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

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

PART II

United States NATIONAL INSTITUTE OF DENTAL RESEARCH

ANNUAL REPORT of program activities.

NATIONAL CARIES PROGRAM

October 1, 1978 - September 30, 1979

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

Compiled By

Dental Research Data Officer

National Institute of Dental Research

National Institutes of Health

Bethesda, Maryland

CONTENTS

PART I - OFFICE OF THE DIRECTOR

PART II - NATIONAL CARIES PROGRAM — PAGE

Section I
Report of the Associate Director — 2-1

Strategy Area I:
Combatting the Microbial Agent — 2-3

Strategy Area II:
Increasing the Resistance of the Teeth — 2-10

Strategy Area III:
Decrease the Cariogenic Potential of the Diet — 2-15

Strategy Area IV:
Improved Delivery and Acceptance of
Caries Preventive Methods — 2-17

Section II
Direct Operations — 2-21

Section III
Contract Activities — 2-131

PART III - EXTRAMURAL PROGRAMS

PART IV - INTRAMURAL RESEARCH

PART V - COLLABORATIVE RESEARCH PROJECTS

National Caries Program

Report of the Associate Director

Noteworthy advances were made during the year in all four Strategy Areas of the National Caries Program:

I: Combat the Microbial Agent

II: Increase Tooth Resistance

III: Alter Harmful Properties of the Diet

IV: Improve Delivery and Acceptance of Caries Prevention Methods.

A grantee had reported that human volunteers vaccinated with killed cells of Streptococcus mutans via the gastric route had increased salivary antibody titers against this organism. NCP scientists repeated this experiment and confirmed that antibody levels increased in both serum and saliva following three vaccinations, per os. Thus the principle of oral immunization against dental caries, previously established in laboratory animals, appears feasible in humans also.

Analysis of final pre-clinical data from tests of a slow release device for intra-oral administration of fluoride showed that fluoride release rates in dogs were remarkably stable, at about 1 mg F per day, over an eight month period. Acute and chronic toxicity evaluation of the device has shown no contraindications for human use, and Phase I clinical trials have begun. It is anticipated that successful development of this slow release system could substantially decrease the cost of delivery of fluoride and other cariostatic agents.

A major program effort was directed toward the development of a valid, reproducible regimen to screen common food items for their cariogenic potential. For practical and ethical reasons, such assessments cannot be made by long-term human trials. The NCP system involves preliminary assessment of the tendency of a food to produce acids from fermentation by oral microorganisms. This is determined by monitoring plaque pH levels in vivo using intra-oral telemetry. A prototype telemetry system has been developed and is ready for human testing. Phase II of the screening system involves feeding the test food to laboratory rats using a unique protocol developed by NCP scientists. The method involves giving the animals all required nutrients by stomach tube, but feeding the test food at fixed intervals and in measured quantities using a programmed feeding machine. Thus any caries which develops is ascribable solely to the cariogenicity of the tested food. Preliminary results from this food screening system were reported during the year.

Data from the third and final year of seventeen school-based fluoride mouthrinse demonstration programs confirmed earlier evidence of the efficacy, low cost and ready public acceptance of this caries preventive method. We estimate that over 10 million U.S. school children are now participating in such programs, compared to about 1.5 million before the NCP demonstration began.

Efforts to inform the public about school-based fluoride programs and to promote their implementation, were sharply increased last year. Two new exhibits, and four films describing these programs were produced, and plans were made for their wide distribution. In addition, NCP staff gave several dozen talks and seminars on prevention to lay and professional audiences.

Now that caries preventive methods are beginning to be widely implemented, it is essential to have a reliable method for measuring their public health effects, as well as to identify segments of the population requiring priority implementation of preventive programs. With assistance from the National Center for Health Statistics, the NCP designed and implemented a national survey of the prevalence of caries and the need for restorative dental treatment. About 50,000 children, ages 5 - 18, will be selected in a probability sample. By summer, 1980, highly precise prevalence estimates will be available, for the first time, by age and by geographic regions of the country. We intend to repeat this survey at regular intervals to detect changes in the caries status of the population.

NCP research grants and research contracting activities were consolidated into a single administrative structure in the newly organized Caries Research Grants and Contracts Branch. Dr. John Townsley was appointed Chief of this Branch, and Dr. Andrew Vargosko joined the staff as Assistant Chief.

A detailed description of the National Caries Program research and development activities during FY 1979 is given in the following pages.

STRATEGY AREA I: Combatting the Microbial Agent

The Program supports and carries out numerous projects designed to provide fundamental information on the etiology and epidemiology of caries. As this information becomes available, it is being used to develop acceptable public health methods for controlling the responsible microorganisms and preventing the disease. This section of the report illustrates several of the promising approaches being made during FY1979.

Adherence of Plaque Organisms

The NCP supports several investigations of the mechanisms by which oral microorganisms adhere to each other, to other species of organisms, to the tooth surface and to the salivary pellicle covering the tooth. These processes are thought to be important in plaque formation and caries development. Their elucidation should suggest effective approaches to prevent caries. One grantee is studying adhesion between Streptococcus sanguis and Actinomyces viscosus. These organisms are among the first to colonize the teeth as they erupt. When separately suspended in salt solution each of these species settles very slowly; when mixed together they form large aggregates, which settle rapidly. This coaggregation results from a lectin-type reaction between a specific protein on the surface of the actinomycetes and a specific carbohydrate on the streptococci. The coaggregation is inhibited specifically by derivatives of β-galactose, indicating that the surface carbohydrate of S. sanguis possesses terminal β-galactose units, which are required for coaggregation. Calcium is essential for coaggregation to occur; it is thought to maintain the necessary conformation of the protein on A. viscosus. This type of adherence is peculiar to certain species and strains of organisms. Eighty percent of numerous strains of A. viscosus and A. naeslundii coaggregated with a strain of S. sanguis but no strain of S. mutans or S. salivarius coaggregated the actinomycetes. There was evidence of two-way reactions in which an actinomycetes protein reacted with a streptococcal carbohydrate and a streptococcal protein reacted with a carbohydrate on the actinomycetes. If suitable inhibitors of these coaggregation reactions can be found, they might be useful in controlling plaque development.

S. mutans will colonize tooth smooth surfaces in the absence of sucrose. However, adherence is more extensive in the presence of sucrose since this organism produces the extracellular polysaccharide dextran from sucrose and forms copious plaque. Recently, several dextran binding proteins have been isolated from this organism. Two of these proteins have no enzymatic activity but they bind water soluble α-1,6 linked dextran. They differ in molecular weight and their production varies among the serotypes of S. mutans. A second group of dextran binding proteins includes the glucosyltransferases. Two distinct glucosyltransferases are present, one synthesizing water insoluble dextrans with a high proportion of α-1,3 linkages and one synthesizing water soluble dextrans containing predominantly α-1,6 linkages. Antibodies directed against glucosyltransferase inhibited colonization of smooth surfaces by strains of S. mutans in vitro, confirming the important role of this enzyme in adherence. These enzymes bind both water soluble and insoluble dextrans,

whereas the non-enzymatically active proteins, mentioned previously, are unable to bind water insoluble dextran. Since the ability to synthesize and bind water insoluble dextrans endows S. mutans with the capacity to form adherent masses in the presence of sucrose, these enzymes appear to be major molecular mediators of sucrose dependent adherence to the teeth. In addition, these enzymes are capable of binding salivary mucin, raising the possibility that they may also mediate sucrose independent adherence of S. mutans to the glycoproteins of the salivary pellicle. Sensitive physical methods have been developed to monitor adherence. Using a laser light scattering technique, a grantee was able to measure the rate of aggregation of S. mutans in the presence of dextran. The technique is sufficiently sensitive to detect the onset of aggregation when less than one molecule of dextran per bacterial cell is present. These studies showed that, on the average, there are at least 1,000 dextran binding sites on the surface of each bacterial cell.

In addition to the bacterial surface proteins and carbohydrates, which participate in adherence, the teichoic acids are being studied intensively in several laboratories. These polymers are composed of repeating units of negatively charged glycerol phosphate and also contain sugars and fatty acids. Thus, they can be attracted to positively charged groups on the tooth surface, and sugar moieties provide sticky qualities and the fatty acids are attracted to salivary lipids coating the tooth surface. These amphipathic qualities of being attracted to both water soluble and fat soluble groups suggest that the teichoic acids are important mediators of adherence. The conditions under which organisms are grown profoundly affects production and excretion of teichoic acids. S. mutans produces the maximum amounts of teichoic acids when grown at 2-3 generations per day, which is equivalent to growth rates calculated for the oral cavity. Organisms grown in sucrose showed enhanced lipoteichoic acid production compared to glucose grown cells. Subsequent experiments suggested that this enhancement is due to the fructose component of sucrose. These findings parallel those of other workers who have reported higher lipoteichoic acid contents of plaque from subjects participating in sucrose rinsing regimens than from subjects of glucose or xylitol mouth rinsing regimens. Thus the stimulation of plaque formation by sucrose may depend not only on dextran formation, but also on the concomitant production of increased amounts of lipoteichoic acid. It is of interest that the antimicrobial, chlorhexidine, which reduces plaque formation, was shown to make the charge on human enamel surfaces more negative. Since bacterial surfaces are also negatively charged, due in part to the teichoic acids, these organisms would be repelled more strongly by an increase in negative charge of the enamel.

New techniques are being devised to hydrolyze bacterial cell walls prior to isolation, purification, and identification of their components. An enzymatic method was established to completely hydrolyze walls of several strains of S. mutans. The cell wall polymers liberated were isolated by ion exchange chromotography and gel filtration. Each strain was found to contain 1 to 4 rhamnose containing polysaccharides, accounting for about 50% of wall substance, and peptidoglycan, also accounting for about 50% of the wall. These techniques improve our picture of cell wall composition and structure, enhance our understanding of the mechanism of

adherence and provide polymers, which can be used as immunogens. The application of enzymatic methods for immunogen production is desirable since the antigens produced in this way more closely resemble those normally encountered by the host, than when antigens are obtained by chemical degradation.

Saliva and Susceptibility to Caries

The importance of saliva as a vehicle for antimicrobial factors was discussed at a recent NCP sponsored conference (Saliva and Dental Caries, eds. I. Kleinberg, S.A. Ellison, and I.D. Mandel, Microbiology Abstracts Sp. Suppl., 1979). Saliva contains antibodies to specific cariogenic microorganisms, other materials which agglutinate and aid in removal of pathogens and several non-specific factors, which have the potential to inhibit bacterial growth and metabolism.

NCP supported scientists found that apolactoferrin, which occurs in saliva, was bactericidal for numerous oral organisms, including S. mutans serotypes a-g, Streptococcus salivarius, and nonenteropathogenic E. coli. Enteropathogenic E. coli, Staphylococcus aureus, Streptococcus pyogenes and two organisms found in milk where lactoferrin is abundant, Streptococcus lactis and Lactobacillus casei, were relatively resistant. Further studies showing inhibition of microbial growth by lactoferrin also demonstrated that in general inhibition was readily reversible by exogenous iron. However, growth of S. mutans occurred in iron deficient media and with this organism growth inhibition by lactoferrin was not reversible by exogenous iron. Therefore, it is unlikely that the bactericidal action of apolactoferrin can be attributed to iron deprivation. Immunofluorescence techniques showed that apolactoferrin bound to the cell surface and could not be removed by washing. Uptake of uracil, thymidine and lysine by S. mutans was inhibited by apolactoferrin treatment, as was glucose utilization and lactic acid production.

Lactoperoxidase is a second nonspecific salivary factor which has the potential to inhibit cariogenic organisms. Using E. coli as a model, a grantee has studied the antimicrobial action of lactoperoxidase. In the presence of hydrogen peroxide, lactoperoxidase generates hypothiocyanite oxidizes thiol groups, resulting in inhibition of respiration of the microorganism. Glucose and sucrose utilization of S. mutans is inhibited and prolonged incubation with excess hypothiocyanite results in loss of viability. Reports from other laboratories indicate that lactoperoxidase accumulates on the enamel surface which lends support to the suggestion that it may provide an effective barrier to cariogenic attack. Studies on lysozyme interactions with oral bacteria are being supported by the Program. Lysozyme mediated lysis of S. mutans is activated by anions and the degree of activation varies with the concentration and type of anion (e.g., thiocyanate, nitrate and fluoride). Bicarbonate was highly potent in activating lysis of S. mutans; suggesting that this anion may have anti-caries actions in addition to its anti-acid properties. Significant lysis could be achieved at concentrations of bicarbonate reported to occur in saliva under physiological conditions. It appears probable that additional antibacterial systems may exist since salivary histidine-rich polypeptides were found to alter membrane permeability and reduce

viability of S. mutans.

Several investigators are conducting comparative studies on individuals who are susceptible to caries and those who are truly resistant to caries, without cavities or fillings, or caries-free subjects who have no active caries at the time of the study. In subjects not exposed to fluoride during tooth formation, the incidence of caries resistance is only 1 in 750. There are twice as many resistant males as females. Caries resistant females had significantly less periodontal disease than susceptible males. Among males, the levels of periodontal disease were similar. These findings refute the common perception that caries and periodontal disease are inversely related. On the basis of thorough diet histories, it does not appear that the resistant group differs nutritionally from the susceptible group. In fact, the caries resistant group had slightly more sucrose exposures. The potential for plaque acid production was measured intra-orally after a sugar rinse. Plaque samples were also removed and the amounts of plaque acids determined. The pH minimum and the amount of plaque acids were significantly lower in the susceptible group. Differences between plaque of caries free and caries active subjects are being studied with the aid of dental appliances fitted with bovine enamel inserts. The appliances are worn for two weeks and the enamel inserts exposed periodically to either sucrose or saline solutions. In all subjects, sucrose exposed plaque developed higher proportions of S. mutans, S. salivarius, lactobacilli and Veillonella sp., while saline exposed plaques exhibited higher proportions of S. sanguis and Neisseria sp. Sucrose exposed plaques were more active than those exposed to saline, in converting radiolabelled sucrose to lactic acid. The higher the proportion of S. mutans and lactobacilli in the plaque, the greater was the extent of enamel demineralization. Initially, plaque of caries free subjects harbored lower levels of S. mutans and lactobacilli, and higher levels of S. sanguis and Neisseria sp. than that of caries active subjects. However, there was a shift to the cariogenic type of plaque flora following frequent exposure to sucrose. This indicates that host resistance factors were unable to prevent the ecological shift induced by frequent sucrose challenge. Earlier studies by a grantee had established that salivary proteins could be utilized as a nitrogen source by plaque forming oral streptococci. These studies have been extended to salivary proteins from caries active and caries resistant subjects. Both S. mutans and S. sanguis grew well in a synthetic medium containing saliva from caries active patients. Analysis revealed that this saliva contained proteins previously shown to be susceptible to attack by S. mutans. In contrast, the saliva from caries resistant individuals seldom contained these proteins and growth in synthetic medium containing this saliva was scant.

Fluoride as an Antimicrobial Agent

Fluoride is generally thought to prevent dental caries by altering the structure of tooth enamel, so that it becomes more resistant to attack by acidogenic bacteria. Data from several NCP supported projects indicate that fluoride may also prevent decay by affecting the growth and metabolism of plaque microorganisms. Periodic treatment of teeth with gels containing fluoride over a two to three year period has proven

effective in reducing development of new carious lesions by up to fifty percent. Similar treatments are effective in controlling the rampant caries associated with xerostomia. Daily applications of 1% fluoride gel for 5 minutes increased plaque fluoride concentrations ten fold. Although the plaque level of fluoride decreased rapidly following treatment, daily applications resulted in plaque fluoride levels appreciably greater than pretreatment levels. There was a decline in plaque lactic acid production for 24 hours following fluoride treatment and the population of S. mutans was suppressed. Studies with radioisotopically labelled fluoride showed that S. mutans in culture can accumulate fluoride against a concentration gradient. This explains why plaque fluoride concentrations are consistently greater than those of saliva or drinking water. Absorption of fluoride was independent of temperature and did not require the presence of sugars, although it was enhanced by acidic conditions. Rapidly dividing cells absorbed more fluoride than did older, non-dividing cells. Additional studies showed that extracellular fluoride was adsorbed by 12 macromolecular cytoplasmic constituents, one of which was enolase. Enolase is an enzyme involved in glycolysis and consequently in lactic acid production. It has been known for some time that enolase is inhibited by fluoride. When purified enolase preparations were obtained from several strains of streptococci, they all bound fluoride and were equally susceptibel in inhibition by fluoride ions. An additional locus of the antimicrobial action of fluoride has been proposed by a grantee. His evidence indicates that fluoride enhances the turnover of peptidoglycans of the cell wall, making the organism susceptible to autolysis and resulting in cell death. Normally, S. mutans is not sensitive to lysozyme unless cells have been pretreated with damaging agents such as detergents. However, when grown in media containing fluoride, S. mutans becomes susceptible to lysozyme induced lysis. It appears that fluoride can sensitize bacteria to this natural defense factor in the mouth.

Specific Immunity to Caries

Last year it was reported that ingestion of killed, whole cells of S. mutans by experimental animals and humans produced an immune response in saliva and in some instances in serum. Several investigators are examining the hypothesis that the ingested bacterial antigen stimulates gut associated lymphoid tissue (GALT). At the NCP supported conference on the Secretory Immune System and Caries Immunity (Secretory Immunity and Infection, Eds. J.R. McGhee, J. Mestecky & J.L. Babb, Plenum Press, 1978), it was postulated that both T. cells and precursor IgA-B cells, underlying epithelium in the GALT are stimulated by penetrating bacterial antigen. These stimulated precursor cells leave the GALT, via the lymphatics, and enter the circulation. The IgA-B cells selectively home to secretory tissues, including the salivary glands, where they mature into IgA secreting plasma cells with specificity for the bacterial antigen. This hypothesis is supported by the fact that introduction of whole cell antigens by gastric intubation in rats resulted in an equivalent immune response to that produced by local injection in the region of the salivary glands. Last year it was also reported that locally administered, purified, glucosyltransferase from a specific serotype of S. mutans was an effective antigen against other serotypes of S. mutans. Investigators have recently shown that glucosyltransferase,

which synthesizes water insoluble polysaccharides, is also effective in inducing a salivary antibody response, when administered to rats by gastric intubation. The results with particulate antigen were superior to those seen with soluble glucosyltransferase in terms of the timing and extent of response in antibody levels in saliva. A cross-sectional study on humans 18-30 years of age lent support to the feasibility of using a glucosyltransferase vaccine to confer protection against caries. Subjects who had no previous caries experience had little salivary anti-glucosyltransferase antibody. On the other hand, subjects who had moderate to high past experience with caries also had detectable levels of anti-glucosyltransferase IgA in their saliva, indicating that humans have the ability to form salivary antibodies to glucosyltransferase in response to multiple infections with S. mutans.

In addition to glucosyltransferase, two other antigens often proposed for use in vaccines are the lipoteichoic acids and cell wall polysaccharides derived from S. mutans. Improved methods to isolate and purify these materials have been developed over the past year. A new technique for purification of lipoteichoic acid uses synthetic membranes of phosphatidyl choline, which binds the lipoteichoic acid and permits contaminating proteins and polysaccharides to be washed away. The structure of the pure lipoteichoic acid was determined after the liquid membrane was solubilized. This is the first complete purification of lipoteichoic acid and the investigator is optimistic that the procedure can be scaled up for large scale antigen production. The structure of glucose- and galactose-containing polysaccharides from S. mutans cell wall has been determined by gas-liquid chromatography and mass spectroscopy. The antibody binding sites for both the a & d and also the a or d serotypes were determined using precipitin inhibition techniques. The discovery of the common a-d site confirms that one vaccine may be employed to confer protection against several serotypes of this bacterial species.

The cellular interactions involved in synthesis and secretion of IgA were examined in thymectomized rats, which are depleted of thymus-derived lymphocytes. Salivary IgA levels were decreased and these animals failed to respond to antigen administration by producing normal levels of IgA. When immunized thymectomized animals were infected with S. mutans, there was an increase in the levels of carious lesions compared with those in intact immunized rats. Another outcome of these experiments was the identification of a compensatory IgM response in the saliva of thymectomized rats. This IgM could be increased following local immunization and may provide some degree of protection against caries in the IgA deficient animals. Since one in 500-700 humans manifest a selective IgA deficiency, the possibility of compensatory responses to afford some protection in an important concept.

Other NCP Initiatives

In addition to general announcements calling for grant proposals in all strategy areas, the NCP has issued specific requests for applications concerning the immunological cross-reactions between S. mutans and mammalian tissues, and on the streptococcal IgA protease in the human oral cavity. As indicated earlier, one of the principal

strategies of the Program is to combat the responsible microbial agent. This includes efforts to induce or possibly enhance host immunity mediated by oral immunoglobulins. The safety, effectiveness and acceptability of any vaccine used for this purpose must be assured. Grantees have reported an immunological cross-reaction between S. mutans and human heart tissue. Identification and elimination of any cross-reacting materials is essential to ensure the safety of vaccines derived from S. mutans. Recent studies have shown that the antibody activity of IgA may be impaired due to inactivation of the immunoglobulin by a bacterial enzyme. Additional information is required concerning this specific protease, which has the potential to diminish the ability of immunoglobulins to control cariogenic organisms in the mouth. Although the Program is already supporting work on these topics, consultants and staff consider that additional effort on those subjects should be stimulated and supported.

During the year 64 grants, 11 contracts and 24 direct operations projects were active in Strategy Area I, representing 53 percent of National Caries Program research projects.

STRATEGY AREA II: Increasing the Resistance of the Teeth

This Strategy Area, because it includes several techniques that have reached the stage of being communicated to the public, provides examples of all the major steps required to develop practical techniques to prevent caries. These examples include fundamental research on enamel structure and the physico-chemical phenomena occurring on the tooth surface, studies on the feasibility of employing these phenomena to control caries-related processes, animal models and clinical trials to evaluate and improve caries preventive techniques, and finally studies of the utility of techniques in public health settings. The strategy of increasing tooth resistance has four components (use of topical fluoride, use of systemic fluoride, use of adhesive sealants and improvement in inherent tooth resistance) of which each provides examples of this R&D continuum. The report that follows illustrates the continuum with projects concerned with developing caries preventive techniques based on topical fluoride.

The National Caries Program long has been committed to the principle that clear understanding of phenomena occurring at the site where caries initiates and occurring under the conditions of caries attack and repair will lead eventually to preventive techniques that are more effective than those that are the outcome of empirical studies. Due to the complexities, however, of the systems at the interface, the development of useful information concerning their major properties has taken many years of research. Nevertheless in the last year or two, descriptions of the interface have been suggested by several grantees that appear to provide strong conceptual bridges linking laboratory measurement of processes with clinical observation of effects on caries and associated tooth surface phenomena. These descriptions are major steps forward for caries research.

Investigators have found that crystals of hydroxyapatite, the mineral phase of teeth, have two major dissolution sites that operate at markedly different rates. Dissolution from site #1 is thought to correspond to the "unwinding" of the hydroxyapatite crystal along a c-axis defect, resulting in the formation of a hole. Site #2 dissolution, perhaps corresponding to processes occurring over the entire basal plane, would not be expected to occur so readily. It has been found that crystallites can be recrystallized successfully, i.e. "rewinding" the hydroxyapatite in the c-axis hole, if weak remineralizing solutions are used. Under specific conditions involving fluoride, the apatite that is formed has hydroxyl ions partially substituted by fluoride which is postulated to prevent further proton exchange along the c-axis. Furthermore, it appears that if remineralization occurs in the presence of high concentrations of calcium and phosphate,

the c-axis hole is blocked with amorphous material, which is quickly lost leading to further demineralization when acidic conditions recur. There are many ramifications of these ideas that help to explain clinical observation of sub-surface softening of enamel in early caries and successful rehardening of these white spots that is achieved with certain remineralizing regimens.

Because demineralization and remineralization of the tooth surface involves multiple phases, numerous complex chemical species, and many dissolved constituents, research on these phenomena usually is carried out with simple model systems in which there is close control over small numbers of interacting factors. Thus in a study of the conditions under which the five possible calcium phosphate phases form and interconvert, stable though supersaturated solutions of calcium phosphate are mixed with seed material and as formation of crystalline material ensues the activities of all ion species in the solution are maintained constant by continuous potentiometric monitoring and simultaneous addition of up to six reagent solutions. The nature of the solid phase is studied chemically, by x-ray diffraction, infrared spectroscopy, scanning electron and ion microscopy and by measurement of specific surface area. Through this approach direct evidence has been established for the kinetically favored precursors in calcium phosphate precipitation at physiological pH.

