Investigation of chemical and physical reactions on tooth structure.
3. Standardization and adaptation of new biophysical instruments for work on mineralized tissues.

Methods Employed:

For studies with mature teeth, negative collodion replicas and positive collodion-carbon replicas and pseudoreplicas are made from the outer surfaces and from the surfaces of ground sections. The specimens are pretreated in various fashions, including simple polishing, acid etching, and removal of organic components by treatment with suitable solvents. For studies with developing teeth, samples are embedded without demineralization in methacrylate mixtures and sectioned with either glass or diamond knives. The various types of preparations are examined under the electron



microscope, subjected to electron diffraction, or studied micro-radiographically.

Major Findings:

1. Following previous studies in this laboratory by the present investigators and by Professor J. G. Helmcke (NIDR-33, 1960), increasingly more detailed determinations of crystalline orientation in human enamel have been made. The use of developing, incompletely mineralized human specimens, not previously available in adequate states of preservation, has made it possible to confirm and extend the observations made in mature fully calcified material, that the majority of the crystals inside individual enamel prisms are laid down along an axis, not necessarily central but parallel to the prism axis. Peripheral to this axial core, the remainder of the intraprismatic crystals lie at angles to this axis which may reach as high as 30 to 40°. Between prisms, crystals are laid down at even more oblique angles to the basic prism axis. The widths and thickness of the crystals have been quite accurately determined, but the problem of lengths (c-axis) still remains unsettled. From the latest data, it now appears as if previous estimations may have been too low. Based on prior experiments which showed that apparently long crystals could be reduced to smaller components by treatment with organic solvents, it was thought that the long ribbon-like structures might be linear crystalline aggregates. Present observations indicate that this interpretation may have to be revised, inasmuch as it appears quite possible that the soluble organic matrix material may be present as an inclusion within very long single crystals. As an interesting sidelight, Moiré patterns, indicative of atomic planes, have been photographed in areas of overlapping crystals. Additional information on crystal structure, particularly perfection, may be derived from this type of micrographs. All of this work has been made possible by the installation of a new electron microscope, capable of the required high resolution and minimization of specimen contamination by cold-stage techniques.
2. Studies on the effects of chemical agents, such as fluoride compounds on outer enamel surfaces have been continued. Special attention has been paid to the effectiveness of stannous fluoride-containing dentifrices in reducing acid-solubility of the enamel. Certain variations in experimental results have been found which are thought to be related to inherent instabilities in the compounded preparations.

Along the line of physical properties of the enamel and dentin,



a project was undertaken to determine the type of surfaces which are developed as a result of cutting with various types of rotary instruments (carbide burs and diamond points). Although it was expected that the microanatomy within enamel and dentin would influence the ultrastructural appearance of flat planes cut in different directions, the configurations found were unexpected. Surfaces which appeared quite smooth exhibited little if any recognizable detail, such as enamel prism outlines or dentinal tubules, and only in very limited areas were crystalline or fibrillar structures seen. Apparently the structure and hardness of mature tooth structure renders the cutting procedure a massive series of micro-fractures dependent upon the point to point friability of the tissues.

3. Considerable time this year had to be spent in moving and reestablishing existing physical equipment in the new building, and in settling up a new electron microscope, projection microradiographic equipment, microdensitometer, and other apparatus. These have now been put into satisfactory operation, and the staff is adequately familiar with their operation.

Significance to Dental Research:

These continuing experiments are directed toward developing information needed for an understanding of the normal composition of enamel and its reaction to external, physical, and chemical influences.

Proposed Course of Project:

Continuation along the same lines.

Part B. included: Yes



1. Histology & Pathology
2. Bethesda, Md.

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Individual Project Report  
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Scott, D. B. and Nylen, M. U. Enamel and Dentin (two revised chapters for new ed. of Orban's Oral Histology and Embryology textbook, H. Sicher, Ed., in press).

Scott, D. B. and O'Neil, J. R. The Microstructure of Enamel and Dentin as Related to Cavity Preparation (in press).

Honors and Awards relating to this project:

None





1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Studies of the Development of Calcified Tissues  
Under Pathologic Conditions.

Principal Investigator: Dr. M. U. Nylen

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

Comparative studies between dentin development in normal rats  
and in rats fed a rachitogenic diet.

Methods Employed:

Female rats, placed 3 weeks prior to mating on a D-deficient diet with a calcium-phosphorus ratio of 4-5:1, were maintained on the same diet during pregnancy and lactation. The offspring and normal controls were killed when four and eight days old, and the specimens were fixed, dehydrated and embedded for electron microscopy and optical microscopy. Alternate series of thin and thick sections were cut on an LKB ultratome or a Porter-Blum microtome. The thick sections (1 -3u thick) were examined without staining under the phase microscope and the polarization microscope. The thin sections were studied in the electron microscope either directly or following staining with lead acetate or phosphotungstic acid.



Major Findings:Macroscopic Changes

Compared with the controls the 4 and 8 day old offspring of female rats fed a rachitogenic diet were much smaller and showed evidence of gross physical changes. Unfortunately, in the past year, the Food and Drug Administration, which supplied us regularly with the rachitic animals, has had serious difficulties in keeping even the mothers alive. Because of this our experimental material has been somewhat limited during the last few months.

Microscopic Findings

Initial cell differentiation, predentin formation and mineralization of the first formed predentin take place in a completely normal manner in the rachitic animals. Once this first layer of calcified dentin is laid down, however, pathologic changes become evident. At the light optical level these changes are characterized by a retardation in mineralization associated with alterations in the odontoblastic and subodontoblastic cell layers. Comparisons between rachitic and normal teeth reveal little difference in the width of combined dentin-predentin layers, while marked disturbances are evident in the relative thicknesses of these layers. Thus in the control animal the width of the predentin layer remains constant while the dentin layer gradually expands. In the rachitic animals the predentin becomes wider and wider and there is only a very slow increase in the thickness of the dentin layer. These findings indicate that although collagen continues to form it does not become mineralized as readily as in the control animals.

At the electron optical level the most pronounced changes are found in the odontoblasts and their immediate vicinity. Studies of the control animals show that the odontoblasts become shorter and more closely packed as dentin formation progresses and the size of the pulpal cavity gradually diminishes. Individual cells are, however, easily discernible, and they exhibit the characteristic long profiles of the endoplasmic reticulum parallel to the long axes of the cells. At the distal ends of the odontoblasts the collagen fibrils of the predentin appear to aggregate in the immediate vicinity of the cell membrane. In the rachitic animals the odontoblasts are much shorter, and it is impossible to differentiate between individual cells. Instead of the flattened parallel profiles of the endoplasmic reticulum the organelle comprises numerous vacuoles. In addition the cytoplasm is dominated by large cisternae filled with a finely granular substance. A similar granular substance is found forming a thin continuous



layer along the distal ends of the cells separating them from the fibrillar elements of the predentin. The presence of this layer indicates a disturbance in the normal aggregation of collagen fibrils. Possibly the cells are capable of producing the collagen precursor, yet formation of fibrils is delayed. Once fibrils appear they exhibit the normal crossbandings, and mineralization, when it takes place, seems to follow the normal pattern.

Two observations seem to warrant closer inspection. One is the normal initiation of dentin development and the sudden change to pathologic development at a certain growth level. A similar phenomenon was observed in dentin formation in humans suffering from dentinogenesis imperfecta. Apparently the odontoblasts are resistant to pathologic conditions in the initial stages of differentiation and dentin development. The reason for this is not clear although it is tempting to believe that it is in some way due to the barrier formed between the connective tissue and the enamel epithelium by the first layer of mineralized dentin.

The other observation of interest is the seemingly causative role locally of the odontoblasts. It is when these cells first become pathologic that alterations in dentin development are observed. In addition to the previously recognized mineralization disturbance, electron microscopic studies have also shown a disturbance in collagen formation. Since the odontoblasts are assumed not only to take part directly in collagen elaboration, but also indirectly in the actual mineralization process, changes in cell function might well cause a slow down of this process. Certainly, in the earliest stages of dentin formation and during all of enamel formation calcification progresses normally, which would indicate that unless accompanied by cellular alterations the Steenbock diet is not prohibitive.

#### Significance to Dental Research:

A study of the relationship between pathologic cell changes and defects in either matrix formation or mineralization may lead to a better understanding of these processes and may add to our knowledge of general cell function.

#### Proposed Course of Project:

It is hoped that the difficulties met with in keeping the experimental animals alive will soon be overcome so that the study on rachitic tooth development can be expanded to include investigations of the changes which take place following injection of rachitic animals with Vitamin D and/or phosphorus.



1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Ultrastructural Studies of Dental Cementum.

Principal Investigator: Dr. Knut A. Selvig

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/3
Professional:	1/3
Other:	0

Project Description:

Objectives:

The cementum on human and animal teeth has in the past been studied by optical microscopy. It is the objective of this project to expand our knowledge of the mechanisms involved in formation of the cementum matrix, and its mineralization, by employing the electron microscope. Another objective of this project is to compare the structure of mature human cementum from teeth involved in periodontal disease with that from teeth with healthy periodontal structures. Earlier studies by this investigator have indicated that the mineral content of the cementum from human teeth involved in periodontal disease in some cases is different from that of teeth with healthy periodontal structures. In this project an attempt is being made to correlate the distribution of minerals in the cementum and underlying root dentin with the extent of periodontal involvement to which the teeth have been exposed.

Methods Employed:

1. Growing incisors and molars have been obtained from 8-24 day old mice, and processed for optical and electron microscopic examination.





2. Replicas have been prepared from the root surface and from cross sections of human teeth of various age groups.
3. Microradiographs have been prepared from semi-serial ground sections of human teeth of various age groups with normal periodontal structures, and from teeth involved in periodontal disease.

Major Findings:

At the growing root end of 18-24 day mouse molars the matrix of the dentin initially is deposited against the basement membrane between Hertwig's epithelial sheath and the odontoblasts. The basement membrane disappears as the epithelial cells separate from the dentinal matrix. The first fibrils of the cementum are formed in close contact with the dentinal fibrils, and no cemento-dentinal junction can be distinguished at this early stage. Microradiographs of ground sections from human teeth have revealed a zone of increased mineral content on the surface of the cervical cementum in many teeth extracted because of periodontal disease. This confirms the results from earlier chemical analysis of such teeth by the investigator.

Significance to Dental Research:

Basic knowledge as to the normal formation and mineralization of cementum and root dentin is necessary before the ultrastructure of the root structures from teeth involved in periodontal disease can be studied. Knowledge about alterations in the structure of the root surface connected with periodontal disease is also important for the understanding of the etiology and pathology of this disease.

Proposed Course of Project:

This project was initiated in September, 1961, and will continue along the lines described.

Part B. included: No



1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Studies on Tooth Mineralization.

Principal Investigator: Dr. Karl-Åke Omnell

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

Extensive studies of tooth formation and mineralization utilizing ordinary light microscopy, fluorescence and polarizing microscopy, radioisotopes, electron microscopy, x-ray diffraction, and micro-radiography have been presented during the last few decades. Despite the effort of numerous investigators we do not have a clear cut picture of the mineralization of enamel and dentin. One reason for this might be that the pattern of mineralization, at least in enamel, differs in different species, a fact which does not seem to have been taken under consideration to any great extent.

The aim of the investigation discussed below is to study, by qualitative and quantitative microradiography and electron microscopy, the mineralization of normal teeth from various species.

Major Findings:

The work was started in the middle of August, 1961. More than two months were consumed in working out a technique with a new x-ray projection microscope. The results obtained during the last month, therefore, are of preliminary character. In the early stage of tooth mineralization there are striking differences between the mineralization pattern of teeth from humans, monkeys and dogs, as reported in the literature from those of rats as found in the present investigation.



Significance to Dental Research:

A detailed knowledge and understanding of the development of normal teeth in humans and in laboratory animals is of paramount importance as a background for studies on pathologic teeth. Also important is the understanding of the differences in tooth formation between humans and laboratory animals. This is especially true of the rodent, which is most commonly used in experimental studies on calcification.

Proposed Course of Project:

The preliminary results described above are encouraging. A detailed mapping of the pattern of mineralization in rat teeth utilizing high resolution microradiography and electron microscopy is therefore planned and under way.

Part B. included: No



1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Electron Microscopic Studies of Dental Calculus.

Principal Investigator: Dr. Jørgen Theilade

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total: 3/4

Professional: 3/4

Other: 0

Project Description:

Objectives:

Further investigation of the organic and inorganic morphology of developing dental calculus in humans and of mature dental calculus in conventional and germ-free rats.

Methods Employed:

1. At the Dental Clinic, the Clinical Center, plastic strips were wired to the lingual surfaces of the lower anterior teeth in human subjects. The strips were removed after intervals ranging from 2 hours to 30 days.
2. Samples of dental calculus were removed from conventional and germ-free rats.

All specimens were fixed in osmic acid, embedded in methacrylate, sectioned with either glass or diamond knives, and examined under the electron microscope.

Major Findings:

1. Developing human calculus: Investigation of one and two day specimens showed an initial deposition of a thin dense layer (500-1000A) along the mylar strip. In some areas this layer





was the only structure found, in others bacteria or epithelial cells were attached to its outer surface. In older deposits the dense layer appeared slightly thicker and undulated. Calcification was always noted in nine day or older strips and occasionally as early as the two day strips. It generally started around the bacteria adjacent to the dense layer and progressed outward as the deposit grew. The types of microorganisms involved could not be determined too well, although it was possible to differentiate between filamentous organisms and other forms. The former were often absent in one and two day deposits and when present did not appear in any definite location. It does not seem likely that these organisms should be responsible for the attachment of the deposit to the tooth as has been claimed. Such a function should more likely be attributed to the dense inner layer observed in even the earliest specimen. The origin of this layer is not apparent from the present work, but it is assumed it is identical to "the acquired pellicle," a thin membrane which readily forms on the smooth enamel surface, and which is supposed to originate from the saliva.

2. Rat calculus: Sections of calculus from conventionally bred rats revealed a structure similar to that of mature human calculus, e.g. the organic components comprised the microorganisms and a fibrillar intermicrobial matrix with apatite crystals present in both areas. Calculus from the germ-free rats, on the other hand, contained apatite crystals deposited in a fairly homogeneous organic matrix with no microorganisms. In both conventional and germ-free rats non-calcified deposits in the nature of thick pellicles were also observed, and it is possible that these pellicles form the organic matrices of the calcareous deposits seen in germfree animals. At least it has been shown that microorganisms are not necessary for either the formation of an organic matrix or for the initiation of mineralization.

#### Significance to Dental Research:

Apart from the basic knowledge which may be obtained as to the calcification process in general, information on the attachment and formation of dental calculus is of importance for our understanding of etiological factors in periodontal disease.

#### Proposed Course:

Dr. Theilade has returned to Denmark and is completing several manuscripts which will terminate the present aspects of the calculus work.

Part B. included: No



1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Secondary Collaborative Projects and Training Activities.

Principal Investigators: Dr. D. B. Scott  
Dr. M. U. Nylen

Other Investigators: None

Cooperating Units: See below

Man Years (calendar year 1961):

Total: 3 1/2  
Professional: 1  
Other: 2 1/2

Project Description:

Objectives, Methods Employed and Major Findings:

Each year a relatively large number of requests is received for applications of biophysical methods to problems under investigation in other laboratories. It has long been accepted that such operations as electron microscopy cannot be adapted to routine service function, primarily because each type of specimen to be studied presents new and usually difficult problems in handling, and because the microscopic observations must be made by an experienced professional investigator. Inasmuch as many of the requests represent worthwhile problems, and since most relate in some fashion to the general program of this Laboratory, the staff has undertaken to offer as much secondary collaboration as seems feasible. The magnitude and success of these projects, listed below, have reached a level at which it is now necessary to consider this activity as a regular part of the Laboratory program.

1. Studies of calcuogenesis: primarily electron microscopy and diffraction of intraoral deposits formed in vivo on polyester strips attached to teeth. With Dr. H. A. Zander, Eastman Dental Dispensary, Rochester, New York. To be continued.



2. Study of dental calculus isolated from antibiotic-treated and germ-free rats; electron microscopy, and electron and X-ray diffraction of scrapings, suspensions and thin sections. With Dr. R. J. Fitzgerald, Laboratory of Microbiology, NIDR. To be continued.
3. Mineralization of collagen, bacteria and other substances, in peritoneal transplants; electron diffraction and X-ray diffraction of material implanted intraperitoneally in animals; particle suspensions or sectioned material. With Drs. S. E. Mergenhagen and A. A. Rizzo, Laboratory of Microbiology, NIDR and Dr. G. Martin, Laboratory of Biochemistry, NIDR. To be continued.
4. Study of the calcareous corpuscles in tapeworms; electron microscopy, X-ray diffraction, electron diffraction and microradiography of isolated corpuscles and sectioned specimens, for purposes of determining composition and morphological characteristics. With Dr. T. vonBrand, Laboratory of Tropical Diseases, NIAID. To be continued.
5. Study of demineralization at neutral pH in molluscs by glandular action of the bivalve boring organ; electron microscopy, optical microscopy and electron diffraction of replicas and pseudoreplicas. With Dr. M. R. Carricker, Fish and Wildlife Service, U. S. Department of the Interior, Oxford, Md.
6. Study of the effects of omission and subsequent repletion of a single essential amino-acid on the ultrastructure of tissue culture cells; electron microscopy of sectioned pellets of cells centrifuged from suspensions. With Dr. E. B. Cohen, Laboratory of Infectious Diseases, NIAID. Completed.
7. Investigation of the status of preservation of collagen fibrils after freeze-drying and rehydration of fascia prepared and banked for surgical procedures; electron microscopy of sectioned material. With Dr. R. Gresham, Tissue Bank, N.M.R.I., N.N.M.C. To be continued.
8. Study of cultured cells infected with tumor-producing virus; electron microscopy of sectioned material. A preliminary screening study with Dr. S. Stewart, NCI. To be taken over by electron microscopy group at NCI.

Because of the preceding lack of space and free time, as well as



the move to the new building, no requests for training, other than very short stays, were granted. It is anticipated that this activity will be reinstated, as in previous years.

Significance to Dental Research:

The importance of making collaborative effort available is self-evident, as is the benefit derived by those receiving training. In addition, it is felt that opportunities are provided to the staff of our Laboratory, professional and technical, both for broadening their experience in the general field of biophysical instrumentation and for contributing useful information related in most part to our interests in the oral tissues. It should be of particular interest that many of the collaborative projects are directed to various facets of the general program on studies of the mechanisms involved in mineralization.

Proposed Course of Project:

As indicated above collaborative work will continue on several problems. If this program is to be continued and any attempt made at meeting the number of requests, it is urgent that at least one additional staff member be assigned to the project. The type of individual required is most likely an intermediate level professional, with a working knowledge of histology, and, if possible, electron microscopy. We feel that real opportunities for development exist for a young worker, not yet established to the point of specialized interest, who would profit by the varied associations offered. An individual of this type has been recruited, and it is planned that he will undergo the necessary training and participate in the program as indicated above.

Part B. included: Yes





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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Martin, G. R., Mergenhagen, S. E., and Scott, D. B. Relation of Ionizing Groups to the Structure of the Collagen Polymer. *Biochim. et Biophys. Acta.* 49:245-250 (1961).

Cohen, E. P., Nysten, M. U., and Scott, D. B. Microstructural Changes Induced in Mammalian Cell Cultures by Omission and Replacement of a Single Essential Amino Acid. *Exp. Cell Res.* 23:443-451 (1961).

Rizzo, A. A., Martin, G. R., Scott, D. B., and Mergenhagen, S. E. Mineralization of Bacteria (in press, *Science*).

Honors and Awards relating to this subject.

None



1. Histology & Pathology
2. Bethesda, Md.

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Part A.

Project Title: Crystal Chemistry of Calcified Tissue and Related Compounds.

Principal Investigator: Dr. Aaron S. Posner

Other Investigators: Drs. E. David Eanes, Isadore Zipkin, Harald Schraer, Robert C. Likins.

Cooperating Units: Department of Physics, The Penn State University (Dr. R. Schraer).

Man Years (calendar year 1961):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

Elucidation of the crystal chemistry of the mineral and protein portions of bone and teeth. This project includes work on the atomic arrangement (i.e. crystal structure) and the crystal texture (i.e. crystal size, shape and orientation) of these materials and their effect on the properties and behavior of hard tissue crystals. This project will also be devoted to a corollary study of the crystal chemistry of synthetic and mineral calcium phosphates related to calcified tissue.

Methods Employed:

The principal techniques are those of X-ray diffraction. Single crystal X-ray techniques are employed to study isomorphous substitution in the mineral apatite series. Powder X-ray diffraction methods, using both film and counter diffractometers, are used for phase studies, crystal size and shape and crystal strain (i.e. perfection of atomic alignment) studies. Polarizing microscope techniques are used as an adjunct to the X-ray methods.



Major Findings:

1. New light was shed on the action of fluoridation on the chemical stability of hard tissue. It was shown by X-ray diffraction that bones of humans and rats increased in crystallinity (i.e. increased in crystal size and/or decreased in crystal strain) as the fluoride content increased. The work on rat bone showed that the fluoride affects the bone crystallinity at a comparatively early age but there is a minimum period before which fluoride does not measurably change the crystal texture of the bone apatite.
2. X-ray studies showed that both in synthetic hydroxyapatites and in biological apatites (in vivo) the larger more slowly grown crystals reject more strontium than the smaller, less perfect, more quickly precipitated crystals. For example when  $\text{Sr}^{89}$  and  $\text{Ca}^{45}$  were injected intraperitoneally in a series of rats after an hour the ratio of  $\text{Sr}^{89}/\text{Ca}^{45}$  in epiphyseal bone apatite (quickly precipitated, poorly crystallized) was unity while the ratio was 0.76 for incisor and molar enamel (more perfectly crystallized) apatite.

Significance to Dental Research:

1. These results seem to indicate that fluoridated hard tissue may resist chemical attack because of improved crystallinity. A larger and/or more perfect crystal of enamel apatite, or bone apatite, will resist chemical attack more per unit of time than will a less perfectly crystalline biological apatite. This may indicate that fluoridated bone will resist resorption more than unfluoridated bone, and suggests fluoridation for a possible preventative measure for bone disorders due to loss of bone mineral (osteoporosis, etc.).

Another significant finding is the fact that crystal perfection leads to comparative rejection or exclusion of Sr from hard tissue. This result coupled with the finding that fluoride ion improves the crystallinity in hard tissue may be very significant. Fluoridation may result in a lower retention of  $\text{Sr}^{90}$  from fall-out in the hard tissue as a result of the improvement in crystal texture.

Proposed Course:

The effect of fluoride on crystal texture and strontium retention will be further investigated. Low angle X-ray diffraction techniques will be used to supplement the present data on crystal size and shape and strain content.



Further work is planned on the general crystallography of substituted apatites and other compounds whose structures relate to hard tissue chemistry.

A full study of the cause of the increased crystallinity of hard tissue upon fluoridation will be started. X-ray diffraction methods are going to be developed to separate the effect of crystal size and crystal imperfection. It will be necessary to use electronic computation for this work and it is planned to write programs for this study.

Part B. included: Yes





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Part B: Honors, Awards, and Publications

Publications:

Likins, R. C., Posner, A. S., and Paretzkin, B. Effect of Crystal Growth on the Comparative Fixation of  $\text{Sr}^{89}$  and  $\text{Ca}^{45}$  by Calcified Tissue. *J. Biol. Chem.* 236, 2804 (1961)

Honors and Awards relating to this project:

None



1. Histology & Pathology
2. Bethesda, Md.

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Part A.

Project Title: Infrared Absorption Spectrophotometric Studies on Calcified Tissues and Related Synthetic and Natural Compounds.

Principal Investigator: Dr. Aaron S. Posner

Other Investigators: None

Cooperating Units: Department of Chemistry, University of Maryland, (Prof. E. R. Lippincott); Dental School, Tufts University (Dr. W. H. Emerson).

Man Years (calendar year 1961):

Total: 3/4  
Professional: 1/4  
Other: 1/2

Project Description:

Objectives:

To delineate the chemical structure of the mineral portion of teeth and bones and to study the chemical and physical relationship between the mineral and protein phases of these calcified tissues. The investigation includes the properties of normal and pathological tissue as well as the effect of certain factors such as diet, age and disease on the structure and properties of these tissues.

Methods Employed:

Major emphasis is placed on infrared spectrophotometry coupled with other physical chemical techniques to observe chemical structure (i.e. the spatial arrangement of the constituent ions) in the biological calcium phosphates and other compounds studied.

Major Findings:

1. A method was developed for the quantitative analysis of hydrogen appearing as a hydrogen bond between two oxygens of adjacent orthophosphate groups in synthetic and biological apatites (i.e. calcium phosphates).



2. The presence of hydrogen bonds in synthetic and enamel (human and rat) apatites was found to be consistent with the earlier general formula proposed by the senior investigator to be:  
 $\text{Ca}_{10-x} \text{H}_{2x} (\text{PO}_4)_6 (\text{OH})_2$ .

Significance to Dental Research:

These results supplement the earlier X-ray diffraction and index of refraction data upon which the structural postulate was based. At this point it seems reasonable to suppose that the major phase of all biological apatites is some calcium deficient hydroxy-apatite with compensatory hydrogen bonding. The higher chemical reactivity of young calcified tissue compared to older calcified tissue may be a result of the gradual perfection of this calcium deficiency. Published data by the senior investigator has shown that the more perfect (i.e. less calcium deficient) synthetic and (rat) enamel apatites are less soluble in weak acid than the more deficient apatites. Hydrogen bond analysis may now be employed to study the nature of hard tissue under varying conditions.

Proposed Course of the Project:

It is thought that infrared analyses can be done on micro samples and this method may afford a tool for in vivo studies utilizing microscopic biopsy of hard tissue. A refinement of the infrared method will be sought to reduce the sample size. In the near future work will continue on hard tissue to delineate the distribution of calcium deficiencies in the various tissues and their parts (e.g. epiphyseal vs diaphyseal apatite calcium deficiency in the same bone).

Part B. included: Yes



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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Stutman, Joel M., Posner, Aaron S., and Lippincott, Ellis R.  
Hydrogen Bonding in the Calcium Phosphates. Nature (in press)

Honors and Awards relating to this project:

None.





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Part A.

Project Title: The Influence of Fluoride on the Metabolism of Ca<sup>45</sup>  
and Sr<sup>89</sup> by Bone.

Principal Investigator: Dr. H. Schraer (Visiting Scientist)

Other Investigators: Dr. J. Menczel and Dr. R. Likins

Cooperating Units: The Pennsylvania State University, Biophysics  
Laboratory.

Man Years (calendar year 1961):

Total: 1/4  
Professional: 1/4  
Other: 0

Project Description:

Objectives:

To determine if a difference exists in the metabolism of Ca<sup>45</sup> and Sr<sup>89</sup> by bone from animals maintained on different levels of fluoridated water.