In research of a more applied nature scientists are studying the minerals of the tooth surface to learn ways to protect these surfaces from caries. It is known that hydroxyapatite in the presence of fluoride converts to fluoroapatite extremely slowly. Some scientists suggest that rapid conversion of the surface of the crystallite might be achieved by dissolving the surface under special conditions that form dicalcium phosphate dihydrate and reacting this with fluoride or calcium fluoride to form fluoroapatite as a new surface layer. To evaluate the feasibility of this suggestion a National Caries Program scientist has tested the proposed technique in rats and found that it achieved greatly increased incorporation of fluoride in enamel. Currently scientists are analyzing the phosphate species just beneath the surface layer of enamel and investigating new treatment regimens in an effort to optimize this conversion.

Other scientists are studying use of calcifying solutions for repairing the sub-surface softening of enamel that occurs as a first stage in caries. These scientists point out that if there is sufficient time between periods of acid demineralization, calcifying properties of saliva provide important caries protection. To supplement the repair capacity of the saliva scientists are studying synthetic calcifying solutions containing low levels of calcium and fluoride and find the solutions have marked capability of decreasing the size and porosity of sub-surface lesions and of limiting the extension of the lesions when there is fresh

exposure to acid. An important technique has been developed for following these changes *in vivo* and for evaluating calcifying solutions. The technique, currently being used with bovine enamel slabs mounted in the mouth and potentially used with teeth *in situ*, measures the amount of iodide that can be imbibed by and then withdrawn from a standard area of the lesion. The technique also can be employed in following the maturation of teeth in the oral cavity and in measuring the decalcification of enamel caused by bacterial fermentation of foods.

These types of research eventually will provide a theoretical explanation for the action of fluoride and other agents in prevention of caries and will lead without question to improved capability in this regard. Nevertheless, numerous fluoride regimens already are in use. Fluoride is added to table salt, to milk, and to vitamin-mineral supplements as well as to municipal water supplies. It is provided as tablets, as mouthrinses, and incorporated in dentifrice and chewing gum. The regimens differ furthermore in the concentration, type, and amount of fluoride provided and in the frequency and other characteristics of suggested use. Because large numbers of children use or potentially will use certain of these regimens and because there is little information upon which parents or community health officials can make reliable decisions concerning their use, there is a clear responsibility for the Program to provide this information. However, because even the shortest of clinical trials to evaluate caries preventive effectiveness requires approximately three years, the development of information on effectiveness, cost, and other characteristics of even a few of the more important of the alternative regimens entails much staff effort. Examples of a few of these projects carried out by staff of the Community Programs Section or conducted by contract are described below.

One of the most important techniques that can be used by school children that do not receive benefits of fluoride in their water supply is daily consumption of fluoride tablets in school. In Wayne County, North Carolina, NCP staff have recently carried out the final examination of caries prevalence in children who, ten years ago commenced a program of chewing an acidulated phosphate fluoride tablet and rinsing their mouth with and swallowing the resulting mixture. The children did this every school day for six years. At that time they had 28 percent less caries than a control group that similarly used a placebo tablet. Examinations two years later showed that benefits largely had been retained. Final data on retention of benefits four years after the end of treatment currently are being analyzed.

In addition to programs based on tablets, mouthrinsing with fluoride can provide important benefits for children in fluoride-low areas. The tablets or rinse solutions usually contain 1 mg. fluoride in the form of acidulated phosphate fluoride or sodium fluoride. Two years ago the Program contracted for a clinical study to compare the protection provided by these four regimens. Approximately 1600 school children, 12 to 14 years of age, are participating in the three-year project in Pittsylvania County,

Virginia. On each school day the children chew a tablet containing 1 mg. fluoride and rinse with and swallow the resulting mixture or they rinse with and swallow 5 ml. of solution containing 1 mg. fluoride. Approximately two years from now, based on the results of this clinical trial, the Program will be able to advise on which of these commonly used regimens is best.

Another compound of fluoride that has been reported to be effective in a mouthrinse as well as in dentifrice is stannous fluoride. This compound also is claimed to remove plaque from the teeth, a property that might make it more effective than sodium fluoride against caries and provide protection against periodontal disease as well. Unfortunately solutions of stannous fluoride are reported to stain teeth and to have an unpleasant taste. To weigh the potential benefits of this compound relative to the problems associated with its acceptance in school-based programs, the National Caries Program contracted in 1978 for two clinical trials, each involving 200 children in which stannous and sodium fluoride are being compared in daily one-minute rinse programs in schools. In these projects in Texas and New York, extrinsic tooth staining, gingivitis, and dental plaque is being measured at frequent intervals. Information from these trials concerning the feasibility of using stannous fluoride rinsing in schools to prevent caries and gingivitis will be available early in 1982.

Though both daily and weekly rinse programs are widely used in schools, delivering a daily program significantly increases costs and detracts from acceptance of the program among both teachers and students. An important question, therefore, for school and community officials is whether daily rinsing is worth the extra cost. The cost question is even more critical when a school-based rinse program is being employed to supplement the benefits obtained from a municipal water fluoridation program. To answer these questions NCP staff in 1976 implemented fluoride rinse programs in schools in Biddeford, Maine, a low-fluoride area, and in Des Moines, Iowa, an optimally fluoridated area. Daily rinsing with 0.05% sodium fluoride and weekly rinsing with 0.2% sodium fluoride are being compared at both sites and results showing the relative benefits of these commonly recommended rinse procedures will be available in 1980.

Similar questions concern the necessity to clean teeth prior to fluoride treatment in preventive programs suggested for school-based use. Prophylactic cleaning is attractive but reliable data is not available on the benefit that it imparts and Program staff believe that problems involved in classroom administration of tooth cleaning would markedly detract from the acceptability of a simple rinse procedure. It is even reasonable that tooth cleaning, by removing a reservoir that would slowly release fluoride, might decrease the effectiveness of topical fluoride treatment. So that it may be in a position to advise properly on these procedures, the Program contracted in 1978 and 1979 for three-year clinical

trials involving weekly fluoride mouthrinsing or semi-annual topical treatment with phosphate fluoride gel. In each program the treatment without prior teeth cleaning is compared to treatment with different types of prior cleaning. Data from these projects will be available in 1982.

The projects described above and those described in Strategy IV to educate the public on available caries preventive techniques document the stages of investigation through which the NCP is translating basic and empirical findings into practical public health methods of protecting the teeth.

During the year 29 grants, 11 contracts, and 14 direct operations projects were active in Strategy Area II, representing 29 percent of National Caries Program research projects.

STRATEGY AREA III: Decrease the Cariogenic Potential of the Diet

In FY 1979 a plan was developed so that 11 major objectives in this Strategy Area would relate to each other and mutally support achievement of the goal, "American diets and dietary habits with decreased cariogenic potential." Underlying the organization of the plan is the belief that the consumer will make improved decisions about the foods that he or she selects if better information about food properties are available. It is similarly believed that improved information can be the basis for decisions of manufacturers in favor of less cariogenic foods and beverages.

One of the objectives in the plan is to develop information on noncariogenic sweeteners that have potential for commercial use in terms of their sweet intensity and taste quality, cost, dependability of supply, compatability with foods and other characteristics. Thus the NCP contracted with Dynapol Corporation, Palo Alto, California, in 1976 to synthesize analogs of neohesperidine dihydrochalcone, a highly sweet product from citrus rind, and evaluate the taste and other characteristics of these analogs for possible use in sweetening foods. During FY 1979 Dynapol Corporation increased the number of analogs that it had prepared and characterized to over 40. Several of these were found to have taste properties at least as good as the starting compound but none has the sharp onset and rapid disappearance of sweetness that is considered essential for wide replacement of sucrose. Currently NCP staff are reviewing all the known compounds that are sweet to evaluate the potential of further synthesis of compounds to achieve a commercially useful sweetener. During FY 1979 the Program also has contracted with American Dental Association Research Foundation to screen the available sweeteners for carcinogenicity by bacterial mutagenesis. These assays did not reveal carcinogenic potential.

A second major objective in the plan is to obtain information on the cariogenicity of specific foods and food classes. Two assays of food cariogenicity are being developed by staff with the expectation that if the reliability of the tools can be demonstrated, the tests will become widely used by food manufacturers and in dental research and accepted as good indicators of food cariogenicity in man. The first of these is based on the changes in plaque acidity that occur when different foods are eaten. These changes are detected by a microelectrode within the mouth and transmitted to recording devices by radio or wired telemetry. The technique was developed and is currently used by Professors H. Muhlemann and H. Graf at the Dental Research Institute, Zurich, Switzerland, but largely because of difficulties in microelectrode fabrication and in obtaining acceptable electrode life the technique has not been established elsewhere. Since 1977 NCP staff have been collaborating with Microelectrodes Co. of Londonderry, N.H., a firm with unique capability in the fabrication of these

sensors, with staff at the University of Utah, and more recently with the NIH Division of Research Services to develop standardized intraoral measuring procedures and establish a resource from which electrodes may be obtained. The second of these assays involves the measurement of caries development in rats that receive the essential components of their diet by stomach tube and eat cariogenic supplements provided at intervals by a feeding machine. This approach to cariogenicity testing is being outstandingly successful. In FY 1979 numerous snack-type foods were evaluated using it and dramatic results were obtained confirming that the frequency of eating affects both the level of caries and the level of infection by Strep. mutans. National Caries Program scientists also are collaborating with industry in screening the effect of feeding animals non-sugar sweeteners.

A third major objective in the plan is to obtain food consumption patterns in U.S. demographic groups. If cariogenicity of individual classes of foods can be determined by the methods described above it might be possible to combine that data with data on average consumption of those foods and obtain an estimate of total cariogenic challenge of each class of food. During FY 1979 NCP staff initiated discussions with the National Center for Health Statistics to explore the possibility of obtaining dietary intake information from the next NCHS national surveys. Thus it appears possible that the National Caries Program might obtain nutritional data from the NCHS studies for Hispanic groups or the nationwide HANES III study.

During the year 5 grants, 2 contracts, and 3 direct operations projects were active in Strategy Area III, representing 5 percent of National Caries Program research projects.

STRATEGY AREA IV: Improved Delivery and Acceptance of Caries Preventive Methods

Dental caries remains of epidemic proportions as a public health problem despite the fact that proven cariostatic agents and techniques are available. It is clear, therefore, that the public needs to be more widely informed of the availability and advantages of these agents and techniques. Certain of these techniques are specifically designed for use in non-fluoridated areas to reduce the incidence of dental caries among children who, being most susceptible to the disease, will derive the greatest benefit. Because most children attend grade school and because increasing numbers of children attend various pre-schools, the school environment provides an excellent setting for self-applied fluoride programs.

Many self-applied techniques have been evaluated for use in schools but only two--weekly rinsing with a 0.2 percent neutral sodium fluoride solution and daily use of dietary fluoride supplements (1 mg. F tablets) --have been actively promoted by the National Caries Program. These two procedures have been selected because they are effective, inexpensive, and highly accepted by school personnel, parents and students. Activities during the year have largely focused on expanding public and professional awareness and implementation and continuation of these two school-based self-applied fluoride regimens.

The National Caries Program's seventeen projects demonstrating community use of weekly fluoride mouthrinsing for children in kindergarten through the eighth grade of school were completed during the year. Reports covering three years have been received from the individual projects and show that participation of children averaged 82 percent and total costs, averaged $3.33 per child per school year. It is particularly noteworthy that of the twelve demonstration projects terminating in January 1979, all are continuing the weekly rinse regimen with local funding.

The other five projects were extended to enable the National Caries Program to obtain information on use of the rinse regimen by high school students. It should be pointed out that most school-based oral health programs have been limited to kindergarten through the sixth grade because of easier management and scheduling at these school levels. Nevertheless adolescents also are prone to dental caries and it is important to develop caries preventive procedures for them. The extension of five of the original seventeen demonstration projects will provide additional information on the effectiveness of the agent as well as on the acceptance of procedures both by students and school personnel in the higher grades.

The National Caries Program is conducting a study in Nelson County, Virginia, to determine the level of caries prevention that is achieved when children use three major fluoride regimens on a long-term basis. The regimen consists of: (1) daily ingestion of the 1 mg. fluoride tablet, (2) weekly rinsing with a 0.2 percent solution of neutral sodium fluoride, and (3) ad libitum use of a fluoride toothpaste at home. The results continue to demonstrate efficacy of the regimen plus high acceptance on the part of school personnel, students and parents. Six-year findings, for instance, show that among continuous participants the prevalence of new dental caries was reduced 45 percent for all tooth surfaces and 85 percent for approximal surfaces. The level of continuous participation also was impressive; among elementary school children (kindergarten through sixth grade) the rate was 96 percent. It is important to note that as participants have advanced to upper grades the participation rate has remained high.

Through a contract with the University of Michigan the National Caries Program has been carrying out a study to determine whether a combination of incremental restorative dental care, topically applied fluoride and sealants would eliminate the residual caries incidence in optimally fluoridated communities. Results after five years show that the caries (DMFS) increment was reduced by 65 percent in older children and 40 percent in younger children. These benefits are particularly impressive when one considers that they are in addition to the 40-60 percent reduction in caries achieved from water fluoridation alone. By incorporating in the study a group of children that was treated only for the first three years, the clinical trial also provides information that significant levels of benefits are retained after termination of these treatments.

The National Caries Program has been deeply involved during the last year in bringing information about the benefits of such programs to educators and dental and community health leaders. The NCP's original large exhibit on school-based self-applied fluoride programs, first used in the fall of 1976, has been shown at 26 annual sessions of health and/or school related meetings. In FY 1979, the exhibit was replaced by a freshly designed and expanded one which includes use of NCP's new films on fluorides. This exhibit, staffed by NIDR, is used primarily at national meetings of organizations such as the: American Association of School Administrators, National School Boards Association, National Congress of the PTA, and American Dental Association. During exhibit hours, brochures and pamphlets on school-based self-applied fluoride programs are distributed and consultation is provided on ways to establish and maintain these programs.

Because NCP staff cannot accompany exhibits to all state and local meetings concerned with dental health, three smaller exhibits on the use of self-applied fluorides in schools have been developed for loan use. NCP staff actively promote the loan of these exhibits to state and local

groups to assist in educating the public and professionals about the use of fluorides. These exhibits, which continue to be much in demand, have been used in more than 33 meetings in 22 states.

The film produced for television, "Reading, Writing, and Rinsing," has been shown approximately 353 times in the past year to an audience estimated at 6.2 million. During the year the film also was made available for loan through the American Dental Association's film library.

Four new films on fluorides have been produced this year. Three of these ("Smile Makers: Self-applied Fluoride Programs for Schools," "The Daily Tablet for Healthier Smiles," and "The 0.2 Percent Solution") are for use in establishing self-applied fluoride programs in schools. The fourth ("Prescribing Fluoride Supplements in Medical and Dental Practice") discusses the reasons for and prescription of dietary fluoride supplements. The latter film will be used with both dental and medical students and practitioners. All four films soon will be available for loan and three ("The Daily Tablet for Healthier Smiles," "The 0.2 Percent Solution," and "Prescribing Fluoride Supplements in Medical and Dental Practice," have been reduced in film size for use with the new large exhibit.

The forty-page publication, "Preventing Tooth Decay: A Guide to Implementing Self-Applied Fluoride Programs in Schools," is being used in every state. Over 18,000 copies of the publication have been distributed. To evaluate use of this manual and to improve the value of information that the National Caries Program provides to health leaders, a survey was carried out to determine the extent to which individuals requesting a copy of the guide have established self-applied fluoride programs in schools and what additional help they needed to start programs. The response was over 50% and questionnaire results make clear that respondents found the publication very useful. Respondents also provided many excellent suggestions concerning additional assistance that would be beneficial in establishing programs. During the year over 79,000 copies of the leaflet, "Fluoride Mouthrinsing in the Schools...Protection for Children's Teeth," and 30,000 copies of the leaflet "Fluoride Tablets...A Healthier Smile for School Children," have been sent to communities for use in explaining to parents the nature of the children's caries preventive program. A new leaflet, "A Healthy Start... Fluoride Tablets for Children in Pre-School Programs," was produced this year and thus far over 23,000 copies have been requested.

A series of posters promoting the use of fluorides are in production. Some of these are designed for use in schools where self-applied fluoride programs are under way. The other posters are for use in dentists' and physicians' offices and focus on the use of dietary fluoride supplements. All posters will be available free of charge.

During the past year, staff members have provided numerous hours of instruction on caries prevention to dental students and graduate students in various dental schools across the country and to students in public health curricula as well. Staff have also given lectures, papers, continuing education courses, and in-service training at dozens of international, national, state, and local meetings. In addition, many staff members have been invited to write papers on the prevention of dental caries for special issues of several journals.

During the year 3 grants, 19 contracts, and 2 direct operation projects were active in Strategy Area IV. This represents 13 percent of the National Caries Program's projects.

Section II

DIRECT OPERATIONS

The following reports describe NCP direct operations projects staff that were active in FY 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00029-12 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979 CT-0600057

TITLE OF PROJECT (80 characters or less)
The effect of school water fluoridation on dental caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
	Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR
	Brunelle, Janet A.	Chief, BS	NCP	CPR	NIDR
	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Dental Health Division, North Carolina State Board of Health, Division of Water Hygiene, Environmental Protection Agency

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.04	.03	.01

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorides were added to the water supply of a school in Seagrove, North Carolina. The concentration of fluoride used was higher than the level considered optimal for the community water fluoridation in the geographic area. Children attending the Seagrove school live in an area without a central water supply and where the various sources of well water contain negligible levels of fluoride. Children are exposed to the higher fluoride level only while at school in an attempt to approximate the total fluoride intake of children who drink optimally fluoridated water on a fulltime basis. Baseline dental examinations for dental caries were made prior to the installation of fluoridation equipment. Follow-up examinations are conducted after four, eight, and twelve years to determine the extent of caries protection as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering the first grade. Results of the four-year examinations showed appreciable decreases in caries prevalence compared with baseline findings. On the eight-year examinations, an assessment of the possible prevalence of dental fluorosis was made along with the regular examinations for dental caries. No children showed any definite signs of the condition.

1. Project Description:

Objective:

The purpose of the study is to determine the decay preventive benefits derived by children who when at home drink water that is essentially devoid of fluoride but when at school consume water fluoridated at 7 times the level considered optimal for community water fluoridation in the same geographic area.

Methods:

In 1968, fluorides were added to the water supply of a consolidated school (grades 1-12) in Seagrove, North Carolina. The target level of fluoride, 6.3 ppm, is seven times the level considered optimal for community fluoridation in the same geographic area. Children are exposed to the higher fluoride level in an attempt to approximate the total fluoride intake of children who drink optimally fluoridated water on a full-time basis. Prior to the installation of fluoridation equipment dental examinations using the DMF tooth and surface index were conducted on approximately 1100 children to determine baseline caries prevalence. Surveillance of the fluoride levels maintained is provided by school personnel under the supervision of the North Carolina State Board of Health. Follow-up examinations are conducted at four-year intervals to measure the extent of caries protection as increasingly larger segments of the study population become continuously exposed to fluoridated water at school since entering in the first grade.

Findings:

Eight-year follow-up examinations were conducted in 1976. The interim data showed that children 6 through 14 years of age, the full beneficiaries of the procedure after eight years of exposure, had an overall 40% difference in age-specific DMF surface scores compared with those of their counterparts of the baseline. Examinations for dental fluorosis were also conducted on the 1976 examinations. None of the children examined exhibited any signs of the condition.

Sigmificance:

Currently, about 23% of the U.S. population resides in areas that lack central water systems. These persons are deprived the benefit of community water fluoridation. School fluoridation is an alternative method of preventing dental caries in children living in such area. The present study will help determine the optimal concentration of fluoride for school water fluoridation. Currently, a level of 4.5 times the optimum is used.

Proposed Course:

Fluoride will be maintained at the target level until the final examinations in 1980, at which time children in all grades (1-12) will have been continuously exposed to the higher fluoride level at school since the first grade. Comparison of findings after 12 years of school water fluoridation at 7 and at 4.5 times the optimum will be made to determine if greater anticaries protection is conferred at the higher fluoride concentration.

2. Publications:

Heifetz, S.B., Horowitz, H.S., and Driscoll, W.S.: Effect of school fluoridation on dental caries: results in Seagrove, N.C. after eight years. J. Amer. Dent. Assoc. 97:193-196, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00032-11 CPR

PERIOD COVERED

October 1, 1978 to September 30, 1979

CT 0600042

TITLE OF PROJECT (80 characters or less)

Effects of chewable fluoride tablets on dental caries in school children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
	Brunelle, Janet A.	Chief, Biometry Section	NCP	CPR	NIDR

COOPERATING UNITS (if any)

Wayne County, North Carolina, Public School System

LAB/BRANCH

Caries Prevention and Research

SECTION

Community Programs

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.15	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study was initiated in October 1969 with 1034 children in the first and second grades of nine schools located in Wayne County, North Carolina, an area that has negligible amounts of fluoride (F) in its supplies of drinking water. Following baseline dental examinations, in which the DMF surface index was used, the children were stratified according to certain variables and then randomly assigned to one of the following three study groups: Group A (controls) chewed a placebo tablet, rinsed their teeth for 30 seconds with the resulting salivary solution, and then swallowed the material; Group B followed an identical procedure using an acidulated phosphate-fluoride (APF) tablet that contained 1 mg. F; Group C followed the same procedure as Group B except that, after at least 3 hours, the procedure was repeated with a second APF tablet that also contained 1 mg. F. The procedures were carried out each day in school under the classroom teacher's supervision for a period of six years. Interim follow-up examinations were conducted in April 1972, May 1974, September 1975 and May 1977. Final examinations were conducted in May 1979.

1. Project Description:

Objective:

To evaluate the caries-preventive effect of the daily use in school of acidulated phosphate-fluoride (APF) chewable tablets.

Methods Employed:

The study, a longitudinal double-blind clinical trial, was initiated in October 1969 on 1034 children attending the first and second grades of nine public schools located in Wayne County, North Carolina, an area that has negligible amounts of fluoride in its sources of drinking water. Children were stratified according to selected variables and then were randomly assigned to one of the following three study groups: Group A (controls) chewed a placebo tablet for 25 seconds, rinsed their teeth for 30 seconds with the resulting salivary solution and then swallowed the material; Group B followed an identical procedure using an APF tablet that contained 1 mg. F; Group C followed the same procedure as Group B except that, after at least 3 hours, the procedure was repeated with a second APF tablet that also contained 1 mg. F. The procedures were carried out each day in school under the classroom teacher's supervision for a period of six school years. Baseline dental examinations, using the DMF surface index, were conducted just prior to treatment initiation. Follow-up examinations were carried out during the treatment phase of the study in April 1972, May 1974 and September 1975. Examinations to evaluate post-treatment effects were conducted in May 1977 and May 1979, two years and four years, respectively, after discontinuation of treatments.

Findings:

Examinations conducted in May 1977, two years after treatments had been discontinued, showed statistically significant reductions in DMFS increment for both treatment groups (B and C), compared with the controls (Group A). The two treatment procedures were about equal in effectiveness. Percentage reductions were approximately 25% for early erupting teeth (incisors and first molars), 45% for late erupting teeth (cuspids, bicuspids and second molars) and 32% for all teeth combined. These percentage figures are of the same magnitude as those found in September 1975, shortly after treatments were terminated, indicating that benefits derived during the treatment period are retained thereafter for at least a two year period. Analysis of data from the May 1979 examinations has not yet been completed. Findings thus far have clearly demonstrated that administration of fluoride tablets in school should be recommended in nonfluoridated areas as an effective and feasible public health measure for the prevention of dental caries.

Significance:

Approximately 50% of the U.S. population resides either in areas that have no central water supplies or in areas that do have central water supplies but have not implemented community water fluoridation. Because these persons are deprived of the benefits afforded by community water fluoridation, other methods of caries prevention must be developed and utilized. One method that offers considerable potential is administration of fluoride tablets to children in school. The present study has proved important in demonstrating both the efficacy and the feasibility of the procedure. By continuing to do dental examinations after treatments were terminated, valuable information with regard to retained benefits are being gained.