Methods Employed:

Male rats 355 days old which had been maintained on prepared diets and on water containing different levels of fluoride (0, 10, 25, 50, 100 ppm) since 35 days of age were provided from the Pennsylvania State Laboratory of the senior investigator (Visiting Scientist, H. Schraer). The animals were brought to the National Institute of Dental Research and injected with a mixture of Ca<sup>45</sup> and Sr<sup>89</sup>. The animals were sacrificed one hour after injection; the tibias were removed and cut to separate the diaphyses from the ends, dry-defatted and weighed. Still to be completed are ashing, preparation of the samples for detecting radioactivity and counting.

Major Findings:

Experiment still in progress.



Significance to Dental Research:

In previous studies at N.I.D.R. it has been found that the more crystalline the mineralized tissues the greater the discrimination against strontium. Current observations in these laboratories have shown that in human bone, there was a decided correlation between the fluoride content and the degree of crystallinity. In rats, increasing crystallinity was observed with the level of fluoride in the water, the fluoride content of the bone and the duration of the fluoride treatment. These observations led to the present experiment. If discrimination against strontium by these fluoridated bones should be demonstrated, the data may be of importance as applied in the relation between fluoridation of communal water and the fallout problem.

Proposed Course of the Project:

No decision to be made until the final results of this experiment are evaluated.

Part B. included: No



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2. Bethesda, Md.

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Part A.

Project Title: Histochemical Studies of Connective Tissues.

Principal Investigator: Dr. Harold M. Fullmer

Other Investigators: Dr. George Martin

Cooperating Units: Dr. L. Kurland, Epidemiology Branch, NINDB,  
cooperated in certain facets of these investigations.

Man Years (calendar year 1961):

Total:	3 1/4
Professional:	1
Other:	2 1/4

Project Description:

Objectives:

To determine the chemical composition and the reactive groups in normal and diseased connective tissues through the use of histochemical methods.

Methods Employed:

Human and animal tissues are obtained from autopsy and surgical material. Specimens are then prepared for histochemical analysis and optical microscopy. Sections may be cut (a) fresh frozen, (b) frozen after short or prolonged fixations in various fixatives, (c) after freeze-drying, or (d) after routine chemical fixation, dehydration and paraffin embedding. Under controlled conditions, the sections are exposed to various reagents designed to determine the site and quantity of an enzyme, reactive group or substance.

Major Findings:

In 1958 two new stains, i.e. the peracetic acid-aldehyde fuchsin-Halmi and the peracetic acid-orcein-Halmi, were developed which led to the discovery of a new connective tissue fiber which has been named oxytalan. It has been found in periodontal membranes, tendons, ligaments and mucous connective tissues. Thus, certain



specialized connective tissues have oxytalan fibers as a normal constituent. The new tissues that develop subsequent to the loss of periodontal tissues as in periodontal disease or in the development of radicular cysts and dental granulomas contain oxytalan fibers. It has also been found that when periodontal membranes thicken as the result of scleroderma, oxytalan as well as collagen fibers develop in the new tissue. It has been found that the connective tissue stroma of ameloblastomas contain oxytalan fibers. These data serve to indicate that oxytalan fibers are inherently produced in connective tissue structures that involve periodontal membranes.

Comparative anatomical, embryological, and histochemical studies of the periodontia of the dog, hog, sheep, cow, deer, rabbit, guinea pig, mouse, rat, monkey and man indicate that oxytalan fibers probably belong to the elastic family of tissue fibers. The peracetic acid-aldehyde fuchsin-Halmi stain is the first stain to demonstrate pre-elastic fibers.

A histochemical study of periodontal disease in human autopsy material has been progressing for several years. This work culminated in a report on a study of 135 autopsies in 1960. The report called particular attention to the observation that connective tissues and bone are undergoing degradation at a site distant from an inflammatory focus, and without concomitant inflammation. Most pathologists have tended to favor the view that hard and soft connective tissues are lost in periodontal disease as a direct result of an inflammatory process. This year, attention was directed to the loss of collagen and bone in critical areas of the periodontia not manifesting inflammation. Collaborative work with Dr. George Martin has been progressing for several years. We have been engaged in several experiments designed to determine the mechanism of action of Vitamin C. These experiments have involved the use of scorbutic guinea pigs which have been given various drugs to determine anti-scorbutic activity. Agents found to have Vitamin C activity have been d-ascorbic acid, 3-methyl-cholanthrene and 5-benzpyrene. Although the carcinogens have some anti-scorbutic action, the animals die. We have been unable to advance a hypothesis whereby the carcinogens have Vitamin C action.

In 1961 we reported for the first time that 60% of individuals afflicted with ALS have a dermal connective tissue disorder characterized by (a) a distinctive elastosis, (b) an increase of mucopolysaccharide, (c) degeneration and an elastosis of arrector pili muscles, (d) focal areas of apparently regenerated connective tissues, and (e) several characteristic changes of collagen. This year we were able to examine a few skins of people afflicted with ALS. Stains for phosphorylase, aminopeptidase and esterase were





within normal limits.

Preliminary work indicates we have developed the most selective stain for nucleoprotein. We (Dr. R. D. Lillie and myself) have reported our work on this stain at the 1961 meeting of the American Society for Cell Biology. The method involves the acid hydrolysis of sections which are subsequently stained in unmordanted hematoxylin at pH 8.2 in .2N tris buffer. We expect this stain to completely supersede Alfert and Geschwind's only other stain for nucleoprotein.

Some time was spent in efforts to develop a specific stain for collagen. We were unsuccessful.

Significance to Dental Research:

Periodontitis and periodontosis are diseases of connective tissues. It is believed that information gained about the nature of normal connective tissues and their alterations in the diseased states will contribute to a better basic understanding of the pathological processes involved in periodontal disease. Work on oxytalan fibers has considerably broadened our view of elastic fibers. It is now apparent that a "spectrum" of elastic tissues exists. Our histochemical analysis for glycolysis ( $\alpha$ -glycerophosphate dehydrogenase, alcohol dehydrogenase and lactate dehydrogenase), activity of the citric acid cycle (succinic, malic and isocitric dehydrogenase), DPN and TPN diaphorases, fatty acid oxidation and other methods that appear appropriate as the problems arise. The use of histochemical methods will permit cellular localization of these activities, and allow one to judge the physiological state of individual cells and groups of cells. It is hoped that a precise definition of the physiological states of cells in various areas of the periodontium will permit a judgement as to factors fostering deleterious effects and how to alleviate or prevent them.

Part B. included: Yes



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1. Histology & Pathology
2. Bethesda, Md.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Fullmer, H. M., Martin, G. R., Burns, J. J. Role of Ascorbic Acid in the Formation and Maintenance of Dental Structures. Ann. New York Acad. Sci. 92:286, 1961.

Fullmer, H. M. A Histochemical Study of Periodontal Disease in the Maxillary Processes of 135 Autopsies. J. Periodontol. 32:206, 1961.

Martin, G. R., Fullmer, H. M., Burns, J. J. Observations of Effect of 3-methyl-cholanthrene in Scorbutic Guinea Pigs. Proc. Soc. Exper. Biol. Med. 106:157, 1960.

Fullmer, H. M. A Critique of Normal Connective Tissues of the Periodontium and Some Alterations with Periodontal Disease. J. Dent. Res. (in press).

Fisher, A. K., Fullmer, H. M. Oxytalan Fibers in Ameloblastomas. J. Oral Surg., Oral Med., Oral Path. (in press).

Fullmer, H. M. Oxytalan Fibers in Health and Disease. Proc. First International Congress of Histochem. Cytochem. 1961.

Fullmer, H. M., Witte, W. E. A Histochemical Study of Periodontal Membrane Affected by Scleroderma. A.M.A. Arch. Path. (in press), 1961.

Honors and Awards relating to this project:

None



1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH  
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Part A.

Project Title: Experimental Dental Caries.

Principal Investigator: Dr. P. H. Keyes

Other Investigators: Dr. R. J. Fitzgerald and Dr. H. V. Jordan,  
Gnotobiotics Section, NIDR.

Cooperating Units: None

Man Years (calendar year 1961):

Total: 3 1/4  
Professional: 1  
Other: 2 1/4

Project Description:

Objectives:

This project has been a continuation of NIDR-34 (1960) in which various factors in the host-parasite-diet combination have been studied in relation to dental caries in hamsters. We have directed our attention to various microbiological and dietary factors which affect the course of the carious process.

Methods Employed:

1. Study of the transmission of streptomycin- and erythromycin-resistant strains of streptococci in animals, which have received the organism either by an inoculation of pure culture or by natural transmission from the mother animal has been continued.
2. Study of the development of caries in non-infected animals by the transfer of appropriate plaque material from infected donors has been continued. This technique has been extended to studies of periodontal disease.
3. Caries activity in hamsters, actively immunized with a vaccine prepared from a known cariogenic streptococcus has been investigated. This has been a cooperative project with Dr. R. J. Fitzgerald (Gnotobiotic Section, NIDR).



4. In cooperation with Dr. H. V. Jordan the role of oleates and stearates on caries activity has been investigated.
5. The roles of various ingredients in our experimental diet have been assessed to determine their contribution to carious activity.
6. In cooperation with Dr. H. V. Jordan (Gnotobiotic Section), preliminary studies have been undertaken to determine the roles of various organisms isolated from animals which have shown extensive periodontal disease and little caries.

#### Major Findings:

1. The labelled cariogenic streptococci have continued to prove useful in the study of caries activity and its transmission. A new strain of streptomycin-resistant organisms (B 14 C) seems to establish itself somewhat more effectively than previously used strains. The potential of this organism as a tracer is being explored.
2. The transfer of plaque material from animals either with caries or with periodontal disease seems to be a technique with a promising potential. Either type of disease has been induced by the transfer of appropriate plaque.
3. In cooperation with Dr. R. J. Fitzgerald an attempt was made to promote an active immunity in hamsters by injections of a vaccine prepared from known cariogenic streptococci. It was disappointing, but not surprising, to find that this treatment did not affect the course of caries activity.
4. In cooperation with Dr. H. V. Jordan an attempt was made to alter the course of caries by adding sodium oleate to the experimental diet. Control animals received sodium stearate. The animals which received the oleate compound showed no difference in caries activity from that observed in controls.
5. Experimental work related to caries has been considerably handicapped this year because the disease has failed to progress following unmistakable initiation. In other words, caries has started in animals and then stopped. There has been no reason to postulate that this response is due to the development of resistant factors within the host animal. Various studies and evidence indicate that a cariogenic microflora is present. Work in the past several months implicates ingredients in the diet, and particular attention has been focused on the role of the liver powder and yeast. This problem is now under investigation and may lead to interesting findings. The role of the salt mixture in the diet has also been studied, but this fraction does not seem to be responsible for the difficulty.





6. In the course of our observations we have observed that some animals develop extensive plaque which involves the periodontium. Caries may be inactive in these animals. In several trials the transfer of this type of plaque material has induced periodontal disease in the inoculated animals. Since control animals have remained relatively free of this disturbance, and since other animals simultaneously inoculated with plaque from caries-active animals have developed caries, we are led to postulate that there may be a specific microbiota responsible for certain type of experimental periodontal disturbances. This observation has stimulated a collaborative investigation with Dr. H. V. Jordan and Dr. R. J. Fitzgerald. The possibility of inducing the disease with pure cultures of organisms isolated from this plaque material is now under investigation.

Significance to Dental Research:

An understanding of caries depends upon disclosures of contributory factors in areas related to host, parasite, and ingesta. The type of interaction between these three factorial groups determines whether the disease is active or not. It is not possible to consider caries comprehensively or analytically without a consideration of all three areas. In many experiments investigators have considered only two combinations. For example in many nutritional studies the relationship between diet and host has received prime consideration. The important and essential microflora has been overlooked, taken for granted, or considered a constant. Limitations in experimental methods may be due to an inability to control simultaneously correlated variables in all three of the contributory areas. Because this problem is an inherent part of the system and is not avoidable or easily solved, recognition of it means that little basic information can be disclosed merely by challenging experimental animals with various diets.

In regard to oral substrate, the quality (physico-chemical), the quantity, and the time the ingesta are in the mouth affect the course of the disease. Furthermore the following combinations should be recognized: (1) substrates may favor host and not favor the cariogenic microbiota (an ideal situation); (2) vice versa; (3) substrates may favor both host and parasite; (4) or neither one. When consideration is given to the above possibilities in experimental design and attention is focused on the dynamic ecological factors involved, experimental design can be improved and of more value in furthering an understanding of this disease. Our work emphasizes the three areas of variables, and indicates that many previous conclusions are open to re-evaluation.

The significance of this work reaches beyond that related to dental research and into the area of therapy. Some form of chemo-therapeutic control of the cariogenic microbiota is clearly indicated as



a logical and potentially effective type of caries control, i.e. a direct attack in the parasitic area. Also no agent has shown a more consistently anti-caries potential than fluoride. There may be considerable benefit in a more direct application of trace amounts of this substance to bolster resistance of the host. A logical next step would be a clinical testing program in which an antibiotic and a trace amount of fluoride are combined and simultaneously applied.

Proposed Course of Project:

To explore factors in the host-parasite-diet combination which influence the course of either caries or periodontal disease.

Part B. included: Yes



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1. Histology & Pathology
  2. Bethesda, Md.

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project.

Keyes, P. H. A New Method for Assessing Caries Inhibitory Agents.  
Dental Progress 2:46-50, 1961.

Honors and Awards relating to this project.

None



Annual Report of the Epidemiology and Biometry Branch  
National Institute of Dental Research  
Summary Statement

In the past, most studies in oral epidemiology have been concentrated upon dental caries. In the United States this disease strikes early in life and can destroy the dentition before adulthood unless checked by preventive or treatment measures. Principally because of earlier and more adequate dental care, tooth mortality from this cause has been reduced to a point where more teeth are actually being lost to the periodontal diseases than to caries. In some other areas of the world, caries is low in prevalence but periodontal disease is widespread and so severe that many young adults are virtually edentulous from this cause. For these reasons periodontal diseases were studied as closely as dental caries during the past year of activity in the Epidemiology and Biometry Branch.

During the calendar year 1961, principal investigators of the Branch examined about 24,000 persons, most of them adults. These persons were participants in a series of broad programs, including (a) nutrition surveys in cooperation with the Interdepartmental Committee on Nutrition for National Defense, in Lebanon, Trinidad, Burma, and with American Indians in Montana; (b) school children in Montgomery and Prince Georges counties, Maryland, where the domestic water has been fluoridated since January, 1952; (c) clinical trials of phosphates as caries inhibitors, added to food in a study with Dakota Indian children, and in a dentifrice tested with children in Pennsylvania; (d) tests of the Keyes-Fitzgerald hypothesis of the communicability of dental caries in animals, to determine whether it is applicable in human caries; and (e) a survey of mottled enamel in Grand Rapids, Michigan, as part of that fluoridation study.

#### Nutrition Surveys

Investigators from the Branch participated as dental examiners in nutrition survey teams of the ICNND sent to Lebanon, Trinidad, Burma, and to Montana to study American Indians. This Interdepartmental Committee was constituted in 1955 to coordinate nutrition surveys of armed forces in friendly countries. Its field survey teams are made up of specialists in nutrition, medicine and dentistry, biochemistry, food technology, agriculture, and other disciplines according to the opportunity presented by the specific situation. Within this framework observers from the Branch have been encouraged to carry out specific and general research relating nutritional states to the oral diseases.

Data from the 1961 surveys are being added to data previously gathered in similar fashion in Alaska, Ethiopia, Ecuador, Chile, Colombia, South Vietnam, and Thailand. These studies have been analyzed as individual units and as components of a whole program, on the general hypothesis that populations depleted in any nutrient will exhibit more or more severe signs of disease if such deficiency is etiologic for that disease.





The prevalence of dental caries has been shown to vary widely; from levels as high as or higher than those seen in adults of continental United States to virtual absence of disease. High prevalences were reported from the larger villages of Alaska, throughout Trinidad, and in some areas in South America. Caries was virtually absent in Ethiopia, affecting an average of less than one tooth per person at all ages up through 40 years, and nearly as rare in Eskimos of some remote Alaska villages, in South Vietnam, and in Palestinian refugees in Lebanon. Preliminary analyses have elicited no consistent relation between dietary or nutrition findings and dental caries, except a very general tendency for low caries prevalence to occur in areas where calorie intake is marginal or low. Further evidence that dental caries is inhibited by fluoride ingestion was adduced in Thailand, Ecuador, Colombia, and Chile.

Taken as a whole, periodontal disease constituted a much greater problem than caries in these national groups. Disease levels lower than those observed in the United States were seen only in remote areas of Alaska and in primitive Jivaros of Ecuador. Gingival disease with relatively little tooth loss from this cause was reported from Ethiopia. Elsewhere the onset of periodontal disease was early, prevalence was virtually universal, and severity was extreme. Despite their favorable experience with dental caries, as many Lebanese as United States citizens were edentulous in middle life, due to tooth loss from destruction of supportive tissues. Equally high levels of disease were seen in Trinidad and the three countries in Southeast Asia. There was a general tendency for periodontal disease scores to be higher in populations in which vitamin A intakes, or vitamin A levels in serum, were low. Persons within a population, however, who were subsisting on inadequate intakes of vitamin A were no more apt to exhibit disease than persons whose intake was adequate or high.

One known etiologic factor in periodontal disease is local irritation, which is usually the result of subgingival deposits of calculus. The influence of calculus deposits, for example, accounted for nearly 90 per cent of the variance in group periodontal scores in Lebanon. But it seems obvious that calculus is much more damaging in the mouths of Lebanese or Vietnamese civilians than in the mouths of Ethiopians or Eskimos. The proper analysis for these additional factors is one which cancels out the effects of such known factors as calculus. This can be done by multiple correlation-multiple regression technics. These are simple in principle but entail enormous numbers of arithmetic calculations. Multiple correlation analysis of the 20 items of information considered in a study of Alaskan Eskimos, for example, would require that each value be used as a multiplier about 250 times. A superficial analysis already carried out, in which each item was used as a multiplier about 40 times, required several months of hand computation. Such problems as these are readily amenable to solution by electronic computer methods. Computer programs for simultaneous consideration of factors which may affect periodontal disease or dental caries as elicited in these ICNND studies are now under preparation.

Several leads for further study have been uncovered. Some populations seem to be free of signs of oral malignancies. In some (notably the Thai) the



excretion of relatively large amounts of fluorides in urine by persons using a domestic water very low in fluorides suggests that there may be unknown sources of fluoride in Thai foods, or that fluoride metabolism may be influenced by unknown factors not normally operating in North America. A lacquer believed by the Vietnamese to protect teeth from disease was found actually to be associated with lesser caries attack and better periodontal tissues. Christian women in Lebanon were notably more susceptible to dental caries than Moslem women living in the same areas. Periodontal disease is ordinarily more destructive in males than in females but in a matriarchal society of hill tribesmen in Vietnam, in which women carried the dominant roles in social and other activities outside the home, disease was more destructive in females than in males.

### Fluorine and Dental Caries

The pioneer study of the practicality of domestic water fluoridation was carried out in Grand Rapids, Michigan, where fluorides were added to the community water beginning in January, 1945. The final field examination required by design of this study was carried out in November, 1961, with a survey of dental fluorosis in children aged 12-14 years, with maximum usage of this community water since birth. Less fluorosis was found than had been predicted on the basis of the observations on children in 21 cities with varying levels of fluoride in the water, a series of studies which had given rise to the hypothesis tested in Grand Rapids. This lessened degree of fluorosis may have been due to rigid control of fluoride levels in the fluoridation plant. Most "natural" fluoride waters fluctuate in fluoride content, with peak levels much higher in many instances than the average levels over the course of months or years. The finding in this survey supports the original estimate of the safety of controlled fluoridation at the recommended level, and suggests, in fact, that safety-factor calculations were conservative.

The Grand Rapids study was intended to determine whether fluoridation of water would induce the same inhibition of caries seen with naturally-fluoridated waters, and was designed on the assumption that first effects would be seen in children born after fluoridation was begun. For this reason examinations during the first few years of study were limited mainly to children aged 5, 6, 9, or 13 years. Efficacy of fluoridation was obvious after four years of observation. A corollary study was then begun to determine how and in what manner the inhibition of caries was effected. This was designed on a longitudinal plan, to run for 10 years following fluoridation of water in Prince Georges and Montgomery counties, Maryland, in January of 1952. Examinations of children aged 5-15 years were carried out in 1961, the ninth year of study. Repeated observations of the same children indicated that most of the assumptions accepted in conventional horizontal ("one-shot") field study are valid. Teeth in eruption at the time of fluoridation continue to decay at essentially the same rates as before. Caries was arrested on smooth surfaces of teeth fully calcified, but not in eruption, at the time of fluoridation; there was little effect on pit-and-fissure caries in these teeth. Caries on all tooth surfaces was inhibited in teeth still undergoing calcification at the time of fluoridation. There was a general decrease in



the prevalence of early periodontal disease in older children as the study progressed. Final analysis after the tenth year is expected to yield a family of findings ranging from actuarial-type tables which will permit estimation of the impact of fluoridation upon a public health dental program for children, to inferences with the sequence and mechanics of development and calcification of the teeth.

### Other Studies

The Keyes-Fitzgerald hypothesis that dental caries is a specific, infectious and transmissible disease in laboratory animals raises obvious questions about dental caries in man which can be answered only through the study of human populations. Organisms which induce caries in rats are benign in the mouths of hamsters, and organisms which induce caries in hamsters do not affect rats. Both are streptococci which can be eradicated completely by penicillin in adequate oral concentration. Assuming a parallel process in humans, it was postulated that young rheumatic fever patients who had been receiving oral penicillin daily, beginning at a time prior to the eruption of permanent teeth, would show no lesions of caries in those teeth after some years at risk. In a pilot field study such children were examined in Pennsylvania. Lesions of caries were demonstrated in their permanent teeth, indicating that the original hypothesis was false or that the actual dosages of penicillin had been inadequate to suppress the cariogenic flora in toto. However, the affected numbers of teeth and tooth surfaces were less than in the mouths of randomly-matched children in the public schools of the same community who had not received penicillin. Once an organism specific for man is identified its role can be verified by demonstrations of absence in such caries-free populations as those in Alaska and Ethiopia (described earlier in this narrative) and uniform presence in caries-susceptible groups.

Findings in some animal studies have suggested that phosphate may be an effective inhibitor of dental caries under certain conditions. A clinical trial of the effect of phosphates added to flour used in baking was carried through its second year in Indian school children in South Dakota. This is a double-blind study, with the clinical observations being made by an independent examiner. Data are collated and analyzed in the Branch. This study has an additional year to run. Besides analysis of the dental caries data, statisticians of the Branch have helped tabulate and analyze collateral data on growth in height and weight, and skeletal calcification in the same children during the term of trial.

A clinical trial of phosphates added to a dentifrice was begun with institutionalized children in Pennsylvania. This study is designed for a term of two years, involving a minimum of three clinical and x-ray examinations.

In addition to direct research activities, a considerable amount of time was spent in consultation on the design and conduct of field studies being made by others, particularly NIDR research grantees, officers of the Navy Dental Corps, and Public Health Service regional dental officers. Biometrics services were furnished in about 20 studies involving NIDR investigators from other laboratories.



1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases among Alaskan Natives

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To determine the prevalence of dental caries, periodontal diseases, and malocclusion among Alaskan natives and to investigate possible relationships of these disorders to certain nutritional and geographic characteristics of this group.

Methods Employed:

Direct examination of members of the Alaskan National Guard. Subsequently, statistical analysis of data obtained to determine relative risks under varying conditions.

Major Findings:

Dental examinations of approximately 700 Eskimo men of the Alaskan National Guard revealed significant information concerning oral health in this population group. Preliminary analysis of data indicated interesting differences when findings from "primitive" groups were compared with those from groups living under more civilized conditions. Dental caries was virtually non-existent in individuals from remote villages. Many of the primitive groups were essentially free of periodontal diseases. Other similarly primitive groups exhibited a uniformly prevalent, severe gingivitis. Contrary to generally accepted theory, this gingivitis demonstrated





little tendency toward progression into chronic destructive periodontal diseases even in the absence of personal oral hygiene or professional dental care. In contrast to these findings in the more "primitive" groups, Eskimos that had lived for some time under relatively "civilized" conditions, demonstrated prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States.

Significance to Dental Research:

The spectrum of living conditions within this Eskimo group affords a unique opportunity to study the influence of "civilization" on oral diseases. Findings from the "primitive" groups are relatively uninfluenced by professional dental care. An opportunity is also present for evaluating oral diseases in terms of the various nutritional patterns included within this population sample.

Proposed Course of Study:

The present phase of study has been concluded. This population, however, can be employed in a crucial test of the hypothesis (established in animals) that dental caries is an infective and transmissible disease once the organisms active in human caries can be identified.

Part B included: Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L., Consolazio, C. F., and White, C. L. Dental caries and nutrition in Eskimo scouts of the Alaska National Guard. J. Dent. Res., 40:594-603, May-June 1961.

Russell, A. L., Consolazio, C. F., and White, C. L. Periodontal disease and nutrition in Eskimo scouts of the Alaska National Guard. J. Dent. Res., 40:604-13, May-June 1961.

Honors and Awards relating to this project:

None



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Vietnam

Principal Investigators: Dr. A. L. Russell and Dr. E. C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total: 1  
Professional: 1/2  
Other: 1/2

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Vietnam and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Analysis of these data are still in progress. However, preliminary tabulation indicates that prevalence of dental caries was generally low. While the prevalence of periodontal diseases was widespread in the general population, these conditions were extremely severe in individuals living in certain mountainous areas. These findings occurred in a population subsisting on a soft, rice diet with indications of marginal intakes of thiamine, riboflavin, and Vitamin A.



Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical and nutritional factors.

Proposed Course of Project:

Electronic data processing will be used to explore numerous multiple correlations between oral disease and nutritional and physical findings developed by other members of the ICNND examination team. Data from this study will be collated with data from others in an attempt to improve present knowledge concerning the occurrence and etiology of oral diseases in these population groups.

Part B included: No





1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases among Ethiopians

Principal Investigator: Dr. N. W. Littleton

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

1. To determine the oral health status in a representative sample of Ethiopians; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion and attrition.
2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of an Ethiopian sample with subsequent analysis of examination data to determine relative risk under varying conditions.

Major Findings:

The prevalence of dental caries in the Ethiopian sample was very low. This low prevalence occurred in a population apparently subsisting on a diet traditionally high in natural carbohydrates but low in sugar. Information on fluoride levels tended to indicate that the relative freedom from dental caries was not entirely dependent on fluoride ingestion. Periodontal diseases were highly prevalent and severe in this population. Nutritional factors



appeared to exert only a minor influence on the status of the periodontal tissues. Positive associations existed between the severity of periodontal diseases and the state of oral cleanliness in this group.