Proposed Course:

The examinations conducted in May 1979 were the final examinations and marked the completion of the study.

2. Publications:

Driscoll, W.S., Heifetz, S.B. and Korts, D.C.: Effect of Chewable Fluoride Tablets on Dental Caries in Schoolchildren: Results After Six Years of Use. J. Amer. Dent. Assoc. 97:820-824, 1978.

Driscoll, W.S., Heifetz, S.B. and Brunelle, J.A.: Treatment and Post-Treatment Effects of Chewable Fluoride Tablets on Dental Caries: Findings After Seven and One Half Years. J. Amer. Dent. Assoc. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00070 07 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979 CT 0500045

TITLE OF PROJECT (80 characters or less)
Combined self-applied fluorides for caries prevention in a non-fluoridated area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
OTHER:	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR
	Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Nelson County, Virginia, Public School System

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.54	.34	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Baseline dental examinations were conducted in October 1972, on approximately 2200 children (grades K-12). All participants in grades K-6 chew daily in school under supervision an acidulated phosphate-fluoride (APF) tablet containing 1 mg. F, and swish and swallow the resulting solution. Once a week in school the same children also swish 10 milliliters of a 0.2 percent sodium fluoride solution for 60 seconds and then empty the contents of the mouth into a cup. A fluoride-containing dentifrice and toothbrushes are distributed to the same children for use at home. These combined preventive procedures will continue in the elementary schools for a minimum of ten years. Follow-up dental examinations are carried out biennially. Final examinations will be made in the fall of 1982 when all senior high school students will have participated continuously in the elementary school program since entering first grade. Children in the 7th grade of Nelson County's junior high school began to participate in the program in the fall of 1978. New grades will be brought into the program in junior high school on an incremental basis in future year.

1. Project Description

Objective:

The purpose of the study is to determine the total effectiveness of a combination of some of the most feasible methods of self-administering fluorides in a non-fluoride area.

Methods:

In October 1972, a self-administered dental health program was started in Nelson County, Va., a fluoride-deficient community. Children in the County's 7 elementary schools, under teacher supervision, chew and ingest daily a 1 mg. F tablet and rinse weekly with a 0.2% NaF solution; a fluoridedentifrice is provided for ad libitum use at home. Baseline DMFS examinations were made of 2135 children in the County's elementary (grades 1-6), junior (grades 7 and 8) and senior high schools (grades 9-12). Follow-up examinations are conducted at two-to-three year intervals to determine the effectiveness of the program as increasingly larger segments of the participants become exposed to the F treatments since entering school in the first grade.

Major Findings:

The most recent follow-up examinations that have been analyzed are those done in 1978. Those findings showed that continuous participants in grades 2-7 (ages 7-12) had 15 percent fewer DMF surfaces in 1978 than their cohorts in 1972. These children in 1978 had 85 percent fewer DMF mesiodistal surfaces than comparable children in 1972. Children in 1978 who had participated in the preventive program at any time had 29 percent fewer DMFS than their cohorts.

Significance:

Self-administered procedures, unlike traditional, professionally administered fluoride applications, can be implemented extensively with few demands on dental manpower, school personnel, facilities and financial resources. From the standpoint of optimizing dental health programs in areas where community water fluoridation is not possible, there is a compelling need to determine the impact of various combinations of feasible, self-administered methods of fluoride delivery.

Proposed Course

Treatment procedures will continue in Nelson County Schools for a minimum of ten years, or until the spring of 1982. Continuing the treatments for this length of time will enable a final evaluation in 1983 of a senior high school population that has received the full benefits of the program. Interim findings after 2 and 4 years have been presented at scientific meetings and have been published. Findings after 6 years were presented at a symposium at the 1979 meeting of the International Association for Dental Research. A report of those findings are being prepared for publication.

There are plans to continue some phase of the program in high school beginning in the 1980-81 school year.

2. Publications

Horowitz, H.S., Heifetz, S.B., Meyers, R.J., Driscoll, W.S. and Korts, D.C. Evaluation of a combination of self-administered fluoride procedures for the control of dental caries in a nonfluoride area: findings after four years. JADA, 98:219-223, February 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00081 06 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Development of an anticaries vaccine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
OTHER:	Kuzmiak-Jones, Harriet M.	Biologist	NCP	CPR	NIDR
	Gomez, Irma M.	Microbiologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.05	.60

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A colony of primates, Macaca fascicularis, which has been infected with Strep. mutans and is being fed a diet rich in sucrose is being used to develop a vaccine against dental caries. Pneumococcal polysaccharides types II and IX, glucosyl transferase, and heat killed Strep. mutans cells have been administered to age matched monkeys intraductally or intramucosally with aluminum hydroxide as adjuvant. No protection was observed in the monkeys vaccinated using the pneumococcal polysaccharide or in the animals vaccinated intramucosally. Monkeys vaccinated with glucosyl transferase had fewer lesions than the other animals.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The purpose of this study is to determine whether monkeys can be vaccinated against dental caries using antigens derived from pneumococci and Strep. mutans. Pneumococci were used because it has been observed that types II and IX induce antibodies which cross-react with polysaccharide from Strep. mutans.

Methods:

Pneumococcal polysaccharides, glucosyl transferase and killed cells were administered intraductally into the parotid gland or intramucosally. The animals were infected with Strep. mutans and fed a diet all the components of which are normally purchased for human consumption.

Major Findings:

Animals which were vaccinated intraductally developed substantially more caries than animals receiving the same immunogen by another route. Monkeys vaccinated with glucosyl transferase developed fewer lesions than other animals. Serum from the animals vaccinated with glucosyl transferase inhibited the enzyme by up to 90%.

Significance:

The prospect of developing a vaccine against caries is attractive and success would represent a major advance in public health. The results presented here confirm and extend the observations that it is in principle possible to prevent dental caries by vaccination.

Proposed Course:

The effect of novel routes of vaccination such as gastric inhibition on the types of immune response and caries development will be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00098 06 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

An Evaluation of Knutson's Formula for Estimating Age Specific DMFT

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Kingman, Albert Statistician - Health NCP CPR NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH
Caries Prevention and Research

SECTION
Biometry

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.04	.04	--

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In 1944 Knutson described a formula for estimating age specific DMFT means from the percent of individuals in the age groups having one or more decayed, missing or filled teeth. This formula was evaluated for both different age ranges, and for different allowable maximum percentages using 14 NCP data sets. The method of weighted least squares was used to fit the formula. Results indicate that restrictions of age range, and maximum percent of individuals with one or more DMF teeth, are necessary before applying the formula. It was determined that for K=100, and B=0.542 the formula could be applied to age groups in the 5-11 year old range which also had not more than 70% of the children with one or more DMFT.

PHS-6040

1. Project Description

Objective:

In 1944 Knutson described a formula for estimating age specific DMFT (X) from the percent of individuals in an age group having one or more decayed, missing, or filled teeth (Y). This study was designed to evaluate Knutson's formula for estimating age specific DMFT using 14 NCP data sets. More recently several manuscripts on this topic have suggested that such an estimation procedure might be more applicable for a restricted range of ages, and/or for age groups with not too large a percentage of individuals having one or more DMFT. This study was designed to investigate these questions.

Methods:

The method of weighted least squares was applied to 148 age specific means and corresponding Y percentages. This method was subsequently reapplied to data sets restricted by either age range, Y percentage or both simultaneously.

Major Findings:

Restricting applicability of the estimating formulas to specific age ranges and maximum Y percentage age groups produced significant improvements in the accuracy of the estimation. The age groups giving best results were the 5-11 year olds, while the most appropriate maximum Y percentage cut-off was 70%.

Significance:

This procedure could be used to estimate DMFT prevalence with simpler shorter and less expensive examinations.

Proposed Course:

The project is completed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00112 06 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Preclinical Screening of Anticaries Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shern, Roald J.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Kingman, Albert	Statistician (Health)	NCP	CPR	NIDR
	Kline, Linda A.	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)
American Dental Association Health Foundation, National Bureau of Standards, Gaithersburg, Maryland. Drs. W.E. Brown and L.C. Chow

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.61	.28	.33

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to identify anticaries agents suitable for short-term clinical trials. Screening is conducted both in vitro and in animals. The in vitro studies measure minimal inhibitory concentration, and modification of enamel dissolution rate. Other measurements are used electively depending on the nature of the agent. The animal studies measure the effects of an agent on dental caries and plaque. Several bisguanides screened favorably providing marked inhibition of dental caries and plaque. A solution that changes some surface enamel to $CaHPO_4 2H_2O$ (dical) promoted uptake of fluoride by the enamel with no change in its surface morphology. The effects of the dical forming solution on dental caries is being studied.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective

The objective of our project is to identify anticaries agents suitable for clinical testing and to improve screening methods.

Methods:

The methods of evaluation are based on the postulated properties of the agents and the therapeutic objectives sought. Most methods have been described in the NCP booklet, "Procedures for Screening Antiplaque Compounds."

In the animal studies staining, plaque, caries and fluoride uptake of rat teeth is measured following regimens of oral rinsing with test agents.

Major Findings:

Bisbiguanides and silane derivatives have been screened and the former found very effective against dental caries and plaque when used at low levels once or twice daily as an oral rinse. Pretreatment of teeth with a solution that converts some surface enamel to $CaHPO_42H_2O$ (dical) appears to promote caries restriction and fluoride uptake from dietary and waterborne fluoride. Those interim studies further suggest that the pretreatment can be used in several regimens.

Method development has concentrated on determining the limitations and advantages of various methods of fluoride analysis described under project number Z01-DE-00262-01.

Significance:

The safety and effectiveness of the bisbiguanides that were studied is in accord with our earlier findings as well as other published reports. One of these agents, chlorhexidine, has been reported to be safe and effective in European clinical studies. One or more of the bisbiguanides should be tested clinically in this country. The dical forming solution appears to be versatile. It might enhance the benefits of fluoride provided by professional application, tablets, rinses, drinking water and sustained release sources. However, this interpretation is tentative because the relationship of enamel-bound fluoride to caries is unclear.

Proposed Course

Additional testing of bisbiguanides will be influenced by the nature of proposed clinical investigations. New types of agents will be tested. Additional testing of the dical forming solution will be delayed pending completion of ongoing studies. Study design will be influenced by the proposed clinical usage of the dical forming solution.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00113 06 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979 CT 0600075

TITLE OF PROJECT (80 characters or less)
Short-term clinical trials of antiplaque agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shern, Roald J.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Brunelle, Janet A.	Statistician (Health)	NCP	CPR	NIDR
	Kline, Linda A.	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Department of Periodontology, School of Dentistry,
University of Pennsylvania, Philadelphia, Pennsylvania
Drs. S.L. Yankell, P.A. Green, and N. Stolle

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.15	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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

PHS-6040

1. Project Description

Objectives:

The objectives are twofold: to identify, adapt and pretest methods of measurement of oral responses, and to conduct short-term clinical studies of agents which might be capable of restricting dental plaque and caries.

Methods:

Because the shortness of these trials preclude measurement of caries, dental plaque parameters are evaluated that are believed associated with eventual caries formation. Untoward effects on oral soft tissues and standard clinical parameters also are measured.

Major Findings:

Methods have been partly developed for micro-measurements for hydrogen and fluoride ions in plaque.

A study of SnF_2 is in progress.

Significance:

Short-term clinical testing provides assurance that agents are safe and potentially effective prior to commitment to long-term testing in large groups of subjects. This project is to conduct these short-term trials and to develop and rigorously evaluate techniques for measuring parameters associated with safety and effectiveness.

Porposed Course:

A short-term study of an antiplaque agent will be done pending receipt of an IND number by the sponsor of the agent.

2. Publication

Yankell, S.L., Paskow, G.W., Rann, R.M., Green, P.A., and Shern, R.J. Evaluation of tiodonium chloride as an antiplaque and anticaries agent. V. Effects on plaque microbiology and plaque and saliva pH. Journal of Pharmacology and Therapeutics in Dentistry. (Accepted for Publication)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00117 06 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Fluoride effect on incorporation of radioactive ^{45}Ca into hard tissues of developing rat teeth

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Stiles, Horace M. Microbiologist NCP CPR NIDR

COOPERATING UNITS (if any)
University of Maryland Dental School, Departments of Anatomy and Physiology

LAB/BRANCH
Caries Prevention and Research Branch

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.32	.12	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pregnant rats were given intraperitoneal injections containing various concentrations of fluoride and ^{45}Ca. Pups from these dams or pups injected similarly when they were 5, 9, or 12-30 days of age were sacrificed just prior to eruption of the first molars and their jaws were resected and teeth examined for radioactivity. A group of rat pups treated in the same manner using non-radioactive Ca were used to study the development of dental caries. In addition, a Ca blocking agent was used on one group of rats.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives:

The research is being conducted to determine whether systemic fluoride during tooth development has an effect on: (1) the uptake of Ca into the teeth of rats and (2) the resistance of tooth enamel to acid dissolution. The research also is being conducted to examine the effect of a hypocalcemic agent on tooth calcification.

Methods:

The teeth of rat pups were labeled via two methods with radiolabled calcium (^{45}Ca). A group of pregnant rats (Osborne-Mendel) was given IP injections of ^{45}Ca two days prior to expected delivery. Another group of rat pups was given IP injections of ^{45}Ca on the 5th, 10th, and 12-20th day of life. The rats also received IP injections of various concentrations of fluoride. The rat pups were sacrificed just prior to eruption of their first molars, their jaws were resected and the teeth were examined for radioactivity. A group of rat pups treated in the same manner using non-radioactive calcium was used to study the development of dental caries.

Possible effects of a thymus factor on tooth calcification was examined in this model. The factor, (IADR Abs. 256, 1974; 378, 1977) has a definite hypocalcemic effect in plasma and an effect on ^{45}Ca in calvaria and long bones. Assays of ^{45}Ca in first and second molars were performed after 10, 15, and 20 days.

Major Findings:

Results suggest that as the concentration of fluoride injected postnatally increases, there is a decrease in radioactive calcium incorporated into the tooth structure. However, the concentration of fluoride injected appears to have little effect, if any, on the uptake or loss of ^{45}Ca from the teeth when incubated in solutions having terminal pH of 4.5.

The experimental variations in fluoride administration did not appear to effect the fluoride content of the outer layers of enamel of the first and second molars of the rats teeth.

Little or no differences existed among all groups as to caries scores.

The thymus factor was found to have a significant effect in the model. The effect on tooth calcification appears opposite to that observed in bone.

Significance to Program

The caries preventive effect of fluoride is well established; however, the mechanisms by which this is accomplished are unclear. The model employed in the study is designed so that systemic effects of fluoride during tooth development can be distinguished from topical effects.

Proposed Course

We have no plans to pursue research based on the described regimen at this time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00147-05 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Lectins in the study of plaque and caries development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mirth, Dale B.	Senior Staff Fellow	NCP	CPR	NIDR
OTHER:	Adderly, Donna D.	Chemist	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Kingman, Albert	Statistician (Health)	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.157	.77	.80

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lectins, which are proteins capable of interacting with certain macromolecules and/or cell types via specific sugar moieties, are being used to investigate the interactions between saliva and/or bacteria in order to better elucidate the role these interactions play in plaque and caries development. Findings to date support the conclusion that 4 lectins, wheat germ agglutinin, concanavalin A, fucuose binding protein and soybean agglutinin, can reversibly bind to and inactivate by complexation and/or precipitation the aggregating factor in saliva that is responsible for inducing the aggregation of Streptococcus mutans cells. These results provide evidence that the salivary aggregating factor contains N-acetyl-D-glucosamine, D-mannose and/or D-glucose, L-fucose and N-acetylgalactosamine and/or D-galactose. Chromatographic and immunochemical techniques are being investigated for the isolation and characterization of the salivary aggregating factor contained in the lectin-induced precipitates.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective:

Lectins, which are naturally occurring proteins capable of interacting with certain macromolecules and/or cell types via specific sugar moieties, are being used to investigate the interactions between saliva and oral bacteria in order to elucidate the nature of the substances responsible for these interactions and the role they play in plaque formation and caries development. Lectins are also being assayed for direct effects on oral bacteria.

Methods:

The ability of saliva to induce the aggregation of various strains of Streptococcus mutans and the effect of lectins on this process was determined using a spectrophotometric assay procedure. Aggregation was detected by measuring the decrease in absorbance at 700 nm over 2 hours of a suspension of S. mutans mixed with saliva samples that had been treated with various concentrations of lectin and comparing this to the change in A_{700} of control suspensions of S. mutans.

Standard immunization techniques were used to immunize rabbits with lectin-induced precipitates from saliva in order to produce antibodies to the salivary aggregating factor for S. mutans.

The ability of lectins to aggregate various oral bacteria was determined using standard microtiter techniques.

Major Findings:

Anti-aggregating factor antiserum has been produced in rabbits by immunizing them with a lectin-induced precipitate from saliva. Treatment of saliva with this antiserum inhibits the subsequent aggregation of S. mutans by the saliva. The antiserum also disaggregates saliva-aggregated S. mutans cells.

Several lectins have been found that can aggregate various strains of oral bacteria. The amount of aggregation was influenced by the media in which the cells were grown.

Significance:

This investigation has shown that lectins are useful for elucidating the structure of salivary aggregating factors and cell surfaces. Earlier findings that at least 4 lectins can precipitate the salivary aggregating factor for S. mutans and the observation that the lectin-aggregating factor interactions can be reversed by the sugars for which the lectins are specific, suggest that these lectins could be useful for

purifying salivary aggregating factors by precipitation and/or affinity chromatography. The development of a purified anti-aggregating factor antiserum could lead to a convenient method of assaying saliva for aggregating activity by rocket immunoelectrophoresis or similar techniques. The finding that lectins can aggregate oral bacteria suggests that lectins will be useful in elucidating the cell surface of these microorganisms and may lead to the development of a therapeutically useful lectin.

These studies will help to elucidate the role of saliva-induced bacterial aggregation in plaque and caries preventive measures.

Proposed Course:

Various chromatographic, immunochemical and electrophoretic techniques will be used in an attempt to isolate and identify the substance with aggregating activity that lectins precipitate from saliva. The purification of the antiserum with anti-aggregating factor activity will be investigated. The effect of lectins on *in vitro* and/or *in vivo* plaque development will also be studied.

2. Publications:

Mirth, D.B., Miller, C.J., Kingman, A., and Bowen, W.H. Inhibition of saliva-induced aggregation of *Streptococcus mutans* by wheat germ agglutinin. *Caries Research*. 13: 121-131, 1979.

Mirth, D.B., Miller, C.J., Kingman, A., and Bowen, W.H.: Investigation of salivary aggregating factors with lectins. In Kleinberg, I., Ellison, S.A., and Mandel, I.D. (Eds.): *Saliva and Dental Caries*. Sp. Supp. Microbiology Abstracts, pp. 255-266, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00154 05 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Biochemical Product and Energy Requirements of Plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robrish, Stanley A.	Microbiologist	NCP	CPR	NIDR
OTHER:	Kemp, Christopher W.	Microbiologist	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Eberlein, Doreen	Biological Aide	NCP	CPR	NIDR
	Emilson, Claes-Goran	Visiting Scientist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.42	.72	.70

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have completed the analysis of three adenine nucleotides, calculated the function of adenylate energy charge (AEC), and found it directly related to the percent viable cells in a pure culture of bacteria. Use of AEC analysis of viable cell mass is being assessed in dental plaque samples obtained from monkeys which have been treated with antiplaque agents. Protein content of plaque, conventional viable counting on bacteriological media, fluorescent antibody analysis for streptococci and actinomyces, and adenine nucleotide and AEC values are being compared. A computer program has been written to do the calculations for the nucleotide analyses.

1. Project Description

Objective:

The objective for this project is to develop essential assays for measures of metabolism in mixed bacterial systems. Though specifically designed for the analysis of dental plaque associated with caries, these assays potentially have wide use in studying other diseases and in enhancing basic understanding of microbial ecology.

Methods:

We have evaluated the use of individual adenine nucleotides to measure viable cell mass and found them not to be reliable indicators of this important plaque parameter. For instance when Escherichia coli was allowed to incubate for a prolonged period of time without nutrients, the ATP content of the cells was directly related to the viable count only after constant intracellular conditions were established. Therefore we have explored the possibility of using adenylate energy charge (AEC) because this ratio of adenine nucleotides is more stable in the presence of external nutrients for cellular storage products than is cellular content of ATP. Adenylate energy charge is defined as AEC = ATP + ½ ADP / ATP + ADP + AMP. We found that when a culture of bacterial cells was deprived of nutrients or heat treated the drop in AEC coincided with the reduction in viable count of the suspension.

A computer program was written to process the large amount of data which is essential for this type of assay. The program is interactive and may be used by personnel with relatively little training. The data obtained from standards is analyzed by linear regression and the percent recovery of each adenine nucleotide is calculated and printed as well as the final AEC calculation.

In collaboration with Dr. Emilson, we are now testing the use of these assays in vivo. Dental plaque samples are being obtained from monkeys which have been treated with a variety of antiplaque agents. The monkeys have been maintained on a high sugar diet and treated with different antiplaque agents over a period of six months. A plaque index has been obtained and organisms have been enumerated by conventional microbiological methods. The ratio of actinomyces and streptococci also has been determined using fluorescent antibody analysis. Currently the plaque samples are being analyzed for the content of the individual adenyne nucleotides and for the AEC value.

We have also continued work on a chemical assay to replace the difficult determination of dry weight of small plaque samples. The basis for the potential assay is the light-emitting reaction of luminol with iron prophyrins such as FMN. In the course of these studies we found two discrete classes of luminol reacting material in microorganisms: streptococci having a relatively low specific luminol reaction content and the other gram positive rods including actinomyces having a 500-fold higher specific luminol reacting content. Further it was found that the plaque samples had a higher luminol reactivity than any of the pure cultures which we had tested. The luminol reactive material bound to the plaque appears to be lactoperoxidase from the saliva. It is clear, therefore, that the luminol reaction as currently employed cannot be used for the analysis of microbial parameters of dental plaque.

Major Findings:

Adenylate energy charge (AEC) appears to provide the basis for a relatively simple chemical method to analyze the viable cell mass of mixed microbial populations such as in dental plaque.

Significance:

Determination of the relative effectiveness of alternative antimicrobial and antiplaque agents is an important objective of the National Caries Program. Currently one can measure the fraction of cells in plaque that remain alive only by tedious plating and colony counting procedures. Development of techniques to measure the effect of agents on viable cell mass would be a major step forward for the Program.

Proposed Course:

In collaboration with Dr. Emilson we will continue assessing the relative value under in vivo conditions of alternative chemical methods of assaying important dental plaque parameters.

2. Publications

Robrish, S.A., Kemp, C.W., Chopp, D.E., and Bowen, W.H. Viable and total cell masses in dental plaque as measured by bioluminescence methods. Clin. Chem., 1979. In press.

Kemp, C.W., Robrish, S.A., and Bowen, W.H. An interactive computer program for the computation of adenylate energy charge. Clin. Chem., 1979. In press.

Kemp, C.W. Adenylate energy charge; a method for the determination of viable cell mass in dental plaque samples. J. Dent. Res., 1979. In press.

Robrish, S.A., Kemp, C.W., Adderly, D.C. and Bowen, W.H. The flavin mononucleotide (FMN) content of oral bacteria related to the dry weight of dental plaque obtained from monkeys. Current Microbiol. 2:131, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00164-04-CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

CT 0600115

TITLE OF PROJECT (80 characters or less)
Effect of school water fluoridation and fluoride mouthrinsing on dental caries.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR
	Brunelle, Janet A.	Chief, BS	NCP	CPR	NIDR
	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR

COOPERATING UNITS (if any)
North Carolina State Board of Health and Duplin County, North Carolina, Public School System

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.26	.16	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

About 1500 children attending grades K through 12 at five schools in Duplin County, North Carilina, are exposed to fluoridation of the school water supply at four and one-half times the level estimated to be optimum for community fluoridation in the same geographic area. In addition, weekly mouthrinsing with a 0.2% NaF solution is done by children in kindergarten through eighth grade. Teachers dispense 10 milliliters of solution to each participant-child and supervise the one-minute rinsing procedure which is carried out in the classroom. To evaluate the long-term benefits of the combined treatments on the prevalence of dental caries, pre-treatment clinical data will be compared with the data obtained every two years until the completion of the program in 1987. Baseline examinations using the DMF tooth and surface index were conducted in October/November 1975. The project is entering its fifth year. The second follow-up examination will be made in September 1979.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective:

The purpose of the study is to evaluate a combined program of school water fluoridation and mouthrinsing with sodium fluoride for the prevention of dental caries.