Significance to Dental Research:

This project presented an opportunity to study a population in which little was known concerning oral health status, and one in which the effects of personal oral hygiene and professional dental care would be minimal. Findings have been evaluated in terms of certain environmental, medical, biochemical, and nutritional data in an effort to establish possible associations.

Proposed Course of Project:

1. To complete the tabulation and analysis of the data.
2. To utilize findings to formulate possible suggestions for improving oral health conditions within Ethiopia.
3. Through correlating these findings with those of other surveys, attempt to expand information concerning the epidemiological characteristics of oral diseases - particularly dental caries and periodontal diseases.

Part B included: No



- Serial No. NIDR-42  
1. Epidemiology & Biometry  
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Ecuador

Principal Investigators: Dr. E. C. Leatherwood and Dr. J. C. Greene

Other Investigators: None

Cooperating Units: Office of the Chief Dental Officer, USPHS,  
Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total: 3/4  
Professional: 1/2  
Other: 1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Ecuador, and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risks under varying conditions.

Major Findings:

Tabulation of findings indicates that dental caries prevalence in Ecuador varied in different regions of the country. This appeared to be associated with differences in fluoride intake and to be somewhat related to the reported frequency of sugar consumption. There appears to be a direct relationship between oral debris and calculus and dental caries and periodontal disease. The prevalence and severity of periodontal disease were quite high.



Significance to Dental Research:

This project presents an opportunity to expand information on the epidemiological characteristics of oral diseases. Further, possible associations may be established between the occurrence of oral disease and nutritional, medical, biochemical, and environmental factors.

Proposed Course of Project:

Tabulation and analysis of data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: No





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Colombia, South America

Principal Investigator: Dr. C. J. Donnelly

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1961):

Total: 1  
Professional: 1/4  
Other: 3/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Colombia and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

The prevalence of both caries and periodontal disease was high in the civilian and military groups examined in Colombia. Most water supplies sampled were deficient in fluoride. Caries prevalence was lower where appreciable amounts of fluoride was found in the drinking water. Mild and very mild fluorosis was seen in areas where the fluoride content of the water would in the United States be considered too low to cause any degree of fluorosis. Oral cleanliness correlated well with periodontal disease but no positive correlation have been found between periodontal disease and any of the nutrients examined.



Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

Tabulation and analysis of the data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups. Associations between dental findings and the various nutritional, physical and environmental characteristics of the population under study will continue to be examined.

Part B included: No



1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Thailand

Principal Investigator: Dr. E. C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartment Committee on Nutrition for  
National Defense and Walter Reed Army Medical  
Center, Institute of Dental Research

Man Years (calendar year 1961):

Total: 1/2  
Professional: 1/4  
Other: 1/4

Project Description:

Objectives:

1. To determine the oral health status in military and civilian samples in Thailand; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion, and attrition.
2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of military and civilian samples in Thailand with subsequent analysis of data to determine relative risk under varying conditions.

Major Findings:

1. Prevalence of dental caries was extremely low especially in the younger age groups and even through the age of forty.
2. Prevalence and severity of periodontal disease were quite high and considered to be the major oral health problem.



3. Evidence of fluoride ingestion throughout the country with the prevalence of fluorosis varying from one region to another.

Significance to Dental Research:

This project presented an opportunity to evaluate the prevalence of oral diseases in the population under study. It also offered the opportunity to evaluate the oral health status in terms of certain biochemical, nutritional, and environmental data in an effort to establish possible associations.

Proposed Course of Project:

To tabulate and analyze the data using electronic computer and attempt to establish associations within these data between dental findings and the various nutritional, physical, and environmental characteristics of the population under study. Attempt to correlate these findings with those of other surveys in Vietnam and Burma and of other areas of the world.

Part B included: No





1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Lebanon

Principal Investigator: Dr. A. L. Russell

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total: 3/4  
Professional: 1/2  
Other: 1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in military personnel, civilians, and Palestinian refugees and to relate these findings to environmental, medical, biochemical, dietary, and nutritional data in an effort to establish relations of possible importance in etiology and control.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk in varying circumstances.

Major Findings:

Analysis of these data is still in progress. Preliminary study indicates that dental caries attack rates were low in all three groups studied. Christian civilian women experienced considerably higher caries attack rates than Moslem women. Dental caries experience of civilian and refugee Moslem men and women did not differ to any extent. Caries attack of Christian refugees were about the same as for Moslem refugees. Periodontal disease was universally prevalent and very severe, particularly in refugees.



These differences are associated with differences in custom, diet, and nutrition; some of these associations may have etiological significance.

Significance to Dental Research:

Study of these associations may lead to a better understanding of etiology, or to more effective methods of control, of dental caries and the periodontal diseases.

Proposed Course of Project:

Statistical analysis of the data will be completed and findings correlated with data on environmental condition, biochemical levels of such nutrients as vitamin A, ascorbic acid, thiamine, and riboflavin, and others. Comparisons will then be made with data from similar studies in other countries to determine whether there are factors which tend consistently to modify the pattern or development of disease.

Part B included: No



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Montana Indians

Principal Investigators: Dr. E. C. Leatherwood and Dr. J. C. Greene

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for  
National Defense and Dental Health Center,  
Division of Dental Public Health and Resources,  
PHS

Man Years (calendar year 1961):

Total: 1/2

Professional: 1/4

Other: 1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases among certain tribes of American Indians and to relate these findings to environmental, medical, biochemical, nutritional, and anthropological data in an attempt to establish possible associations.

Methods Employed:

Clinical examinations of population groups to be followed by statistical analysis of findings to determine relative risks under varying conditions.

Major Findings

Tabulation and analysis of data has not begun on these findings.

Significance to Dental Research:

This project presents an opportunity to expand information on epidemiological characteristics of oral disease as related to environmental, medical, biochemical, anthropological and nutritional factors.



Proposed Course of Project:

1. To complete the tabulation and analysis of the data.
2. To utilize findings to formulate possible recommendations for improving oral health conditions among certain tribes of American Indians.
3. Through correlating these findings with those of other surveys, attempt to explain information concerning the epidemiological characteristics of oral diseases.

Part B included: No





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Epidemiology of Oral Diseases in Burma

Principal Investigator: Dr. N. W. Littleton

Other Investigator: None

Cooperating Unit: Interdepartmental Committee on Nutrition for  
National Defense

Man Years (calendar year 1961):

Total: 1/4  
Professional: 1/4  
Other: none

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in the people of Burma, and to relate these findings to environmental, medical, mental, biochemical, dietary, and nutritional data in an effort to establish relations of possible importance in etiology and control.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk in varying conditions of life.

Major Findings:

Field examinations in this study were concluded late in December, 1961. Analysis of these data are still in progress.

Significance to Dental Research:

The prevalence of dental caries was found to be very low, and the prevalence of periodontal disease very high, in neighboring Thailand and South Vietnam. This study will test whether certain observed associations are accidental or of possible etiological significance.



Proposed Course of Study:

Statistical examination of the data and comparison of findings with patterns developed in similar studies in other parts of Asia and of the world.

Part B included: No



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Clinical Trial of a Dentifrice Containing 10% Soluble Phosphate

Principal Investigators: Dr. C. J. Donnelly and Dr. F. J. McClure

Other Investigator: Mr. C. L. White

Cooperating Units: Lancaster Cleft Palate Clinic and  
Milton J. Hershey School

Man Years (calendar year 1961):

Total: 1  
Professional: 1/2  
Other: 1/2

Project Description:

Objectives:

To determine if a dentifrice with a soluble phosphate will reduce the incidence of dental caries.

Methods Employed:

A dentifrice containing 10% soluble phosphate is being tested in a double blind study at an institution with 900 boys residing in approximately 50 cottages. The cottages were matched by caries prevalence and equal numbers were randomly assigned to study and control groups. The control dentifrice is similar but without 10% soluble phosphate. A base line of caries prevalence was established by a bite wing radiograph and visual dental examination. Caries incidence will be determined from periodic repetition of the bite-wing and visual examination. Mean incidence of carious teeth and tooth surfaces are to be compared for study and control groups.

Major Findings:

Data currently being obtained.



Significance to Dental Research:

Dietary control of caries is impractical for large groups. Water fluoridation is not readily available to those who are not served by a communal water supply and has been denied to many others by the action of antifuoridationists or by the inaction of local authorities. It would be desirable to have available other effective methods for the mass control of dental caries to supplement fluoridation. Because toothbrushing is a widespread established habit available to all, therapeutic dentifrices offer promise as a mean of inhibiting caries. There is some evidence from animal experimentation that a soluble phosphate is capable of inhibiting caries. Animal experiments further indicate that the action of soluble phosphates may be topical and hence its incorporation in a dentifrice to assure appreciable contact with tooth surface.

Proposed Course of Project:

The study was initiated in 1961 and will be continued for 18-30 months.

Part B included: No





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: The Epidemiology of Dental Caries - Including the  
Fluoride-Dental Caries Relationship

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Board of Education, City of Grand Rapids; City-  
County Health Department, Grand Rapids, Michigan;  
Michigan Department of Health; Montgomery and  
Prince Georges Counties, Maryland

Man Years (calendar Year 1961):

Total: 1-1/2  
Professional: 1  
Other: 1/2

Project Description:

Objectives:

1. To observe the effects of fluoridation of a community water supply on children whose initial exposure to fluoride occurred at different age levels.
2. To determine the epidemiological characteristics of dental caries.
3. To obtain basic information concerning the nature of dental caries and the dental tissues susceptible to attack so that present methods of control may be perfected and, possibly, new methods may be evolved.

Methods Employed:

Direct examination of population groups with subsequent analysis of findings to determine relative risks under various conditions.



Major Findings:

1. Children who use a fluoridated water from birth are protected from dental caries to the same extent and in the same way as children using a natural fluoride water.
2. There was substantial inhibition of caries in teeth which were undergoing calcification at the time of first exposure to water-borne fluoride.
3. There was inhibition of smooth-surface caries, but little of caries in pits and fissures, in teeth which had calcified but which had not yet erupted at the time of fluoridation.
4. Caries inhibition was substantially nil in teeth which were in eruption at the time of fluoridation.

Significance to Dental Research:

Findings from this study are expected to expand present knowledge of the beneficial effects of controlled fluoride ingestion on the dentition. Through detailed analysis, an attempt will be made to secure information on the action of fluoride during the various formative periods of the teeth. Inferences can be drawn on the duration of the inhibitory effects of fluoride on dental caries.

Proposed Course of Project:

The field phase of the Grand Rapids study was ended in December, 1961, with a fluorosis survey of children aged 12-14 years. 1962 is to be the tenth and final year of field examinations in the Maryland study.

Detailed analysis by electronic methods is planned for data from the Maryland study. This was organized on a longitudinal design. A study of year-by-year changes in the status of teeth, surface by surface, is expected to expand present information on the stages of tooth development and the mechanics and duration of the caries-inhibitory effect of fluoride ingestion.

Part B included: Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, A. L., and White, C. L. Dental caries in Maryland children after 7 years of fluoridation. Pub. Health Rep., (for publication in December, 1961, issue).

Russell, A. L., and Hamilton, Peggy M. Dental caries in permanent first molars after eight years of fluoridation. Read before the Eighth Congress of the European Organization for Research on Fluorine and Dental Caries Prevention, London, July 6, 1961. To be published in Archives of Oral Biology.

Honors and Awards relating to this project:

None



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Study of Periodontal Disease and Caries in Adventists Families

Principal Investigator: Dr. C. J. Donnelly

Other Investigators: None

Cooperating Units: Conferences of Seventh Day Adventists in Indiana, North Dakota, Wisconsin, Michigan, Oklahoma, Southern New England, Chesapeake, and Georgia-Cumberland

Man Years (calendar year 1961):

Total: 1  
Professional: 1/4  
Other: 3/4

Project Description:

Objectives:

1. To investigate the influence of familial factors and of geographic location on periodontal diseases and dental caries among Adventists families.
2. To determine if the dental caries rate among Adventists children is lower than among comparable non-Adventists children.
3. To study the prevalence and severity of periodontal disease in children and adults from a related group of individuals.

Methods Employed:

Direct examination of parents and children attending Seventh Day Adventist Camp Conferences in different parts of the United States. The data will be treated statistically to determine the influence of familial factors, geographic location and Adventists health reforms. Direct examination of Adventist and other children residing in the same non-fluoride area. The data will be treated statistically to determine if caries prevalence is different in Adventist and other children.





Major Findings:

1. The caries prevalence of children examined at Adventist camps in different parts of the United States was lower than expected and did not show as marked geographic variation as expected.
2. Adventist children who were continuous residents in a non-fluoride area had consistently lower caries prevalence than other children who had resided continuously since birth in the same non-fluoride area.
3. Children generally tend to reflect the periodontal status of their parent. In those families in which the parents were dissimilar in periodontal score or in education background, there was no tendency for the periodontal scores of the children to more closely resemble the score of one parent or the other.
4. The periodontal scores of Adventist examined in different parts of the country did not differ significantly.
5. Urban residents had periodontal scores which were slightly but consistently better than the scores of rural residents.
6. Levels of periodontal disease among Adventist are probably quite similar to non-Adventist with equivalent educational backgrounds.

Significance to Dental Research:

1. The low-caries rate consistently found among Adventist children may be related to the dietary recommendations of the "health reform" suggested by the Adventists Church. Further study might add to our information on the influence of diet on caries rates.
2. By examining Adventists in different parts of the country, some information will be obtained on the influence of geographic location on caries and periodontal disease. By determining if one parent exerts more influence than the other on the periodontal status of the offspring, additional information may be gained about the factors influencing the severity of periodontal disease.

Proposed Course of Study:

1. The controlled study of caries prevalence among Adventist and public school children will be extended to a nonfluoride area to eliminate the influence of fluoride on teeth.



2. Data collected at the Adventist camps will be analyzed for any possible influence of familial or geographic factors.

Part B included: Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Donnelly, Charles J. A Comparative Study of Caries Experience in Adventist and Other Children. Public Health Reports. 76: 209-212, March 1961.

Honors and Awards relating to this project:

None



1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Relation Between Dental Caries and Periodontal Diseases

Principal Investigator: Mr. C. L. White

Other Investigator: Dr. A. L. Russell

Cooperating Units: None

Man Years (calendar year 1961):

Total: 3/4  
Professional: 1/2  
Other: 1/4

Project Description:

Objectives:

To study possible correlations between dental caries, fluoride, and periodontal diseases.

Methods Employed:

Analysis of data obtained from studies on caries and periodontal diseases conducted by the Epidemiology and Biometry Branch.

Major Findings:

1. Preliminary findings indicate that there is no significant difference in the occurrence of periodontal diseases about teeth with or without dental caries.
2. Periodontal tissues are not harmed by use of a fluoride-bearing domestic water.

Significance to Dental Research:

This project expands the program of the Epidemiology and Biometry Branch through statistical investigation of data available from other projects.





Proposed Course of Project:

To continue to expand the study as outlined.

Part B included: Yes



PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Accepted for publication by New York State Dental Journal:  
White, Carl L., and Russell, A. L. Some Relations Between  
Dental Caries Experience and Active Periodontal Disease in  
Two Thousand Adults.

Honors and Awards relating to this project:

None



1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A.

Project Title: Transmissibility of Human Dental Caries

Principal Investigator: Dr. N. W. Littleton

Other Investigators: Dr. P. H. Keyes and Dr. R. J. Fitzgerald

Cooperating Units: Children's Heart Haven Hospital, Lancaster, Pa.

Man Years (calendar year 1961):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

Drs. Keyes and Fitzgerald have shown that dental caries in rats and hamsters is an infectuous disease, due to a streptococcus specific for each species of animals. This study is the first move in a project to determine whether human dental caries is similarly infectuous and transmissible.

Methods Employed:

Examination of a population of children with rheumatic heart disease, who had been receiving penicillin by mouth for some years, beginning at an age prior to the eruption of permanent teeth. Dental caries does not develop in rats or hamsters on an analogous regime.

Major Findings:

Lesions of dental caries were observed in the study children, so that the hypothesis of complete suspension of caries attack was not supported. However, caries attack was consistently less severe in the study children than in children of the same ages not receiving penicillin, living in the same community.



Significance to Dental Research:

If dental caries has a specific etiology it is amenable to specific measures for control.

Proposed Course of Project:

Definitive field study can be carried out once the specific organism or group of organisms responsible for human caries has been identified by microbiological technics. Strong supportive evidence will be adduced if this organism is universally absent in population groups free of caries (such as Nunivak Island Eskimos, Ethiopians, Vietnamese) and universally present in groups in which caries is endemic (as in continental United States). Final demonstration, if the hypotheses survive these tests, may require longitudinal observation and clinical trial with narrow-spectrum antibiotics.

Part B included: No





Annual Report of the Clinical Investigations Branch  
National Institute of Dental Research  
Summary Statement

By  
Dr. Robert M. Stephan

## INTRODUCTION

The scope of research activities in the Clinical Investigations Branch, National Institute of Dental Research, while originally centered around oral disease problems, has become more extensive and now includes problems of speech, hearing, swallowing, and respiration, as well as a continuously developing program on a variety of inherited disease conditions and abnormalities of genetic origin. For purposes of this review, we may describe the various research projects under four headings or sections: (1) Studies on oral diseases, (2) Medical investigations, (3) Studies on oral pharyngeal development and function, and (4) Familial and isolate population studies in human genetics.

The opening of the new building for the National Institute of Dental Research furnished an opportunity for considerable growth in the Clinical Investigations staff, even though most of the staff have their laboratories in the Clinical Center. Thus, the professional staff increased from 20 to 28, the supporting staff from 17 to 26, for a total staff increase from 37 to 54 persons. This increase was chiefly in the beginning of a new section on "Oral Pharyngeal Development and Function," headed by Dr. James F. Bosma, with 6 professional and 5 subprofessional staff members, and an increase in the Genetics Section, headed by Dr. Carl Witkop, from 6 to 8 professional and from 4 to 7 subprofessional staff members. These changes have increased the broad range of projects in the Clinical Investigations area, even though the National Institute of Dental Research staff is small, compared with the staffs of the other National Institutes of Health institutes. While some of the research projects are definitely aimed at helping the dental profession solve some of its more difficult clinical problems, the general aim of most of these projects has been to develop new and more adequate research methods and data to elucidate the underlying mechanisms of the normal and abnormal biological processes as they are related to health and disease.

## ORAL DISEASES

Research projects on oral diseases include those concerned with the teeth, such as caries, erosion, periodontal disease, and pulpitis, and those concerned with pathological conditions of the mucous membranes and underlying oral structures, such as aphthous stomatitis, leukoplakia, infections and painful temporomandibular joints. It is recognized that one cannot dissociate the health of the oral structures from the health of the entire body, and that one cannot dissociate the health of the individual patient from that of the family and the social and genetic community from which he has come. It is also recognized that clinical investigations should include related laboratory studies on physiological and biochemical mechanisms, as well as studies of similar disease conditions in model animal experiments.

(a) Dental Caries: Two research projects are being conducted in this area: The first is a comprehensive clinical and laboratory study of the



multiple factors involved in the etiology of dental caries by Dr. Stephan. This has included the use of advanced instrumentation for making intra-oral observations and measurements on the caries process, the evaluation of predisposing systemic and familial factors in children with rampant caries, and multifactorial experiments in laboratory animals to determine the effects of strain of animal, the cariogenic properties of various dietary materials, and the cariogenic and infections potentialities of specific types of oral bacteria derived from patients with rampant caries, using the fluorescent antibody technique to trace specific microorganisms. Although this study deals with the etiology of dental caries in all of its complexities, it is leading to a clarification of the relative importance of different basic factors involved in the caries process, and particularly to the host-parasite relationship as affected by diet. As more knowledge is gained on the parasitic and cariogenic properties of different microorganisms, particularly in tracing specific organisms using the fluorescent antibody method, a more specific control of potentially cariogenic microorganisms may be developed. From a practical standpoint, the dental profession has been very happy to refer patients with rampant caries for this study, since it represents one of their most difficult and expensive treatment problems. From the results achieved thus far by prescribing new dietary habits for patients, it appears that caries activity has been greatly reduced in patients who have followed this control program.

The second caries research project, by Dr. Ship, Dr. Mickelsen (NIAMD) and others, is on the "Effect of Dietary Phosphates on Dental Caries in Children." This study is a field study testing a single dietary factor, calcium phosphate, for its effects when added to bread on the dental caries incidence in Indian school children in South Dakota. It was based on earlier laboratory studies by Dr. McClure, in which the addition of certain phosphates to the diet of rats decreased the development of caries in these animals when they were fed cariogenic diets. The plans for this study have been widely discussed in previous reports, and the present report gives the results of examinations to the spring of 1961. No difference in the increments of either decayed, missing and filled teeth or tooth surfaces was found between the children in the control and the children in the phosphate-supplemented groups. Since the data so far suggests that dietary phosphate supplements are inactive in preventing dental caries in children, the major findings of the project will be on the effects of the supplements on growth and development of Indian children. This study will be terminated at the end of the present school year.

(b) Dental Erosion: The study on the Etiology and Control of Dental Erosion, which was begun this year by Dr. Stephan, is developing a comprehensive picture concerning the mechanisms operating in the production of this little understood condition. It appears that the local effects of specific foods, physical as well as chemical, have been operative in some cases.

(c) Periodontal Disease: Three project reports have been made in this area. The first is a study by Dr. Baer on the therapeutic evaluation of a periodontal dressing used following surgical gingivectomy in the treatment of advanced periodontal disease. It was found that a periodontal dressing containing eugenol produced a greater inflammatory response in healing tissues than did a dressing without eugenol, and that a hydrogenated fat zinc bacitracin dressing was the most useful of those tested for postoperative healing. This work was



published in the Journal of Dental Research.

The second report by Dr. Baer is on the effect of the milk factor during nursing on the development of periodontal disease in two different strains of mice, one of which is "susceptible" and the other "resistant" to periodontal disease. Experiments in which young mice of the "susceptible" strain were nursed by a mother of the "resistant strain, and vice versa, failed to change the subsequent susceptibility of the young mice to periodontal disease. It was concluded that a "milk factor" did not appear to affect the susceptibility of mice to periodontal disease.

The third report on periodontal disease is a morphohistological study of changes in the periodontium in human autopsy material by Dr. Stanley. In this study, an attempt has been made to reconstruct in three dimensions the microscopical picture of pathological processes of the periodontium. The picture developed indicates that a single histological section may not furnish an adequate description of the periodontal process for a given specimen, and that step-serial sections are needed for a suitable diagnosis.

Dr. Wertheimer has also reported studies related to periodontal disease, chiefly histochemical staining of the secondary dental cuticle, and a field study on periodontal disease in the West Indies.

(d) Pulpitis: Another project by Dr. Stanley is concerned with the histopathology of the human dental pulp, particularly in studying the pathological changes induced by dental drilling procedures and the placement of filling materials. This study has furnished the dental profession some very practical information on operative procedures, particularly in regard to optimal cutting speeds, the use of coolants and methods for the placement of amalgam and certain temporary filling materials. An interesting observation has been the finding of occasional mitotic figures in cells approximating the predentin.

Dr. Archard and Dr. Stanley report a current study on the histopathology of the buccal membranes in normal individuals and in patients undergoing cancer chemotherapy and in patients with multiple myeloma. Dr. Kakehashi and Dr. Baer have reported a study on the Influence of Several Dietary Materials on the Deposition of Calculus in the Sprague-Dawley rat. Dr. Baer has also reported a "positive pressure" appliance for the treatment of gingival hemangiomas and hyperplastic gingival tissue in patients treated with dilantin.

The project on recurrent aphthous stomatitis, which was formerly conducted by Dr. Ship, has been renewed by Dr. Graykowski. This is a fairly common and painful disease of unknown etiology, and the current project seeks to determine if any relationship exists between the presence of recurrent aphthous oral lesions and the metabolism of individuals in which they occur. There is suggestive evidence that the various mucosal changes associated with iron deficiency anemia may in some ways be correlated with aphthous stomatitis.

(e) Anesthesia: A study of general anesthesia in ambulatory dental patients by Dr. Driscoll, with Dr. Christenson and Dr. Hebert (CC), is developing important information concerning the physiological effects of anesthesia and oral surgical procedures. This includes continuous data on pulse, blood



pressure, arterial O<sub>2</sub> saturation, respiratory phenomenon, cortical brain activity and the electrical activity of the heart EKG. The accumulated data from this study has been and will continue to be used as a baseline of comparison for new anesthetic drugs which are being proposed for use in oral surgery. Since in some areas there are almost as many general anesthetics administered in dental offices as in the local hospitals, the basic physiological data from this study will prove very important for the dental profession.

A new study was initiated on the physiological responses of the dental patient under hypnosis by Dr. Drury and Dr. Driscoll. Although the use of hypnosis in dental practice has become more widespread in recent years, particularly in the treatment of apprehensive and fearful dental patients, no formal research has been done on the physiological responses of the dental patient to this procedure. This study will be similar to the anesthesia study just described, and will include measurements of blood pressure, respiration, oxygen saturation of the blood, EKG, EEG, body temperature, galvanic skin response, and psychological data recorded in the Minnesota Multiphasic Personality Inventory Test. The findings in this study will be compared with those in the general anesthesia study for an objective evaluation of these procedures in oral surgery and will furnish much needed basic data, which should prove of great value to the dental profession.

Another new study initiated in 1961 by Dr. Gamble, Dr. Driscoll, Dr. Swerdlow and Dr. Lloyd, with the cooperation of the Psychological Testing Center at NIH, is on the use of subperiosteal implants for stabilization of artificial dentures. It is estimated that one out of five edentulous patients is unable to wear conventional mandibular dentures, generally due to advanced resorption of the alveolar ridge. A thorough psychological evaluation of patients who are unable to wear conventional dentures will be made. In these cases, the use of subperiosteal implants offers the possibility of securing greater stability for artificial dentures. This study is aimed at finding a satisfactory solution for a very difficult prosthetic problem, which becomes particularly important for people as they advance in age.

#### ORAL PHARYNGEAL DEVELOPMENT AND FUNCTION

As was mentioned earlier, a new Section on Oral Pharyngeal Development and Function was organized this year, with Dr. James Bosma as Chief. He reports that the activities of this group have been devoted principally to its own orientation and to the introduction and adaptation of investigative methods. A major experimentation is in the interaction of the different professional specialists whose interests converge in the "portal area" of the mouth, pharynx, nose and larynx. In the first nine months of the Section's existence, research investigators in the dental specialties of orthodontics and prosthodontics and the medical specialties of pediatrics and otolaryngology have been brought into close working relationship with specialists in speech therapy and physiology. The most significant interactions thus far have been between speech and orthodontics. Further efforts will be made toward the introduction of methods and personnel of the basic scientific disciplines, particularly anatomy, physiology and anthropology.

These three major disciplines also afford appropriate categorization of the





efforts in this Section since its inception.