Methods:

The study, a cross-sectional clinical trial, was initiated in October 1975, on approximately 1500 children attending grades K-12 in five public schools located in Duplin County, North Carolina. The County has negligible amounts of fluoride in its sources of drinking water.

All study schools, including high schools, were fluoridated at 4½ times the level estimated to be optimum for community fluoridation in the same geographic area. Surveillance of the fluoride levels is provided by school personnel under the supervision of the North Carolina State Board of Health. In addition, children in grades K-8 rinse weekly, under the supervision of the classroom teacher, with a 0.2 percent sodium fluoride mouthrinse. Children swish 10 milliliters of the solution between their teeth for 60 seconds and then empty the contents of their mouths into a paper cup. Baseline dental examinations, using the DMF tooth and surface index were conducted prior to the installation of fluoridation equipment and the start of the rinse procedures. Follow-up examinations will be compared with baseline findings.

Findings:

The most recent follow-up examinations that have been analyzed are those done in 1977. After 2 years, findings for 1249 subjects in grades 1-10 (ages 6-15) who had participated in the combined program were compared with those of their counterparts at the baseline. Results showed that the prevalence of dental caries (DMFS) for each age group in 1977 was lower than in 1975. Compared with the overall average of 6.74 DMFS at the baseline, children averaged 5.41 DMFS at the follow-up examination, a reduction of about 20%. Because the systemic benefits of school fluoridation after only 2 years are difficult to demonstrate, the caries reduction observed to date must be primarily attributed to the topical effects of the two procedures.

Significance:

Populations in the United States residing in areas that have no central water supply are deprived of the benefits afforded by community water fluoridation. For children in these areas a combined program of

school water fluoridation and weekly mouthrinsing with sodium fluoride should produce a marked cariostatic effect. These procedures combine the systemic effects of fluoride provided by the ingestion of school water and the topical effects of fluoride provided by the weekly mouthrinses.

Both preventive methods offer a number of advantages for school health programming: simplicity, economy, feasibility, safety, acceptability and minimal requirements for professional personnel.

Proposed Course:

Treatment procedures will continue until the Spring of 1987. Follow-up examinations will be made every two years, for the first six years of the study, and every three years during the final six years, ending in the fall of 1987.

2. Publications:

Meyers, R.J., Driscoll, W.S., Murphy, R.F., Brunelle, J.A., and Horowitz, H.S.: Effect of school water fluoridation and fluoride mouthrinsing on dental caries: results after two years. Abstracted, IADR Program and Abstracts of Papers: J. Dent. Res. 58: 294, 1979.

Meyers, R.J., Driscoll, W.S., Brunelle, J.A., Horowitz, H.S., and Murphy, R.F.: Evaluation of school water fluoridation and fluoride mouthrinsing for the prevention of dental caries: findings after two years. Program and abstracts, 26th Congress, European Organization for Caries Research, Stirling, Scotland, June 28-30, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00185-04 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Antimicrobial Susceptibility of Streptococcus mutans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Little, Wayne A.	Microbiologist	NCP CPR NIDR
Other:	Thomson, Lynn A.	Dental Surgeon	NCP CPR NIDR
	Bowen, W.H.	Chief, CPR	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.51	.31	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The antibiotic susceptibility of Strep. mutans has been examined. From the results of screening experiments with Oxoid multidiscs, twelve antibiotics were selected for MIC determinations using a broth microtiter technique.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The objective of the study is to obtain information on the antibiotic susceptibility profiles of Streptococcus mutans strains representing serotypes a through f.

Methods:

The minimal inhibitory concentration (MIC) of each antibiotic was determined by a broth microdilution procedure.

Findings:

Several differences in antibiotic susceptibility were observed in strains representing the different serotypes. Bacitracin was most inhibitory for serotype a strains, having an MIC of less than 0.78 U/ml. Strains representing serotypes b through f were inhibited by bacitracin at concentrations ranging from 0.78 to 50 U/ml. Serotype a and b strains were sensitive to polymyxin B at concentrations of 50 U/ml or less. Four of the 10 serotype b strains had MICs of less than 6.25 U/ml. The majority of strains representing the other serotypes were inhibited by polymyxin B at levels between 25 and 400 U/ml. Strains of serotypes a and d were generally less sensitive to methicillin, with 60% of the a strains and 75% of the d strains having an MIC greater than 0.39 µg/ml. On the other hand, nearly 100% of the other serotypes were susceptible to methicillin at this concentration.

Significance:

The involvement of S. mutans in dental caries and bacterial endocariditis has prompted interest in the susceptibility of this organism to antimicrobial agents. Furthermore, the inclusion of certain agents into selective media has produced preliminary evidence that there may be differences in antimicrobial susceptibility among the different serotypes of S. mutans.

The differences in profiles among the serotypes, although not sufficient to be a practical means of identification are nevertheless significant when applied to laboratory methodology. Antibiotics such as bacitracin, polymyxin B and neomycin have been incorporated into media to increase selectivity without first thoroughly testing the agent against representative strains of S. mutans. The results of this study demonstrate that such testing is essential in order to avoid possible selection against one or more of the serotypes.

Although the actual mechanisms responsible for differences in antibiotic susceptibility observed among the S. mutans serotypes are not

understood, it is notable that bacitracin, polymyxin B, and methicillin utilize the cell wall as the site of action. These findings indicate that certain antibiotics, and perhaps other agents which act on the bacterial cell surface, may not be equally effective against representative strains of S. mutans.

It has been noted by other investigators that there has been a worldwide increase in the number of Lancefield group A streptococci resistant to tetracycline and that the incidence of resistance among S. mutans may also be increasing. In the present study, only animal isolates were resistant at clinical levels to some of the more active antibiotics, including tetracycline and erythromycin. Animal diets are often supplemented with tetracyclines and macrolides, suggesting that prolonged exposure to these antibiotics leads to the appearance of resistant strains of S. mutans in dental plaque. Although a human population study failed to demonstrate resistant S. mutans strains following long term penicillin therapy, similar results might not be observed with tetracycline or macrolide antibiotics. Resistant strains have been supplied to several investigators studying the mechanisms of antibiotic resistance.

Proposed Course:

The project is completed.

2. Publications

Little, W.A., Thomson, L.A. and Bowen, W.H.: Antibiotic Susceptibility of Streptococcus mutans: Comparison of Serotype Profiles. Antimicrobial Agents and Chemotherapy, 15:440-443, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00195-04 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Adherence and Coherence of Cariogenic Streptococci in Dental Plaque.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ciardi, Joseph E.	Research Chemist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Reilly, John A.	Biologist	NCP	CPR	NIDR
	Lee, Terry	Biological Aid	NCP	CPR	NIDR
	McAllister, Paul	COSTEP (Dental)	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Dr. Gunnar Rölla, Department of Pedodontics and Caries Prophylaxis, University of Oslo, Oslo, Norway

LAB/BRANCH
Caries Prevention & Research

SECTION
Etiology

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.73	.38	.35

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms by which oral streptococci interact with teeth, saliva, and with each other to form cariogenic dental plaque are under investigation. Human or rat saliva depresses the adsorption of S. mutans serotype b and c cells to hydroxyapatite. Both types of saliva cause aggregation of only serotype c cells and appear to favor the adsorption of type c cells to hydroxyapatite. A rapid quantitative method to screen substances with a cariogenic potential has been developed. It measures growth, adherence and acid production by S. mutans. Mutants of S. mutans with diminished virulence and decreased ability to synthesize water-insoluble glucans adhered poorly to glass surfaces in the presence of sucrose. They were rendered as adherent as the parent strain following their exposure to glucosyltransferases that synthesized water-insoluble glucans. Human dental plaque formed in the presence of sucrose contained more lipoteichoic acid than plaques formed in the presence of glucose or xylitol. The importance of lipoteichoic acid in cariogenic dental plaque is being explored.

PHS-6040

1. Project Description

Objectives

The objective of the project is to elucidate the mechanisms by which oral streptococci adhere to teeth and to each other in the formation of cariogenic dental plaque. The information obtained from in vitro studies on adherence of bacteria to various smooth surfaces and the effects of oral secretions and cariostatic agents on adherence should eventually lead to the formulation of relevant in vivo experiments.

Methods

Existing and newly developed methods for measuring the adherence of bacteria to solid surfaces and to each other are utilized. Radioisotope methods are employed to produce radioactive bacterial cells for adherence studies.

Major Findings

S. mutans serotype c is most commonly found in humans whereas serotype b appears to predominate in rats (Thompson et al., Caries Res. 13:9 1979). The present study was carried out to determine if saliva from specific animals species might influence the S. mutans serotype that colonizes dental plaque. An improved assay technique was used to measure the influence of human or rat saliva on the adsorption of radiolabeled cells of serotypes b and c to hydroxyapatite (HA) powder. The ability of the salivas to aggregate the S. mutans cells was also assessed.

Preliminary results show that human and rat salivas depress the adsorption of both serotype b and c cells to HA and that the rat saliva is significantly more inhibitory. Both types of saliva appear to favor the adsorption of serotype c cells. They also caused aggregation of serotype c cells. Serotype b cells were not aggregated by either rat or human saliva.

A rapid, quantitative method has been developed to screen substances for cariostatic properties. The method requires only a single 20-hour growth of radiolabeled S. mutans and uses disposable glass tubes and rods. It measures the effect of test agents such as antiseptics, enzyme inhibitors, enzyme modifiers, hydrolytic enzymes, microbial products, and antibodies on growth, acid production, and sucrose-mediated adherence of S. mutans 6715. Because several concentrations of each of a large number of agents can be examined in a single experiment, it should be highly useful as an initial screening tool. In the assay the primary effect of chlorhexidine was bacteriostasis. On the other hand, fluoride inhibited acid production, dextran inhibited dextranase, and specific antiserum inhibited sucrose-mediated adherence. (Ciardi, J.E., Rosenthal, A.B. and Bowen, W.H. J. Dent. Res. 58:IADR Abs. 630, 1979.)

Two mutants of S. mutans 6715 which exhibited diminished virulence and a significantly decreased ability to adhere to glass surfaces in the presence of sucrose were found to synthesize primarily water-soluble glucans in their extracellular glucosyltransferase (GTF) systems. The parent extracellular enzymes synthesized water-insoluble glucans. When the mutants were exposed to the parent extracellular GTF's and subsequently washed they were both rendered as adherent to glass surfaces as the parent strain. The results suggest that GTF's that synthesize water-insoluble glucans play a significant role in the formation and pathogenicity of dental plaque by S. mutans.

Our previous results indicated that the inhibition of glucan synthesis by bis-biguanides and quaternary ammonium salts was significantly potentiated in the presence of certain dental plaque constituents (organic amine derivatives) which are not inhibitors themselves. Mixtures of inhibitors and amines are also more effective bacteriostatic agents than inhibitors alone. In vivo studies in rats are now in progress and will be reported under a new NIDR project.

Collaborative studies with Dr. G. Rolla (Univ. of Oslo, Norway) have shown that human dental plaque formed in the presence of sucrose contains substantially more lipoteichoic acid (LTA) than dental plaque formed after exposure to either glucose or xylitol. These results lend support to our hypothesis that LTA-glucan complexes in association with acid producing bacteria in dental plaque form a diffusion barrier which renders the bacteria more cariogenic. (Rolla, G., Ciardi, J.E., Bowen, W.H. and Knox, K. High amounts of lipoteichoic acid (LTA) in sucrose induced dental plaque. Abstract, 26th ORCA Congress, Stirling, Scotland 1979.)

Significance

A more thorough understanding of the mechanism by which oral streptococci adhere to solid surfaces and to each other and of the influence of oral secretions on these interactions is essential to developing more effective inhibitors of dental plaque formation. Accurate and reproducible methods for assaying adherence and coherence of cariogenic streptococci will prove useful in testing potential inhibitors of plaque formation.

Proposed Course

Studies will continue on the influence of human and animal saliva and on the adsorption of pure or mixed suspensions of oral bacteria to hydroxyapatite. The effect of saliva on growth and sucrose-mediated adherence of bacteria also will be assessed. Dental plaque will be analyzed and relevant in vitro experiments carried out to determine if

lipoteichoic acid-glucan complexes render dental plaque more cariogenic. Our in vitro assay methods that measure growth, adherence and acid production by cariogenic bacteria will continue to be used to test substances with a cariostatic potential.

2. Publications

Ciardi, J.E., Reilly, J.A., and Bowen, W.H. Inhibition of sodium fluoride of ^{3}H-glycerol incorporation into lipoteichoic acid in Streptococcus mutans. Caries Res. In Press, 1979.

Ciardi, J.E., Rosenthal, A.B., and Bowen, W.H. Rapid quantitative determination of the effect of antiplaque agents and antisera on the growth, acid production and adherence of Streptococcus mutans. J. Dent. Res., Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00206-03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

CT-0600118

TITLE OF PROJECT (80 characters or less)
Effect of daily and weekly rinsing with sodium fluoride solutions in a non-fluoride area (C)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR
	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
	Kingman, Albert	Statistician	NCP	CPR	NIDR

COOPERATING UNITS (if any)

Biddeford School Department, Biddeford, Maine

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .50	PROFESSIONAL: .35	OTHER: .15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In October 1976, a sodium fluoride (NaF) mouthrinse study was started in Biddeford, Maine, a non-fluoride area. Baseline dental examinations (DMFS Index) were made of 825 children in grades 5-7 attending seven schools in the community. Participants were randomly divided into three groups. Under teacher supervision, they rinse either weekly with a 0.2% NaF solution or a 0.1% sodium chloride solution (Placebo) or daily with a 0.05% NaF solution. Treatments are to be carried out for three school years. Follow-up dental examinations are scheduled annually to determine the anti-caries effectiveness of the two fluoride mouthrinse procedures. The third and last year of treatments was completed in June 1979. Final examinations will be made in October 1979.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective

To determine the comparative effectiveness of weekly rinsing with a 0.2% sodium fluoride (NaF) solution and daily rinsing with a 0.05% NaF solution in a non-fluoride area.

Methods:

In October 1976, parental consent to participate in the F mouthrinse study was obtained for 825 students in grades 5-7 attending seven schools in Biddeford, Maine, a non-fluoride area. Baseline caries prevalence was registered and participants were randomly divided into three comparable study groups: Group 1 rinses weekly with a placebo solution containing 0.1% sodium chloride; Group 2 rinses weekly with a 0.2% NaF solution; and Group 3 rinses daily with a 0.05% NaF solution. The mouthrinse procedures are carried out in school under teachers' supervision. Treatments will extend over a period of three school years. Follow-up dental examinations are conducted annually for a period of three years.

Findings:

Findings after one year of study showed that each frequency of fluoride rinsing was effective in controlling decay. However, no difference in effectivemess between the two fluoride rinse procedures could be distinguished. Two-year findings are currently being analyzed.

Significance:

Although studies have shown that both fluoride mouthrinse procedures can reduce the incidence of dental decay, there is insufficient evidence to determine if one regimen of fluoride mouthrinsing is clearly more effective than the other. Information on the comparative benefits of the two procedures will be helpful to school and health officials interested in implementing a school mouthrinse program.

Proposed Course:

Three-year follow-up examinations are scheduled to be carried out in October 1979. The number of treatments that each child receives during each year of study will be tallied and recorded. Children judged to have "inadequate exposure" will be excluded from interim and final analyses. Interim and final reports will be prepared.

2. Publications:

Kingman, A., Heifetz, S.B. and Meyers, R.J.: A comparison of the anticaries effectiveness of daily and weekly rinsing with sodium fluoride solutions. In IADR Program and Abstracts of Papers: J. Dent. Res. 58:295, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00220-03-CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979 CT 0600121

TITLE OF PROJECT (80 characters or less)

Comparison of daily and weekly rinsing with sodium fluoride in a fluoridated community

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Driscoll, William S.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Swango, Philip A.	Clinical Investigator	NCP	CPR	NIDR
	Horowitz, Alice M.	Public Health Educator	NCP	CPR	NIDR
	Kingman, Albert	Statistician	NCP	CPR	NIDR

COOPERATING UNITS (if any)

Des Moines Independent Community School District

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.61	.41	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study was initiated in September 1977 with 1000 children in the seventh grade of nine junior high schools located in Des Moines, Iowa, a community that has optimal amounts of fluoride in its supply of drinking water. The children were randomly assigned to one of the following three study groups: Group I (controls) rinse their mouths once every week in school for 60 seconds with a placebo solution; Group II follows an identical procedure using a 0.2% neutral sodium fluoride solution (0.9% F). Group III rinse their mouths once every day in school for 60 seconds using a 0.05% neutral sodium fluoride solution (0.023%F). The procedures are carried out under the classroom teacher's supervision for a period of three years. Baseline dental examinations, using the DMF surface index, were conducted in November 1977. The first follow-up examination was conducted in April 1979. Final examinations will be conducted in May 1980.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective:

To compare the caries-inhibiting effect of weekly rinsing with a 0.2 percent sodium fluoride solution (0.09%F) and daily rinsing with a 0.05 percent sodium fluoride solution (0.023%F) in children who have been reared during the years of school attendance in an optimally fluoridated community.

Methods Employed;

The study, a longitudinal double-blind clinical trial, was initiated in September 1977, with 1000 children in the seventh grade of nine junior high schools located in Des Moines, Iowa, a community that has optimal amounts of fluoride in its supply of drinking water. The children were randomly assigned to one of the following three study groups: Group I (controls) rinse their mouths once every week in school for 60 seconds with a placebo solution; Group II follows an identical procedure using a 0.2% neutral sodium fluoride solution (0.09%F); Group III rinse their mouths once every day in school for 60 seconds using a 0.05% neutral sodium fluoride solution (0.023%F). Under the supervision of the classroom teachers the procedure is to be carried out for a period of three years. Baseline dental examinations, using the DMF surface index, were conducted in Novermber 1977. One follow-up examination was conducted in April 1979 and the second and final follow-up examination will be conducted in May 1980.

Findings:

Analysis of data from the first follow-up examination has not yet been completed.

Significance:

Although the practice of water fluoridation makes a sizable inroad into the widespread problem of dental decay that exists in most communities, complementary public health measures must be developed to prevent the dental decay that still persists despite fluoridation. There is good reason to believe from recently reported data that fluoride mouthrinsing in school may confer significant additional decay preventive benefits when practiced by children who consume optimally fluoridated drinking water. It is the intent of the proposed study to determine the extent of benefit derived from the procedure and then compare the efficacy and feasibility of the weekly procedure with that of the daily procedure. The information that can be gained from this study is needed before fluoride mouthrinsing programs can be recommended and promoted for wide scale use in optimally fluoridated communities.

Proposed Course:

Two years of fluoride mouthrinsing have been completed. The final year of mouthrinsing will begin as soon as possible after school starts in September 1979. A report presenting findings of the first follow-up examination will be prepared for publication. The second, final, examination will be conducted in May 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00222-03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Specific and non-specific immune factors in plaque fluid and saliva

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Cole, Michael F.	Visiting Scientist	NCP CPR NIDR
OTHER:	Bowen, William H.	Chief	NCP CPR NIDR
	Hsu, Su-Cheng D.	Biologist	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.36	.16	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The free aqueous phase was obtained from individual samples of dental plaque and the plaque matrix was then eluted with a chaotropic buffer in an attempt to remove bound protein. The fluid and the low pH phases were assayed for secretory immunoglobulin A (sIgA), IgG, IgM, the third component of complement (C'3), lysozyme, lactoperoxidase and lactoferrin. The presence of these specific and non-specific immune factors in the free and bound phases suggest they are important in host defense at the plaque-enamel interface.

PHS-6040

1. Project Description

Objective

The purposes of this study are to measure specific and non-specific defense mechanisms in dental plaque and saliva and to determine their role in host defense at the enamel plaque interface.

Methods

Supragingival plaque free of visible blood was centrifuged at high speed to obtain the free fluid phase. The plaque was then washed with neutral pH buffer until no protein remained. The plaque was then treated with buffer in order to elute proteins bound to the matrix. The proteins were measured by single radial diffusion, rocket immunoelectrophoresis, solid phase fluoroimmunoassay and spectrophotometry.

Major Findings

The levels of the specific and non-specific immune factors found in plaque fluid suggest that predominantly saliva and, to a lesser extent, gingival exudate contribute to the protein pool. Significant levels of these immune factors were bound to the plaque matrix suggesting that they participate in host defense at the enamel plaque interface.

Significance

Despite extensive studies of the composition of saliva in caries active and caries free subjects, it has not been possible to correlate the concentration or activity of any salivary protein with resistance or susceptibility to dental caries. This observation may suggest that the ability of host defense factors to control dental caries depends more on their concentration and ability to retain biological activity in dental plaque than in their concentration and activity in saliva.

The study of the qualitative and quantitative composition of plaque fluid in populations with high and low caries experience could help in understanding the mechanisms by which the host protects the tooth surface.

Proposed Course

1. Design *in vitro* systems to study the interactions of these proteins.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 DE 00223 03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

An Improved Method for Analyzing Caries Clinical Trials Data

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Kingman, Albert Statistician - Health NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Biometry

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.04	.04	--

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In many clinical trials the groups being compared at the end of the trial are not balanced as to initial DMFS scores, even though subjects were randomized to treatment groups initially. Two analytical methods have been used to adjust for these imbalances: the blocking analysis and the covariance analysis. The blocking analysis is the preferred method in that it requires fewer assumptions made of the data.

In this study an adaptation of Grainger's severity index, one method of summarizing the initial DMFS scores, was used as a blocking factor in the analysis of caries clinical trial data. This blocking factor has the advantage of being independent of the data set on which it is used. Further, its high correlation with initial DMFS scores insures that it is utilizing the existent information within subjects at the beginning of the trial. This analytical method was compared with 3 other methods, including the covariance analysis and other blocking techniques to see what increase in efficiency would be realized by using this technique.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives

The purpose of this study is to identify a blocking or stratification factor which could be used in caries clinical trials to adjust the group increment scores for DMFS imbalances, and which is capable of being defined a priori and is based on objective criteria that can be used by any clinical investigator.

Methods:

An adaptation of Grainger's severity index (one way of summarizing a subject's initial DMFS experience) was compared to three other methods of summarizing a subject's initial DMFS experience. The properties of this index were investigated to see how it would compare with other methods in terms of correcting for differences in risk among subjects who participate in these trials. Six clinical trials data sets were examined by each method and the results compared.

Major Findings:

The blocking or stratification method using Grainger's severity index resulted in the greatest efficiency among the methods tested for these six trials data. This suggests that more information exists in knowing the types of decay present in the subject's dentition than in knowing the subject's total DMFS score.

Significance:

A common problem occurring in caries clinical trials is the existence of an imbalance in the initial DMFS scores among the study subjects remaining at the end of the study. Two analytical procedures have been used to adjust the increment scores for this risk imbalance: the blocking or stratification analysis and covariance analysis. The blocking method is the preferred method in that it requires fewer assumptions from the trial data.

2. Publications

Kingman, A. A method of utilizing the subjects' initial caries experience to increase efficiency in caries clinical trials. Community Dentistry and Oral Epidemiology, 7:87-90, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00225-03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cost analysis of implementing school-based community mouthrinse programs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Brunelle, Janet A.	Chief, BS	NCP CRP NIDR
OTHER:	Miller, Ann J.	Health Science Administrator	NCP CGC NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Biometry

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on costs of program, student participation and caries experience are being collected from seventeen communities throughout the U.S. and Guam which are conducting three-year demonstrations of school-based mouthrinse programs. Analysis of implementation, acceptance of community and cost-benefit will be performed during and after the three years.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The objective of the project is to obtain detailed data on the cost of implementing a school-based mouthrinse program in a variety of community settings.

Methods:

Data on costs of personnel, consumable materials, permanent equipment and building overhead were collected from 17 sites in the United States and Guam where mouthrinsing is being demonstrated. Participation statistics from consent forms, attendance, and drop-out records are also being collected and evaluated.

Findings:

The participation after three years of the study averaged 83% of the total eligible population. Dental caries activity was less after three years of mouthrinsing in all seventeen sites with grades one, two and three receiving the greatest protection on their permanent teeth. The costs per child per year for supplies, equipment and personnel ranged from 40¢ to $9.52. The method of delivery and the differences in administrative levels used to dispense, deliver or supervise account for the cost differential between sites. Cost-benefit estimates were made for supplies and equipment after three school years. Caries prevalence in a particular geographic area affects the cost effectiveness. The lower the disease level the higher the cost. For areas with initial DMFS ranging from 4 to 6 affected surfaces, the cost of supplies and equipment only was approximately 40¢ to $3.50. For areas having a much lower DMFS--1.5--2.5 DMF surfaces the cost ranged from approximately $4.00 to $6.00.

Significance:

Early estimates of cost-benefit indicate that the procedure is highly cost-effective in a variety of settings and suggest that any community can afford the school-based mouthrinse program.

Proposed Course:

Final analyses of cost-benefit will be made for the total three and one-half year study using the differences in caries scores from the first and final clinical examinations as the measure of effectiveness.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00228-03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Association of Streptococcus mutans with Dental Caries Reduction in a School Population.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomson, Lynn A.	Dental Surgeon	NCP CPR NIDR
COPI:	Little, Wayne A.	Microbiologist	NCP CPR NIDR
	Bowen, William H.	Chief, CPR	NCP CPR NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.71	.41	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dental plaque is being studied to determine if changes in microorganisms, specifically Strep. mutans, occur during fluoride preventive procedures. The school population consists of children using fluoride rinses either daily or weekly, as well as some children using a placebo solution. Dental plaque samples are obtained periodically and analyzed using cultural methods and fluorescent antibody reagents specific for bacteria under study. Alterations in plaque composition during the study period will be related to the changes in the caries incidence.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives

The objectives of the project are to:

a. determine any changes that occur in the Strep. mutans population in the plaque of children using fluoride rinses in a school program.

b. associate changes in the Strep. mutans populations with the reduction in dental caries or alteration in plaque fluoride level.

c. evaluate the effect of transportation and storage methods on the viability of bacteria in the plaque specimens and

d. examine the apparent absence of Strep. mutans serotype d or g organisms in the dental plaque samples of this school population (St. Joseph's School, Biddeford, Maine).

Methods:

The present investigation employed improved FA examination methods and reagent grade FA conjugator prepared by the Biologic Reagent Section, Center for Disease Control, Atlanta, Georgia. The conjugates and FA methods are described in the following papers:

a - J. Dent. Research 55 (Special Issue A): A80-86 (1976).

b - Caries Research 13:9-17 (1979)

c - Article submitted to: J. Dent. Research (1979).

Thirty students for the plaque study were selected randomly by the Biometry Section, NCP. Three plaque samples were obtained from each child. These samples included one specimen for fluoride determination and two for bacterial examination at specific tooth sites. All samples were immediately frozen and stored at <-70^{0}C until removed for analysis.

The fluoride level in the plaque samples was determined with a fluoride electrode using a method developed by Dr. R. Shern.

Major Findings

Using cultural methods, 63.3% of the Maine children were determined to have S. mutans. When FA methods were employed, the number of Maine children with detectable levels of S. mutans increased to 70.17%. The S. mutans colonies comprised about one-third of the colony forming units on the MS agar plates. In comparison, the FA/phase contrast results indicated that S. mutans averaged 1.9% of the total cell count. Serotype c

was the most frequently detected serotype of S. mutans (89.5% culturally and 76.1% with FA). Serotypes a, b, d and g were not detected in this population. Serotype e and f were encountered in 21.1% and 10.6% of the specimens which were positive for S. mutans. Strep. sanguis, Actinomyces israelii and Actinomyces naeslundii Actinomyces viscosus were found to be present in over 98% of all children studied and were observed to account for 9.6%, 10.4% and 38.4% (respectively) of all cells visible in the plaque specimens.

Plaque fluoride levels were determined for samples representing each of the three groups of children. The wide variation, however, in the fluoride values at baseline and subsequent examinations precludes meaningful comparisons with the prevalence of Strep. mutans or dental caries experience.

During the course of this study, plaque samples from one sampling period were observed to have a lower viability of bacteria than expected. Other studies have also reported a reduction in the viability of plaque bacteria stores at -70°C for even short periods of time. One purpose of the present study is to investigate the adequacy of these sampling methods and to determine their limitations.

Significance

Efforts to determine the etiologic significance of Strep. mutans, Actinomyces and lactobacilli in the initiation of dental caries have been hampered by time-consuming cultural procedures. The new procedures used here, including improved FA reagents, will considerably assist scientists in studying this question.

Data on the prevalence of these species in this school-age population provide valuable information on the frequency of these bacteria and their variation. Of particular interest is the absence of Strep. mutans serotypes d and g organisms.

Proposed Course

The significance of not finding serotypes d and g organisms in this population will be assessed.

2. Publications:

Thomson, L.A., Little, W.A., Bowen, W.H., Sierra, L.I., Aguirre, M., and Gillespie, G. Prevalence of Streptococcus mutans Serotypes, Actinomyces, and Other Bacteria in the Plaque of Children. J. Dent. Research: Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00229 03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Plaque variations in populations ingesting different levels of water fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stiles, Horace M.	Microbiologist	NCP	CPR	NIDR
COPI:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
Other:	Brunelle, Janet A.	Statistician (Health)	NCP	CPR	NIDR
	Wittig, Anna B.	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.14	.49	.65

CHECK APPROPRIATE BOX(ES)

☒ (c) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three communities having 4.2, 2.3 and less than 0.1 ppm fluoride in the drinking water were chosen as study sites. Children 12-18 years of age who had been lifelong residents in the communities comprised the study populations. Plaque and saliva samples, collected from each participant, are being analyzed for microbial and fluoride content. DMF surfaces also were recorded for each participant. Data will consist of comparisons of various parameters among the three groups.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives:

The purpose of this study is to examine associations among DMFS, plaque fluoride concentration and certain microflora of dental plaque from populations with different concentrations of naturally-occurring fluoride in the drinking water. The microbiologic phase of the study is still in progress.

Methods:

Three population groups residing in Texas were selected for study, each with a different concentration of fluoride naturally occurring in the drinking water. The sites were designated as low (L), mid-range (M) and high (H) in fluoride, with water concentration of fluoride less than 0.1, or 2.3 or 4.2 ppm. The water fluoride concentrations had been at these levels for at least 18 years. The recommended optimal fluoride concentration for the geographic region is 0.7-0.9 ppm.

Major Findings:

a. Mean age for the 3 groups was essentially the same, 14.6, 14.3 and 14.- years. As expected the caries experience of the group consuming less than 0.1 ppm fluoride was higher than in those groups consuming 2.3 or 4.2 ppm fluoride. This difference was significant ($P<0.001$).

b. No significant difference in caries scores was seen between the participants consuming 2.3 or 4.2 ppm fluoride in their drinking water.

c. The mean plaque dry weight for the three groups was essentially the same 0.91, 0.92 and 1.19 mgms.

d. The fluoride concentration in the dry plaque specimens was significantly different in each group.

e. Positive correlations were found between age of subject and plaque fluoride concentration. However, these correlations were signficiant only for groups I and III, the low (<0.1 ppm F) and high (4.2 ppm F) water fluoride groups.

f. Results from the microbiological studies from the participants are incomplete.

Significance to Program:

A major problem for the Program is to distinguish the various mechanisms through which fluoride prevents caries and evaluate their significance. Fluoride in saliva and drinking water is concentrated by dental plaque. This project should provide important data on the fluoride levels reached in plaque and on associated changes in the numbers and species of cariogenic microorganisms.

Proposed Course:

The study will continue to examine the microbiology of plaque from these three populations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 DE 00231 03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Radiation caries in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar, William M.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.50	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Plaque fluid and saliva samples from 4 irradiated monkeys (Macaca mulatta) and from groups of non-irradiated controls, all receiving a cariogenic diet, were analyzed on an individual basis for inorganic and organic constituents. Basic information on the composition of plaque fluid from animals with contrasting caries and on the effects of salivary gland irradiation is accumulating, giving some insights which may be relevant to caries in man.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The project is being carried out to obtain information on: (1) the chemical environment of the plaque in animals with contrasting caries experience, and (2) the effects on the composition of saliva of radiation damage to the glands.

Methods:

Four Macaca mulatta monkeys, irradiated as described previously, were compared with controls (M. cynobalus) from other experiments. All animals received the same cariogenic diet. Plaque and saliva were sampled 11 times over 6 months. Plaque fluid was separated by centrifugation (40,000 X g, 60 min, 4°C) and, together with clarified saliva, stored at -20°C. The following constituents were analyzed:

Inorganic: Calcium, phosphate (total and inorganic), magnesium, sodium, potassium, zinc, iron, strontium, fluoride, iodide, calcium activity, thiocyanate, pH.

Organic: Total protein, electrophoretic and electrofocusing profiles, albumin, amylase, immunoglobulins, complement, lysozyme, acid end-products of bacterial metabolism. Most components were determined on an individual basis; occasionally pooling was required.

Major Findings:

The study is the first to compare samples of saliva and plaque from individuals with contrasting caries, with regard to a wide range of important constituents. Differences between individuals and between groups suggest primary and secondary results of irradiation and consequent caries development.

Significance:

The basic information generated on plaque fluid composition in animals with contrasting caries and on salivary effects of gland irradiation may be extrapolated to radiation caries and caries pathogenesis in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00234 02 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Develop method of intraoral telemetry of various ions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shern, Roald J.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Schuette, William H.	Chief, ACE		BEI	R
	Tipton, Harold W.	Technician		BEI	R
	Talbot, Thomas L.	Mechanical Engineer		BEI	R

COOPERATING UNITS (if any)
Microelectrodes, Londonderry, NH Dr. Normand Hebert

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.18	.18	--

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Oral biotelemetry is being developed which enables direct and continuous measurements of intraoral responses such as $\underline{H}^+$ and $\underline{F}^-$ levels following ingestion of various foods and therapeutics. Measurements of ionic levels may be conveyed to the recording equipment by radio or wire transmission. Clinical studies will be initiated soon.

Data from telemetry coordinated with data from other projects will aid in the evaluation of various food stuffs for their relative cariogenicity.

PHS-6040

1. Project Description

Objectives:

The goal of this project is to develop a method which will be useful in predicting the cariogenicity of foods, in measuring the bioavailability of fluoride, and in measuring other biological phenomena in the mouth.

Methods:

Equipment needed for making the various intraoral measurements is not commercially available and must be fabricated. This project which was initiated several years ago remains in the pretest phase. However, substantial progress has been made in developing a workable wire telemetry apparatus and conventional and transitor sensors (CHEMFET) have been tested for intraoral use.

Major Findings:

The CHEMFET and the pH microelectrode responded accurately and reliably in in vitro tests. Both the CHEMFET and the radiotelemetry device failed after prolonged contact with moisture. The signal from the wire telemetry device that used a conventional glass pH electrode was degraded by inadequate shielding and other deficiencies. However, wire telemetry using a glass electrode shows enough promise to warrant further testing.

Significance:

Telemetry of pH, F^- and other ions would be useful to government, industry and universities. Physiologists could gain much useful information concerning interactions in the mouth of bacteria, host, and diet. As an example, government and industry could study the cariogenicity of various foods and snack items using telemetry and coordinated animal studies. This information would be useful for developing less cariogenic diets.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00235 02 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Induction of secretory immunity in the gnotobiotic rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Cole, Michael F.	Visiting Scientist	NCP CPR
OTHER:	Hsu, Su-Cheng D.	Biologist	NCP CPR
	Kemp, Christopher	Microbiologist	NCP CPR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.16	.10	.06

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Germ free rats were immunized orally, intragastrically and intramucosally with Streptococcus mutans 6715-15. Unimmunized animals served as controls. All rats were infected with S. mutans 6715-15. After 8 weeks the rats were sacrificed and serum, saliva, gut and pulmonary washes were collected. In addition, splenic, salivary, mesenteric and Peyer's patch lymphocytes were assayed. Serum and salivary antibodies were detected in all groups. Primed lymphocytes were detected in spleen and salivary lymph nodes.

1. Project Description

Objective

The purpose of this study was to investigate the relative importance of the oral lymphoid tissue and the gut associated lymphoid tissue in the induction of a secretory immune response in the oral cavity.

Method

Weanling Osborne Mendal rats were divided into four groups. One group was immunized orally by including 1×10^8 colony forming units (CFU) per ml S. mutans in the drinking water. A second group received 0.2 ml of 5×10^9 CFU/ml S. mutans by stomach tube. Group three also received 0.2 ml of 5×10^9 CFU/ml, but in the vicinity of the salivary glands. The final group served as unimmunized controls. After 8 weeks the animals were sacrificed and serum, saliva, gut and pulmonary washes were collected and assayed for antibodies to S. mutans by microagglutination. The ability of single cell suspensions of spleen, salivary lymph node, Peyer's patch and mesenteric lymph to undergo blastogenesis in the presence of S. mutans was also determined.

Major Findings

Serum and salivary antibodies were detected in all groups including the unimmunized controls. Intramucosal immunization elicited the greatest response. Primed lymphocytes were detected in the spleen and salivary lymph nodes. Peyer's patch and mesenteric lymph nodes gave poor responses.

Significance

Results of previous experiments have indicated that rodents and primates can be protected against dental caries by active immunization by a variety of routes. It has also been demonstrated that rabbits and primates hyperimmunized with S. mutans exhibit antibodies reactive with human heart. Although it is unclear whether such antibodies are pathogenic, it is clearly undesirable to induce heart-reactive antibodies in humans as a result of immunization with S. mutans. There is evidence that local mucosal immunity can be induced in the absence of a humoral immune response. If these preliminary findings can be confirmed the problem of heart cross-reactivity may be circumvented. The success of a vaccine against dental caries will depend upon the induction of high levels of antibody in the oral cavity. Studies of the type outlined above may help to establish the best route, dose and schedule to achieve this goal.

Proposed Course:

1. Specifically label cells to determine where the committed salivary lymphocytes originate.

2. Study the immunogenicity of purified components of S. mutans in an attempt to identify protective antigens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00236 02 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Purification of rat immunoglobulins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Hsu, Su-Cheng D.	Biologist	NCP	CPR	NIDR
	Monell-Torrens, Esteban	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.70	.30	.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunoglobulins M and G (IgM and IgG) were purified from rat serum and secretory IgA from rat colostrum by molecular sieve, ion exchange and affinity chromatography. Purification of IgM was complicated by contamination with α_2, macroglobulin.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The purpose of this study was to purify immunoglobulin A, G and M in order to raise monospecific antisera to these proteins.

Methods:

Rat serum was collected and the immunoglobulins precipitated with 20 volumes of 2.5% boric acid. The precipitate was resuspended in 0.1 M Tris HCl pH 8.0 containing 0.01 M glycine and 0.15 M NaCl and chromatographed on Sephadex G200. The exclusion peak containing IgM was concentrated and chromatographed on Sepharose 6B. Immunoglobulin M was found in the second peak and was free of detectable α_2 macroglobulin.

The supernatant from the precipitated serum was dialyzed and chromatographed on QAE Sephadex A-50 to purify IgG. Colostrum was obtained from rat dams following stimulation with oxytocin and was precipitated with 50% $(NH_4)_2\ SO_4$. The precipitate was redissolved, dialyzed and chromatographed on Sephadex G200. The exclusion peak was rechromatographed on Sepharose 6B to yield pure SIgA.

Recently the immunocytoma bearing rat strain LOU/WSL has been obtained from Dr. Hervé Bazin, Experimental Immunology Unit, University of Louvain B-1200, Brussels, Belgium. The following tumors are maintained in rats as solid subcutaneous or intraperitoneal ascitic tumors or the neoplastic cells have been frozen in liquid nitrogen:

IR 202 IgM
IR 22 IsA
IR 677 IgG_1

Single cells suspensions of the solid tumors are made by mincing the tissue and passing it through stainless steel screens. The erythrocytes are lysed and the numbers of viable cells enumerated by Trypan Blue exclusion. The cell density is adjusted to $1X10^7$ cells/ml and 1 ml of the cell suspension in RPMI 1640 tissue culture medium injected intraperitoneally. The ascidic fluid is collected by aspiration after 7-10 days and serves as a rich source of monoclonal immunoglobulin.

Significance

Antiglobulin reagents, i.e. antibodies directed against immunoglobulin isotypes are indispensable for the assay of class specific antibody

in secretions and eserum. Without these well-characterized reagents it is impossible to measure class specific antibody. These reagents can be utilized in antiglobulin augmentation assays, enzyme linked immunoabsorbant assays, fluoroimmunoassays and radioimmunoassay.

The production of monospecific antiglobulin reagents will allow the detection of antibody forming cells and the quantitation of secretory antibodies in the rat caries model.

Proposed Course:

(1) Immunize rabbits and goats in order to produce antisera.

(2) Render antisera monospecific by solid phase absorption.

(3) Conjugate antisera with fluorescein or tetrarhodamine isothiocyanate, alkaline phosphatase or horseradish peroxidase.

(4) Devise sensitive assays to measure mucosal antibodies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00237 02 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Immunoglobulins and antibodies in plaque fluid and saliva in two populations with contrasting levels of caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Hsu, Su-Cheng D.	Biologist	NCP	CPR	NIDR
	Kingman, Albert	Statistician	NCP	CPR	NIDR
	Brunelle, Janet	Chief, BS	NCP	CPR	NIDR
	Rodgers, Patricia	Statistical Assistant	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Univ. of Antiquia, Medellin, Colombia, S.A. Drs. Sierra, Espinal and Aguirra
PAHO, Wash., D.C. Dr. G. Gillespie

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.29	.36

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Plaque fluid and saliva were collected from 25 children aged 7-12 years from two communities with contrasting levels of caries and assayed for secretory immunoglobulin A (SIgA), IgG, IgM, complement (C'3) and lysozyme. Significantly more IgA was found in the saliva and lysozyme in the plaque fluid from subjects with low caries experience. Subjects exhibiting antibody reactive with S. mutans serotypes a and d had a lower prevalence of caries than those who failed to demonstrate antibodies.

PHS 6040

1. Project Description

Objective

The purposes of this study are to measure immunoglobulins, antibodies and non-specific immune factors in saliva and plaque fluid and to determine their relationship to the level of caries.

Methods

Supragingival plaque, free from overt blood was collected from each subject and centrifuged at 38,000 .g to obtain the free aqueous phase. Whole saliva was also obtained from each subject. Secretory IgA, IgG, IgM and complement were assayed by rocket immunoelectrophoresis. Lysozyme was quantitated by the lysoplate technique.

Major Findings

Significantly more SIgA was found in the saliva of subjects from the low caries community. Low levels of IgG and trace amounts of IgM and complement C'3 were found in saliva from all subjects. No difference in the concentration of SIgA in plaque fluid between the two populations was detected. However, significantly more lysozyme was found in the plaque fluid from the population with low caries experience. Subjects exhibiting antibody reactive with serotypes a and d had a lower prevalence of caries than those who failed to demonstrate antibody.

Significance

Study of the levels of specific and non-specific immune factors in saliva and plaque fluid from subjects with high and low caries experience could aid in understanding the role of host defense factors in protection against dental caries.

Proposed Course

1. Expand the numbers of subjects in the study populations in order to better understand the role of host defense in the etiology of dental caries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00240 02 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Cariogenicity of foodstuffs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
OTHER:	Monell-Torrens, Estaban	Lab Technician	NCP	CPR	NIDR
	Amsbaugh, Suzanne M.	Biologist	NCP	CPR	NIDR
	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR
	Gomez, Irma M.	Biologist	NCP	CPR	NIDR
	Emilson, Claes-Goran	Visiting Scientist	NCP	CPR	NIDR
	Edgar, William M.	Visiting Scientist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.59	.08	.51

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The public, dental profession and regulatory agencies have considerable interest in identifying those foodstuffs which have a high cariogenic potential. The purpose of this study is to develop a simple animal model which can be used to determine and to rank the cariogenic potential of common snack foods in a reproducible manner.

PHS-6[illegible]
(Rev. 10-76)

1. Project Description

Objective:

The purpose of this study is to determine whether a method could be developed which would permit the ranking of the cariogenic potential of foodstuffs. A secondary purpose is to study the effect of particular foodstuffs on the Strep. mutans population in animals.

Methods:

Groups of rats were fed their essential nutrition by means of gastric intubation. The test foodstuffs were powdered and passed through a standard mesh and fed to the animals on a Konig-Hofer feeding machine 17 times daily. Thus, any caries which developed can be ascribed solely to the interaction of the test food with oral bacteria on the tooth surface. All the animals were infected with Strep. mutans 6715.

Major Findings

A wide variety of foods has been tested ranging from potato chips to cookies, breakfast cereals and caramels. Differences in cariogenicity are readily detected and can be related to the cariogenicity of sucrose. It was found that the number of carious lesions which the animals developed is directly related to the frequency of exposure to sugar. Animals which received their entire diet by gastric intubation remained caries-free. The number of Strep. mutans which became implanted in the animals was influenced by the number of exposures to sugar and the food offered.

Significance:

These results show that it is possible to detect differences in the cariogenic potential of foodstuffs, and that this model may fill the need for a simple reproducible test. The model may also be used to investigate the influence of foods on the oral microflora.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00243 02 CPR

PERIOD COVERED: October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Growth Energetics and Interaction of Plaque Microorganisms .

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robrish, Stanley A.	Microbiologist	NCP	CPR	NIDR
OTHER:	Kemp, Christopher W.	Microbiologist	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Eberlein, Doreen	Physical Science Asst.	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH: Caries Prevention and Research

SECTION: Etiology

INSTITUTE AND LOCATION: NCP, NIDR, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.67	.27	.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pure cultures of bacteria were prepared under highly controlled conditions to be used as antigens for a variety of immunologic experiments. Antigens are to be prepared to be used for antiserum preparation to quantitate Streptococcus mutans and Streptococcus sanguis in mixed culture. Experiments have continued on the growth properties of S. mutans and S. sanguis in batch and continuous modes.

PHS-6040

1. Project Description

Objective:

The objectives of this project are: (a) to obtain pure cultures of bacteria grown under highly controlled conditions to be used as antigens for a variety of immunologic experiments and (b) to prepare antisera based on these antigens to quantitate Streptococcus mutans and Streptococcus sanguis in mixed culture.

Methods:

Bacteria grown under controlled conditions for experiments testing the immune response of S. mutans have been supplied to the Branch. It is important for these studies that the cells be grown under highly defined conditions so that the antigenic content of the cells may be reproduced. The pH of the medium and g-s atmosphere are controlled. The growth medium for the cells is made free of high molecular weight components which may behave as antigens. In addition to usual growth assays the turbidity of the culture is monitored continuously using fiber optic probe. Cells from a large batch of a strain of S. mutans grown under these conditions have been used as antigenic material to stimulate secretory immunoglobulin in human volunteers. Organisms prepared similarly also have been supplied to Dr. Cole for in vitro immunological experiments.

The growth of S. mutans and S. sanguis in mixed culture also is being studied. The organisms are being grown under energy limitation at several growth rates in a continuous culture apparatus. The experiments which are planned are to grow each of the organisms separately and together in limiting energy conditions to determine if there is any competition between the organisms for the same substrate.

Findings:

The production of immunoglobulin and response to homologous test organisms now are being studied in human volunteers.

Data from the mixed culture experiments will be fully evaluated when the analyses for residual glucose and formation of lactic acid and other volatile fatty acids is completed.

Proposed Course.

In order to analyze the ratio of the two organisms while grown with limited sources of energy, antisera are being prepared against each.

These antisera will be conjugated with fluorescein isothiocyanate and used to quantitate each of the organisms in the mixed culture.

Significance:

Preparation of these antigenic materials will allow initial testing of the possibility of immunizing per os against caries.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00244 03 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The Evaluation of a Medium for the Isolation of Actinomyces from Dental Plaque

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Little, Wayne A.	Microbiologist	NCP	CPR	NIDR
OTHER:	Thomson, Lynn A.	Dental Surgeon	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.12	.02	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

CNAC-20 agar was evaluated as a differential medium for strains of Actinomyces viscosus and Actinomyces naeslundii. Human and monkey plaque specimens, in addition to stock strains of actinomyces and other oral bacteria were cultured on CNAC-20 agar. Plaque isolates were made from colonies judged to be actinomyces or non-actinomyces on the basis of morphology. Verification of these isolates was made with fluorescein labeled antisera specific for A. viscosus and A. naeslundii.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The objective of the project is to develop simple, reliable procedures for identifying A. viscosus and A. naeslundii in human plaque samples. Use of CNAC-20 agar will be evaluated as a selective growth medium for these species.