(a) Anatomy: Initial efforts of this area have been in anatomical dissection. On the basis of comparative studies in infant and adult humans and in four other species, Y. Takagi, J. Waters and J. Bosma have developed a concept of how the muscles of the cervical spine interact with the muscles at the upper end of the pharynx. The particular effects of this interaction upon the cavity of the pharynx in the palate area is now under study in normal humans and in those having impairment in this area by reason of neurologic disease or malformation.

The anatomical growth of the pharynx and face in the human is also under continuing study. Dr. Peter Coccaro has submitted reports on two items of this study: (1) growth of the soft palate in cleft palate persons, and (2) growth in height of the face in impaired children wearing dentures. Dr. Richard Grossman has developed implementation for comparing the physical softness or elasticity of tongue, lip and face tissues at different stages of development, and in pathologic conditions.

(b) Physiology: Neurophysiological studies of the pharynx region in respiration and in feeding have been initiated. The implementation of this now includes an 8-channel ink-on-paper recorder and/or a 7-channel tape recorder, with each system capable of combining information from several muscles and from sensors of the muscular effects, including motions or pressures or sounds accomplished. With this apparatus, Drs. Bosma, Irwin, Takagi and Lifschitz are engaged in study of sensations from the pharynx which influence its own actions and also those of the larynx and the trunk in respiration.

The muscular actions of the mouth area of the human are also under study in normal and in impaired subjects by Dr. Richard Grossman, employing observation techniques of very small strain gauges and soft tissue displacement indicators. This instrumentation is designed to provide information about the physical forces accomplished by the tongue and face muscles. In impaired persons, these muscles are capable of accomplishing marked deformity with displacement of teeth and incapacity of oral actions in eating and in speaking. Identification of these abnormal muscle actions is essential for proper definition of causes of oral deformity in individual subjects and for the design of their appropriate therapy.

Studies on speech adaptation of respiration have also been initiated. A remarkably sensitive and informative method of airflow perception, developed by Dr. Svend Smith, a ten-week guest from Denmark, has been introduced--the first such instrument in America. By this instrumentation, the minute air pulses of speech are recorded, along with the sounds. This highly sensitive and discriminate recording makes possible the study of the fine articulations of human speech. Currently, Dr. Smith is preparing an adaptation for simultaneous and differential recording of airflow from the nose and from the mouth under a developmental contract from this Section. This dual instrument will aid in demonstration of the actions of the soft palate in speech.

As a separate speech physiological observation, the influence of obturators upon palatal patencies have been observed by Drs. Ralph L. Shelton and Ralph



Lloyd. This observation has been made by speech recording on tape and also by cinema observation of facial movements. The effect of surgical removal of parts of the soft or of the hard palate are highly similar, and these nasality distortions are abruptly improved by plastic obturators. The nasality of congenitally cleft persons is acoustically similar to that of those having surgical removals, but the placement of an obturator does not afford abrupt and uniform improvement in nasality. In palatal deficiency of either kind, the subjects variably employed similar patterns of movement distortions in the face of exaggerations of closing actions about the mouth and the nose. This observation is being prepared for cinema publication.

Drs. Shelton and Bosma also employed cinema and tape recording to study the actions of the soft palate, tongue and pharynx in twenty subjects who had surgical removals of parts of the face by reason of local cancer. These patients, most of whom were at the Sloan Memorial Hospital in New York, afforded unique opportunity for study of these motions, which are otherwise not accessible to observation. Particular details of related motions of palato-pharyngeal wall and tongue have been discussed for the first time. This material is in preparation as an extensive documentation cinema.

(c) Anthropology: We shall add an anthropologist, Dr. Melvyn Baer, to this group in July 1962, on a Visiting Scientist appointment for a period of one year. In anticipation of his work with us, preliminary and methodological studies are in progress in which immature experimental animals are being injected with alizarin red-S, which stains the portions of skeleton currently being calcified. By appropriately scheduled studies of these animals in litters, it is possible to define the portions of facial and cranial skeleton currently growing. The general schedules of growth in these areas can thus be described in normal animals and in those impaired by local surgery or imposed neurological disease.

#### MEDICAL INVESTIGATIONS

At the beginning of the year, Dr. A. D. Merritt, who had been Chief of the Medical Investigations Section, left NIDR to become associate Professor of Medicine at Indiana University. His project report "Genetic Studies in a Population Isolate (Brandywine) with Particular Reference to Hemoglobin and Haptoglobin Patterns" covers work done with Dr. Witkop. Sickle cell hemoglobin has been found in approximately 20% of the tri-racial isolate population (approximately 10% of the national Negro population), and a 2:1 "modified" haptoglobin pattern was found in a correspondingly increased percentage. These findings will serve as a correlation point for other disease states in the tri-racial isolate study.

Other studies include "Familial Neonatal Hepatitis - Study of a Large Family," and "Hereditary Renal Dysfunction with Associated Anomalies," by Dr. Cassady. These include genetic, clinical and laboratory studies on a family of 35 members with a high frequency of "giant cell hepatitis of the newborn" and a study of six families with hereditary renal dysfunction. In addition, studies have been begun at Gallaudet College for the Deaf on a number of individuals with hearing impairment and abnormal urinalysis. These studies will be further described in the part listed under the Human Genetics Section.



## HUMAN GENETICS

During the last year, the Human Genetics Section directed considerable attention to program expansion and the recruitment of qualified personnel to round out a program in human genetics.

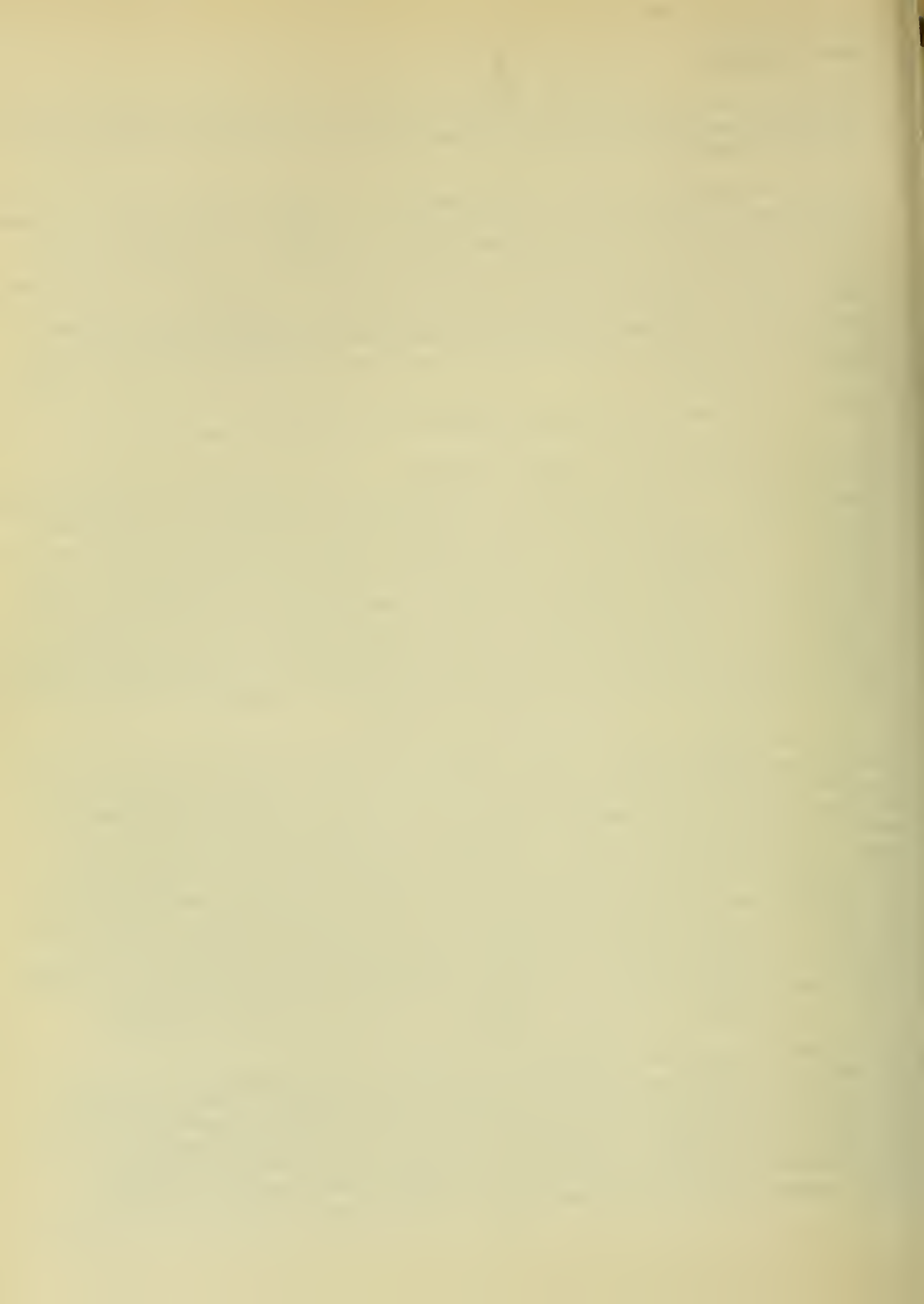
Two new program areas have been developed in the Human Genetics Section through recruitment of new personnel. This includes activities in cytogenetics and statistics. Dr. Herbert Cooper came on duty on December 1 to take charge of the cytogenetics unit, where he will primarily deal with defects of chromosomes, translocations and the genetics of tissue culture. In the area of statistics and research in genetic analyses, we have been able to recruit Dr. Bertram Hanna and Dr. C. Chung. These men are developing high-speed computer programs of analysis of various types of genetic data, especially those involving large population studies.

Brief descriptions of the primary projects of this Section are given below.

(a) Hereditary Defects in Enamel and Dentin: This project was initiated by a survey of 100,000 school children in the state of Michigan for the prevalence of these defects. It was determined that dentinogenesis imperfecta occurs about once in 8,000 individuals and that amelogenesis imperfecta occurs about once in 16,000 in the general population of the state of Michigan. At least two distinct genetic diseases of dentin exist, dentin dysplasia and opalescent dentin. At least five distinct diseases of enamel formation exist: hypoplasia of enamel, inherited as a sex-linked dominant trait; hypocalcification, as an autosomal dominant trait; hypomaturation, as a sex-linked recessive trait; pigmented hypomaturation, as a recessive trait; and local hypoplasia, as an autosomal dominant trait. Methods of restoration and treatment have been compared in a large series of patients by Dr. Lloyd and Dr. Driscoll.

(b) The Brandywine Study: The Brandywine Study was the first large population study of the Human Genetics Section. This is a study of 5,000 individuals of Caucasian, Amerindian and Negro ancestry, who reside in Southern Maryland and who have had their marriage patterns restricted to this small isolate for several hundred years. To date, information has been collected on the approximately 5,000 living individuals, as well as some 12,000 deceased predecessors. This group essentially furnishes us with a genetic population laboratory. At the present time, most of the historical, genetic, social, physical, dental and laboratory data have been accumulated and are now being processed for machine analysis. A complete genetic, medical and dental history was obtained from each individual. Each individual was given a physical and dental examination. Laboratory procedures included a complete genotyping, abnormal hemoglobins, serum electrophoresis and saliva studies.

To give some idea of the mutational load present in this population, 20.7% of its members are sicklers, the highest known rate outside of Africa; 1.6% are albinos, the highest known rate of albinism in man; 3.7% have opalescent dentin, the highest known rate of this condition in man; as well as 23 other well-defined, simply inherited conditions. A study of the biochemical defect in albinism by Dr. C. Witkop, NIDR; Dr. E. Van Scott, NCI; and Dr. G. Jacoby, NIAMD, showed that the most common type of this disease is not due to a lack of



the enzyme tyrosinase, but appears to be a defect in a tyrosine transport system. These findings strongly suggest that this disease can be treated - in fact, these workers have induced local pigmentation in these subjects.

(c) A study of Tri-racial Isolates in Eastern United States: This project was developed from the Brandywine project when the question was asked, whether or not other inbred populations of a similar nature existed in the United States.

Through the cooperation of Dr. Calvin Beale of the Department of Agriculture, formerly of the Bureau of the Census, it was determined that approximately 100,000 people are members of such isolates residing along the eastern seaboard. These extend from New York to Louisiana, and we have identified 26 such populations, all of whom show some type of hereditary abnormality. Investigations of these isolates and their genetic diseases led to the next study of hereditary dyskeratoses.

(d) Cytological Investigation of Hereditary Dyskeratoses and the Effects of Cancer Chemotherapeutic Agents on Cell Division: In the Haliwar isolate of North Carolina, a unique disease leading to blindness, associated with a lesion of the bulbar conjunctiva and a white lesion of the oral mucosa, was studied to determine whether or not this was a genetic disease or an environmentally induced condition. A cytological investigation of this disease and other hereditary oral dyskeratoses revealed a new method of diagnosis by exfoliative cytology. The cell defect in two of these diseases appeared to arise from abnormal cell division. A study of epithelial cell division found that this cell change could be experimentally induced in humans by certain cancer chemotherapeutic drugs. Through this work a method of predicting toxicity with those drugs and a better method of drug selection shows hope of reducing drug trial times from three months to three days. Results of these investigations led not only to the description of a new hereditary disease of mucosa and conjunctiva, but also to investigations of a new neurological condition which is inherited as a recessive trait, causing mental deficiency, spasticity, and ichthyosis. This latter condition is of unusual interest to the dentist because of the oral neurological abnormalities. These have been recorded in motion picture form. It was determined that loss of sensation in the oral cavity resulted in abnormal motor function. This concept of sensory loss has led us to reevaluate other apparent oral motor disturbances and may offer a solution to some of these defects and the reevaluation of certain types of speech therapy. This disease appears to be a biochemical error involving amino acid metabolism - the aminoaciduria is being investigated.

(e) A Familial Study of Kidney Disease: Three observations concerning certain oral diseases led to a study of a familial form of kidney disease: (1) The oral-neurological ichthyosis disease was known to have an associated kidney defect with aminoaciduria; (2) pedigrees of certain tooth defects indicated peculiar genetic ratios that appeared to violate Mendelian segregation; and (3) certain oral clefts with ear defects were associated with an abnormal number of chromosomes. Dr. George Cassady, Mr. Maimon Cohen, Dr. Bertram Hanna and Mr. Ronald Robinette found indications that a type of hereditary renal dysfunction involved similar mechanisms. Because sufficient patient material appeared available, a study of this disease was undertaken to define this peculiar type of inheritance in man. At first this kidney disease appeared to be a rare condition which was





manifested by hematuria, beginning in childhood; a peculiar loss of hearing, which was evident early only on audiograms in the range of 4,000 cycles; associated defects of the eye, such as astigmatism and myopia; a possible increase in the frequency of middle ear infections; and an abnormality of the external ear. The first point of attack on this illness was the epidemiological aspect. Closely associated with this were the clinical aspects. As a result, seven large kindreds from the general population were obtained, and field studies were set up to investigate all known relatives. Patients were admitted to the Clinical Center for detailed study of the clinical aspects of the condition. These studies have shown the following: (1) The condition is not uncommon. For example, routine examination of the out-patients admitted to the Dental Clinic revealed five new propositi within one month; (2) additional families were accumulated by contacting local physicians; (3) Because loss of hearing was one of the outstanding manifestations of the illness, children attending Gallaudet School and Gallaudet College were examined, and additional cases were found in this population; (4) foam cells are found in the kidneys of affected persons; (5) thus far, lipo protein electrophoresis patterns appear to be abnormal in affected persons; (6) for the first time a "new" type of inheritance has been reported in man, involving preferential segregation of the autosome carrying the defective gene with the X-chromosome.

While we have no direct estimate of the frequency of this disease, it becomes obvious, when compared to other fairly rare conditions, such as opalescent dentin, which occurs about once in 8,000 individuals, that this kidney disease is much more frequent - perhaps in the order of one in 400 individuals.

(f) A Study of Consanguineous Marriages in Nagasaki and Hiroshima: This study was initiated by Dr. James Neel of the University of Michigan, and Dr. Jerry Niswander from our Section was invited to do the oral aspects of the study. This involved examination of the offspring of 5,000 consanguineous matings who were unirradiated, and a comparable control group to determine the effects of consanguinity. One of the important pieces of information that this study attempted to determine was the average number of deleterious recessive genes carried by man. This figure is important in determining safe levels of total lifetime radiation exposure.

The data for this study have now been collected and are being processed at the University of Michigan, where Dr. Niswander is obtaining a Ph.D. degree in genetics. One of the important observations that he made was that developmental time, as indicated by tooth eruption time, can be changed markedly in the same population by environmental factors.

(g) A Nutritional Survey of the Chilean Population: In 1960 a nutritional survey of the Chilean population was conducted under the sponsorship of the ICNND. Dr. Witkop of this Section participated as the American representative, and Dr. Luis Barros of the Chilean Army participated as the Chilean dental representative. Dr. Barros is at present a Visiting Fellow in the Human Genetics Section; his primary duty is to process the data obtained in this study. The objective of this study was to determine the nutritional status of 3/4 of 1% of the entire civilian population and 10% of the military population. Every fifth person in this original sample was given a dental examination.



Every other person who obtained a dental examination had a blood sample drawn for nutritional determinations, hemogram, genetic abnormalities of the erythrocyte and hemoglobin, and a genotyping. In addition to information collected on caries and periodontal disease, prevalence of 14 oral anomalies was determined on this sample. This sample was selected to represent a low and medium socio-economic, geographic and age distribution equivalent of the entire Chilean population. Three significant findings can be reported at this time. These have been described in detail in the recent publication, "Nutritional Survey of Chile, March-June, 1960, Interdepartmental Committee on Nutrition for National Defense, August, 1961."

(h) A Study of Genetic Factors in Saliva: This project is being conducted by Dr. Wolf of the biochemistry unit and consists of an electrophoretic and immunochemical study of salivary proteins. The initial approach in this study has been to determine the occurrence and factors that govern the genetics of secretor factor in saliva, localization of the secretor factor in the various glands concerned, and the relationship of secretor factor titrations to other genetic markers and diseases. The second aspect of this study concerns the use of electrophoretic techniques to separate from the saliva the various constituent protein elements and to determine if these are under genetic control. At the present time, Dr. Wolf has developed a knowledge of the various factors that cause variation between individuals.

(i) A Study of Hemoglobinopathies: This study was conducted by Dr. Rucknagel and Dr. Schneiderman. It is now nearly complete, as far as the field and laboratory data are concerned. These data are now being analyzed for selective factors in the populations, which apparently are such as to maintain a very high frequency of this gene in the Brandywine population. In addition to this, several new hemoglobinopathies have been under investigation. These apparently do not coincide with any of the known defects reported to date.

The above include the major studies of the Section. In addition to these, numerous families and patients are being followed for what appear to be genetic defects, such as familial neutropenia, causing a severe periodontal condition in the patients in whom it appears; various disorders of connective tissue, such as osteogenesis imperfecta, where enzyme studies have shown a phosphatase abnormality in developing teeth and bone; a familial form of neonatal hepatitis; and a hereditary form of osteoarthritis, associated with extensive degenerative disc disease. This latter disease is common in a large Caucasian population residing in Southern Maryland.



Serial No. NIDR-53 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies on the Etiology and Control of Rampant Dental Caries.

Principal Investigator: Dr. R. M. Stephan

Other Investigators: None

Cooperating Units: Dental Clinic, Clinical Center, NIH

Man Years (calendar year 1961)

Total: 1 1/2  
Professional: 1/4  
Other: 1 1/4

Project Description:

Objectives:

Rampant dental caries is a very severe form of dental caries in which practically all of the teeth are attacked by decay in a relatively short period of time. It is found chiefly in young children, but may develop in adults who previously had little or no caries experience. Under suitable experimental conditions comparable forms of rampant caries can be developed in laboratory animals such as rats and hamsters. From a research standpoint rampant caries offers a most favorable opportunity to study the basic factors which activate or control the caries process because the usually prolonged time element is reduced to a minimum and the determination of caries activity can be much more certain than in caries of usual severity. The purpose of this project is to evaluate in clinical studies the many factors which may be important in different cases of rampant caries, and to study the more important of these factors in laboratory and animal experiments. Efforts have been concentrated on isolating specific microorganisms from carious lesions and tooth surfaces in humans, determining the occurrence of these organisms in different cases, and testing the possible cariogenic properties of the organisms by inoculation in multifactorial animal experiments. Special attention is also being given to an evaluation of the cariogenic properties of the dietary materials consumed by patients with rampant caries, and the further testing of these dietary materials



Part A (continued)

in animal experiments.

Methods Employed:

In addition to the usual oral and medical examinations and laboratory tests, a detailed history is taken in regard to the family, to diet and illness during the period of tooth formation, to the eating habits and habits affecting oral hygiene during the period in which caries developed, and to a comparison with these factors in siblings. Direct stereomicroscopic observations in the oral cavity are made at 6X-40X magnifications with a Zeiss "Otoscope." Intra-oral pH measurements are made with micro-antimony and glass electrodes and with microapplicators containing colorimetric indicators. Measurements of parotid salivary flow rates are made with the "Lashley Cup" apparatus. Samples of plaques and other material from the teeth, periodontal pockets, and mucous membranes are taken with dental explorers, swabs, and specially designed microscalers for quantitative samples which can be weighed on a microbalance. These samples are studied in micro culture slides by phase microscopy, in smear preparations using the indirect fluorescent antibody technique to label specific microorganisms, in gram stained smears, and in selective culture media.

In animal experiments, the mouths of rats and hamsters are inoculated with pure cultures of bacteria isolated from caries to test the potential cariogenic effect of these organisms. Pretreatment of weanling animals with antibiotics is used in some of the experiments to reduce the resident flora before inoculation. Also diets of different cariogenicity and strains of animals of different caries susceptibility are employed in multifactorial arrangements so that all combinations of these factors are equally represented and so that any particularly favorable combination of animal strain, diet, and microorganism, may be found.

Major Findings:

During the past year significant progress has been made in bringing to a focus the clinical, laboratory, and animal experimental observations in regard to the etiology of caries. Concerning the infectious nature or transmissibility of caries among humans, from humans to laboratory animals, and among laboratory animals the following findings are of particular interest.

- (1) Rampant caries frequently occurs in one child in a family but not in the other children or in the parents with whom the child is in daily contact.





Part A (continued)

- (2) Under the phase microscope, different forms of microorganisms can be found in plaque material from different carious lesions in the same mouth at one time. Thus massed colonies of coccal forms may be seen to make up the bulk of plaque material in one carious lesion, masses of filamentous forms in another lesion, and masses of short rods forms in still another carious lesion. Quantitative cultural studies have indicated the first to be predominantly streptococci, the second, leptotrichia, and the third, lactobacilli, respectively. Monilia forms predominate in some cases of rampant caries.
- (3) Six different strains of streptococci isolated from cases of rampant caries have been studied by the fluorescent antibody technique. When inoculated into animals fed cariogenic diets an increase in caries was found in animals inoculated with one strain but not with the other five strains.
- (4) One important clinical finding in regard to diet is that children who have developed rampant caries generally eat essentially the same meals as their siblings who have not developed rampant caries, but their between meal eating habits often are entirely different. Almost all of the children studied who had developed rampant caries had a habit of frequent between meal eating of foods such as cookies, candy, soft drinks, and sandwiches.

Significance to Dental Research:

The dental profession can generally treat caries successfully by operative and restorative means, but most dentists and patients feel overwhelmed when confronted with the need for filling and refilling all of the teeth in children with rampant caries. They have tried to apply various theories and methods for prevention of caries, but many of these are conflicting and have not solved the problem so that dentists in this area are generally happy to refer patients with rampant caries to N.I.D.R. for study. We are now in a position to make a much more comprehensive study of caries etiology that has previously been done, and study the local oral and the general systemic condition of patients, together with advanced methods for laboratory investigation and animal experimentation to test clinical findings.

Proposed Course of Project:

It is planned to follow through on the studies in progress, to develop further the studies using the fluorescence antibody to show specific microorganisms, and to set up cooperative studies



Part A (continued)

with other investigators who wish to study particular areas of bacteriology or biochemistry in these patients with rampant caries.

Part B included: No



Serial No. NIDR-54 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Effect of Dietary Phosphates on Dental Caries in Children.

Principal Investigators: Drs. I. I. Ship and O. Mickelsen, NIAMD

Other Investigators: Drs. F. J. McClure, R. C. Likins, I. Zipkin  
A. L. Russell, and Mr. C. L. White, NIH  
Dr. H. Schraer, The Pennsylvania State University  
Drs. G. E. Waterman, B. Bosley, and Miss H. G.  
Olson, Division of Indian Health  
Misses M. Talcott and B. Wenberg, South Dakota State  
College of Agriculture and Mechanic Arts

Cooperating Units: Division of Indian Health, DI  
Home Economics Department, South Dakota State College  
of Agriculture and Mechanic Arts Department of Physics,  
the Pennsylvania State University Bureau of Indian  
Affairs, PHS

Man Years (calendar year 1961) :

Total:	1/3
Professional:	1/3
Other:	None

Project Description:

Objectives:

To determine whether the addition of phosphates to the diets of children will reduce the incidence of dental caries; whether the addition of phosphates to the diets of children will affect growth and development; and to evaluate the safety of the phosphates and calcium added to the diets.

Methods Employed:

Two percent calcium phosphate has been added to the bread in four boarding schools and the addition of a placebo (flour) to the bread in four control schools. Neither the subjects nor the investigators know the identity of the schools. Total number of children in the study is 1800. Routine examinations are: an annual dental examina-



Part A (continued)

tion including bilateral bite-wing radiographs on all children; quarterly measurements of height and weight on all children; annual bone density determinations using hand radiographs on a sample of 200 children from both the control and supplemented schools; annual saliva collection and analysis on a sample of 200 children from both the control and supplemented schools; regular food intake surveys including collection and analysis of representative diets in all schools; annual physical exams for nutritional status on a sample of children in both control and supplemented schools; and annual blood and urine collections on a sample of children in both groups of schools for hemoglobin, calcium, phosphorus, and magnesium determinations. During the past summer 9 girls (9 to 11 years of age) from two schools receiving the calcium phosphate supplement and 9 girls from two control schools spent four weeks in the metabolic ward of the Clinical Center. Balance studies were carried out on these children for calcium, phosphorus and magnesium.

Major Findings:

1. At the time of the spring 1961 dental examinations, no X-rays were secured. On the basis of visual examinations only, there was no difference in the increments of either decayed, missing and filled teeth (DMFT) or surfaces (DMFS) between the children in the control and supplemented schools.
2. Since there have been reports that experimental animals develop deficiencies of trace minerals when extra calcium is added to the diet, hemoglobin determinations were done on about 100 children in two control schools and an equal number in two supplemented schools. Preliminary results suggest no difference in hemoglobin levels of the children receiving the extra calcium from those in the control schools. Less than one percent of the children had hemoglobin levels below 10 g. per 100 ml. with the average for these 8 to 11 year olds being 14 g.
3. The reports of the bread consumed by the children during the second half of the last school year suggested that the calcium intake in the supplemented schools was about 2.5 g. per day. To validate the bread consumption figures, a system has been established for collecting data on bread and milk waste. This involves the weighing of all bread and milk left by the children on their trays.