Methods:

Human and monkey plaque specimens, in addition to stock strains of Actinomyces and other oral bacteria, were plated on CNAC-20 and incubated 48 hours anaerobically followed by 48 hours in 90% air, 10% CO_2. Verification of plaque isolates was made with fluorescein labeled antisera specific for A. viscosus and A. naeslundii.

Major Findings

Strains of Rothis dentocariosus, Bacterionema matruchotii, and Propionibacterium avidum produced colonies similar in morphology to A. viscosus and A. naeslundii colonies. In addition one A. naeslundii strain produced an atypical colony.

All of the human plaque isolates which were identified as A. viscosus or A. naeslundii on the basis of colonial morphology were also positive by the fluorescent antibody technique (FA). Twenty percent of atypical (non-Actinomyces) isolates gave positive FA staining for A. viscosus or A. naeslundii. All of the 'atypical' Actinomyces colonies were of a similar morphology and readily detectable.

None of the atypical isolates from monkey plaque gave positive FA staining for A. viscosus or A. naeslundii. In addition, only 54% of the typical Actinomyces isolates were positive by FA. It is likely that some of these isolates belong to additional serotypes of A. viscosus and A. naeslundii which are not stained with available antisera.

CNAC-20 agar can prove useful as a differential medium for A. viscosus and A. naeslundii in human plaque as long as its limitations are recognized. The occurrence of other genera which give rise to Actinomyces-like colonies, necessitates the use of FA or some rapid backup method for positive identification. The experience gained from this study provides a tool for NCP investigators to use in the detection of A. viscosus and A. naeslundii in human dental plaque.

Significance:

Little is known about the ecology of A. viscosus and A. naeslundii and their relationship to human dental diseases. Part of the problem is that the identification of Actinomyces is difficult and does not lend itself to processing numerous samples from clinical studies. Use of CNAC-20 agar described by Ellen et al. has potential for greatly facilitating the identification of this species.

Proposed Course:

CNAC-20 will be used to determine levels of A. viscosus and A. naeslundii in plaque samples.

Plaque isolates from CNAC-20 will continue to be monitored to determine if additional genera give rise ot Actinomyces-like colonies and to determine if there are other atypical morphological types of A. viscosus or A. naeslundii.

A comparative media study will be carried out with CNAC-20 and selective media developed by Loesche et al. and Beighton et al.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00256-01

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
The effect on dental caries of varying periods of school water fluoridation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Heifetz, Stanley B.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Horowitz, Herschel S.	Chief, CPS	NCP	CPR	NIDR
	Meyers, Rhea	Clinical Investigator	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Division of Dental Health, Department of Health, Commonwealth of Virginia

LAB/BRANCH
Caries Prevention and Research

SECTION
Community Programs

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.24	.24	--

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluoride will be added to the water supplies of public schools in three Virginia counties to study the anti-caries effectiveness of fluoride ingestion: throught grades K-6 (Cumberland Co.); throughout grades K-8 (Matthews Co.); and, throughout grades K-12 (Amelia Co.). All school populations live in areas that have no central water supplies and where the drinking waters contain negligible concentrations of fluoride. In May, 1979, baseline dental examinations (DMFS Index) were made of a total of approximately 4,600 students in grades K-12 in the three counties. In September 1979, schools containing the required grade levels for study fluoridated at a concentration of 4.5 times that recommended for community fluoridation in the geographic area. Follow-up examinations will be conducted after 3,7,9 and 13 years of study as increasingly more subjects become continuously exposed to fluoridated water at school for the desired periods of time.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective:

Previous studies have shown that children who consume fluoridated water in school throughout grades 1-12 have a 40 percent reduced prevalence of dental caries. The present study will determine if similar benefits can be obtained by exposure for shorter periods of time, i.e., only during elementary school (grades K-6), elementary and junior high schools (grades K-8), and elementary, junior and senior high schools (grades K-12) in the Virginia counties of Cumberland, Matthews and Amelia, respectively. A concentration of 4.5 times the optimum recommended for community water fluoridation in the geographic area will be maintained in the schools' water systems. Prior to the installation of fluoridation equipment, baseline dental examiniations using the DMF surface index were made of all school children (grades K-12), in each of the three counties--a total of approximately 4,600 students. Three Public Health Service investigators and two public health dentists on the staff of Virginia's Division of Dental Health comprised the examination team. Results of follow-up examinations after 3, 7, 9 and 13 years of study will be compared with baseline findings to determine the benefits received from varying periods of consumption of school water fluoridation. Fluoride concentrations will be monitored by school personnel under the supervision of the Virginia Department of Dental Health.

Findings:

Baseline findings are currently being analyzed to determine the comparability of dental caries prevalence among children in the three selected counties.

Significance:

Currently, it is recommended that school fluoridation programs be conducted throughout grades K-12. If it were necessary to fluoridate only elementary schools (grades K-6) or even only through junior high schools (grades K-8) to obtain decay preventive benefits that approximate those conferred by exposure through senior high school (grades K-12), then the cost of implementing a nation-wide school water fluoridation program would be considerably reduced.

Proposed Course:

School fluoridation units will be installed in 7 study schools located in the three Virginia counties. Fluoride at a concentration of 4.5 "times the optimum" will be maintained at the study schools until the final examinations in 1992. A comparison of results of interim and

final examinations among the three county school populations will indicate if it is necessary to expose children to fluoridated water at school throughout grades K-12 in order to produce maximum caries inhibitaion or if near maximum benefits can be obtained by exposure throughout only grades K-6 or K-8.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00258 01 CPR

PERIOD COVERED
October ., 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Passive immunity against dental caries in the rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Amsbaugh, Suzanne M.	Biologist	NCP	CPR	NIDR
	Monell-Torrens, Esteban	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.61	.21	.40

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antiserum against formalin killed whole cells of Streptococcus mutans was raised in rabbits. The antiserum was added to the drinking water of one group of weanling conventional Osborne Mendel rats. Their controls received drinking water containing non-immune rabbit serum. Five days after the initiation of passive immunization all animals were infected with the homologous S. mutans strain. After 8 weeks the experiment was terminated, the teeth scored for caries and the numbers of S. mutans determined. There was a significant reduction in the number of S. mutans in the immunized group, however, no significant difference was detected in the level of caries. This probably resulted from the high level of non-S. mutans background caries activity seen in these animals.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

If protection against dental caries is mediated by salivary antibody or antibody derived from crevicular fluid, it should be possible to elicit protection by passive immunization. The purpose of this study was to determine whether passive immunization with rabbit antisera to Streptococcus mutans could protect conventional rats against dental caries.

Method:

High titer antiserum to formalin killed S. mutans 6715-15 was raised in rabbits. The pooled antiserum was titered by whole cell agglutination. Formalin killed cells were employed so as to avoid the induction of antibodies to glucosyltransferase (GTF). This allowed the efficacy of anti-GTF antibodies to be tested in a separate experiment. The antiserum was added to the drinking water of one group of 12 conventional weanling Osborne Mendel rats so as to give a final titer of Log_2 2 (1:4). The control groups received non-immune rabbit serum at the same dilution or plain drinking water. Five days after the antiserum was placed in the drinking water all animals were infected with S. mutans 6715-15 and fed diet NIH 2000. The experiment was terminated after 8 weeks and the level of caries and colonization with S. mutans evaluated.

Major Findings:

Significantly fewer S. mutans were recovered from the teeth of the animals provided antiserum in the drinking water than from the teeth of animals given the non-immune rabbit serum or the controls. Lower numbers of S. mutans were recovered from the teeth of animals receiving the non-immune rabbit serum than the controls.

A significant reduction in dental caries was not detected in the passively immunized group. The failure to effect a reduction in dental caries despite a significant reduction in the numbers of S. mutans is probably attributable to the high non-S. mutans caries activity seen in these conventional rats.

Significance:

Studies of this type aid in understanding the role of local antibody in the mouth. The inclusion of antibody in cows milk may be an effective means of preventing dental caries in humans.

Proposed Course:

Repeat the study in gnotobiotic rats.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00262 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Study of an intraoral device designed for providing sustained low levels of fluoride

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Shern, Roald J.	Clinical Investigator	NCP	CPR	NIDR
OTHER:	Mirth, Dale B.	Staff Fellow	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Emilson, Claes G.	Visiting Scientist	NCP	CPR	NIDR
	Kingman, Albert	Statistician (Health)	NCP	CPR	NIDR
	Kline, Linda A.	Chemist	NCP	CPR	NIDR
	Adderly, Donna D.	Biologist	NCP	CPR	NIDR
	Li, Shou-Hua	Statistician (Health)	NCP	CPR	NIDR

COOPERATING UNITS (if any)
Southern Research Institute (SRI), Birmingham, Alabama Dr. D.R. Cowsar
Hazleton Laboratories, Oral Research section, Vienna, Virginia, Dr. D. Dalgard

LAB/BRANCH
Caries Prevention and Research

SECTION
Preventive Methods Development

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.53	.72

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation supplements earlier studies done by SRI in collaboration with Dr. Bowen and Dr. Mirth to develop a fluoride releasing device for intraoral use. The safety and pharmacokinetics of the device is being evaluated preliminary to possible clinical studies of its anticaries benefits. Preliminary studies in the subhuman primate suggest that the appliance will adhere to the teeth for releasing fluoride at the appropriate rate.

PHS-6040

1. Project Description

Objectives:

The long-range objectives of this study include providing maximum anticaries benefits with minimum fluoride dosages. The current subhuman primate and clinical investigations are designed to measure the pharmacokinetics of fluoride in order to verify the performance and safety of the fluoride release devices.

Methods:

Fluoride levels were measured in the saliva, plaque, blood and urine of the subhuman primate before and after attachment of a fluoride releasing device. Published methods of direct measurement were used for estimating fluoride levels in urine, saliva and blood. It was necessary to develop a microdiffusion method for measuring the fluoride levels in the plaque. The evaluation and development of analytic procedures was augmented by project No. Z01-DE-00112-06.

Major Findings:

Fluoride levels in most of the biologic samples followed the expected time course. Fluoride was at increased levels while the device was in place and returned to pretest levels when the device was removed.

Pretest evaluation showed that the microdiffusion method was able to detect tightly bound fluoride and complexed fluoride. Fluoride was readily measured from submilligram samples of dental plaque. Extremely high levels of ions including Al^{+++} and Fe^{++} failed to interfere with the accuracy of the fluoride analysis.

Significance:

This controlled release delivery system appears to provide a more effective and efficient way to deliver fluoride because it provides a continuous therapeutic level of F^- to the caries site for long periods of time.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-DE-00263-01-CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Cariogenicity of the Different Serotypes of S. mutans in gnotobiotic rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Little, Wayne A.	Microbiologist	NCP	CPR	NIDR
OTHER:	Thomson, Lynn A.	Sr. Dental Surgeon	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR

COOPERATING UNITS (if any)

NIH Germfree Section

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.12	.02	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of experiments is underway with gnotobiotic rats to determine the relative cariogenicity of the different serotypes of S. mutans.

PHS-6040
(Rev. 10-76)

1. Project Description:

Objective:

Results of studies on the prevalence of S. mutans in human populations suggest that serotype c is most prevalent and serotypes a and b are essentially absent. Serotypes d, e, f, and g are usually present but to varying degrees of prevalence.

Evidence on the relative cariogenicity of the different S. mutans serotypes is sparse. A human population study by Keene et al. demonstrated a correlation between high caries scores and the presence of biotype e in plaque. Using antibiotic suppressed SPF Sprague-Dawley rats, Hameda et al. observed the highest caries scores with serotype d strains. They also observed significantly reduced caries activity in serotype b reference strains compared to caries reports on the original isolates. Our long term goal is to examine fresh human plaque isolates of serotypes c, d/g, e, and f and to see if differences in cariogenicity are related to serotype or merely variations in the strains.

The second objective of this experiment involves comparing the caries activity of S. mutans isolates from Don Matias and Heliconia, Columbia, S.A. These two communities have been observed to have substantially different caries levels; furthermore, in vitro tests have demonstrated that Don Matias plaque is significantly more acidogenic than plaque from Heliconia. However, there appear to be little or no differences in the prevalence of S. mutans or the distribution of serotypes.

We propose to include strains from these communities in this series of experiments to see if S. mutans isolates from Don Matias plaque are more cariogenic than Heliconia isolates.

Methods:

Two isolators at a time are used; each containing twelve Osborne-Mendel rats (18 days old). Animals are provided with fluoride-free deionized water and diet 2000 vs. The breeder animals are maintained on diet MIT 200 and fluoride-free deionized water. Animals are inoculated orally on two consecutive days with 50 µl of broth culture containing approximately 10^8 CFU. Cultures will be grown for 24 hours in dialized Todd Hewitt broth and adjusted to proper cell density. Experiments will be of 8 weeks duration so that less cariogenic strains will have sufficient time to develop detectable caries levels.

Major Findings:

In the first experiment, serotype c and d strains are being compared.

Significance to Biomedical Research:

The results of this study could have potential significance in the selection of strains for whole cell vaccines to be used in immunization studies. Furthermore, insight may be gained into a possible relationship between DMFT scores and S. mutans serotype prevalence in human populations.

Proposed Course:

A number of strains will be examined to determine if there are differences in cariogenicity among the serotypes of S. mutans. In addition, strains from two communities in Columbia, S.A. will be examined for differences in cariogenicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00264 01 CPR

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

Fluorescent Antibody Methods to Determine the Prevalence of Certain Plaque Bacteria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomson, Lynn A., Jr.	Dental Surgeon	NCP CPR NIDR
OTHER:	Little, Wayne A.	Microbiologist	NCP CRP NIDR

COOPERATING UNITS (if any)

Center for Disease Control, Atlanta, Georgia
Roger McKinney, William Harrell and Sandra Bragg

LAB/BRANCH

Caries Prevention and Research

SECTION

Etiology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.51	.31	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several laboratories are collaborating in developing, evaluating, and improving "reagent grade" fluorescent antibody (FA) conjugates for specific dental plaque bacteria considered important in caries etiology.

These conjugates are being used to determine the prevalence of certain organisms in plaque samples from various locations.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives

The objective of the project is to develop methods and materials permitting the detection and enumeration of specific dental plaque bacteria directly in clinical specimens. The objectives include the FA identification of procedures which can be recommended as sensitive, specific and reliable for use in determining the prevalence of selected plaque bacteria.

Methods

Efforts to improve FA procedures for examining dental plaque have involved: (a) comparing effects of light sources on the intensity of specimen fluorescence, (b) adapting and refining bacterial-immuno-absorbent columns to improve the specificity of conjugates, (c) modifying immunization schedules to obtain higher-titered antisera, (d) improving of microscope slide design to conserve antisera, and (e) reformulating FA mounting medium and conjugate diluent.

Major Finding:

The improved specificity of FA conjugates achieved with the bacterial-immunosorbent columns recommended by Dr. Roger McKinney, has permitted direct FA examination of Streptococcus mutans in plaque smears. The Biological Reagents Section of the Center for Disease Control has followed production protocols prepared during this project and prepared "Reagent Grade Conjugates" in quantities which have permitted major population studies.

Significance

The development of methods and FA conjugates which permit the detection and enumeration of certain oral bacteria in direct plaque smears avoids the difficulties associated with conventional cultural methods. The extensive manipulation of specimens for cultural study and the inhibitory effect of selective media are not required in the examination of plaque samples with FA methods. These improvements in monitoring plaque organisms will permit the study of specific bacteria with the increased sensitivity and specificity associated with FA methods.

2. Publications

Thomson, L.A. and Hageage, G.J. Evaluation of Excitation Light Sources for Incident Immunofluorescence Microscopy. Applied Microbiology, Vol. 30, No. 4., p. 616-624, October 1975.

Thomson, L.A., Hageage, G.J., and Little, W. Application of Fluorescent Antibody Methods in the Analysis of Dental Plaque Samples. J. Dent. Res. 55 (Special Issue), A80-A86, 1976.

Thomson, L.A. Preparation of Antiserum for Use in the Fluorescent Antibody Identification of Certain Plaque Bacteria. J. Dent. Res., 55 (Special Issue A), A28-A32, 1976.

Wong, M.C., McKinney, R.M. and Thomson, L.A. Soluble Antigen Extract Used as Blocking Agents to Obtain Specificity in Serotyping of Streptococcus mutans. J. of Immun. Methods, In press, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00266-01 CPR

PERIOD COVERED
October 1, 1978 to September 1, 1979

TITLE OF PROJECT (80 characters or less)

Comparison of the retention of two bis-GMA pit and fissure sealants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Li, Shou-Hua	Visiting Associate	NCP CPR NIDR
OTHER:	Swango, Philip A.	Clinical Investigator	NCP CGC NIDR
	Heifetz, Stanley B.	Clinical Investigator	NCP CPR NIDR

COOPERATING UNITS (if any)

Fairfax County Health Dept., Fairfax, Va.

LAB/BRANCH
Caries Prevention and Research

SECTION
Biometry

INSTITUTE AND LOCATION
NIH, NIDR, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.35	.25	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The retention of bis-GMA pit and fissure sealants polymerized chemically (Delton) or by ultraviolet light (Nuva-Seal) is being compared. Different analytic methods for categorical data will be used to determine the effect of different treatments, tooth types and length of time on percent retention.

PHS-6040

1. Project Description

Objective:

During the last ten years, a number of clinical trials have demonstrated the effectiveness of pit and fissure sealants. The majority of these studies has evaluated a bis-GMA system (Nuva-Seal) which uses ultra-violet light to induce polymerization. Recently a bis-GMA system (Delton) that employs a chemical catalyst to polymerize the sealant has been developed. Initial reports of studies sponsored by the manufacturer indicate high levels of complete retention: 94% after one year and 90% after 2 1/2 years. These impressive results need to be evaluated through side-by-side clinical comparison of the two systems.

Methods:

From September, 1976-March, 1977, 200 patients aged 6-16 years who visited the Fairfax County Health Department and were eligible to receive care were invited to participate in the study. Those found to have one or more pairs of homologous permanent posteria teeth free of decay or fillings on their occlusal surfaces and free of decay on all other surfaces were included in the study. One half the mouth of each participant was treated with Nuva-Seal and the other half was treated with Delton. In addition sound permanent posteria teeth that had a decayed or filled homologue were treated with the adhesive designated for use on that half of the mouth. Treated teeth were examined for sealant loss approximately every six months for a period of two years. On the 6-, 12-, 18-month examinations, teeth diagnosed as having lost any or all of the sealant were retreated. All sealant treatments were administered by a dental hygienist.

Findings:

The two year study was completed in March, 1979, and findings are currently being analyzed. Different analytic methods for categorical data are being used to determine the effect of different treatment, type of teeth, and length of time on percent retention.

Significance:

Although studies have shown that either of the bis-GMA sealants can have good levels of retention, there is insufficient evidence to determine if one is clearly better than the other. Information on the comparative benefits of the two sealants will be helpful to dentists as well as in estimating acceptance of these techniques in public health use.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00267 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)

Antibody response in humans to Strep. mutans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
OTHER:	Cole, Michael F.	Visiting Scientist	NCP	CPR	NIDR
	Emilson, Claes-Goran	Visiting Scientist	NCP	CPR	NIDR
	Ciardi, Joseph E.	Chemist	NCP	CPR	NIDR
	Robrish, Stanley A.	Microbiologist	NCP	CPR	NIDR
	Stiles, Horace M.	Microbiologist	NCP	CPR	NIDR
	Shern, Roald J.	Dental Surgeon	NCP	CPR	NIDR
	Mirth, Dale B.	Staff Fellow	NCP	CPR	NIDR
	Thomson, Lynn A.	Dental Surgeon	NCP	CPR	NIDR
	Gomez, Irma M.	Microbiologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.41	.11	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was carried out to determine whether humans would develop serum and salivary antibodies after swallowing large doses of Strep. mutans in enteric coated capsules. It was observed that it was easier to infect those humans who harbored Strep. mutans than those who were mutans-free. Antibodies were found in saliva and serum following vaccination per os.

1. Project Description

Objective:

The purpose of this study is to determine whether antibodies could be induced in humans following oral ingestion of encapsulated Strep. mutans and to determine whether implanted strains of Strep. mutans could be cleared rapidly from the mouth following vaccination.

Methods:

Ten human volunteers were screened repeatedly over several weeks for the presence of Strep. mutans in their mouth, antibodies (including immunoglobulin class) to Strep mutans in parotid and whole saliva and serum. Volunteers rinsed their mouth with streptomycin resistant mutant of Strep. mutans. Saliva and plaque samples were taken over several months to monitor its clearance. Nine subjects swallowed S. mutans encapsulated in enteric coated capsules over a 3-day period. Antibody response was noted using indirect FA techniques. Each subject was again challenged with streptomycin resistant mutants of Strep. mutans and their clearance monitored.

Major Findings:

Strep. mutans became established more readily in those who naturally harbored high levels of Strep. mutans than in those who were mutans-free. Several months elapsed before Strep. mutans was eliminated from most subjects. Antibodies were detected in saliva and serum from most subjects who were vaccinated and Strep. mutans disappeared rapidly from subjects who were vaccinated.

Significance:

These results show that it is possible to induce antibodies in humans by means of oral vaccination.

Proposed Course:

The study is being repeated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00268 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of eating patterns on dental caries in rodents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar, William M.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Amsbaugh, Suzanne M.	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human studies have indicated the importance of food sequence in pH changes in dental plaque in situ. This concept has been extended by feeding rats according to a programmed sequence of foods, interspersing foods of low potential cariogenicity (peanuts, cheese) between exposures to cariogenic diet. With cheese (but not peanuts) caries and Strep. mutans counts were reduced compared with controls fed cariogenic diet alone, in partial agreement with the human studies.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The objective of the project is to obtain information on the effect of food sequence on caries development in rats and to correlate this with previous observations in man.

Methods:

Four groups of 10 OM rats received the following programmed sequence of foods:

A. Diet 2000, 22x/day
B. Diet 2000, 34x/day
C. Diet 2000, 22x/day + cheese 12x/day
D. Diet 2000, 22x/day + peanuts 12x/day

All animals were inoculated per os and in the drinking water at the start of the experiment with S. mutans 6715/15. After 35 days, the animals were anaesthetized, pilocarpine-stimulated saliva collected, and submandibular salivary glands dissected out and weighed. After sacrifice, S. mutans counts and caries were assessed by conventional methods.

Major Findings:

Smooth surface caries was increased by increased frequency of exposure to Diet 2000 and by peanuts; and was abolished by introduction of cheese into the feeding sequence. S. mutans counts were depressed by cheese. Gland function and size varied between groups, being greatest with cheese.

Significance:

Besides frequency of sugar consumption, the presence of other foods eaten in sequence is shown to be an important factor in smooth surface caries.

Proposed Course:

Scoring of sulcal caries will complete this study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00269 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of calcium glycerophosphate and fluoride on dental caries in rodents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Edgar, William M.	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Amsbaugh, Suzanne M.	Biologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.12	.07	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous studies showing synergism between the effects of fluoride and calcium glycerophosphate in reducing dental caries in rats have been reinvestigated. Suboptimal levels of fluoride and calcium glycerophosphate gave a greater reduction in caries in combination than separately. Further statistical analysis will reveal whether or not this interaction is significant.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives:

The project is being carried out to reinvestigate the claim that calcium glycerophosphate (CaGP) potentiates the effect of fluoride in reducing dental caries.

Methods:

Four groups of 18 OM rats received:

A. Diet 2000 + deionized water
B. Diet 2000 + 2.5 ppm fluoride solution
C. Diet 2000 + 1% CaGP + deionized water
D. Diet 2000 + 1% CaGP + 2.5 ppm fluoride solution

Animals were inoculated with S. mutans 6715/15 at the start of the experiment. Diet and drinking water were available ad libitum. At sacrifice after 35 days, caries scores and S. mutans counts were carried out using conventional methods.

Major Findings:

2.5 ppm F in the drinking water reduced smooth surface caries, but CaGP had no effect by itself. Together, however, the two agents reduced smooth surface caries more effectively than NaF alone. S. mutans counts were not significantly affected.