Significance to Dental Research:

1. Since the data so far suggest that dietary phosphate supplements are inactive in preventing dental caries in children, the major findings of the project will be those pertaining to the effects of a high calcium intake for a period of almost two years. It is for this rea-





Part A (continued)

son that the studies of that part of the program are being intensified.

2. The study will also provide considerable information on the growth and development of Indian children. This aspect of the study will include heights, weights, eruption of teeth and dentition.

Proposed Course of the Project:

1. The study will be terminated at the end of the present school year (May 1962).

2. A general report or a survey of the entire project will be prepared for publication possibly as a supplement to the Public Health Reports. This publication will only foreshadow other more detailed reports covering the various aspects of the work.

Part B included: No



Serial No. NIDR-55 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies on the Etiology and Control of Dental Erosion.

Principal Investigator: Dr. R. M. Stephan

Other Investigators: None

Cooperating Units: Dental Clinic, Clinical Center, NIH

Man Years (calendar year 1961)

Total: 1/4  
Professional: None  
Other: 1/4

Project Description:

Objectives:

Dental erosion is a condition in which enamel and dentin are worn or dissolved from certain limited areas of exposed tooth surfaces, and which may lead to practically complete destruction of the tooth crown. It generally develops in adults and progresses with increasing age. Certain forms of experimental dental erosion can be developed in laboratory animals by feeding acid diets and acid drinking fluids, but in humans the etiology is not always apparent, and in some cases the use of abrasive dentifrices, excessive tooth brushing, or some abnormal metabolic process has been suspected. The objective of this study is to determine the etiological factors in patients who have developed severe forms of dental erosion and to find means for prevention of its progress.

Methods Employed:

In addition to the usual oral and medical examinations and laboratory tests, a detailed history is taken in regard to dental erosion in the family, disturbances during the tooth formative period, diet and eating habits, tooth brushing habits, and the use of dentifrices and mouth washes, unusual illness, chronic vomiting or metabolic disturbances, the use of medication in any form, and the presence of any chemical fumes in the environment.



Serial No. NIDR-56 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Further Studies on Periodontal Dressings

Principal Investigator: Dr. Paul N. Baer

Other Investigators: Dr. F. W. Wertheimer

Cooperating Units: Dr. Charles L. Sumner III  
Walter Reed General Hospital  
Washington, D.C.

Man Years (calendar year 1961)

Total: 1/3  
Professional: 1/3  
Other: None

Project Description:

Objectives:

1. To study the effects of several periodontal dressings in periosteal covered and denuded bone.
2. To determine the efficiency of a periodontal dressing in treating the acute phase of necrotizing ulcerative gingivitis.

Methods Employed:

1. Eugenol and non-eugenol containing periodontal dressings were placed against periosteal covered and denuded bone of rats skulls. After one and two week periods the animals were sacrificed and the involved areas were studied histologically.
2. Soldiers in the field suffering from necrotizing ulcerative gingivitis, were treated for the acute phase by covering the involved areas with a hydrogenated fat zinc bacitracin periodontal dressing. A total of 300 patients were treated in this manner at Army camps.

Major Findings:

1. (a) Periodontal dressings, when placed against bone denuded



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Baer, P. N. and Wertheimer, F. W.: A Histologic Study of the Effects of Several Periodontal Dressings on Periosteal covered and Denuded Bone, Jour. Den. Res. 40:858:1961.
2. Sumner, C. F. and Baer, P. N.: Necrotizing Ulcerative Gingivitis: Treatment of the Acute Phase. Oral Surg., Oral Med., Oral Path. 14:1391:1961.

Honors and Awards Relating to this Project: None





Serial No. NIDR-57 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies on Periodontal Disease in the Mouse

Principal Investigator: Dr. Paul N. Baer

Other Investigators: None

Cooperating Units: Dr. Lyman B. Crittenden  
United States Department of Agriculture  
Regional Poultry Research Laboratory  
East Lansing, Michigan

Man Years (calendar year 1961)

Total: 1/3  
Professional: 1/3  
Other: None

Project Description:

Objectives:

In a previous study on the genetic aspects of periodontal disease in mice we reported that the offspring of reciprocal crosses exhibited a degree of alveolar bone loss which was dependent on that found in the maternal line. Therefore, we designed an experiment to determine whether this effect was transmitted through the mother's milk.

Methods Employed:

Pairs of STR/N mice (susceptible to periodontal disease) and DBA/2JN mice (resistant to periodontal disease) which had litters born on the same day, had the litters divided so that each STR/N mother nursed half her own litter as well as an equal number of newborn DBA/2JN mice. The DBA/2JN mother in turn, nursed equal numbers of her own offspring and STR/N newborn mice.

Major Findings:

1. The difference in periodontal scores between the DBA/2JN mice and the STR/N mice was highly significant and of the same order of magnitude as observed in previous experiments.



Part A (continued)

2. A "milk factor" does not appear to be the cause of reciprocal cross differences previously observed.

Significance to Dental Research:

These studies should contribute to our knowledge of maternal influences on periodontal disease.

Proposed Course of Project:

To continue the study of dietary and maternal influences on periodontal disease.

Part B included: Yes



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Baer, P. N. and Crittenden, L. B.: Studies on Periodontal Disease in the Mouse. V The Milk Factor, Arch. of Oral Biology (in press).

Honors and Awards Relating to this Project: None



Serial No. NIDR-58 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: "Three Dimensional Studies of Periodontal Tissues."

Principal Investigator: Dr. H. R. Stanley

Other Investigators: None

Cooperating Units: Pathologic Anatomy Laboratory of National Cancer  
Institute

Man Years (calendar year 1961)

Total: 1/2

Professional: 1/4

Other: 1/4

Project Description:

Objectives:

1. To establish more completely the array of morphohistological changes that occur within the buccal and lingual surfaces of the periodontium.
2. To evaluate the concept of the "Cyclic Phenomenon."
3. To evaluate and determine the incidence of the interproximal periodontal "col".

Methods Employed:

Human jaw specimens are obtained at autopsy, photographed, X-rayed, and measured grossly. Gingival crevices are measured on the buccal, lingual, mesial, and distal surfaces of the teeth. Impressions and casts are constructed. Specimens are then fixed in 10% formalin for at least 72 hours and dimensional changes recorded. After decalcification in 5% formic acid, the specimens are trimmed and blocked through the crowns of the teeth to preserve the interproximal tissues. The tissues are processed routinely, embedded in paraffin and cut serially, keeping track of every stroke of the microtome in order to know the exact depth of penetration. Every fifth and sixth section is mounted and every 20th section is stained with eosin and hematoxylin. Specific distances are measured bet-





Part A (continued)

ween certain histologic and pathologic landmarks of the periodontium employing a stage micrometer at x35. Also the following landmarks are plotted on graph paper: the level of the gingival crest, the lowest point of the calculus plaque, the bottom of the epithelial attachment, and the level of the alveolar crest. When the respective points of every 20th section are connected on the graph, a "profile" of a periodontal surface is produced which enables an investigator to see at a glance all the histologic and pathologic variations and relationships that occur within this heterogenous structure.

Major Findings:

Previous studies have shown that essentially the same results are obtained by the measurement of every 20th section as every section.

Statistical relationships have been established between local pathologic factors as well as their influence upon certain measured distances. For instance we have found that the shape of the subgingival calculus significantly influences three distances that extend respectively from the bottom level of the subgingival calculus to the bottom of the epithelial attachment, to the alveolar crest, and to the deepest level of the inflammatory cells in the periodontal membrane. Their average values were greater when approximating scaly subgingival calculus and smaller when approximating nodular subgingival calculus. A hypothesis termed the "Cyclic Phenomenom" has been proposed to describe an interplay of actions occurring between the gradual build-up of scaly and nodular calcular formations and a diminishing tissue tone within the interdental papilla. When the tissue tone of the papilla is reduced by chronic inflammation to a point that it can no longer determine the shape of the calculus, as exemplified by a shift of the calculus mass from a scaly to a nodular form, the calculus then begins influencing the shape of the interdental papilla. The build-up of the nodular calculus masses on approximating tooth surfaces impinges upon the papilla until the tissue above the impinging masses is severed. Beneath a bridge of calculus formed by the approximating nodular masses a new papilla regenerates which eventually is again subjected to the same turn of events.

Significance to Dental Research:

This study has vividly demonstrated how certain histological characteristics appearing in one histologic section can encourage very misleading assumptions and interpretations in respect to the total microscopic picture and especially to treatment planning.



Part A (continued)

ween certain histologic and pathologic landmarks of the periodontium employing a stage micrometer at x35. Also the following landmarks are plotted on graph paper: the level of the gingival crest, the lowest point of the calculus plaque, the bottom of the epithelial attachment, and the level of the alveolar crest. When the respective points of every 20th section are connected on the graph, a "profile" of a periodontal surface is produced which enables an investigator to see at a glance all the histologic and pathologic variations and relationships that occur within this heterogenous structure.

Major Findings:

Previous studies have shown that essentially the same results are obtained by the measurement of every 20th section as every section.

Statistical relationships have been established between local pathologic factors as well as their influence upon certain measured distances. For instance we have found that the shape of the subgingival calculus significantly influences three distances that extend respectively from the bottom level of the subgingival calculus to the bottom of the epithelial attachment, to the alveolar crest, and to the deepest level of the inflammatory cells in the periodontal membrane. Their average values were greater when approximating scaly subgingival calculus and smaller when approximating nodular subgingival calculus. A hypothesis termed the "Cyclic Phenomenom" has been proposed to describe an interplay of actions occurring between the gradual build-up of scaly and nodular calcular formations and a diminishing tissue tone within the interdental papilla. When the tissue tone of the papilla is reduced by chronic inflammation to a point that it can no longer determine the shape of the calculus, as exemplified by a shift of the calculus mass from a scaly to a nodular form, the calculus then begins influencing the shape of the interdental papilla. The build-up of the nodular calculus masses on approximating tooth surfaces impinges upon the papilla until the tissue above the impinging masses is severed. Beneath a bridge of calculus formed by the approximating nodular masses a new papilla regenerates which eventually is again subjected to the same turn of events.

Significance to Dental Research:

This study has vividly demonstrated how certain histological characteristics appearing in one histologic section can encourage very misleading assumptions and interpretations in respect to the total microscopic picture and especially to treatment planning.



Part A (continued)

It has become obvious that before an investigator can draw any conclusions associating certain characteristics of a peridontal lesion with the presence of calculus, bone resorption, the length and migration of the epithelial attachment, or loss of principle fiber attachment and the pathogenesis of the disease, he must be certain that the associations are maintained through a reasonable thickness of tissue since the characteristics change so frequently.

- Proposed Course of Project:

1. Having established the minimal number of step-serial sections needed to include all the morphohistologic variations of the periodontium, many more periodontal "profiles are to be constructed. This appears to be the best mechanism for revealing the three-dimensional aspects of periodontal lesions. Many more profiles in various situations, both normal and diseased, are to be charted to increase our knowledge and understanding of the total concept of the periodontium and neutralize or destroy many of our erroneous, preconceived notions.
2. The profile technic will also permit us to evaluate the "Cyclic Phenomenon" and the "col", recently described by Cohen of England.

Part B included: Yes



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Stanley, H. R.: A three dimensional morphologic perspective of periodontal tissues. To be published by the J. D. Res. in 1962.
2. Stanley, H. R.: The value of periodontal surface "Profiles" in the three dimensional study of periodontal lesions. To be published in the J. of Periodontology.

Honors and Awards relating to this project:

None





Serial No. NIDR-59 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies Related to Periodontal Disease  
1. Histopathology and Histochemistry  
2. Epidemiology

Principal Investigator: Dr. F. W. Wertheimer

Other Investigators: Dr. Harold M. Fullmer, Dr. Robert H. Brewster

Cooperating Units: Armed Forces Institute of Pathology, Interdepartmental Committee on Nutrition for National Defense Governments of Trinidad and Tobago, St. Lucia, St. Kitts, Nevis, and Anguilla, The West Indies.

Man Years (calendar year 1961)

Total: 1/2  
Professional: 1/2  
Other: None

Project Description:

Objectives:

During the year 1961 work was continued in attempting to identify by histochemical and routine staining methods the composition of the human secondary dental cuticle. Part of this time was spent at the A.F.I.P. in Oral Pathology training status. During this time odontogenic cyst material was obtained to study "hyaline bodies" that had been reported as occurring in the walls of odontogenic cysts. The suggestion that these hyaline bodies might have the same composition and a similar derivation as the secondary dental cuticle prompted this comparative study. A dental survey was conducted of the West Indies in conjunction with the ICCND. Data were gathered about periodontal disease and dental caries.

Major Findings:

The human secondary dental cuticle was found to be unique in its reaction to histochemical and routine staining procedures. Within the limits of histochemical techniques on such fixed and decalci-



Part A (continued)

filled material the cuticle was found to contain protein, carbohydrate, and presumably lipid. Although obviously not a true keratin it is suggested that the secondary dental cuticle is a keratin like substance and is probably a product of the odontogenic epithelia. This hypothesis was further strengthened by finding that the hyaline bodies in the epithelial linings of radicular cysts and a cuticle on the apex of tooth roots involved by radicular cysts reacted to the battery of stains and histochemical reactions in the same unique way as had the secondary dental cuticle. All of these three widely separated structures were associated with epithelia from odontogenesis.

The data from the West Indies survey are being analysed at present. The information obtained to date indicates that the D.M.F. rate is high and is comparable to that found by a group of dental examiners in Baltimore. However the dental needs are much greater than in Baltimore. For example the ratio of filled to decayed teeth (F/D) in Baltimore is 1/1.5 for non-whites, 8/1 for whites, while the West Indies ratio varies from 1/3.5 to 1/121 in the several islands surveyed.

Periodontal disease was significantly higher in the West Indies than in Baltimore. This is attributed in part to the lack of both oral hygiene and professional dental care in the West Indies. No oral lesions were found that could be directly attributed to nutritional deficiency.

Significance to Dental Research:

Since periodontal disease is initiated in the area of the secondary dental cuticle, any information pertaining to the composition and derivation of the cuticle may add to a better understanding of the initiation and progression of periodontal disease.

Data from the West Indies ICNND survey will add new information to the increasing collection of epidemiological data on periodontal disease and will also allow a comparison with extensive nutritional biochemical and dietary information.

Proposed Course of Project:

Further comparative studies are in progress to shed light on the function of the human secondary dental cuticle. Attempts to obtain fresh material for frozen sections for more reliable histochemical data, especially lipid reactions are proposed for the future.

Further analysis of the dental data obtained from the West Indies



Part A (continued)

survey will be made and compared with biochemical and dietary information.

Part B included: Yes



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Individual Project Report  
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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Wertheimer, F. W., Fullmer, H. M.: Morphologic and Histochemical Observation on the Human Dental Cuticle. J. Periodont. (To be published).

Honors and Awards Relating to this Project: None





Serial No. NIDR-60 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Histopathology of the Human Pulp

Principal Investigator: Dr. H. R. Stanley

Other Investigators: Drs. H. Swerdlow and R. Lloyd

Cooperating Units: Dental Department, Clinical Center

Man Years (calendar year 1961):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

1. To determine the healing capacity of normal and diseased pulps following drilling procedures per se.
2. To determine the irritating qualities of permanent and temporary filling materials compared to zinc oxide and eugenol. This study will include the effects of silicate cements, self-curing acrylics, and pulpdent. The effects of various cavity liners and sterilizing agents will also be considered.
3. To determine the characteristics of pulp pathology which are the result of pressure changes when frictional heat is neutralized.
4. To determine the range of pathologic findings in the pulp resulting from various degrees of carious involvement.
5. To determine the amount of pathology that might be produced by supercooling of teeth by air-water spray technics.
6. To determine the mitotic frequency of the odontoblasts and other less differentiated cells of the pulp and the time sequence of pulpal regeneration.
7. To determine the contribution of argyrophilic granules within



Part A (continued)

odontoblasts to the formation of Korff's fibers.

Methods Employed:

In the filling material, cavity liner, and sterilization studies, patients with intact teeth to be extracted are selected and Class V cavity preparations cut. The teeth are restored and extracted post-operatively to meet the requirements of the study. After fixation in formalin the teeth are decalcified in 5% formic acid, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin. Masson's trichrome, Wilder's reticulum stain, Feulgen's reaction, periodic acid-Schiff, toluidine blue, and other special stains are also utilized. Every attempt is made to standardize the categories to be compared in respect to age of the patients, tooth size, postoperative extraction interval, and remaining dentin thickness. The pulpal reactions are compared by recording the incidence of the inflammatory response, lesions predominating in leukocytes, and cellular displacement. The intensity of the inflammatory response and the cellular displacement are also recorded using an arbitrary scale of 0-3.

In the study concerned with the range of pathologic findings in carious teeth, the teeth with various degrees of caries but without restorations are selected for study and extracted without cavity preparations.

Major Findings:

1. Speeds of 50,000 rpm and over, with instruments utilizing the belt-driven or turbine principle, have been found to be less traumatic to the human pulp than technics utilizing 6,000 and 20,000 rpm with the conventional, ball-bearing handpiece. The value of coolants became more significant at the higher speeds. In the absence of adequate coolants, intermittent grinding was of no appreciable benefit. Preparation time at the higher speeds was of little consequence provided frictional heat was controlled. The combination of high speed, controlled temperature, and light load was conducive to minimal pulpal pathologic alteration.
2. When the effects on the pulp of manual versus mechanical condensation of amalgam were compared no significant histopathological differences were found. In the high speed technics, the amalgam raised the intensity of the initially mild response to cavity preparation to a level comparable to that obtained with the lower speed technics followed by zinc oxide and eugenol restorations. The mechanical aspects of amalgam condensation apparently



Part A (continued)

play a role in producing an increased pulpal response regardless of the physical properties of the restorative material itself.

3. When temporary fillings were compared, it was found that gutta percha produced the greatest number of responses with leukocytes predominating in 50% of the lesions as compared to only 6.3% in the ZNOE category. When eucalyptus oil was used with the gutta percha a definite decrease in response was noted. Since heat and pressure in the placement of this filling material are minimized by using this oil, it would appear that these factors contribute to the intensity of the pulpal irritation. The pulpal response with zinc phosphate cement was similar to that seen with temporary stopping. The reaction was not as severe as previously reported by others, but still was much greater than the baseline ZNOE category.
4. When teeth were surveyed for quantity of collagen, it was found that anterior teeth possessed more collagen than posterior teeth and that root pulp contained more collagen than coronal pulp tissue. The amount of collagen did not increase in the coronal pulp after 20 years of age. Bundle collagen increased in the root pulp to 39 years. Diffuse collagen in the root decreased between 10 and 49 years. Teeth containing more than 1.0 mm of irregular dentin filling the pulp chamber had more collagen than did similar teeth with no irregular dentin. From these findings it appears that increased collagen is not a direct result of aging but is a reflection of previous irritation or stimulation to the pulp. A tooth which is stimulated to produce irregular dentin also contains more collagen.

Significance to Dental Research:

1. Contradictory findings in the dental literature leave the profession in a quandary concerning the use of certain operative technics and filling materials. Much of this confusion apparently has emanated from the lack of appreciation of various investigators for the response of the pulp to operative trauma per se. Pulpal response can vary in incidence and intensity according to the speed and pressure employed in instrumentation, the thickness of remaining dentin, the post-operative extraction period, the type of coolant, and the size of the cutting tool. All these factors must be considered before evaluating accurately the additional irritating properties of permanent and temporary filling materials, cavity liners, and sterilizing agents.
2. Since in clinical practice carious teeth are usually treated, some attempt must be made to relate these studies to carious



Part A (continued)

teeth. Before we can determine the pulpal responses of carious teeth to various controlled technics we must determine some code whereby we can predict the quantity of pathology to be found in the carious teeth prior to experimentation. Some carious teeth present little or no pulpal pathology, whereas others present severe changes. This problem is most acute to the dental profession.

Proposed Course of Project:

1. Since we have shown that high speed technics are biologically safe for the human pulp, emphasis will continue on studies related to the additional effects of filling materials, cavity liners and sterilizing agents.
2. Odontoblasts have been considered incapable of mitotic division. Preodontoblastic cells in the cell rich layer are, however, believed capable of dividing into cells which differentiate into odontoblasts as needed. With the accumulation of a number of specimens revealing mitotic divisions approximating predentin, this whole problem of pulpal regeneration will be re-evaluated.

Part B included: Yes





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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Stanley, H. R. Factors of age and tooth size in human pulpal reactions. J. of Oral Surg., Oral Med., and Oral Path. April 1961, 14:498-502.
2. Stanley, H. R. The traumatic capacity of high speed and ultrasonic instrumentation (a review of the histopathological literature - requested by the Council on Dental Research of the American Dental Association) 63: Dec. 1961.
3. Swerdlow, H. and Stanley, H. Response of the human dental pulp to amalgam restoration. Accepted for publication by the J. of Oral Surg., Oral Med., and Oral Path.
4. Stanley, H. R. The cells of the dental pulp. Accepted for publication by the J. Oral Surg., Oral Med., and Oral Path.
5. Dubner, R. and Stanley, H. Response of the human dental pulp to temporary filling materials. Accepted for publication by the J. of Oral Surg., Oral Med., and Oral Path.

Honors and Awards:

Elected to an Associated in the International Academy of Oral Pathology, October 16, 1961.



Serial No. NIDR-61 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Histopathology of Buccal Mucous Membrane

Principal Investigator: Dr. H. O. Archard, Jr.

Other Investigators: Dr. H. R. Stanley

Cooperating Units: N.C.I., Medicine Branch, Dr. Paul Carbone and  
Dr. Robert Scoggins

Man Years (calendar year 1961):

Total: 1/2  
Professional: 1/4  
Other: 1/4

Project Description:

Objectives: To determine:

1. Normal variations in the histologic appearance of buccal mucous membrane through various ages.
2. Histologic changes seen in patients undergoing cancer chemotherapy.
3. Frequency of amyloid deposition in multiple myeloma patients and application of fluorescent stain technics to this problem.

Methods Employed:

Buccal mucosa specimens, obtained in vivo and at autopsy, are collected and preserved in 10% formalin. After routine staining procedures are carried out, the specimens are subjected to various special staining technics. The buccal mucosa specimens from multiple myeloma patients and autopsies are examined for amyloid with the crystal violet and Congo red methods. They are also examined with the use of a fluorescent dye (thioflavin-T) and ultra-violet light microscopy. In autopsied cases of multiple myeloma, sections of heart, kidney, and bowel (rectum, where available) are subjected to these same histochemical examinations for determination of amyloid deposition.



Part A (continued)

Major Findings:

The methods noted above are still in progress. However, it is expected that significant findings will be available within the coming year. Histologic variations have already been noted in buccal mucosa taken from autopsies, particularly in patients receiving the newer anticancer chemotherapeutic agents.

Significance to Dental Research:

1. Considerable confusion exists at present concerning what constitutes histologically normal and diseased buccal mucous membrane. It is expected that the normal range of histologic variation will be determined for human buccal mucosa. Incipient changes in buccal mucosa, prior to the development of any one of its several recognized disease entities (lichen planus, leukoplakia, hyperkeratosis, and carcinoma), will in the future be better recognized.
2. It is hoped to determine the relative efficacy of biopsy of buccal mucosa as compared with rectal mucosa in demonstrating amyloid in multiple myeloma. Also, it is hoped to determine the relative occurrence of amyloid in certain other tissues in cases of multiple myeloma. Further, evaluation of standard and new staining technics for amyloid will be done in order to fine a method to better characterize this metachromatic material.
3. The evaluation of buccal mucosa in patients on chemotherapeutic agents for carcinoma may lead to some understanding of the reasons for ulcerative breakdown of the buccal mucosa in this and other related conditions.

Proposed Course of Project:

After completion of the present residency at AFIP and collection of additional specimens it is expected that more information will be obtained on both the incidence of amyloid in oral tissues and also the variations seen in normal buccal mucosa.

Part B included: No



Serial No. NIDR-62 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Influence of Several Dietary Minerals on the Deposition of Dental Calculus in the Sprague-Dawley Rat.

Principal Investigator: Dr. Samuel Kakehashi

Other Investigators: Dr. Paul N. Baer

Cooperating Units: None

Man Years (calendar year 1961):

Total: 1/4

Professional: 1/4

Other: None

Project Description:

Objectives:

To evaluate the effects of various dietary levels of calcium, phosphorus and bicarbonate on the deposition of dental calculus in the albino rat.

Methods Employed:

Two hundred and sixteen Sprague-Dawley rats were divided into nine groups and fed similar diets varying only as to calcium, phosphorus and bicarbonate content. Animals were sacrificed after 30 days, the jaws were defleshed for microscopic examination and scored for calculus. The results of the experiment were subjected to statistical analysis.

Major Findings:

1. Calculus deposition occurred with all diets including those deficient in calcium and phosphate.
2. Calcium-phosphorus ratios of 1:1, 2:1 and 1:7 showed no significant differences in calculus deposition.
3. The presence of bicarbonate in the diet enhanced the deposition of calculus.





Part A (continued)

4. Calculus deposition occurred bilaterally symmetrical.
5. No significant sex difference in the deposition of calculus was noted.

Significance to Dental Research:

The data from this experiment contributes evidence to the probable influence of diet on the deposition of dental calculus.

Proposed Course of Study:

Follow-up experimental studies as a direct result of this experiment will be designed to differentiate calculus deposition as affected by local oral phenomenon and as affected by general physiologic processes.

Part B included: Yes



PHS-NIH  
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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Takehashi, Samuel, Baer, Paul N. and White, Carl: Studies on  
Experimental Calculus Formation in the Rat. II. Effect of Calcium,  
Phosphate, Bicarbonate, J. Periodont., in press.

Honors and Awards relating to this project: None



Serial No. NIDR-63 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Treatment of Hemangiomatosis and Hyperplastic Lesion  
in Patients with a "Positive Pressure" Appliance.

Principal Investigator: Dr. P. N. Baer

Other Investigators: None

Cooperating Units: Lawrence Stanwich, Clinical Center, Dental Department

Man Years (calendar Year 1961):

Total: 1/3  
Professional: 1/3  
Other: None

Project Description:

Objectives:

Since the therapy of gingival hemangiomas and dilantin hyperplastic gingivitis is so unsatisfactory, we decided to evaluate the use of a Positive Pressure Appliance in treating these lesions.

Methods Employed:

Patients with either dilantin hyperplastic gingivitis or gingival hemangiomatosis were used on the subjects. After surgical removal of the affected tissues positive pressure appliances were made to help maintain the result. These appliances are worn at night indefinitely.

Major Findings:

Gingival hemangiomatous and dilantin hyperplastic gingival lesions can be successfully treated with the use of a prosthetic "Positive Pressure" appliance which is capable of maintaining positive pressure over the involved tissues for a prolonged period of time. No recurrences have been observed during the two year period that the appliance has been used.



Part A (continued)

Significance to Dental Research:

A new method of treating cases in which recurrences were the rule, has been established. This should give improved health to countless numbers of patients.

Proposed Course of Project:

An attempt will be made to use this appliance in treating other forms of resistant gingival lesions, such as those in which mouth-breathing is a major contributing factor.

Part B included: Yes





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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Baer, P. N., Stanwich, L., Alloy, J., Merritt, A. D. and Lewis, J. R.: Gingival Hemangioma Associated with Sturge-Weber Syndrome. Oral Surg., Oral Med. and Oral Path. 14:1383: 1961.

Honors and Awards relating to this project: None



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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Baer, P. N., Stanwich, L., Alloy, J., Merritt, A. D. and Lewis, J. R.: Gingival Hemangioma Associated with Sturge-Weber Syndrome. Oral Surg., Oral Med. and Oral Path. 14:1383: 1961.

Honors and Awards relating to this project: None



Serial No. NIDR-64 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: "Biochemical and Histochemical Studies in Recurrent  
Apthous Stomatitis"

Principal Investigator: Dr. E. Graykowski

Other Investigators: Dr. E. Driscoll

Cooperating Units: Dental Clinic, Clinical Center

Man Years (calendar year 1961)

Total: 1  
Professional: 3/4  
Other: 1/4

Project Description:

Objectives:

Our objective is to determine if a relationship exists between the presence of recurrent apthous oral lesions and the iron metabolism of individuals in which they occur.

There are many types of mucosal changes associated with iron deficiency anemia: such as cheilosis, glossitis, atrophy of the lingual papillae and various types of nonspecific stomatitis. Could iron deficiency at the cellular or hematological level be associated with apthous stomatitis? This is an interesting speculation worthy of investigation, since there is some clinical evidence that apthous ulcers and deficiencies in serum iron (certain anemias) are sometimes correlated.

Methods Employed:

1. The following tests are performed: red blood cell count, white blood cell count and differential, wintrobe indices, sedimentation rate, complement fixation, urinalysis, serum iron, total serum proteins and albumen-globulin ratio.
2. The oral mucosa of all patients is biopsied, part of the tissue is sent to the histopathology laboratory and a part is prepared



Part A (continued)

for viral studies. When vesicles are present in the lesion the fluid is aspirated for viral studies.

Patient Material:

Patients are selected from those on record from the previous stomatitis studies and those being referred at the present time to the Clinical Center Dental Clinic. About fifty patients will comprise the study group.

Major Findings:

This study has just been started and there are not yet any results.

Significance to Dental Research:

In view of the fact that the cause of aphthous stomatitis is not known, obtaining hematological and biochemical data from patients with aphthous stomatitis may prove useful in determining the etiology of this oral disease.

Proposed Course of Project:

The accumulated data from this study will be used as a basis for further investigations into the etiology and treatment of aphthous stomatitis.

Part B included: No





Serial No. NIDR-65 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: General Anesthesia (Dental) Baseline Data

Principal Investigator: Dr. E. J. Driscoll

Other Investigators: Dr. G. Christenson and Dr. C. Herbert

Cooperating Units: Anesthesiology Department, Clinical Center

Man Years (calendar year 1961):

Total: 1  
Professional: 3/4  
Other: 1/4

Project Description:

Objectives:

It is estimated that in many parts of the country, particularly in the East and far West, there are nearly as many general anesthetics administered in dental offices as there are in the local hospitals. This is also true in some foreign countries, i.e. England.

1. Dental general anesthesia is different from ordinary hospital anesthetics in several important respects:
  - (a) the patients are ambulatory and not usually sedated;
  - (b) the operations are performed in as light a plane as possible;
  - (c) the operations are performed in or about the airway; and
  - (d) Since the patients expect to be incapacitated only for short periods of time because of these major differences, the background of knowledge in general dental anesthesia is incomplete and not accurate or comparable. Thus, a great need exists for fundamental data concerning alterations in the physiological mechanisms and a definition of the limits beyond which hazards are faced.



Part A (continued)

2. The accumulated data from this study has been and will continue to be used as a baseline of comparison for the many new anesthetic drugs which have been and are being proposed for use in oral surgery. These data will also be valuable if and when electronarcosis is ready for evaluation in dental anesthesia.

Methods Employed:

1. For the purpose of standardization of surgical trauma, full mouth extractions have been utilized rather than random oral surgical cases. The teeth are extracted in quadrants and the entire operation systematized for time of procedure and traumatic experience. Under these conditions the following agents have been undergoing evaluation:
  - (a) Sodium methanhexatol and Pentothal.
  - (b) The parasympatholytic antisialogogues, atropine and hexocyclium.
  - (c) Various proportions of nitrous oxid oxygen as adjuvants. (75%-25%) (80-20) (85-15), these being the usual proportions employed by oral surgeons.
2. Physiologic data being recorded are pulse, blood pressure, arterial O<sub>2</sub> saturation, respiratory phenomena, cortical brain activity and the electrical activity of the heart (E.K.G.). Likewise recorded are such important technical data as amount of drug used in units of time, length of operation, duration of sleep, mental alertness on awakening, age, race, sex, emotional status in relation to the conduct of anesthesia, and post-anesthetic sequelae (headache, nausea and vomiting, depression and hiccoughs).

Patient Material:

This year another 150 detailed studies were completed on about 50 adult patients. With this increment we now have detailed data on over 600 cases with an average operating time of 20 minutes.

Major Findings:

1. Sufficient data is now available to determine objectively from brain wave activity the precise anesthetic levels at which various procedures are being carried out with methahexital. The addition of N<sub>2</sub>O and O<sub>2</sub> of various proportions does not significantly alter these E.E.G. patterns, nor does short per-



Part A (continued)

iods of apnea, as some investigators have indicated in the literature. Thus, it is definitely established that we are working in an extremely light plane.

2. The E.E.G. is making it possible for us to state with certainty the precise anesthetic plane which is present at specific intervals when other physiologic measurements such as pulse and pressure are made and studied in relation to trauma.
3. A slight pharmacologic difference has been found in the behavior of atropine and Tral (hexocyclium) when employed as an antisialogogue. Both of the agents are given before the barbiturate is administered. Atropine causes an acceleration of the pulse almost immediately whereas there is no increase in pulse rate when Tral is used until the barbiturate is added. The relative increase is also slightly greater during the anesthesia when atropine is the drying agent.
4. Hexocyclium was found to be a far superior drying agent than atropine. Another practical feature about hexocyclium as compared to atropine is that its action is of short duration; i.e. it clinically reduces secretions very effectively for 20-30 minutes. Thus, in the post-operative period when the patient becomes oriented, there is no annoying problem of a dry mouth.
5. The significant blood pressure elevation and pulse acceleration continues to be our most important finding from a practical standpoint. It was found that the blood pressure and pulse elevation with Methahexitol was slightly greater than that we reported with Pentothal and Neraval. Although the values are greater for both pulse and blood pressure, the pattern of rise is identical with the two previously described drugs, i.e., the pulse peaks in one to two minutes whereas the blood pressure peaks in six minutes indicating a slightly different physiological mechanism in operation in these closely allied physiologic measurements.
  - (a) Alarming tachycardias are being recorded and their onset parallels the first E.E.G. changes which occur as soon as the drug methahexitol passes through the blood brain barrier.
  - (b) The previously reported hypertension was principally of the systolic variety. We are now ready to report an almost as significant elevation in the diastolic system.



Part A (continued)

The diastolic rise is probably related to the inability of the vascular system to obtain any measure of relaxation because of the rapidly beating heart.

Significance to Dental Research:

1. The search is constantly being made in dentistry for safer general anesthetic agents. However, without baseline data on physiologic responses, this search has no foundation. Methahexitol is a good example of the new drugs which have been recently introduced, and our studies on this drug demonstrate the value of our previously accumulated data on intravenous barbiturates as far as baselines are concerned. With our previously acquired baselines on Pentothal and Neraval, we are in a good position to evaluate not only Methahexitol but any other new anesthetic drug which may be introduced for use in ambulatory oral surgery.
2. The possibility also exists for improvement and refinement of present methods of anesthesia as well as laying a sound foundation for further anesthesiology research. An example of refinement in methods is indicated in our studies of N<sub>2</sub>O and O<sub>2</sub> supplementation and demerol premedication, both of which help to control adverse rises in blood pressure. Pulse control has so far eluded our therapeutics and technical investigation.

Proposed Course of Project:

1. The study will continue on both good risk (ASOS class I) and medium risk (ASOS class II) outpatients. These medium risk patients have nothing wrong with their cardiovascular or respiratory systems which would make them unsuitable candidates for general anesthesia. Additional baseline and comparative data will be accumulated, studied, and evaluated.
2. The studies of Methahexitol and the usual supplements will continue. An additional supplement, fluothane (which is described as causing hypertension and bradycardia) is being considered for study as a possible antagonist to the forces inducing hypertension and tachycardia.
3. Position, (supine vs upright) in the operating chair will also be investigated, as possible determinant of the adverse pulse changes.
4. The effects of the vagolytic agents will be further recorded





Part A (continued)

and observed. Studies of arterial oxygen saturation by use of the air oximeter has been found to be very effective and will be continued.

5. Studies on continuously recorded respiratory data will be continued. The recordings are essential to the proper interpretation of arterial oxygen values.
6. Consideration is being given to the study of galvanic skin. Response as a continuous measure of pre-operative and post operative apprehension and anxiety.

Part B included: Yes



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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Driscoll, E. J. and Christenson, G. R. and White, C. L., Physiological studies in general anesthesia on ambulatory dental patients.

Part II - In preparation.

Honors and Awards Relating to this Project: None



Serial No. NIDR-66 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Physiological Responses of the Dental Patient Under Hypnosis.

Principal Investigator: Dr. Robert E. Drury

Other Investigators: Dr. E. J. Driscoll

Cooperating Units: National Institute of Mental Health

Man Years (calendar year 1961)

Total:	1/3
Professional:	1/3
Other:	None

Project Description:

Objectives:

A search of the literature reveals no formal work has been done in the area of the physiological responses of the dental patient under hypnosis.

1. It is necessary that many of the measureable physiological responses are studied in order that we may learn more about the effects of hypnosis on the dental patient.

2. To compare the results of such a study with previous studies involving general anesthetics, local anesthetics, and other methods of treating patients in the dental setting. The final result being a method of choice for a particular patient.

3. An inquiry into the psychological make-up of the fearful dental patient will be made, and from these findings further work in the psychological area may be attempted.

Methods Employed:

1. Basically procedures of similar trauma and time will be carried out under hypnosis and then without the aid of hypnosis. With a random selection, some patients will have the first procedures done



Part A (continued)

without the benefit of hypnotic states and then with hypnosis.

Patients will have a quadrant of operative dentistry done under routine conditions, and then a quadrant of similar work under hypnosis utilizing suggestions for anesthesia, relaxation, and arrest of salivation.

2. Physiological data to be recorded: E.K.G., E.E.G., blood pressure, oxygen saturation of the blood, respiration, galvanic skin response, body temperature.

3. Psychological data to be recorded: Minnesota Multiphasic Personality Inventory (MMPI) to be administered. Pre-study psychological evaluatory screening. Patient attitudes during the course of the study. Follow up studies on unusual cases.

Patient Material:

Presently being organized.

Major Findings:

Pending study.

Significance to Dental Research:

An attempt is being made to find suitable methods to treat the dental patient with a pre-existing physical defect requiring minimum stress on the body. Development of methods to diminish psychological stresses concerning the fear of treatment expressed by many dental patients, would render these patients acceptable for research studies in all fields of dentistry. The whole program of dental research will be broadened by inquiry into the psychosomatic aspects of dental treatment.

Proposed Course of Study:

1. Upon analyzing the data obtained from the initial study, further areas of investigation may be determined along the physiological line.

2. Hypnosis being just one adjuvant in handling the difficult patient, the whole field of the psychology of the fearful or difficult patient remains for future extension of this study.

Part B included: No





Serial No. NIDR-67 (c)

1. Clinical Investigations

3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Project in Oral Surgery and Prosthodontics "Subperiosteal Implants".

Principal Investigator: Dr. J. W. Gamble

Other Investigators: Drs. E. J. Driscoll and H. Swerdlow

Cooperating Units: Dental Department, Clinical Center

Man Years (calendar year 1961)

Total: 1/2  
Professional: 1/2  
Other: None

Project Description:

Objectives:

It is estimated that one out of every five edentulous patients is unable to wear a conventional denture. The reasons for this inability to wear conventional dentures are many, but advanced resorption of the supporting structures of the mandible is the leading reason.

The subperiosteal mandibular implant denture has been used by prosthodontists for over ten years and differs from the conventional mandibular denture in that the supporting structure is provided by surgically placing and attaching a metallic casting beneath the mucoperiosteum directly to the mandibular bone by screws and/or wires. Support or retention is afforded for the denture by four metallic posts protruding into the oral cavity to which the denture is attached.

The purpose of this study is to determine the extent to which subperiosteal implants increase masticatory function, and to evaluate the bacterial seepage around the metallic posts and the possible subsequent infection. Also a thorough psychological evaluation of patients who are unable to wear conventional dentures will be made using the Minnesota Multiphasic Personality Inventory before and after placement of the implant dentures, to obtain a more clear understanding of the psychological problems of these patients.



Part A (continued)

Methods Employed:

1. Patients are being interviewed concerning their desire for implant dentures and a total of 12 have been selected for study. Patients will be screened by a prosthodontist and an oral surgeon. Those patients accepted will have:
  - (a) History, laboratory work, physical exam, X-ray evaluation, impressions and study models of maxilla and mandible, photographs intra and extra-orally, and construction by NIH prosthodontist of conventional dentures.
2. After adequate time has elapsed following construction of new conventional dentures to determine patients' inability to wear these dentures the following procedures will be done:
  - (a) First stage surgical procedure and taking of impressions of the mandibular bone.
  - (b) Second stage surgery and the placement and attachment to the mandibular bone of the metallic substructure.
  - (c) Insertion of the superstructure over the metallic posts.
  - (d) Evaluate the stability of the sub and the superstructure, tissue healing over and around the substructure, and the bone changes beneath the substructure.

Patient Material:

Healthy adult edentulous patients who have been unable to wear conventional dentures.

Major Findings:

It is too early to report findings since the study is still in preliminary stages.

Significance to Dental Research:

1. The search is constantly being made for adequate replacements of lost parts. If a more physiological procedure for replacement of lost dentition can be effected, then results would be most rewarding. In the future thousands of unfortunate patients who are for one reason or another unable to wear conventional dentures may be aided by implant procedures.



Part A (continued)

Proposed Course of Project:

1. Following the placement of ten or fifteen implant dentures, data from this study will be evaluated, and a similar study for victims of malignancies might be feasible.
2. It is planned to investigate the possibility of using maxillary implants to stabilize maxillary dentures.

Part B included: No



Serial No. NIDR-68 (c)  
1. Clinical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Temporomandibular Joint and its Relationship to the Masticatory Musculature and the Occlusion of the Teeth

Principal Investigator: Dr. R. Dubner

Other Investigators: Dr. P. N. Baer, Dr. P. J. Coccaro

Cooperating Units: Electromyography Section, National Institute of Neurological Disease and Blindness; Diagnostic X-ray Department, Clinical Center; Normal Volunteer Group, Clinical Center.

Man Years: (calendar year 1961)

Total: 1/4  
Professional: 1/4  
Other: None

Project Description:

Objectives:

1. To determine what role muscular forces and occlusal disharmonies play in temporomandibular joint disorders.
2. Evaluation of various treatment procedures in cases of temporomandibular joint dysfunction.

Methods Employed:

Through the study of patients with temporomandibular joint disorders an attempt is made to learn about the etiologic factors involved in the temporomandibular joint syndrome. Emphasis is placed on the history, physical findings and various diagnostic methods, though therapy is necessarily an important phase of the procedures.

Diagnostic Procedures include:

1. Complete medical history, examination and laboratory workup (blood and urine tests, chest X-ray, EKG, etc.)





Part A (continued)

2. Complete history and clinical examination of the temporomandibular joint illness.
3. Radiographic examination including temporomandibular joint laminographs and lateral plate cephalometrics.
4. Electromyographic studies of the muscles of mastication.
5. Psychiatric consultation when necessary.

Therapeutic procedures include:

1. Double-blind study of muscle relaxant drugs.
2. Occlusal therapy consisting of removal of centric and balancing side disharmonies.
3. Therapeutic exercises of the jaw.

Patient Material:

Patients included in the study are those with complaints of pain in the temporomandibular joint or masticatory areas associated with clicking, subluxation and/or limitation in mandibular movement. Organic disorders such as osteoarthritis, rheumatoid arthritis, fractures and neoplasms are ruled out during the complete medical workup.

Fifty patients will be studied.

Major Findings:

At the present twenty five patients with temporomandibular joint disorders have been studied. No conclusions have been reached at this early date in the study.

Significance to Dental Research:

Disorders related to the temporomandibular joint area have been a concern of modern dentistry for over forty years. Disharmonies of the occlusion of the teeth have previously been implicated as a major cause of these disorders. Since 1950, the trend has been towards emphasis on the role of the mandibular muscle dysfunction in these cases. Greater understanding of the physiology of the inter-relationship of these structures will aid in determining the etiologic factors involved.



Part A (continued)

Proposed Course of Project:

The proposed number of patients in both groups will be studied and evaluated. The knowledge gained will aid in directing us to new investigative approaches of this important problem.

Part B included: No



Serial No. NIDR-69 (c)  
1. Clinical Investigations  
2. Medical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Genetics Studies in a Population Isolate (Brandywine)  
with Particular Reference to Hemoglobin and Haptoglobin  
Patterns..

Principal Investigators: Dr. A. D. Merritt and Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total: 1/2  
Professional: 1/4  
Other: 1/4

Project Description:

Objectives:

1. To utilize the haptoglobin and hemoglobin patterns within the population isolate as markers for the study of disease processes and other biologic data available from these patients.
2. To identify unusual hemoglobin and haptoglobin patterns and to define the types of haptoglobins produced when analyzing sibships in this population.

Methods Employed:

Sera and hemoglobin were collected from the Brandywine population isolate and studied by means of starch gel and paper electrophoresis respectively. The genetic types of haptoglobin and hemoglobin are being defined and will be analyzed by standard statistical methods.

Patient Material:

The patient source material for this study is derived from a major triracial isolate situated in the Brandywine, Maryland area. Most of the samples have been collected in field studies.



Part A (continued)

Major Findings:

Sickle cell hemoglobin is found in approximately 20% of the population (national average approximately 10% in a Negro population) and 2-1 "modified" haptoglobin patterns have been found and in a correspondingly increased percentage. The progeny from 2-1 "modified" haptoglobin parents are being defined.

Significance to Dental Research:

These genetic markers will serve for a correlation point for other disease states, and abnormal and normal findings within this tri-racial isolate. The general significance lies in the further definition of the pattern by which man inherits his normal and abnormal characteristics and the possible influence of genetically determined traits, one upon the other.

Proposed Course of Study:

These findings have been written up and are in the process of publication. This project is discontinued.

Part B included: No





Serial No. NIDR-70 (C)  
1. Clinical Investigations  
2. Medical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Familial Neonatal Hepatitis - Study of Large Family

Principal Investigator: Dr. George Cassady

Other Investigators: Maimon Cohen and Dr. A. Morrison

Cooperating Units: University of Pennsylvania - Department of  
Pathology and Pepper Laboratory

Man Years (calendar year 1961):

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

This is a genetic, clinical, and laboratory study of a family with a high frequency of 'giant cell hepatitis of the newborn'.

Methods Employed:

A field trip to North Carolina and study of thirty-five family members, including genetic analysis, physical examinations, and the obtaining of blood specimens for liver function studies.

Patient Material:

Thirty-five members of a family in North Carolina.

Major Findings:

Laboratory studies have not yet been completed. The blood studies are being performed at the Pepper Laboratory, University of Pennsylvania.

Significance to Dental Research:

The project is related to human genetics.



Part A (continued)

Proposed Course of Project:

The study has been completed. Manuscript is in preparation.

Part B included: No



- Serial No. NIDR-71 (C)  
1. Clinical Investigations  
2. Medical Investigations  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Hereditary Renal Dysfunction with Associated Anomalies  
--Genetic and Clinical Studies

Principal Investigator: Dr. George Cassady

Other Investigators: Maimon Cohen, Dr. William DeMaria, Dr. Bertram  
Hanna, Dr. Kenneth Brown

Cooperating Units: Duke University Medical Center - Department of  
Pediatrics

Man Years (calendar year 1961):

Total:	3-1/4
Professional:	2-1/4
Other:	1

Project Description:

Objectives:

To study the genetic, clinical, and laboratory abnormalities of six families with hereditary renal dysfunction.

Methods Employed:

Field trips to North Carolina and Indiana resulted in the study of approximately 500 family members, with physical examination, dental examination, urinalysis, audiogram, genotyping, blood collection for hemocrit, smear with differential, electrolytes, calcium and phosphorus, cholesterol, protein electrophoresis, lipo-protein electrophoresis, renal auto-antibodies, toxic factor for Hela cells, and urine collected for amino acids, organic acids, and sugar chromatography.

Patient Material:

Five hundred family members from North Carolina and Indiana, as well as a number of individuals from Gallaudet College who have hearing impairment and abnormal urinalysis.



Part A (continued)

Major Findings:

A genetic mode of inheritance involving non-random chromosomal segregation has been postulated.

Clinical features of the syndrome have been clarified. Abstract of the details of the clinical studies has been published in the American Journal of Diseases of Children, November, 1961.

Significance to Dental Research:

This project is related to research in Human Genetics.

Proposed Course of Project:

This project will continue to investigate the etiology of the disorder.

Part B included: Yes





PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cohen, M.M., Cassady, G., Hanna, B.L., A genetic study of hereditary renal dysfunction with associated nerve deafness, Am. J. of Human Genetics, December 1961.

Honors and Awards Relating to this Project: None.



Serial No. NIDR-72 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development  
and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: The Influence of Muscle Forces and Physiological  
Character of Soft Tissues Upon Skeletal Form (Including  
Dental Alignment) in the Oral and Pharyngeal Area

Principal Investigator: Dr. Richard C. Grossman

Other Investigators: Dr. James F. Bosma

Cooperating Units: Clinical Center Dental Department, NIH

Man Years:

Total:	1
Professional:	1
Other:	None

Project Description:

Objectives:

- A. To design muscle force transducing devices for use in the mouth and pharynx.
- B. To obtain initial measurements from these devices and to relate this information to particular muscular actions during function.
- C. To develop a radiographic method for the assessment of muscle "density" within soft tissues of the face, mouth and pharynx.
- D. To develop a volumetric method for the assessment of muscular tone by means of tissue "distensibility" within the soft tissues of the face, mouth and pharynx.

Methods Employed:

- A. In the initial phase of this program, deviant patterns of oral muscular function are being observed in individuals who also exhibit severe malocclusions of the teeth. Before, during and after gross correction of these orthodontic problems,



Part A (continued)

cephalometric radiographs and models suitable for analysis of dental displacements are being collected to ascertain the most significant design and location for placement of force transducers in a later phase of this investigation.

- B. Following preliminary explorations, an arrangement was made for the purchase of two "developmental" models of semi-conductor strain-gage pressure transducers from Micro-Systems, Inc. This investigator participated in the design as well as the trial applications of these prototype models in a joint effort which took place both at the NIH and at the plant of Micro-Systems, Inc. These pressure sensors have been placed in a variety of orientations on the teeth, palate, alveolar processes, and on plastic devices temporarily situated in the mouth and pharynx in an initial attempt to discern forces exerted by the muscles of the tongue, cheeks, lips and pharynx during functional activity.
- C. Preliminary efforts have been undertaken to establish appropriate x-radiation exposure criteria for quantitative roentgenographic determination of the "density" of the muscular mass within the soft tissues in question.
- D. Work has been initiated in which controlled negative pressure is applied to facial, oral, and pharyngeal structures in an effort to determine by means of tissue "distensibility" varying degrees of tone in spastic, normal, and flaccid muscle states.

Patient Material:

Sixteen patients who manifested some degree of discrepancy in oral muscular function associated with severe deviations in the form and relationship of their dental arches have been selected. These subjects range in age from seven to nineteen years. Included in this group are six individuals exhibiting Angle Class I malocclusion, seven with Class II and three with Class III malocclusion. Among these are represented severe mesio-distal and bucco-lingual alveolar discrepancies and anterior and lateral open bites. The muscle problems presented by these individuals include deviations from normal tongue positioning associated with excessive forces exerted against or between the teeth and also with forces of a lesser degree than is necessary for maintenance of normal arch form; deviant swallowing function; greatly diminished total muscular coordination; speech defects possibly related to articulatory malfunction; and diminished gag reflex. Gross correction of each patient's alveolar discrepancies are being attempted by orthodontic treatment. It is anticipated that these severe dental and bony



Part A (continued)

deviations will relapse to some degree following treatment. This tendency will be given close attention.

Major Findings:

At this date the major part of the program is in active progress and some phases of the investigation have only recently begun. Therefore, a statement of results and conclusions would be premature.

Significance to Dental Research:

At the present time there exists no means for making exact determinations of most oral and pharyngeal muscular functions and describing how these relate to the configurations of hard tissue structures of the face and mouth. It is evident that the role of forces exerted by the musculature in question on jaws and teeth must be elucidated for a better understanding of facial development and the etiology of malocclusion. Moreover, to better understand the complexities involved in deglutition and maintenance of the airway for respiration and speech, it is necessary to bring new approaches to bear on the moving mass of musculature concerned with these functions. It is anticipated that this study will provide new knowledge regarding several parameters of function of the musculature of the "portal area."

This information is relevant to the practice of orthodontic treatment of malocclusion and to the pending study by the Oral and Pharyngeal Development and Function Section of persons with catastrophic disabilities of the mouth and pharynx.

Proposed Course of Project:

Upon fulfillment of the initial objectives, the instrumentation and methodology developed will be employed in a comprehensive study of the muscle force phenomena during functional activity of the oral cavity and pharynx.

Part B included: No





- Serial No. NIDR-73 (C)
1. Clinical Investigations
  2. Oral Pharyngeal Development and Function
  3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Behavior of Points "A" and "B" in Immediate Denture Patients Utilizing Tantalum Pins.

Principal Investigator: Dr. Peter J. Coccaro

Other Investigator: Dr. Ralph Lloyd

Cooperating Units: Dental Department, Clinical Center

Man Years:

Total: 1 1/2

Professional: 3/4

Other: 3/4

Project Description:

Objectives:

1. To study the behavior of points "A" and "B" in immediate denture patients, using metallic pins and in patients with an ample pre-insertion period and no pin implants.
2. To define discernible changes (antero-posteriorly) and (supero-inferiorly) noted in the region of the cephalometric landmarks "A" and "B" twelve months following the removal of upper and lower incisors.