Significance:

The possibility of increasing the effectiveness of fluoride (systemically or topically) as an anticaries agent suggests substantial possible benefit in human applications.

Proposed Course:

Scoring of sulcal caries and further statistical analysis is required to substantiate this effect.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00270 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Microbial composition of dental plaque from irradiated primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Emilson, Claes-Göran	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Edgar, William M.	Visiting Scientist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.22	.17	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Plaque samples from four irradiated monkeys and from four controls were analysed by using fluorescent antibody (FA) reagents specific for S. mutans, S. sanguis, S. salivarius, lactobacilli, Actinomyces and B. melaninogenicus. The irradiated animals showed a higher proportion of S. mutans and a lower proportion of S. sanguis when compared with that in the controls.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The purpose of this study was to determine the proportion of some of the major bacterial groups of the plaque flora in irradiated monkeys.

Methods:

Supragingival plaque was collected and pooled from the buccal surfaces of premolars in two opposite quadrants in 4 irradiated monkeys (*Macaca mulatta*) and in 4 monkeys (*Macaca fascicularis*) serving as controls. The samples were diluted and plated on blood and MSB agar for enumeration of the proportion of *S. mutans* and then subjected to FA-examination using serotype and species specific conjugates kindly provided by Dr. Thomson.

Major Findings:

S. mutans constituted a higher proportion of the total count in irradiated animals than in the controls. In all but one control the animals harboured one or two serotypes of *S. mutans* belonging to the groups *c*, *d/g*, and *e*. The population of *S. sanguis* was lower in the irradiated animals than in the controls. No major differences were seen in the proportion of *S. salivarius*, *Actinomyces*, *Lactobacilli*, and *B. melaninogenicus*.

Significance:

The results show that irradiated animals with a low saliva secretion have a plaque flora with a high level of *S. mutans* and a low level of *S. sanguis*. This observation is of interest as plaque samples from caries-active teeth in humans often contain high levels of *S. mutans* and low levels of *S. sanguis*.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00271 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of interaction between amine derivatives and chlorhexidine on rat caries

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Emilson, Claes-Göran	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Ciardi, Joseph E.	Chemist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.30	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study was designed to compare the effect on caries activity in rats by topical application of chlorhexidine with different concentrations and frequencies with and without the addition of the basic amino acid lysine. Plaque score, the proportion of S. mutans of total count in plaque and caries decreased with increasing concentration and frequency of chlorhexidine application. The addition of lysine did not enhance the effect.

PHS-6040
(Rev. 10-76)

1. Project Description

Objective:

The bisbiguanides are potent inhibitors of glucosyltransferase activity from S. mutans. This effect is enhanced in vitro by addition of basic amino acids. The purpose of this study was to examine if the potential of glucosyltransferase inhibition by amino derivatives could render the antimicrobial agent chlorhexidine more effective in preventing cariogenic plaque formation in rats.

Methods:

Seven groups of 12 rats were infected with S. mutans 6715 and A. viscosus T6. The animals were fed diet 2000 and deionized water ad libitum. Topical application was performed 5 days a week with the following solutions: 0.2% chlorhexidine with and without the addition of Lysine once or twice a day and 1% chlorhexidine once a day. Deionized water and Lysine served as controls. After 5 weeks the animals were sacrificed and plaque and caries score and the proportion of S. mutans of the total count in plaque was determined.

Major Findings:

With increasing frequency of applications and increasing concentration of chlorhexidine both plaque and caries score were reduced. This effect was paralleled by a reduction in total bacterial count and in the proportion of S. mutans. The addition of Lysine did not enhance the effect of chlorhexidine on these parameters.

Significance:

The results confirm earlier observations that chlorhexidine is a potent antimicrobial agent against S. mutans pathogenic plaque. It was not possible to use a lower concentration of chlorhexidine thereby reducing some of its observed side effects by the addition of Lysine and still achieve the same caries-reducing effect.

Proposed Course:

The interaction between other amine derivatives and cetylpyridinium on rat caries is now being examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE 00272 01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Effect of topical applications of antimicrobial agents on the plaque flora in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Emilson, Claes-Göran	Visiting Scientist	NCP	CPR	NIDR
OTHER:	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	Robrish, Stanley A.	Microbiologist	NCP	CPR	NIDR
	Kemp, Christopher W.	Microbiologist	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention and Research

SECTION
Etiology

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.28	.18	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Four unknown compounds supplied by manufacturers were tested in primates in the search for promising antimicrobial agents to prevent dental plaque formation and caries activity. Changes in the population of dental plaque organisms were examined by using Fluorescent Antibody (FA) reagents specific for S. mutans, S. sanguis and four species of Actinomyces. An increase in the proportion of S. sanguis and a reduction of Actinomyces was observed with decreasing plaque scores. For two of the agents this effect was equal to or more pronounced than that obtained with chlorhexidine which served as a control.

1. Project Description

Objective:

The purpose of this study was to evaluate the effect of four antiseptics on the plaque flora in primates. The agents were applied sequentially over a period of six months using chlorhexidine as a positive control.

Methods:

Eight monkeys of the species Macaca fascicularis received a high sucrose diet and drinking water containing 4% sucrose. The animals were treated with the test substances once a day for 7 days by topical application to the buccal tooth surfaces. Water and chlorhexidine served as controls. Plaque was scored once a week and samples were collected from the buccal tooth surfaces of the two premolars and first molar in two opposite quadrants. The plaque was transferred to vials containing 2 ml transport fluid and kept on ice until processing. After plating for viable count the samples were prepared for FA examination using reagent grade conjugates specific for the serotypes of S. mutans and for S. sanguis and four species of Actinomyces. The antisera were kindly provided by Dr. Thomson.

Major Findings:

Two of the compounds showed an equal or better plaque reducing effect than that obtained with chlorhexidine. The reduction in plaque score was accompanied by an increase in the proportion of Actinomyces species; especially A. viscosus and A. naeslundii. The baseline composition of the plaque flora was not changed by treatment with deionized water. No S. mutans was detected during the experimental period in any of the animals.

Significance:

These results show that two of the compounds submitted for testing had antimicrobial properties equal to or better than that obtained for chlorhexidine. These agents should, therefore, be further evaluated as promising compounds for possible use in humans. A significant reduction in the plaque proportion of Actinomyces was of special interest because Actinomyces have been correlated with the onset of gingivitis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00274-01 CPR

PERIOD COVERED
October 1, 1978 to September 30, 1979

TITLE OF PROJECT (80 characters or less)
Role of host saliva on implantation of S. mutans in human dental plaque.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ciardi, Joseph E.	Research Chemist	NCP	CPR	NIDR
OTHER:	Emilson, Claes-Goran	Visiting Scientist	NCP	CPR	NIDR
	Bowen, William H.	Chief, CPR	NCP	CPR	NIDR
	McAllister, Paul F.	COSTEP (Dentistry)	NCP	CPR	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH
Caries Prevention & Research

SECTION
Etiology

INSTITUTE AND LOCATION
National Institute of Dental Research, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.09	.07	.02

CHECK APPROPRIATE BOX(ES)

X (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability of exogenous S. mutans to implant and be retained in dental plaque varies considerably among human subjects. Because of differences in susceptibility to implantation by S. mutans, test subjects were divided into three groups: 1) Highly susceptible; 2) Moderately susceptible; and 3) Not susceptible. The purpose of the present investigation is to determine if a significant correlation exists between host saliva and S. mutans implantation by initially assessing the effects of these salivas on bacterial growth, acid production, aggregation, and ability to adhere to surfaces - all activities involved in the caries process. Results will be compared with respect to the three test groups.

PHS-6040
(Rev. 10-76)

1. Project Description

Objectives

The ability of exogenous S. mutans to implant and be retained in dental plaque varies considerably among human subjects. The present study proposes to determine if a significant correlation exists between host saliva and the extent of S. mutans implantation.

Methods

Because of differences in susceptibility to implantation by S. mutans strains Ingbritt (serotype c) and OMZ65 Serotype d/g), test subjects were divided into three groups: 1) Highly susceptible; 2) Moderately susceptible; and 3) Not susceptible. The groups contained 3, 2, and 3 subjects, respectively.

Saliva is collected over ice and stored frozen. Just before use it is thawed, clarified by centrifugation at 12,000xg, and stored over ice. Its pH is measured and in some experiments the pH is adjusted. Protein content of clarified saliva is estimated by measuring absorbance at 280nm with bovine serum albumin as a standard.

The effect of saliva is measured on the following activities:

1. adsorption of radioactive S. mutans cells to hydroxyapatite.

2. the rate of aggregation of S. mutans cells by changes in absorbance at 700nm.

3. growth, acid production and sucrose-mediated adherence of S. mutans. A rapid, quantitative in vitro assay has been developed in our laboratory that measures the effects of chemical and biological agents on growth and sucrose-mediated adherence of radioactive S. mutans. Acid production is measured in the same assay, by change in pH. (Ciardi, J.E., Rosenthal, A.B. and Bowen, W.H. J. Dent. Res. 58:IADR Abs. 630, 1979).

Major Findings

All assay procedures have been found to give reproducible results in tests with individual human saliva. Preliminary results show that all saliva tested depress the adsorption of radioactive Strep. mutans cells to hydroxyapatite and that the extent of inhibition varies among salivas.

Significance

A more thorough understanding of the influence of host saliva, or specific factors in host saliva, on the ability of exogenous cariogenic

bacteria to colonize dental plaque could lead to the discovery of more effective means to prevent caries.

Proposed Course

Initially, the effect of host saliva on bacterial growth, acid production, aggregation and ability to adhere to surfaces will be assessed. The results of these studies will determine the design of future *in vitro* and *in vivo* experiments.

Section III

CONTRACT ACTIVITIES

The following reports describe NCP contracted projects active during FY 1979 that are not collaborative extensions of specific direct operations. The Biometry Section computes data and provides statistical services for many of these projects.

Contract (no intramural research component)	NO1-DE- 42434
Period Covered: FY 79	Contract Period: 5/1/74 - 6/30/79

Title of Project: Experiments in Anti-Caries Immunizations in Sub-Human Primates

Contractor: Hazleton Labs., Vienna, Virginia
Principal Investigator(s): Dan Dalgard
Activity Site: Vienna, Virginia

P.O.: William Bowen
Organizational Position: NIDR, NCP, CP&R Branch

Relevance to NCP Objectives: Moderate protection against animal caries has been achieved by stimulating antibodies against streptococcal glucosyl transferase. There are reasons to believe that other bacterial fractions might be even more potent antigens and that the protective effect of salivary immunoglobulins may be less bacterial-specific than first believed. Both possibilities would greatly enhance the feasibility of this approach to caries prevention and should be tested. The primate is the model of choice for such experiments since its oral microflora, dentition and immune response is very similar to humans and caries can be readily induced by "human-type" high sucrose diet.

Activity: The contractor is providing personnel, facilities and materials necessary to NIDR staff to conduct experiments in anti-caries immunization with monkeys. This includes initial provision of 50 disease-free Macaca fascicularis, (the colony size later was increased), appropriate housing for the animals and facilities and technical assistance to NIDR to enable examination and vaccination of and collection of biological samples from the monkeys. In addition, the contractor, at intervals during the project, measures the incidence of caries in the animals and specified blood, saliva, and plaque constituants.

Results: The monkeys have developed caries as planned and otherwise remain in good health. Groups of animals have been vaccinated with potentially important immunogens at several anatomic sites to study host secretory immune responses. Animals vaccinated intraductally into salivary glands have more caries than animals vaccinated intramucosally. Type II and IX pneumococcol polysaccharide failed to induce protection. Glucosyl transferase appears to hold promise as a suitable immunogen.

Contract (no intramural research component)	NO1-DE-42438
Period Covered: FY 79	Contract Period: 7/1/74-12/31/78

Title of Project: Effects of Local Immunization with Streptococcus Mutans Enzymatic Antigens on Experimentally Induced Dental Caries in Rodents

Contractor: Forsyth Dental Center, Boston
Principal Investigator(s): Martin Taubman
Activity Site: Boston

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Animal studies have established that immunization with cariogenic bacterial cells or extracellular enzymes gives rise to s-IgA that inhibits a cariogenic bacterial challenge. It is essential that the specific cell wall and extra-cellular antigens responsible for these responses be identified and evaluated for potency and that the antibodies be characterized and quantitated. The experiments should be carried out in a well-characterized dental caries model.

Activity: The contractor has prepared extracellular enzymes (primarily glucosyltransferase) of S. mutans, vaccinated rodents including thymectomized rats with these immunogens, studied host mechanisms of secretory immune response, measured levels and specificity of antibody developing in serum and saliva and determined function of the salivary antibody in caries resistance.

Results: Elevated levels of salivary sIgA antibody to the injected immunogens resulted in lowered levels of the challenge Streptococcus mutans and lower caries scores. In vitro studies and heterologous challenge experiments showed that some cross-protection was obtained between various S. mutans serotypes. Synthesis of all immunoglobulin classes was shown in the immunoglobulin-containing cells of the salivary glands and cervical lymph nodes. Neonatally thymectomized rats proved the dependence of sIgA antibody synthesis upon presence of the thymus, although this deficiency was partially compensated by increased synthesis of sIgM.

Contract (no intramural research component)	NO1-DE-42444 CT 0600107
Period Covered: FY 79	Contract Period: 6/28/74 - 7/15/79

Title of Project: Research Study and Clinical Field Trial Aimed at Determining the Effect of Specific Methods of Fluoride Administration in Controlling Dental Caries

Contractor: University of Oregon Dental School
Principal Investigator(s): Kuo Lu
Activity Site: Portland, Oregon

P.O.: Janet Brunelle
Organizational Position: NIDR, NCP, CP&R Branch

Relevance to NCP Objectives: Two main approaches are being employed to treat teeth topically with fluoride--ten consecutive daily applications of concentrated fluoride given once and a daily application of dilute fluoride for two years. The former causes a temporary though marked increase in fluoride concentration in the enamel surface, the latter does not increase the enamel fluoride measurably. It is important to establish which of these approaches is more effective and if the effects are additive.

Activity: A clinical trial is being carried out with 1200 children (initially 12 years old) to determine the effect of high-level short-term and low-level long-term methods of fluoride topical administration in controlling caries. The infrequent high-level treatment consists of a 5-minute topical application of APF (1.23%F) in a custom-fitted tray administered in school for 10 consecutive school days. The frequent low-level treatment consists of a 1-minute mouthrinse with NaF (0.023%F) administered in school on each school day for 2 years. The children are in 4 groups that receive one, both or neither of the treatments. Caries examinations are carried out at appropriate intervals and the results are submitted to NCP.

Results: After 24 months, each procedure was shown to be effective in inhibiting dental caries, with the gel treatment producing a more marked effect. The treatments together appear to be additive with the number of rinses received being a related factor. Measurement of caries a year after treatment stopped showed continued benefit in all treatment groups. However, the contractor feels that the rinse program in the upper grades is difficult to administer and poorly received by students.

Contract (no intramural research component)	NO1-DE- 42446
Period Covered: FY 79	Contract Period: 6/28/74-8/31/79

Title of Project: Development of a Fluoride Releasing System for Prolonged Oral Use

Contractor: Southern Research Institute
Principal Investigator(s): Donald Cowsar and Danny Lewis
Activity Site: Birmingham, Alabama

P.O.: Dale Mirth
Organizational Position: NIDR, NCP, PMD Section

Relevance to NCP Objectives: Fluoridation of drinking water continues to be the simplest and least expensive way to deliver fluoride for the prevention of caries. For areas in which a municipal water supply does not exist or in which there is opposition to municipal fluoridation, other vehicles (school water, tablets and salt) have been used to provide fluoride. It is believed that for these areas controlled release of fluoride from a repository within the mouth might be superior to school water, tablet and salt fluoridation since it would provide fluoride continuously, would not entail daily actions by the participant, and might prevent caries at lower levels of fluoride. Devices that continuously release controlled levels of fluoride are needed for testing the feasibility of this approach in animal models and in humans.

Activity: A biocompatible system for use in the oral environment is to be developed and tested which will release fluoride at a constant predetermined rate for at least six months. These devices are to be evaluated in dogs and other animals and an IND filed with the FDA in anticipation of human clinical testing.

Results: The contractor has developed and delivered devices made of biocompatible copolymers of hydroxyethyl methacrylate and methylmethacrylate. The devices release fluoride intraorally at a constant predetermined rate for several weeks. Acute and chronic toxicity tests have been completed in several species with no indication of adverse reactions. An IND has been prepared by the contractor and filed with the FDA by NCP. A short-term (Phase I-Phase II) clinical study will be initiated by the NCP in September 1979.

Contract (no intramural research component)	NO1-DE-52449 CT 0600094
Period Covered: FY 79	Contract Period: 4/1/75 - 6/30/80

Title of Project: Clinical Field Trial to Assess the Cost Effectiveness of Various Caries Preventive Agents

Contractor: University of Connecticut
Principal Investigator(s): Neville Doherty
Activity Site: University of Connecticut Health Center, Farmington, CT

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: The NCP has initiated several studies to determine the maximum caries-prevention effect which can be achieved with currently available methods and agents. These studies are designed to demonstrate the total effect of such procedures as topically applied fluoride and adhesive sealants when used in combination in school-aged children. Before specific community preventive programs can be recommended, however, it is important to assess the relative cost-effectiveness of each of these procedures, since it is possible that their combined use results in only minor increases in protective benefits compared to the use of a single agent or method.

Activity: A multi-group clinical trial is being carried out to determine the cost and clinical effectiveness of topical fluoride (semi-annual professional application of APF), pit and fissure sealant (bis-GMA) and fluoride mouthrinse (supervised weekly mouthrinse with dilute NaF) when used singly and in combination. There are approximately 200 children in each of the eight groups. Caries incidence is recorded on NIH forms and submitted to NCP Biometry Section for processing. The cost of each procedure and combination of procedures is computed and related to the degree of caries prevention that is achieved.

Results: Interim findings for dental caries have been reported but at this stage, differences between groups are small. Interim cost figures confirm the expected ranking of treatments by cost (sealants? topical gels? rinsing?). Cost effectiveness comparisons cannot be made until the final clincial and radiographic data have been analyzed. A final report should be available by June, 1980.

Contract (no intramural research component)	NO1-DE- 52456
Period Covered: FY 79	Contract Period: 6/20/75-10/19/80

Title of Project: Possible Role of Salivary Immunoglobulin A Deficiency in Humans as Related to Dental Caries

Contractor: University of Alabama, Birmingham
Principal Investigator(s): Jerry McGhee
Activity Site: Birmingham, Alabama

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Partial immunization against caries and increased salivary IgA has been achieved in animal models. To establish evidence that immunization is feasible in man several groups have looked for correlations between caries experience in man and salivary immunoglobulins. Thus Lehner, et al found higher IgA levels in whole saliva of caries-free subjects than in caries-active patients. Zengo, et al also noted higher IgA levels in submandibular but not parotid gland secretions. To obtain further evidence on this possible relationship one could examine the caries level in patients who lack IgA in their serum and external secretions. These patients appear particularly susceptible to diseases involving the oral membranes.

Activity: Dr. McGhee and his colleagues are elucidating the function of salivary IgA in providing protection to caries. They have established a study population of patients with salivary IgA deficiency, obtained complete medical histories on each patient and carried out complete oral/dental examinations. Periodically saliva and plaque samples have been obtained and analyzed for immunoglobulins and enzymes that act in conjunction with immunoglobulins. The numbers of various bacterial species in plaque also have been determined.

Results: Information obtained so far indicates that salivary IgA-deficiency does not necessarily correlate with increased caries activity. This may be due to some compensation with salivary IgM and perhaps some elevation of the innate immune factors, e.g. lactoferrin, lactoperoxidase and lysozyme in these patients. In the absence of any secretory antibody to Streptococcus mutans, there appears to be some increase in caries incidence. Plaque microbial composition shows no significant changes from control subjects.

Contract (no intramural research component)	NO1-DE- 52458
Period Covered: FY 79	Contract Period: 6/27/75-1/31/79

Title of Project: Development of a New World Primate as a Model for Dental Caries Studies

Contractor: Hazleton Laboratories, Vienna, Virginia
Principal Investigator(s): Dan Dalgard
Activity Site: Vienna, Virginia

P.O.: William Bowen
Organizational Position: NIDR, NCP, CRP Branch

Relevance to NCP Objectives: Caries research in experimental animals has been performed largely in rats and hamsters due to the convenience and inexpense of working with these animals. Rodents as models for studying human caries, however, have a number of deficiencies including: coprophagy, dissimilar dentition and tooth morphology and eruption patterns, different oral flora and secretory immunologic patterns and different diet. Because the monkey more closely resembles the human in these factors a simple, reproducible and available monkey caries model should be developed for studies on immunization, anti-plaque and antimicrobial agents, trace element and diet, mechanisms of action of fluoride, etc.

Activity: Techniques have been determined for rapid, reproducible development of caries in squirrel monkeys and a colony of these animals has been established. Saliva, dental plaque and serum are being sampled and analyzed at frequent intervals, caries is monitored and other data is being collected to prepare for use of this model in caries research.

Results: A nutritionally adequate cariogenic diet has been established. Thirteen monkeys have become pregnant (some on the cariogenic diet) and have delivered infants. The animals have been inoculated several times orally with S. mutans but the micrcorganism has not established itself. Microorganisms were isolated from dental plaque of the squirrel monkeys 37 weeks after oral challenge. S. sanguis was the most frequently isolated Streptococcus species and L. acidophilus was the most frequently isolated Lactobaccilus species. Antibody specific for squirrel monkey IgA has been prepared. The animal is probably unsuitable as a caries model.

Contract (no intramural research component)	NO1-DE- 52459
Period Covered: FY 79	Contract Period: 6/30/75-4/30/80

Title of Project: Community Caries Prevention Demonstration Project

Contractor: University of Connecticut Health Center
Principal Investigator(s): James Rule, Howard Bailit and Douglas Macko
Activity Site: Ansonia and Derby, Connecticut

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 3,100 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades range from 21 to 71% initially. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NO1-DE-52460
Period Covered: FY 79	Contract Period: 6/30/75-2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: State University of New York, Stony Brook
Principal Investigator(s): Louis Ripa and Gary Leske
Activity Site: Stony Brook, Old Field, and Setouket, NY

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,600 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by University personnel. The rinse is dispensed by teachers in the schools.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52461
Period Covered: FY 79	Contract Period: 6/30/75 - 4/30/80

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Forsyth Dental Center
Principal Investigator(s): Paul DePaola
Activity Site: Arlington, Massachusetts

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is staffed by volunteers under a paid coordinator.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades range from 21 to 71% initially. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NOI-DE-52462
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Eastman Dental Center
Principal Investigator(s): Dennis Leverett
Activity Site: Watkins Glen, Montour Falls and Odessa, NY

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 2,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by University personnel. The rinse is dispensed by a paid rinse supervisor.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE-52463
Period Covered: FY 79	Contract Period: 6/30/75-2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: University of Alabama in Birmingham
Principal Investigator(s): Joe Thomas
Activity Site: Birmingham, AL

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 1,400 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

There is a paid rinse coordinator; however, the rinse is dispensed by teachers and students.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE-52465
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Wisconsin Department of Health and Social Service
Principal Investigator(s): Betty Krippene
Activity Site: Waushara County, Wisconsin

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 1,500 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by existing health department personnel. The rinse is dispensed by volunteer parents and teachers.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52466
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Michigan Department of Public Health
Principal Investigator(s): Louis Szwejda
Activity Site: St. Joseph County, MI

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 5,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

State Health Department personnel administer the program and dispense the rinse in the schools.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52467
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Montana State Department of Health and Environmental Sciences
Principal Investigator(s): Jack Terrill
Activity Site: Anaconda, Montana

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 1,500 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by State Health Department personnel. The rinse is dispensed by school nurses.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52468
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Virginia Commonwealth University, Richmond, VA
Principal Investigator(s): Sherwin Fishman
Activity Site: Charles City County, VA

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 1,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is coordinated by the school nurse and the rinse is dispensed by teachers and students.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NOI-DE-52469
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Independence Neighborhood Councils, Independence, MO
Principal Investigator(s): Dennis Colombo
Activity Site: Independence, MO

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 5,200 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by Neighborhood Health Council personnel. The rinse is dispensed by paid monitors.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52470
Period Covered: FY 79	Contract Period: 6/30/75 - 4/30/80

Title of Project: Community Caries Prevention Demonstration Project

Contractor: University of California, San Francisco
Principal Investigator(s): Steven Silverstein
Activity Site: Livermore, CA

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,200 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In additipn provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

Teachers or part-time aides dispense the rinse for the lower grades. Paid community aides or trained student aides mix and dispense the solution for upper grades.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades range from 21 to 71% initially. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NO1-DE- 52471
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: University of Texas Health Science Center at Houston
Principal Investigator(s): Lawrence Friedman
Activity Site: Wharton and Conroe, TX

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 3,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The program is administered by school nurses and the rinse is dispensed by the nurses and teachers.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52472
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Colorado Department of Health
Principal Investigator(s): Roy Reger
Activity Site: Towns on Southwestern slope of Colorado

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 1,100 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

There is a paid rinse coordinator; the rinse is dispensed by the teachers.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 received the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings, particularly those with high DMFS levels.