Methods Employed:

The method for this study was based on the analysis of longitudinal cephalometric roentgenograms. X-ray records were taken during the following periods for all patients: pre-surgery, denture insertion, six months and twelve months following denture insertion.



Part A (continued)Patient Material:

The subjects included in this study consisted of two groups varying in size and treatment. The smaller group (two subjects, one male, age forty-six, one female, age thirty-seven) had tantalum pins inserted into the areas normally selected as cephalometric landmarks for points "A" and "B." These patients received immediate full upper and lower dentures following surgery. In the larger group (made up of twenty-two patients) none of the subjects had metallic pin implants or immediate dentures. Pre-denture insertion records showed an average of 3.6 months for patients in this group.

Angular and linear measurements were used to evaluate antero-posterior and supero-inferior changes at points "A" and "B" for all patients in this study. Angles SNA (sella-nasion-point "A") and GPB (gonion-pogonion-point "B") were used for angular readings while linear measurements were made from SN (sella-nasion plane) to point "A" and from MP (mandibular plane) to point "B."

Major Findings:

1. Angular measurements (SNA) for point "A" during the period between pre-surgery and twelve months following surgery indicated that the subjects in both groups reflected some change at point "A"--it appeared to locate more posteriorly. Subjects in both groups showed point "B" also to be located more posteriorly twelve months following surgery.
2. The linear measurements from sella-nasion-plane to point "A" during the interim between pre-surgery and twelve months following surgery showed point "A" to be located slightly higher for both "pin" cases and slightly lower for the twenty-two cases. An evaluation of point "B" in the "pin" cases showed it to locate higher in one instance and lower in the other. The mean of the twenty-two cases reflected a lowering of point "B" during the same period studies.

Significance to Dental Research:

Studies have shown that there is a good chance that maxillary and mandibular apical base relationships can be improved through treatment. These changes result from new locations of points "A" and "B" during bodily movement of maxillary and mandibular incisors and remodeling of alveolar bone in the immediate area after orthodontic treatment.



Part A (Continued)

If the apex of the incisor root is so critically identified with the cephalometric landmark "A" and "B", the present study could contribute additional data in this area particularly since we are removing apical base relationships by the extraction of incisors and evaluating changes about point "A" and point "B."

Proposed Course of Study:

1. To extend the findings in these areas of cephalometric landmarks "A" and "B" to include eighteen and twenty-four months following surgery.

Part B included: No



Serial No. NIDR-74 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development  
and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: A Longitudinal Cephalometric Analysis of the Height  
and Depth of the Epipharynx in Cleft Palate Patients

Principal Investigator: Dr. Peter J. Coccaro

Other Investigators: None

Cooperating Units: Cleft Palate Clinic, University of Illinois.

Man Years:

Total:	1/3
Professional:	1/3
Other:	None

Project Description:

Objectives:

A serial analysis of lateral cephalometric roentgenograms to evaluate the vertical and horizontal changes in the nasopharynx of cleft palate patients.

Methods Employed:

To study the increase in height of the nasopharynx on a time basis, the linear distance from the point of intersection of the palatal plane with the pterygomaxillary fissure to the cranial base plane (nasion-basion) was measured. This line was constructed perpendicular to the cant of the Frankfort horizontal plane and projected to intersect the cranial base line. Changes in depth of the nasopharynx were analyzed by measuring the linear distance along the palatal plane, from the point of intersection of the palatal plane with the pterygomaxillary fissure to the soft tissue of the posterior wall of the pharynx.

All measurements were made with correctional scales.





Part A (continued)Patient Material:

Fifty-seven children with varying degrees of cleft lip and/or palate were selected for this study. Of the fifty-seven, thirty-four were boys and twenty-three were girls. Cephalometric records were taken on these children from birth through seven years of age. During the first year the records were taken every three months. During the second year they were taken every six months; and from the third year on, the x-ray records were obtained annually. The cleft palate sample included eleven cases with an involvement of the lip and alveolar process only. Since the defect did not involve the hard and soft palate in these cases it was possible to employ them as a cleft control group. The data obtained on the cleft palate population was compared with the cleft control group as well as with a group of normal children with no palatal defects, analyzed by Subtelny.

The cleft lip and/or palate population were categorized according to the type of involvement as follows:

1. Posterior or isolated cleft palate--fifteen cases.
2. Unilateral cleft lip and cleft palate--twenty-one cases.
3. Cleft lip and alveolar process--eleven cases.

Major Findings:

Statistical analysis of the measurements permitted the following conclusions:

1. Steady and consistent increase in nasopharyngeal height was noted throughout the periods studied. The greatest increment was recorded during the first two years of life. This was consistent with the findings in the normal group.
2. Shorter nasopharyngeal vertical measurements were observed for the cleft palate group at most of the age levels studied, when compared with the normal group.
3. Little increase in the antero-posterior measurements of the nasopharynx was recorded from birth through the first year of life. Thereafter, until the seventh year of life, there is an apparent upward swing in the growth curve, showing significant increases in the depth of the nasopharynx. During this age span there are periods of



Part A (continued)

apparent increase and decrease in the horizontal dimensions of the nasopharynx the fluctuation in depth may be a direct reflection of adenoid growth and concomitant growth of the upper face.

4. Shorter nasopharyngeal horizontal measurements were noted for the cleft palate group at most age levels studied when compared to the normal group. Greater proportional increments in the horizontal dimensions of the nasopharynx were noted between five and seven years of age for the cleft palate group when compared to the normal group.

Significance to Dental Research:

The cleft palate patient presents many complex oral-dental and facial deformities affecting mastication, speech, respiration and deglutition. In the velopharyngeal region, cleft palate diagnosis seems to center about two fundamental considerations. One is the assessment of existing velar tissue and the other is an evaluation of pharyngeal dimensions as a function of time.

Proposed Course of Study:

Future studies are planned to obtain information on growth in other pharyngeal areas.

Part B included: No



Serial No. NIDR-75 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Dissection and Clinical Studies of Anatomical Relations in the Pharynx, Basicranium and Adjacent Cervical Vertebrae.

Principal Investigator: Dr. Yasuaki Takagi (Visiting Scientist)

Other Investigators: Dr. James F. Bosma

Cooperating Units: Wards 10E and 13E, Clinical Center  
Department of Radiology of Clinical Center

Man Years:

Total:	1 2/3
Professional:	1
Other:	2/3

Project Description:

Objectives:

- A. To obtain detailed anatomical description and familiarity with the upper pharynx, its attachments to the basicranium, and its anatomical relations with the adjacent vertebrae.
- B. To apply and develop methods of clinical study of the junction of pharynx with the basicranium, aimed at further study of this area in anomalous subjects, as this is a site of predilection of malformation.

Methods Employed:

- A. Gross anatomical dissections were performed on nine adult and eight newborn human cadavers. Comparative dissections of this region were performed in dog, cat, rabbit, calf and opossum. Supplementary studies were performed on tissue blocks of this area excised at postmortem of one newborn and one adult. These blocks were stained to differentially demonstrate connective tissue, muscle and mucopolysaccharides.



Part A (continued)

- B. Clinical Studies of the upper pharynx have been performed previously on normal children and adults, by members of this group, at the University of Utah, and on normal infants, in Stockholm. As an extension of these studies, clinical comparisons have been performed on one anomalous subject, having known deformities of the cervical vertebrae, and possible deformity of the upper pharynx. Special radiographic views of the basicranium were devised to permit delineation of the occipital area despite distortions of head and neck position and limitations of their motions. The pharynx was observed in rest position by single lateral films and in swallow action by cineradiography.

Major Findings:

- A. The anatomical dissections demonstrated the longus capitis muscles (of the prevertebral system) penetrate the bucco-pharyngeal fascia through a fenestra--an anatomic intersection that was not previously known.

Also, the pharyngeal aponeurosis, continuation of the constrictor system, attaches to the sphenoid, rather than to the occipital bone, as was previously thought. By these dissections, the pharyngeal aponeurosis-constrictor sleeve or column is distinguished from the pharyngo-visceral fascia, with only the latter attaching to the "pharyngeal tubercle" of the occipital bone. In lower animals, these attachments are all displaced ventrally and further dispersed upon the basilar portions of sphenoid and occipital bone, the degree of this ventral displacement being roughly correlated with their relative extension relations of head at neck.

- B. In the clinical studies of anomalous children by these special radiographic procedures, deformities of the cervical spine and the occipital portion of basicranium and the anatomical relations of these to the upper pharynx were defined. The hypoplasias of the occipital bone and of the upper cervical vertebrae were found to be extensive. The anatomical arrangement of the junction of pharynx with the abnormal basicranium requires further comparison with pathological subjects in parallel clinical observations and in cadaver studies.

Significance to Dental Research:

- A. The dissection studies show the pharynx proper (the pharyngeal aponeurosis-constrictor element) to be related anatomically





Part A (continued)

to the sphenoid, rather than to the occipital bone. This is appropriate, for the basilar portion of the occipital bone is otherwise considered developmentally to be related to the vertebral, rather than the foregut structures. This conceptual point is relevant to clinical studies of the malformations at this junction of foregut and basicranium, for we may hereby anticipate certain differential involvements and disabilities in anomalies separately affecting the vertebral or the foregut elements in this area.

The spatial relation of the longus capitis to the upper pharynx wall implies that this may be a determinant of motions of that wall, particularly in infants and more probably in certain anomaly circumstances. We are currently devising methods to demonstrate this longus capitis influence upon upper pharyngeal contours. This anatomical relation also has implications for our pending electromyographic studies of this area.

- B. The project significance of the single subject studied clinically is that of initiation of efforts, and particularly of interaction with the various portions of the Clinical Center. These studies were of significant contribution to the subject, also. The fundamental precariousness of her cranio-vertebral pedestal was well shown, and on this basis we gave clear recommendation against suggested surgery, displacing her anomalously elevated shoulders downward, for literally this girl's head is resting on her shoulders, as much as on her cervical vertebral support.

Proposed Course of Project:

These dissection observations are now being applied to the living, in direct exposure, for electromyographic observation, in anaesthetized cats, and per cineroentgen observations in humans.

Part B included: Yes



PHS-NIH  
Individual Project Report  
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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

A publication report of the dissection studies has been prepared under the authorship of Yasuaki Takagi, M.D., John Waters, Jr., B.A., and James F. Bosma, M.D., and title of "Anatomical Studies of the Epipharyngeal Wall in Relation to the Base of the Cranium" for prospective submission to the Annals of Otology, Rhinology and Laryngology.

Honors and Awards Relating to this Project: None



Serial No. NIDR-76 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development  
and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Studies of Palatal Perforations and Their Obturation

Principal Investigators: Dr. Ralph Shelton  
Dr. Ralph Lloyd

Other Investigators: Dr. James F. Bosma

Cooperating Units: Dental Department of the Clinical Center, NIH  
Radiology Department of the Clinical Center, NIH  
National Cancer Institute, Clinical Center, NIH  
Sloan-Kettering Memorial Cancer Hospital, New York  
City

Man Years:

Total: 1/3  
Professional: 1/3  
Other: None

Project Description:

Objectives:

To explore nasality and speech articulation resulting from anomalous or surgical cleft of the hard and/or soft palate.

Methods Employed:

- A. Tape recording. Eighteen subjects with either congenital or acquired palatal defects have been tape recorded, each with and without his obturator. Analysis of this tape will provide a statistical statement of the effect of obturation on speech. We will also compare the speech effect of acquired palatal defect with that of congenital cleft palate.
- B. Motion picture photography. Fourteen subjects with maxillo-facial carcinoma have been recorded by cinematography--two of these were pre-operative. Two congenital cleft palate persons have been studied in this manner also. This film is being edited and supplemented by drawings, labels and commentary to



Part A (continued)

illustrate certain items of oropharyngeal physiology.

- C. Cinefluorography. Several subjects have been studied by this means. We have experimented with techniques of marking soft tissue to permit filming of p-a views. Film of normal and cleft palate subjects is being analyzed to determine the effect of obturation on deglutition.
- D. Obturator construction. Four subjects were seen as candidates for obturation of palatal clefts. Three have been fitted with obturators, and the problems of obturator fitting and construction have been recorded by note and photography. This material is being prepared for publication in the speech literature and should contribute to dentistry-speech pathology interaction in treatment and teaching of cleft palate people.

Major Findings:

A specific statement of results and findings would be premature inasmuch as the data compiled has not yet been analyzed.

Significance to Dental Research:

Some of the material gathered gives new information about oropharyngeal physiology; other is useful didactically or clinically. Comparison of speech in persons with congenital as contrasted with acquired palatal defects may provide information for better theoretical understanding of neuro-muscular modification by training.

Proposed Course of Project:

These studies of palatal obturation will be continued in the Dental Clinic of the Clinical Center and also by Dr. Shelton at the University of Kansas Hospitals.

Part B included: No





- Serial No. NIDR-77 (C)
1. Clinical Investigations
  2. Oral Pharyngeal Development  
and Function Section
  3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Cuspid Retraction Forces in Orthodontic Cases.

Principal Investigator: Dr. Julian M. Lifschiz

Other Investigators: None

Cooperating Units: Clinical Center, Dental Department, NIH.

Man Years:

Total: 1/4

Professional: 1/4

Other: None

Project Description:

Objectives:

To establish through clinical studies and laboratory measurements the ideal force range for retraction of cuspid teeth in orthodontic patients whose diagnosis indicated the necessity for extraction of bicuspid teeth.

Materials and Methods Employed:

Approximately fifteen patients exhibiting treatment problems requiring the extraction of bicuspid teeth were selected from routine screening examinations. Identical teeth were banded in all subjects. The amount of force exerted by each cuspid retraction device was measured in order to determine the amount of force each cuspid is receiving. Measure rates of movement in terms of distance, time, pain response and in ability to control tooth position. Evaluate anchorage loss.



Part A (continued)

Major Findings:

A description of results at this time would be premature for the project is in its early stages.

Significance to Dental Research:

There are not in existence at this time precise figures to advise any practitioner of the ideal amount of force to apply to a tooth that requires movement. At present many theories exist that are based on personal opinion. A study of this scope would offer a scientific approach to many clinical and research aspects of orthodontics.

Proposed Course of Study:

This is a continuing project in directions outlined above.

Part B included: No



Serial No. NIDR-78 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Evaluation of Epipharyngeal Mechanisms and Sensations in Cleft Palate Animals.

Principal Investigators: Drs. James F. Bosma and Julian M. Lifschiz

Other Investigators: None

Cooperating Units: Animal Hospital and Care, NIH. (Laboratory Aids Branch)

Man Years:

Total:	1/3
Professional:	1/3
Other:	None

Project Description:

Objectives:

1. To map the varied reflex modifications elicitable by stimulation in the epipharynx.
2. To determine the influence of isolation of the epipharynx by deflection of respiratory stream into the oral portal, employing mechanical obturation.
3. To determine the influence of abnormal exposure of the epipharynx, by cleft and/or excision of the soft palate.

Methods:

1. Surgically split soft palates of normal dogs and other animals for the purpose of access to the epipharynx in acute experiments.
2. Study the exposed area by tactile gross stimulation and by electrical stimulation, motor effects upon respiration by multiple channel EMG, by intra-tracheal pressure, etc.



Part A (continued)

3. Sensory "maps" on plaster impression of the individual dog's epipharynx.
4. Complete, or nearly complete, respiratory obturation at palate.

Materials:

Five normal 20-30 pound long-nosed dogs, mature rabbits and cats.

Major Findings:

To date findings are incomplete, for the experiment is in its early stages. Have observed thus far: Stimulation of epipharynx in particular areas produces nasal dilation, cough, sneeze, swallow, inspiration and gag. These responses require further study.

Significance to Dental Research:

The epipharynx is a highly strategic area of respiration--relevant sensations. The current respiratory stream apparently influences diameters and contours are pathologically modified in cleft palate and other anomalies, and in neurological impairment of pharynx and of branchial area. These pathological modifications are accessible to improvement by prosthetic and orthodontic measurements.

Proposed Course of Project:

This study is still in progress, with repeat observations of sensations elicitable by stimulation at epipharynx surfaces.

Part B included: No





- Serial No. NIDR-79 (C)
1. Clinical Investigations
  2. Oral Pharyngeal Development and Function
  3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Study of Tongue Position in Sustained Vowels, Adaptations to Individual Variations in Oral Form and Alignment.

Principal Investigator: Dr. Svend Smith (Visiting Scientist)

Other Investigators: Dr. Ronald Dubner  
Dr. Julian Lifschiz

Cooperating Units: Institute for the Deaf, Hellerup, Denmark (Dr. Smith), and Dental Clinic of the Clinical Center, NIH.

Man Years:

Total:	1
Professional:	1
Other:	None

Project Description:

Objectives:

1. To define the typical arrangement of the pharynx and mouth cavities during sustained vowel phonations.
2. To discern the various motor adaptations required to accomplish these phonation-relevant contours in subjects having the various basic patterns of alignments as categorized by the Angle orthodontic classification.

Methods Employed:

1. Selected normal volunteer subjects, employed in related orthodontic studies utilizing cephalometric techniques, were observed by single lateral x-rays of the oral and pharyngeal region during practiced sustained phonations of the vowels, "a," "ee," and "u."
2. These subjects were described by orthodontic criteria, from cephalometric films and from standard dental impressions. Standard lateral and frontal photographs were also obtained.



Part A (continued)

3. The differential adaptations of the action accomplishing the "portal area" contours were compared among the subjects having different oral-pharyngeal region arrangements, by detailed tracings from the films, employing consistent orthodontic landmarks for orientation and tracing.

Major Findings:

These subjects of varied oral-pharyngeal region spatial arrangement when in rest position accomplished highly similar portal area contours during phonation of the same vowels. Their motions and sustained position of supporting structures, however, were notably different. These differences are still under study, relating the tongue and pharynx phonation contours to skeletal elements in the films and to the dental impressions.

Significance to Dental Research:

It is already clear from this study that the various patterns of spatial orientation in the oral and pharyngeal region are associated with subject-distinctive motion patterns in these standard phonation performances. We thus have further evidence that the individuality of this region is that of their motor system as well as of their skeletal alignment.

Proposed Course of Project:

This study is continued by Dr. Smith in Denmark.

Part B included: No



Serial No. NIDR-80 (C)

1. Clinical Investigations
2. Oral Pharyngeal Development  
and Function
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year, 1961

Part A

Project Title: Development of Clinical Methods for Evaluation of External Pterygoid Muscle Disability in Patients with Temporomandibular Joint Dysfunction.

Principal Investigator: Dr. Ronald Dubner

Other Investigator: Dr. James F. Bosma

Cooperating Unit: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

1. To develop methods and criteria of clinical observation of the external pterygoid muscle.
2. To apply these findings in evaluation of temporomandibular joint disorders.

Methods Employed:

1. Subjects were examined clinically in the following fashion:
  - a) Evaluation of deviation of the mandible during passive, voluntary, and resisted voluntary opening movements of the mandible.
  - b) Evaluation of lateral displacement of the mandible during passive, voluntary and resisted voluntary movements.



Part A (continued)

- c) Palpation of oral and pharyngeal muscles for tender areas.
2. Cinematography was employed to permanently record defective mandibular movements during opening and lateral displacement.
3. Laminographic roentgenograms were taken of the temporomandibular joint and the pterygoid plates simultaneously by means of a transverse film at the level of the hard palate.
4. Cinefluography studies of the hyomandibular area were conducted during swallowing and vowel speech.

Patient Material:

Patients included in the study are those with complaints of temporomandibular joint dysfunction. Four patients in this category have been studied.

Patients with neurologic and muscular disorders of the head and neck will also be studied.

Major Findings:

1. A new method of pterygoid laminography has been developed.
2. No conclusions can be drawn from the small sample previously studied.

Significance to Dental Research:

- A. A general concept has been developed of an anatomical and performance entity consisting of pterygoid plates, external pterygoid muscles and condyle of mandible. It is suggested that this entity may be in error of form and/or function in patients having the "temporomandibular joint syndrome."
- B. The above entity is distinctly involved in ventrad displacement of the mandible, thus contributing to maintenance of the pharyngeal airway. The external pterygoid muscle action is thus continuous, varying in degree with changes in head and neck posture. The phylogenic development of this musculoskeletal entity suggests that wide variations in anatomic form are present. These anatomic variations and the continuous compensation of the muscle for transient imbalances of head and neck position lead one to suspect that this muscle is liable to disability with resulting pain and limitation of movement.





Part A (continued)

- C. The clinical examination routines developed in this study may aid in comparing these patients, and in evaluating spontaneous variations of their disability in comparison to variations resulting from attempted therapy.

Proposed Course of Study:

The functions of the external pterygoid muscles are under continuing study at the NIDR by Dr. Bosma and group, and at the Department of Orthodontics, University of Michigan School of Dentistry by Drs. Dubner and Moyers. The criteria of disability limitations developed by Dr. Dubner are being employed.

Part B included: No



1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Tri-racial Isolates in Eastern United States

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

1. To determine the number, types, location and size of tri-racial isolates in Eastern United States.
2. To determine the degrees of inbreeding shown by various isolates.
3. To determine the amount and types of hereditary illness shown by the groups.
4. To verify or not the assumption that more individuals will show homozygous recessive hereditary illness in such populations.
5. To investigate the social consequences of isolate formation and their effects on the distribution and prevalence of intrinsic disease.
6. To investigate and compare the prevalence, types and inheritance patterns of intrinsic illness with in-group and out-group marriages, with degrees of consanguinity, and various other tri-racial groups, and with the general population.
7. In summary, the purpose of this investigation is to find, enumerate and describe all tri-racial isolates residing in Eastern United States; to investigate the degree of consanguinity in



## Part A (continued)

these groups; to look for unusual types of matings, rare in human material, that are of special value in determining modes of inheritance, gene interaction, and biochemical pathways; to investigate the hereditary diseases present in these groups; and to utilize the patient material found for further clinical and biochemical genetic investigations. It is assumed that in isolate populations, consanguinity will be prevalent; and it is further assumed that an increased number of individuals in such a population will show homozygous recessive illness. It is also assumed that all cases of a specific hereditary illness from any one group will nearly always be due to the same gene defect, thus providing an epidemiological method for selecting a large sample of individuals with the same gene defect for biochemical investigations in other NIDR projects in other Institutes.

Methods Employed:

This investigation requires three approaches:

1. Analysis of census and other civil records.
2. Survey of groups by letters to physicians and public health officials concerned.
3. Field work by NIDR teams.
  - a. Analysis of census and other records:

Because of the U. S. Census Bureau's wish to further classify people whose race entries were somewhat nebulous, the 1950 Enumerators Reference Manual directed, "Report persons of mixed white, Negro, and Indian ancestry living in certain communities in Eastern United States in terms of the name by which they are locally known." Such persons were then to be classified for publication purposes among "other nonwhite races;" that is, other than Negro, American Indian, Chinese, Japanese, or Filipino. Starting with these data, and tracing the surnames so recorded through previous U. S. and British Colonial Census records, it was determined that such surnames frequently residing in the same household or neighborhood showed variable race entries from decade to decade, even for the same individual (i.e., white, Negro, Indian or other), and for the most part had large families. The existence of suspected tri-racial isolates from these data were then verified from local civil, historical, and social records, such as marriage records, historical accounts, and newspaper articles. A search of the anthropological and social literature frequently revealed studies on such groups. Visits to these communities, to verify the existence and surname data, were



## Part A (continued)

made by collaborators and this investigator.

Of the 116 counties checked, the population of tri-racial character was estimated at 77,000 persons, of which 33,000 were listed as Indian, 29,000 as white, 14,000 as Negro and 1,000 under colloquial race names. Forty percent of the total reside in North Carolina.

Local and census records were used to estimate consanguinity and fertility. For example, census data from one North Carolina group showed the highest fertility ratio for any known racial or ethnic group in the United States. The ratio of children under 5 years old per 1,000 women, 15 to 49 years old, was 825, as compared to 417, the U. S. average. Marriage records of one group showed that 177 required ecclesiastical dispensation for relationship within the first three degrees (i.e., 2nd cousins or closer). Investigation of the marriage records in Barbour and Taylor Counties, West Virginia, showed that 102 of the 112 marriages from 1856 to 1931 were to other tri-racial group members. School records of the same group in 1958 showed an unusual proportion of students from the group in the higher age levels for the grade in which they were placed.

b. Survey of groups by letters to physicians and public health officials:

After determining the number and surnames of persons comprising these isolate populations, letters were sent to all county health officials in the 116 counties where these people reside requesting information on specific listed hereditary illnesses among these groups and any illness that appeared unusually frequent in such populations. Other local physicians and dentists were also contacted. These returns were tabulated. Literature by all writers was scanned for mentioned hereditary illness in these groups, (e.g., microphthalmia in the Moors of Delaware).

c. Field work by NIDR team:

Biological specimens are collected in the field for analysis at the National Institutes of Health. These include bloods, urines, exfoliative cytology smears and biopsy material. In general, this work has been directed along two lines: (1) determining blood group frequencies, linkages and other biochemical or cellular abnormalities of blood fluid and urine; (2) determining the genetic patterns of inheritance of two particular diseases--hereditary benign intraepithelial dyskeratosis and oligophrenia, spasticity and ichthyosis. This latter condition is inherited as an autosomal recessive trait. The clinical features of this latter condition have been recorded in a movie film.





## Part A (continued)

Patient Material:

Patients found in this study of isolates have been admitted from time to time for the study of particular diseases. In addition to the diseases appearing in isolates, it was found necessary to admit certain families from the general population suffering from similar diseases in order to make cytological and cytochemical comparisons of these hereditary defects.

Major Findings:

1. Hereditary benign intraepithelial dyskeratosis was shown to be inherited as an autosomal dominant trait and affects oral mucosa and bulbar conjunctiva, leading to blindness. This new hereditary disease has been described, and publication was made late in 1960.
2. Comparison between the cellular, the cytochemical and the genetic findings in hereditary benign intraepithelial dyskeratosis was made with pachyonychia congenita of Jadassohn and Lewandowsky, white sponge nevus of Cannon, and Darier's disease. It has been determined that Darier's disease, hereditary benign intraepithelial dyskeratosis and white sponge nevus of Cannon are characterized by diagnostic discrete individual cell lesions. A new method of diagnosis of these conditions has been worked out by the exfoliative cytology technique. These methods have been described in the A.M.A. Archives of Dermatology and in a scientific exhibit presented at the annual session of the American Academy of Dermatology and Syphilology, Chicago, December 1-7, 1961.
3. The study of the dyskeratosis led us to theorize that the bases for these cellular abnormalities were genetic defects in the synthesis of nucleic acid. It has been found that patients receiving Methotrexate, 5-Fluorouracil, 5-Fluorodeoxyuridine, have similar cell lesions induced by these drugs in normal buccal mucosal cells. This reaction takes place within three days after administration of the drugs. It is postulated that either genetic or environmental factors which interfere with nucleic acid synthesis will induce this cellular change. This cellular change is manifested by a defect in cytokinesis and abnormal division of the chromosome and the formation of peculiar dyskeratotic cell-within-cell bodies.
4. These studies led to the present study which is now under way to determine whether or not this cellular change can be used to evaluate the efficiency of cancer chemotherapeutic drugs.
5. Investigation of the neurological defect present in the Haliwar



## Part A (continued)

Indians was made by admission of patients to the National Institutes of Health and by recording the findings in a motion picture. This picture has been completed and shows the following: The clinical features of this disease are oligophrenia, spasticity, ichthyosis of the skin of the trunk, and an abnormal pigmentation of the retina. This condition is inherited as an autosomal recessive trait, and all affected individuals are the offspring of consanguineous matings. In addition to the primary symptoms listed, the affected individuals show: (1) Loss of sensation in the maxillary anterior gingiva, (2) loss of tactile sensation in the tongue, (3) loss of normal masticatory function, (4) loss of sensation in the anterior portion of the leg, and (5) amino-aciduria.

Significance to Dental Research:

These groups offer unusual genetic material for the study of oral, connective tissue, eye, and metabolic defects in sufficient quantities so that the geneticist can assure the clinical and biochemical investigator that the genes involved are all of the same type. This offers an epidemiological method for the selection of patients with hereditary disease all with the same metabolic defect.

Proposed Course of Project:

To continue to accumulate information on the hereditary diseases present in these isolate populations. To create two new projects: (1) Concerns the study of the mental deficiency, spasticity and ichthyosis, and (2) to set up a study of exfoliative cytology of the hereditary dyskeratotic mucosal diseases. This latter will include a study to evaluate the responses of patients to cancer chemotherapeutic agents with the possibility that the exfoliative cytology method can predict the patient's responses to anti-cancer drugs within a period of three to four days, rather than the three to four months that are now required.

Part B included: Yes



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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Witkop, C. J., Jr.: Oral Exfoliative Cytology as a Diagnostic and Research Aid. Scientific Exhibit at the 20th Annual Meeting Am. Acad. Derm. & Syph., Chicago, Ill., Dec. 2-7, 1961.

Witkop, C. J., Jr., and Gorlin, R. J.: Four Hereditary Mucosal Syndromes: Comparative Histology and Exfoliative Cytology of Darier-White's Disease, Hereditary Benign Intraepithelial Dyskeratosis, White Sponge Nevus and Pachyonychia Congenita. Arch. Derm. 84:762-777, Nov., 1961.

Honors and Awards Relating to this project: None.



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Part A

Project Title: Genetic Study in Southern Maryland

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. L. J. Schneiderman  
Dr. R. O. Wolf  
Hazel R. Dyson, R.N.  
Dr. A. Donald Merritt  
Dr. B. L. Hanna  
Dr. C. S. Chung

Cooperating Units: School of Dentistry, Howard University;  
Sociology Department, Catholic University

Man Years (calendar year 1961):

Total:	4-1/2
Professional:	1-3/4
Other:	2-3/4

Project Description:

Objectives:

1. To determine the total amount of hereditary illness present in an isolate population of not over 5,000 related individuals of white, American Indian and Negro ancestry as revealed by dental and physical examinations and selected laboratory procedures.
2. To determine the prevalence, mode of inheritance, effects on viability and longevity, clinical manifestations and variations in expressivity of the hereditary illness found.
3. To investigate the relationships and possible linkages between these illnesses and blood types, secretor factor, P.T.C. taste test and other genetic markers.
4. To investigate genetic factors in illness not usually known to have a hereditary component.
5. To select patient and biological material from this population





Part A (Continued)

for clinical and biochemical investigations of specific illnesses and normal factors (these will constitute separate projects).

6. To assess the effects of inbreeding as revealed by in-group and out-group marriages and by degree of relationship (i.e., first cousins, first cousins once removed, second cousins, etc.) as shown by perinatal deaths, viability, longevity, and the presence of apparent homozygous recessive illness.
7. To try to trace the source of outflow of pathological genes in this population.
8. To assess the social and genetic factors in mate selection.
9. To look for correlations between genetic illnesses and other normal and abnormal factors that may have etiological or diagnostic relationships.
10. To try to reconstruct, on the basis of gene frequencies, the source of the group's genetic material.
11. To compare the prevalence of illness, gene frequencies, and vital statistics with other isolate populations and the general population.
12. To detect carriers of recessive genes, if possible.

Methods Employed:

This study is divided into three phases:

1. Field study:

The field study consists of history and dental, medical and laboratory examinations designed to detect as much hereditary illness as possible.

- a. History, genetic and census data are obtained on a questionnaire covering 158 items which arranges the data by family groups and by sibships. Such items as birth order effects, parental age, consanguinity, age of onset, longevity and perinatal mortality are included. A record of hospitalizations and institutionalizations is obtained in addition to a medical and genetic history. A master kindred chart showing the relationship of each individual is made.
- b. A search of existing social and church records for births and marriages is made by Fr. Thomas Harte of Catholic



## Part A (continued)

University. Sociological data on development in marriage patterns in this group are also obtained.

- c. All subjects in this study are given a complete dental and medical examination, designed to detect as much hereditary illness as possible. This includes complete blood typing genetic markers; urinalysis; hematological work-up, especially for abnormal hemoglobins; and special procedures, such as X-rays and ophthalmological examinations, where indicated. These examinations are made during the summer months at mobile units.
- d. All data are coded and tabulated for I.B.M. punch cards.

## 2. Clinical Study:

Selected patients seen in the field study are brought to NIH for detailed clinical studies. These studies are designed to give a description of the hereditary diseases involved; to determine the limits of the signs and symptoms of the illness; and to describe any metabolic abnormalities detectable on routine laboratory examinations, or other associated defects that might be found on X-rays or by special procedures.

## 3. Biochemical investigations:

Selected genetic defects are being investigated biochemically to find the chemical error present. Currently under study are sickle cell disease, dentinogenesis imperfecta, and diffuse goiter. The sickle cell disease is being investigated by tonometry, electrophoresis, and the survival of this abnormal hemoglobin in transfused patients. Dentinogenesis imperfecta is being investigated histochemically and by chemical analysis for abnormal protein constituents. The goiter patients are being studied for the presence or absence of non-thyroglobulin thyroid proteins, the response to thyroid-stimulating hormone, serum electrophoresis, total protein bound and butanol extractable iodide, and incubation of thyroid biopsies with  $I^{131}$ .

Patient Material:

Patient material is selected from the families seen in the field studies. Approximately 200 hospital days have been utilized by this and other Institutes.

Major Findings:

1. A biochemical investigation of albinism was conducted. This study



## Part A (continued)

utilized the albinos found in the isolate, as well as albinos collected from the general population in review of the following:

- a. That most albinos do have tyrosinase activity in the melanocytes of the skin and hair bulbs.
- b. These albinos have normal sera levels of tyrosine.
- c. By stripping off the keratin layers of the skin with repeated Scotch tape strippings and by packing the skin with wet packs of tyrosine, pigmentation can be induced in albinos.
- d. Loading experiments with hair-test positive (tyrosinase positive) albinos did not induce pigmentation where sera levels of 20 mg.% were maintained for 24 to 48 hours.
- e. The hair-test positive albinos have the inability to secrete normal levels of tyrosine in the saliva. This work was done in cooperation with Dr. Zipkin.
- f. Pigmentation cannot be induced by incubating skin and hair in certain types of albinos.
- g. The net results of these studies indicate that there are two types of recessive albinism in man, and that the biochemical defects in each are different. The two types of albinos (i.e., the hair-test positive albinos and the hair-test negative albinos) have not occurred in the same sibship, to date. From this we postulate that one type of albinism is due to a defect or an absence in the enzyme tyrosinase. This is by far the rarer type of albino. The other type of albinism does possess active functional tyrosinase. The defect in this type appears to be in the transport mechanism between the normal sera levels and the tyrosinase-intact melanocyte. It is postulated that this is a defect in a permease or a transferase.

Significance to Dental Research:

An isolate population has been found containing a vast number of hereditary diseases which may shed light on genetics and biochemical pathways of various hereditary defects.

Proposed Course of Project:

1. Data are being processed for machine analysis.



Part A (continued)

2. Paper on the biochemical defect in albinism is now in press in the Proceedings of the Second International Conference on Human Genetics, Rome, Sept. 6-12, 1961.

Part B included: Yes





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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Witkop, C. J., Jr.: Studies of Intrinsic Disease in Isolates with Observations on Penetrance and Expressivity of Certain Anatomical Traits. p. 291-308. (In Pruzansky, S., ed. Congenital Anomalies of the Face and Associated Structures, Thomas, C. C., 1961).

Honors and awards relating to this project: None



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2. Human Genetics
3. Bethesda, Maryland

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Part A

Project Title: Hereditary Defects of Enamel and Dentin  
(Michigan Study)

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/4
Professional:	1/4
Other:	None

Project Description:

Objectives:

1. To define the various hereditary defects in enamel and dentin.
2. To determine the prevalence and mutation rates in the general population.
3. To determine the mode of inheritance.
4. To investigate the histological processes involved in the defects.
5. To describe any associated physical or chemical defect.
6. To investigate possible linkage associations.
7. To determine the various methods of restoration, prevention or treatment, where possible.

Methods Employed:

1. Field Study: A survey of 96,761 school children between the ages of 4 and 12 was conducted in 42 counties in the state of Michigan for hereditary defects and other formations. Subsequent pedigree studies on these individuals were conducted to determine



## Part A (continued)

the mode of inheritance and possible linkage with known genetic markers, such as blood type, secretor factor, and P.T.C. taste test. These defects were classified by clinical descriptions, histological findings, and inheritance patterns. Patients with representative difficulties were brought to NIH for clinical evaluation.

2. Clinical Study: Complete physical and routine laboratory examinations were given to determine if any physical or metabolic defect was also associated with the tooth defect. Except in one particular defect, osteogenesis imperfecta, no genetic defect was found associated with the tooth condition.
3. Laboratory Study: Histochemical studies conducted on fresh ground and decalcified specimens, using normal controls, showed that there was a possible biochemical defect in opalescent dentin. Linkage studies were analyzed.

Patient Material:

Patient material was obtained from: (1) individuals contacted during our field study in Michigan; (2) individuals in our Genetic Study in Southern Maryland, Project No. D-4-002; and (3) in a few instances, from individuals referred by local practitioners.

Major Findings:

1. The fertility of individuals affected with opalescent dentin is from 1/3 to 1/2 greater than their unaffected siblings.
2. The teeth from individuals affected with opalescent dentin have been analyzed by the method of Bonting for their phosphatase activities, and these have been compared with a series of normal controls. These studies have shown that the phosphatase activity of the tooth type is approximately 1/3 that of normal teeth, and approximately of the same activity as that found in hypophosphatasia. The basis for this decreased enzyme activity has not been adequately explained.

Significance to Dental Research:

The etiology of several conditions may now be ascribed to hereditary causes. The clinical description of the oral defects has been given, and experience in surgical correction and restoration has been obtained.

Proposed Course of Project:

Continued accumulation of pedigree material and linkage data is



Part A (continued)

planned. Investigation of the biochemical defects present in these conditions will be continued.

Part B included: Yes





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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and awards relating to this project:

The Human Genetics Section of the National Institute of Dental Research was asked by the American Dental Association to sponsor the First International Symposium on Genetics Related to Dental Health, which was held April 4-6, 1961, at the National Institutes of Health in Bethesda, Maryland: Hill, T. J. and Pearlman, S.: Symposium on Genetics Related to Dental Health: A summary report. J. Am. Dent. Assoc. 63:639-642, November, 1961.



Serial No. NIDR-84 (C)  
1. Clinical Investigations  
2. Human Genetics  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
Calendar Year 1961

Part A

Project Title: Sickle Cell Anemias, Other Inherited Hematological Disorders, and Blood Groups

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. D. L. Rucknagel  
Dr. L. J. Schneiderman  
Dr. Robert O. Wolf  
Dr. A. D. Merritt  
Dr. Luis Barros

Cooperating Units: Department of Human Genetics, University of Michigan

Man Years (calendar year 1961):

Total:	1-1/4
Professional:	3/4
Other:	1/2

Project Description:

Objectives:

To study in detail physiologic, biochemical, and genetic factors in various inherited hematologic disorders in the Brandywine tri-racial isolate. Laboratory and clinical data will be integrated with the pedigrees and analyzed for the purpose of defining biochemical mechanisms and mode of inheritance of disease. In addition, prevalence figures for abnormal hemoglobins will be determined in various surveys in which the Section participates. These include the ICNND Nutritional Survey of Chile, various North Carolina groups and American Indians.

Methods Employed:

Field surveys among the Wesort group have detected individuals with several hematologic disorders. Approximately twenty percent of this group harbor the sickle cell gene. Individuals with sickle cell anemia are hospitalized and evaluated by standard hematologic procedures. In addition, specialized investigations of intravascular



## Part A (continued)

sickling, arterial oxyhemoglobin saturation, and erythrocyte physiology, using tonometric techniques, are utilized.

Relatives of propositi are studied hematologically, using standard hematologic techniques, paper and starch block electrophoresis and, when indicated, oxygenation kinetic studies.

Non-sickleemic anemic individuals and their families are also being studied to elucidate other genetic abnormalities. One individual with pernicious anemia was studied extensively (in cooperation with Drs. Seegmiller, LaDu and Laster) in an attempt to elucidate the effect of vitamin B<sub>12</sub> deficiency upon purine biosynthesis. The prevalence of blood factors and the correlation with genetic disease and nutritional status in various populations was made for blood type, red cell enzymes and abnormal hemoglobin.

Patient Material:

Patients and families (640 persons) were selected from the Brandywine population for haptoglobin study.

In Chile 1906 Chileans residing from Punta Arenas to Arica were examined for dental defects and nutritional status. Of these individuals, 673 were blood typed; the erythrocytes were examined for glucose-6-phosphate dehydrogenase activity; and the hemoglobins were analyzed by paper electrophoresis for abnormalities. Samples from 120 persons of a tri-racial isolate in North Carolina were screened for abnormal hemoglobins and blood type. Three hundred North American Indians and 140 Araucanian Indians of Chile were surveyed.

Major Findings:

1. Preliminary analysis of the Chilean data show that there is an excess of individuals with scrotal tongue who have blood group O.
2. Analysis of the Chilean data show that people who have scrotal tongue also have significantly low serum vitamin A levels. It is postulated that this is not a cause and effect. Currently we are investigating this relationship. A possible explanation is as follows: Scrotal tongue has been shown to be genetically determined as an autosomal dominant trait. It is postulated that the scrotal tongue is only one manifestation of an abnormal gastrointestinal mucosa. This mucosa lacks the ability to properly absorb either vitamin A or the carrier substances, such as fat, resulting in low serum vitamin A levels in these individuals. We are investigating this particular idea by doing absorption studies on a selected group of patients and normal controls.



## Part A (continued)

3. The gene frequencies for blood groups of Araucanian Indians, the general population of Chile, the Haliwar Indians and Ethiopians have been calculated and are now being prepared for publication.
4. The frequencies of haptoglobins occurring in the Brandywine population have been a subject of presentation at the Second International Conference of Human Genetics in Rome, and a new method for characterizing population differences has been utilized. This indicates a vast amount of genetic drift in the Brandywine population.
5. There was no correlation between periodontal index scores and and blood groups ABO, MN and Rh in the Chilean population.
6. There was no correlation between DMF scores and blood groups ABO, MN and Rh in the Chilean population.
7. There was no correlation between blood groups ABO, MN and Rh and vitamin deficiencies in the Chilean population.
8. The oral manifestations of tuberous sclerosis have been documented and photographed and are in the process of publication.
9. The frequencies of oral anomalies determined in the Chilean population have been compared to blood group distributions and are in the process of publication. Significant findings in this particular area indicate: (a) the frequency of torus mandibularis and torus palatinus in the Chilean population is the lowest reported in any population studies to date and (b) evidence for genetic and nutritional balanced polymorphisms have been obtained from the Chilean population.

Significance to Dental Research:

Since it is recognized that genetic factors are important in the study of dental diseases as well as other medical problems, this program is aimed at evaluating genetic mechanisms by as many approaches as are feasible. By studying specific entities as thoroughly as possible and correlating variations of disease and laboratory findings with pedigree data, it is anticipated that conclusions may be forthcoming regarding genetic mechanisms.

Proposed Course of Projects:

Enlargement of the Brandywine pedigree will continue, but hematologic investigations are now being concentrated in areas of the pedigree deemed to be most promising. These are designed to elucidate the presence and nature of modifying hereditary factors





Part A (continued)

in sickle cell anemia, and to evaluate the presence of Thalassemic genes in the non-sickleemic anemia sibships. Data are now being processed for I.B.M. machine analysis.

Part B included: No



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1. Clinical Investigations
  2. Human Genetics
  3. Bethesda, Maryland

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Part A

Project Title: Oral Aspects of the Results of Consanguineous Marriage  
in Hiroshima and Nagasaki, Japan

Principal Investigator: Dr. J. D. Niswander

Other Investigators: Dr. C. J. Witkop, Jr.

Cooperating Units: National Academy of Sciences, Washington, D. C.;  
University of Michigan Department of Human Genetics  
under the direction of James V. Neel, William J.  
Schull, and James N. Spuhler

Man Years (calendar year 1961):

Total:	1
Professional:	1
Other:	None

Project Description:

Objectives:

1. In connection with the genetic program of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki, Japan, the details of which have been presented by Neel and Schull (Neel, J. V. and Schull, W. J.: The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, #461, Washington, 1956), the outcome of pregnancy was determined in 5,033 instances in which the parents reported consanguinity of some degree. The products of these pregnancies ranged in age between five and ten years at the time they were recontacted in the present study. In essence, this study involved an attempt to obtain a detailed follow-up study on each of these 5,033 pregnancy terminations, plus a suitable control material.
2. Of the 5,033 pregnancy terminations, a number were known to have resulted in stillbirths or deaths during the first nine months of life. Starting from the records of the Atomic Bomb Casualty Commission, a trained staff of "field workers" reestablished contacts with all of the remaining children (i.e., registered in



## Part A (continued)

the Genetics Program and not known to have died during the period of time covered by this program). The result was a roster of living children, a roster of now-deceased children, and a roster of children who have moved from the two study cities. An effort was made to establish cause of death for all deceased children. The background of the children who moved from the cities will be analyzed, in a search for biasing factors. The fact of death and, possibly, the cause of death will be analyzed in relation to parental consanguinity.

3. A pilot study carried out in 1956 under the direction of W. J. Schull has already revealed that the death rate among the children of consanguineous marriages is approximately twice the control death rate (cf. Appendix). There is, then, already considerable assurance of significant results from this inquiry.
4. The dental portion of this study proposes to determine the effects of consanguinity on DMF rates, periodontal disease indices, growth and development problems, and specific hereditary diseases affecting the oral cavity.

Methods Employed:

Using ABCC facilities in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, hematology, dentistry, and anthropology began examinations of offspring of consanguineous marriages and appropriate control groups in September, 1958. Examinations were completed in July, 1960. The following examinations were performed:

1. Physical examination: This consists of a standard physical examination, with emphasis on characteristics which can be graded in an objective manner lending itself to statistical analysis.
  - a. Presence of gross physical defect; i.e., abnormalities of skin, musculature, skeleton, etc.
  - b. Defects of the auditory mechanism, with special reference to loss of hearing.
  - c. Defects of the eye, including loss of visual acuity.
  - d. Cardiac abnormality (cardiomegaly, disorders of rhythm, murmurs, etc.).
  - e. Pulmonary abnormality.
  - f. Apparent abnormality of abdominal viscera.



## Part A (continued)

- g. Abnormality of external genitalia.
  - h. Bone age (single wrist film).
  - j. Determination of leucocyte count and hemoglobin level.
  - k. Standard urinalysis.
2. The dental examination includes pathology of the soft tissues, pathology of the teeth and index of malocclusion, index of tooth loss, developmental failures of oral structures, DEF and DEF rates and the association of these conditions with and without generalized hereditary disease.
  3. Anthropometric examination: This included such standard items as weight, sitting and standing height, span, measurements of head and face, subcutaneous fat index, etc.
  4. Psychometrics: Each child was tested at time of clinic visit by means of a portion of the WISC test (maze and trail). In addition, the complete Japanese standardized WISC was administered by the Hiroshima University Department of Psychology.
  5. School performance: Access was obtained to school records for each child and selected information abstracted.

All results were coded and transferred to IBM cards for statistical analysis. As for the Genetics Program data, the statistical analysis will undoubtedly draw heavily on an approach which makes allowance for certain uncontrollable sources of bias. The first result will be a comparison of the different groups of children with respect to a wide variety of characteristics with particular reference to the regression of the findings on degree of inbreeding. Beyond this, however, it is anticipated that the material will permit tentative conclusions concerning the number of "lethal-equivalents" carried by the average Japanese, utilizing the mathematical approach suggested by Morton, Crow, and Muller (1956).

Major Findings:

The field work in Japan was completed in August, 1960. A total of 3,382 children of cousin marriages were examined in addition to a control group of 3,501. For a more detailed breakdown of the study group, see the 1960 Project Report. As anticipated, a large portion of the time during the previous year has been spent in assembling the data and making the necessary preparations for their analysis by high-speed computing machines. A large number of preliminary analyses have been completed at this time. Most of these have been concerned





## Part A (continued)

with checks on consistency of the data, as well as distribution of concomitant variables, such as age, socio-economic level, city effect, etc. These analyses have revealed no major differences between control and inbred groups in most respects. However, certain differences between cities appear to exist. The major portion of the mortality and historical data has been analyzed at this time, as well as certain aspects of the anthropometric and morbidity data.

Aspects of the dental data which are now partially analyzed include dental eruption, malocclusion, caries, and congenital anomalies of the teeth. The most striking finding appears at this time to be an almost complete absence of inbreeding effects on any of these variables. Some other findings not necessarily related to inbreeding are:

1. A significant acceleration in tooth eruption in Hiroshima between 1951 and 1959.
2. An apparent effect of socio-economic status (possibly nutritional) on tooth eruption, indicated by a positive association between the amount of money spent for food by the family and the number of permanent teeth erupted.
3. A greater similarity in dental eruption status between siblings than non-sibling pairs.
4. A significant positive association between caries of the deciduous teeth and structural defects in the enamel of their permanent successors.
5. A higher prevalence of supernumerary teeth in Japanese than in Caucasians, with a marked sex difference in Japanese (4% of all males affected and 2% of the females).
6. Racial differences in frequencies and distribution of congenitally missing teeth and other dental anomalies.

Significance to Dental Research:

1. Properly executed studies on consanguinity effects will yield information on the genetic structure of human populations which at present can be obtained in no other way. Such information is vital to a realistic, quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.
2. The Japanese Study will offer an opportunity to study the genetic effects of a specific type of inbreeding, complementing our



## Part A (continued)

Brandywine Study. Whereas the latter program is assessing the effects of continuous inbreeding, the Japanese Study has the unique advantage of affording an evaluation of one type of mating pattern.

3. It would be expected that certain recessive characteristics determining normal growth and development, as well as pathological traits, would show up in the homozygous state with increased frequency. Many of these conditions are probably unknown, and the Japanese Study consequently offers an opportunity to define both normal factors and pathological traits.
4. The Brandywine population group is descended from relatively few ancestors. Consequently, unless an ancestor carried a hidden trait, it could not appear in the descendants, that is, barring mutation. Therefore, the Brandywine group would present only those inherited conditions carried by predecessors. On the other hand, the first cousins in Japan are drawn from a large unrelated population, and therefore, many characteristics and pathological traits should appear in their offspring.
5. In addition to the study of inbreeding effects, the normative aspects of the data offer numerous opportunities for the investigation of the role of racial and environmental factors in many oral conditions.

Proposed Course of Project:

It is hoped that the major portion of analysis with respect to inbreeding effects will be completed in the forthcoming year. Write-up of the complete consanguinity findings in monograph form is expected to be begun sometime during the next year, and will include a summary of the dental findings. In addition to the consanguinity monograph, a number of other publications on incidental findings are anticipated. It is expected that this project will occupy a major portion of the Principal Investigator's time for at least two additional years.

Part B included: Yes



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Part B: Honors, Awards, and Publications

Publications Other than Abstracts from this Project:

Niswander, J. D., and Sujaku, C. Dental Eruption, Stature, and Weight of Hiroshima Children. J. Dent. Res. 39:959-963, September-October 1960.

Schull, W. J., and Neel, J.V. The Child Health Survey: A Genetic Study in Japan. WHO Seminar on the Use of Vital and Health Statistics for Genetic and Radiation Studies, Geneva, 1960 (in press).

Schull, W. J., and Neel, J. V. The Effect of Inbreeding on Mortality in Japan. Second International Conference on Human Genetics, Rome, 1961 (in press).

Honors and Awards Relating to this Project: None.



Serial No. NIDR-86 (C)  
1. Clinical Investigations  
2. Human Genetics  
3. Bethesda, Maryland

PHS-NIH  
Individual Project Report  
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Part A

Project Title: Saliva Study

Principal Investigator: Dr. R. O. Wolf

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1-1/4
Professional:	1
Other:	1/4

Project Description:

Objectives:

The objectives of this project are:

1. To use various techniques (see methods) which have inherent varying resolving powers to determine and define salivary components.
2. To determine the normal variation of the salivary components between normal individuals as related to environmental conditions at the time of collection.
3. To relate components to genetic control. This includes genetic systems, such as secretor factor and unknown, but suspected, control of salivary proteins.
4. After methods are perfected and normal bases are established, the salivary components of selected disease entities will be studied.

Methods Employed:

1. Investigation of methods best suited for estimating salivary protein concentration of individual samples for electrophoretic methods.





Part A (continued)

2. Paper, starch and cellulose acetate media are being used for saliva electrophoresis.
3. Immuno-electrophoresis in agar gel of saliva is being investigated. Antibodies are made in the rabbit. Saliva is being collected with Lashley cups.
4. The agglutination inhibition type test is used for determining the secretor factor condition of a given saliva sample.

Patient Material:

1. Normal control patients of the Clinical Center. NIH approval for study of normal control patients has been obtained.
2. Selected Clinical Center patients.
3. Individuals from genetically defined populations.

Major Findings:

1. Adequate methods for concentrating salivary proteins have been found. Concentration of protein is under some control. Minimal protein denaturation with consistent similar paper electrophoretic results have been found.
2. Discrepancies in the salivary secretor factor and blood groups of a genetically defined population have been found, as well as variation in the salivary components.
3. A paper electrophoretic technique has been developed for salivary protein. A pilot study is under way to prove the usefulness of the technique.

Significance to Dental Research:

This study has further elucidated the components, biological control and significance of this lesser studied body secretion.

Proposed Course of Project:

We intend to detect and define possible gene-controlled salivary components, which in turn may lead to the definition of new metabolic pathways or corroborate known ones.

Part B included: No













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