Contract (no intramural research component)	NO1-DE- 52473
Period Covered: FY 79	Contract Period: 6/30/75 - 4/30/80

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Research for Health in Erie County, Inc.
Principal Investigator(s): Donald Bissell
Activity Site: Angola, Derby, and North Collins, NY

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 4,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

Teachers and school nurses dispense the rinse solution.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades range from 21 to 71% initially. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NO1-DE- 52474
Period Covered: FY 79	Contract Period: 6/30/75 - 4/30/80

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Geauga County Health Department
Principal Investigator(s): Terry Hull, Carol Sherman, and Frank Kellogg
Activity Site: Geauga County, Ohio

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 7,600 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

In the Amish schools the teachers are responsible for the entire program. In the other schools the school nurses conduct the program with assistance from teachers and students.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. For the five sites with high school programs the participation rates for these grades range from 21 to 71% initially. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NO1-DE- 52475
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: Harvard School of Dental Medicine
Principal Investigator(s): Leon Dogon
Activity Site: Roxbury - Boston

P.O.: Ann Miller
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 2,600 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The rinse is coordinated by University personnel. The rinse is dispensed by paid monitors.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

Contract (no intramural research component)	NO1-DE- 52476 CT 0600108
Period Covered: FY 79	Contract Period: 6/27/75-3/31/80

Title of Project: Development and Evaluation of a Diagnostic System for the Early Detection of Dental Caries

Contractor: Tufts University School of Dental Medicine, Boston
Principal Investigator(s): George White
Activity Site: Boston, MA

P.O.: Ralph Frew
Organizational Position: NIDR, NCP, OD

Relevance to NCP Objectives: Due to the etiology of caries, clinical trials typically require 3 years and several hundred children per group for a significant difference to be measureable between treatment and control groups with meaningful sensitivity and confidence. If caries could be detected earlier, the length of time for a clinical trial could be reduced and NCP comparison of alternative agents or modes of delivery would be markedly expedited. Capability to detect caries at a reversible stage could allow (1) the consideration of clinical research in which caries challenge is experimentally increased, (2) reversal of incipient lesions to be a cost-attractive alternative to prevention programs or operative therapy and (3) study of caries etiology at earlier stages.

Activity: An instrument to detect caries by conductivity measurement (and primarily for use with pits and fissures of molar teeth) is being developed and evaluated. The contractor has designed an instrument, evaluated its safety and ease of use and determined the sensitivity and reproducibility of measurements made with it under laboratory conditions. Currently the accuracy of the instrument in identifying early lesions is being determined in a longitudinal clinical trial.

Results: Sixty-two children, aged 10 to 14, were examined for occlusal carious lesions by: (1) routine clinical examination or (2) electrical resistance measurement, and results were compared to histological findings. An excellent correlation ($r=0.965$) was found between the instrumental diagnosis and histological findings. In 36 instances the instrument indicated the presence of carious occlusal lesions that could not be detected clinically with the dental explorer, but were confirmed by histologic findings. The instrument was found to be more sensitive than, and as specific as, the conventional examination for occlusal caries detection. Moisture contamination which may result in an increase in false positive diagnoses, has required modification of the instrument.

Contract (no intramural research component)	NO1-DE-52484
Period Covered: FY 79	Contract Period: 6/27/75 - 6/26/80

Title of Project: Clinical Screening of Antiplaque and Antibacterial Agents

Contractor: University of Pennsylvania, Philadelphia
Principal Investigator(s): Samuel Yankell
Activity Site: Philadelphia

P.O.: Roald Shern
Organizational Position: NIDR, NCP, CPRB, PMDS

Relevance to NCP Objectives: A promising approach to prevent caries is to use antimicrobial or antiplaque agents that decrease bacterial colonization or erosion of the tooth surface. To identify such agents and to ensure that they are evaluated accurately and comparably, the NCP is providing a service to screen promising compounds in vitro, in animals, and in short- and long-term clinical trials. Contracts and NCP direct operations are used jointly in separate phases of this screening service.

Activity: This "task-order" contract provides short-term clinical evaluation as specifically requested by NCP of IND certified agents. The trials are up to 16 weeks in length and involve not more than 50 male subjects. Agents are closely monitored for untoward effects and compared in their effects on oral microflora and several indices of plaque suppression.

Results: Short-term clinical studies have been carried out to determine effects on plaque of conventional and new topical agents:

a) fluoride gel (SnF_2 and/or APF)--no significant effect
b) calcium glycerophosphate rinses--no significant effect
c) DAPA-1 rinses--significant effects on plaque
d) SnF_2 rinses--significant effects on plaque

New criteria have been established for describing plaque responses.

Contract (no intramural research component)	NO1-DE-62479
Period Covered: FY 79	Contract Period: 4/14/76 - 3/16/79

Title of Project: Synthesize, Physicochemically Characterize and Evaluate the Sensory Properties and Stability of New Dihydrochalcons Analogs

Contractor: Dynapol Corp., Palo Alto, CA
Principal Investigator(s): Guy Crosby
Activity Site: above

P.O.: William Rogers (previously G. Roussos)
Organizational Position: NIDR, NCP, Planning Unit

Relevance to NCP Objectives: Current marketing events suggest convincingly that food manufacturers will readily convert to a non-cariogenic sweetener if its availability, cost, and other characteristics offer them a market advantage. Therefore NCP strategy is to identify, characterize and make information widely available on potentially useful non-cariogenic sweeteners. Dihydrochalcone-type sweeteners are attractive because of low probability of toxicity, intense sweetness, low cost and high stability.

Activity: Synthesize, physicochemically characterize and evaluate the sensory properties and stability of new dihydrochalcone analogs in a search for a superior food sweetener possessing, as nearly as possible, a taste quality comparable to that of sucrose in terms of rapid sweetness impact followed by a sharp cutoff.

1) Synthesize 40 specific new dihydrochalcone analogs and evaluate sweetness intensity, sweetness quality (like sucrose), safety (preliminary studies), and stability (dry and in solution).
2) Physicochemically characterize those sweeteners identified as potentially useful in regards to structure, purity, m.p. hydroscopicity, stability, solubility.
3) Conduct taste panel sensory evaluation (after limited-scale single-dose toxicity study in mice) of sweetness rating with respect to sucrose, saccharin and neohesperidin dihydrochalcone, taste quality, as sweetener for food and beverage porducts, and synergism with food ingredients and flavoring agents.
4) Evaluate stability under conditions of food manufacturing.

Results: (April '79) 43 compounds have been prepared and 39 evaluated by sensory panel. Seven of thse dihydrochalcone analogs have been found to be intensely sweet and of these, two appear to have potential for commercial development. 28 compounds were evaluated in the Ames mutagenicity assay and in the single oral dose acute toxicity assay. The compounds appeared safe. All other prescribed activities were completed.

Contract (no intramural research component)	NO1-DE-62488
Period Covered: FY 79	Contract Period: 5/1/76-4/30/79

Title of Project: Immunologic Cross-Reactivity of Antigens from Oral Streptococci with Mammalian Tissue and Tissue Components

Contractor: Research Foundation, SUNY
Principal Investigator(s): Russell Nisengard
Activity Site: SUNY, Buffalo, NY

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Microbial antigens proposed for use as immunogens may resemble host tissue antigens. Since host response to the bacterial substance may damage host tissue, the possibility of cross-reactivity must be carefully examined and eliminated. In 1972, rabbit antiserum to strains of S. mutans BHT and FA1 was noted to react with human myocardial tissue and skeletal muscle. To establish the dimensions of this phenomenon the Program is screening the oral microorganisms that would be used as sources of immunogens for cross-reactivity with human tissue. For convenience screening is carried out through two contracts, NO1-62488 (oral streptococci) and NO1-52481 (oral non-streptococci).

Activity: Representative strains and serotypes of S. mutans, sanguis and salivarius have been collected. Antisera to these microorganisms have been prepared, labelled with fluorescent dye and tested for binding to human heart (normal and rheumatic), kidney, skeletal muscle, liver and brain tissue. Crude antigen preparations were fractionated to identify antigens responsible for the cross-reactivity and these were defined and characterized.

Results: Contract NO1-52481 (oral non-streptococci) expired on 12/31/76. Rabbit antibody to Lactobacillus casei and Propionibacterium acnes bound to monkey brain and kidney tissue. The significance of this cross-reactivity is not known. Contract NO1-62488 (oral streptococci) and three sub-contract collaborators have found that many details of the methodology affect the final reading of the immunofluorescent reaction of rabbit anti-streptococcal sera to mammalian tissues. The original (1973) report of binding of anti-S. mutans rabbit sera to heart and skeletal muscle remains valid but is now subject to cautious interpretation because of some normal rabbit serum binding and lack of correlation between intensity of binding and anti-bacterial titer. Much further study of this phenomenon is required.

Contract (no intramural research component)	NO1-DE-62491
Period Covered: FY 79	Contract Period: 6/28/76 - 7/27/79

Title of Project: Research Study on the Use of Mutants of Cariogenic Streptococci to Prevent Dental Caries

Contractor: University of Alabama in Birmingham
Principal Investigator(s): Theodore Shiota
Activity Site: Birmingham, Alabama

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Glucan and fructan production by S. mutans is commonly considered to be a critical factor in the accumulation of plaque and bacterial fermentation acids that cause caries. Conceivably one might prevent caries by modifying the amount or nature of these microbial polymers. To achieve this a possible approach is to substitute S. mutans, with mutant polymer synthesis, for the indigenous strain of S. mutans. It is already known that some mutants of S. mutans do have modified capability to synthesize glucan and fructan in vitro and diminished caries virulence in rat models. If this approach is to be feasible, non-cariogenic mutants would have to be found that can supplant the wild type and neither revert to nor be supplanted by the wild type.

Activity: The contractor is employing a variety of techniques to prepare and isolate mutants of S. mutans with altered agglutination and plaque and colony forming ability. Cariogenicity of the mutants, ability to withstand being supplanted by the wild type, and stability to reversion are being determined in rat models.

Results: A large number of gentic mutants of S. mutans has been obtained. They are deficient in one or more or the in vitro characteristics associated with cariogenicity such as adherence, agglutinability with sucrose/dextran and glucan production. Several mutants have caused less caries in gnotobiotic rats and have been transmitted from dams to pups. However, revertants back to the parental type occur at varying rates and cause intermediate levels of caries.

Contract (no intramural research component)	NO1-DE- 62493
Period Covered: FY79	Contract Period: 06/30/76-06/29/79

Title of Project: Enzymatic Prevention and Control of Dental Plaque Formation

Contractor: Abcor, Inc., Wilmington, Massachusetts
Principal Investigator(s): David Williams
Activity Site: Wilmington, Massachusetts

P.O.: John Townsley (Previously G. Roussos)
Organizational Position: NIDR, NCP, CRGCB

Relevance to NCP Objectives: Enzymes which were effective in controlling plaque and preventing caries in animal models proved to be ineffective when tested in humans. This lack of effect is attributed to differences in composition of plaque from test animals and human subjects, differences in rates of inactivation of exogenous enzymes in animals and humans, and the intermittent and brief exposure periods when enzymes were applied to human plaque. To test these suggestions, the NCP is searching for stable enzyme combinations, which are more active in solubilizing plaque, and developing techniques to release small amounts of enzymes continuously in the mouth. Some of the contracts involved in these activities are NO1-22329, 52446, 52447 & 52448.

Activity: In the first phase of the study individual enzymes and selected combinations of enzymes were tested for their ability to solubilize human plaque in vitro. In phase two, the most efficient systems in vitro were tested in rats to establish the optimal formulation for plaque control and prevention of caries development in vivo. Attempts were made to immobilize effective enzyme preparations so that they could be retained in the oral cavity for prolonged periods.

Results: Solubilization of human plaque was measured using 78 enzyme preparations. Protein and carbohydrate solubilization were determined after 24 hour plaque, obtained by brushing, was incubated with enzyme. Protein/carbohydrate ratio averaged 7.1. The most effective enzymes were the proteinases thermolysin, subtilisin, and an endopeptidase. The 14 most effective enzymes were tested in two-way combinations. No combination was better than thermolysin. In rats, where the plaque protein/carbohydrate ratio (2.7) was lower than that of humans, dextranase and thermolysin reduced caries scores by 75 & 50%, respectively. NaF(10ppm) as a positive control reduced caries by 87%. Attempts to immobilize active enzymes were unsuccessful.

Contract (no intramural research component)	NO1-DE-62496 CT 0600109
Period Covered: FY 79	Contract Period: 6/30/76 - 6/29/79

Title of Project: Clinical Study to Evaluate Oral Rinsing with a Sodium Monofluorophosphate Mouthrinse on Preventing Dental Caries in School Children

Contractor: University of Texas Health Science Center at Houston
Principal Investigator(s): Lawrence Friedman
Activity Site: Houston, Texas

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Fluoride used in mouthrinses is chiefly NaF. There is evidence, however, that other fluoride compounds might be more effective, have lower toxicity, etc., and ought to be tested. Among these is Na_2PO_3F which: (1) is the active ingredient in a major dentifrice, (2) has encouraging anticaries activity in other vehicles, (3) has lower toxicity than sodium fluoride and (4) possibly acts by exchanging PO_3F for PO_4 ions in the enamel crystal to form highly insoluble fluoroapatite.

Activity: A three-year clinical trial has been carried out to compare the caries preventive effectiveness of weekly rinsing with Na_2PO_3F and NaF solution. The 300 children, initially approximately 12 years old, in each of the groups (placebo, NaF and Na_2PO_3F) rinsed once per week during the school year under supervision of the classroom teacher. Caries at baseline and at appropriate intervals was recorded on NIDR forms and submitted to NCP Biometry Section for processing.

Results: Results after two years of treatment show a non-sigmificant trend toward effectiveness for both formulations. Third-year data have been collected and are being analyzed.

Contract (no intramural research component)	NO1-DE- 62498
Period Covered: FY79	Contract Period: 09/29/76-03/31/79

Title of Project: Dietary Habits and Dental Plaque Composition of Fructose-Intolerant Patients

Contractor: University of San Francisco Dental School
Principal Investigator(s): Ernest Newbrun
Activity Site: San Francisco

P.O.: John Townsley (previously G. Roussos)
Organizational Position: NIDR:NCP:CRGCB

Relevance to NCP Objectives: Persons who suffer from hereditary fructose intolerance (HFI), due to a deficiency of hepatic fructose-1-phosphate aldolase, may not ingest either fructose or sucrose without at least experiencing nausea. Consequently, they avoid all sweet foods. As long as they avoid fructose they remain healthy and asymptomatic. They have substantially less caries than normal individuals. HFI individuals are ideal subjects for studies on the effects of restricted sucrose intake on the oral flora and the caries process.

Activity: In collaboration with investigators at the University of Berne, Switzerland, the contractor studied 17 HFI subjects and 14 control subjects, who were blood relatives of HFI subjects. Detailed dietary records were maintained and oral hygiene, plaque and caries indices determined. Plaque samples were assayed for specific microorganisms and metabolic parameters (protein, carbohydrate, phosphate, calcium and magnesium) and the fluoride content of drinking water was determined.)

Results: HFI subjects consumed sucrose-containing foods less often than control subjects. HFI diets contained less sucrose than control diets. HFI subjects had lower DMFT and DMFS scores than control subjects. The degree of oral hygiene was similar in both groups. However, potentially odontopathic S. mutans and lactobacilli were isolated less frequently from HFI subjects than from control subjects. S. sanguis was isolated with equal frequency from both groups. No differences were found in the chemical composition of plaque of HFI and control subjects. Findings support the concept that a reduction in dietary sucrose results in a reduction in cariogenic plaque organisms and in caries scores.

Contract (no intramural research component)	NO1-DE- 72402
Period Covered: FY 79	Contract Period: 7/15/77-4/14/79

Title of Project: Determination of Antibody to Streptococcus Mutans from Radiation-Induced Xerostomia Patients

Contractor: University of Texas at Houston
Principal Investigator(s): Peggy O'Neill, Lee Brown, Sam Dreizen
Activity Site: Houston, Texas

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Though many antibacterial and presumably caries inhibitory properties of saliva have been described, the functional significance of individual properties or the full complement of factors has not been established. It is known, however, that following salivary gland extirpation or inactivation by x-ray, caries becomes rampant in humans as well as in animals. Analysis of the changes taking place in saliva and in the oral flora that accompany development of rampant caries (NO1-DE-12377) have provided insight to the relative protection provided by salivary factors and the relative pathogenicity of oral microorganisms. Samples of saliva and serum are available from this unique set of patients. Analysis of these samples might provide evidence linking the rise in secretory immunoglobulins to these acidogens.

Activity: As only small volumes of these saliva samples were available and information was desired on specific antibodies present only in trace amounts in the samples the first stage in the contracted work was to prepare reagents and establish technique necessary for augmented microagglutination assays. Antigens were isolated from various strains of S. mutans and other microorganisms and microagglutination assays augmented with anti-immunoglobulin reagents were carried out.

Results: Antibody levels against various oral bacteria were generally higher in the saliva and serum of cancer patients than in controls. This correlated with the higher levels of salivary IgA and IgG in some cancer patients. Specific antibody was highest against S. mutans c. Patients with high salivary IgA levels had lower caries activity than patients with low saliva IgA levels. Anti-bacterial agglutinating activity in saliva was mainly in sIgA as confirmed by chromatographic separation.

Contract (no intramural research component)	NO1-DE-72404 CT 0600125
Period Covered: FY 79	Contract Period: 8/15/77 - 8/14/80

Title of Project: Clinical Investigation of the Effect of Concentration and Frequency of the Use of a Sodium Fluoride Mouthrinse on Dental Caries Formation

Contractor: University of Florida at Gainesville
Principal Investigator(s): Stanley Lotzkar and A.J.Conti
Activity Site: Polk County, Florida

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: The use of fluoride mouthrinsing in school-based programs for the prevention of dental caries is gaining widespread acceptance in the U.S. It would be of benefit to these programs if the NCP could recommend the most effective combination of frequency of rinsing and concentration of F solution. Unfortunately, the variations in the procedures that have been tested and the diversity of results do not support clear-cut recommendations. To obtain the data that is desired it is necessary to carry out a clinical trial in which the major combinations of frequency and concentration are compared side-by-side.

Activity: A three-year longitudinal clinical trial with approximately 1700 children is being carried out to compare the levels of caries prevention that are obtained with the major variations of rinse frequency and concentration used in school-based NaF mouthrinse programs. There are five groups in the study: a placebo rinse group, a daily and a weekly .05% NaF rinse group, and a daily and a weekly 0.2% NaF rinse group. Caries experience was measured at baseline and is redetermined annually.

Results: Data collected after one year are being analyzed. Results are not available.

Contract (no intramural research component)	NO1-DE-72406
Period Covered: FY 79	Contract Period: 8/15/77 - 2/14/79

Title of Project: Production of Specific Antisera: Anti-Rat IgA and S-IgA and Anti-Monkey IgA and S-IgA

Contractor: University of Chicago
Principal Investigator(s): Richard Newcomb and Barbara Peri
Activity Site: LaRabida, University of Chicago Institute

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Current research to determine the feasibility of caries immunization requires technical capability to measure in saliva the titer of classes of immunoglobulins as well as the titer of antibodies raised to specific antigens. All of the commonly used technics to measure the level of classes of immunoglobulins employ class-specific anti-immunoglobulin. Thus there would be considerable value in making available to scientists studying caries immunity high-titered class-specific antisera to secretory immunoglobulins of the chief animals used in caries immunization research. Use of a standard source of antisera also would increase the comparability of experiments carried out in different laboratories.

Activity: The contractor has produced anti-rat and anti-monkey sIgA, IgA, α-chain, IgM and μ-chain sera. Rat and monkey colostrum, milk, saliva, serum, and ascites were fractionated to obtain the proper immunoglobulin or fragment in relatively pure state. Monospecificity was confirmed by Ouchterlony analysis. Appropriate host animals were immunized with these materials to obtain antisera. These were evaluated, lyophilized in small aliquots and delivered to NCP.

Results: Final lots of goat anti-rat and anti-monkey immunoglobulin-class sera have been delivered to the NIDR. Appropriate titers have been reached but monospecificity has not been obtained in many of the lots as shown by immunoelectrophoresis. NCP is presently re-checking the various batches of antisera.

Contract (no intramural research component)	NO1-DE-72407 CT 0600126
Period Covered: FY 79	Contract Period: 9/28/77 - 1/31/81

Title of Project: Evaluation of Effects of Tooth Cleaning Prior to Weekly Rinsing with NaF

Contractor: Eastman Dental Center, Rochester, New York
Principal Investigator(s): Dennis Leverett
Activity Site: Rochester, New York

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Supervised weekly mouthrinsing with a 0.2% solution of NaF (900 ppm) is increasingly being recommended to communities that wish to implement programs for dental caries prevention. It has been suggested that the removal of plaque and other debris from the surface of the teeth prior to rinsing could enhance the effectiveness of the procedure. This should be investigated. In school programs, however, it is desirable that rinsing procedures occupy a minimal amount of classroom time. Thus any advantages of prior cleaning would have to be significant before it could be recommended as a routine adjunct to rinsing.

Activity: A three-year longitudinal clinical trial is being carried out to establish whether cleaning the teeth prior to customary weekly fluoride rinsing prevents signigicantly more caries than fluoride rinsing by itself. A population of approximately 900 school children 12-13 years of age is involved in the study. All of the children rinse weekly with 0.2% NaF solution. The children in one group do not clean their teeth prior to the weekly fluoride rinse. Children in the two other groups clean their teeth by brushing or by brushing and flossing. Caries scores were determined at baseline, remeasured periodically and reported on NIDR data forms.

Results: First year data have been collected and are being analyzed. Results are not yet available.

Contract (no intramural research component)	NO1-DE-72408
Period Covered: FY 79	Contract Period: 9/1/77 -8/31/79

Title of Project: Search for Cross Reacting Antigens to Acidogenic Bacteria of the Human Oral Flora

Contractor: Research Foundation, SUNY
Principal Investigator(s): Murray Stinson
Activity Site: SUNY, Buffalo, NY

P.O.: Norman Ikari
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: A major NCP strategy is to develop caries immunization for public health use. There would be risks however in using immunogens from acidogenic streptococci, lactobacilli and Actinomycetes because these classes of microorganisms have known pathogenicity. The possibility exists, however, to substitute immunogens from non-pathogenic microorganisms if there is sufficient similarity in antigenic properties. Thus, for example, in other areas of medicine, overt immunization with certain serological types of E. coli and species of staphylococci and bacilli have been found to give rise to antibodies which cross-react with the disease pathogens, pneumococci, meningococci and Hemophilus influenzae "b".

Activity: The Contractor is searching for antigens from non-pathogens that are similar to antigens from oral strains of acidogenic streptococci, lactobacilli and Actinomyces. Bacterial colonies are made into air-dired films, exposed to rabbit antisera and stained by a goat anti-rabbit IgG conjugate. Presumptive identification is made of those bacteria yielding significant cross-reactivity in the indirect immunofluorescence screening. Rabbit antisera is raised to these cross-reactors. The ability of these antisera to inhibit adherence of acidogenic bacteria is measured. Preliminary characterization of the cross-reactive sites in the bacteria is performed by fractionation and extraction of the bacterial cells and further testing of these fractions for cross-reactivity and adherence-inhibitory capability of their antisera.

Results: A total of 51 bacteria isolated from various sources have been found to cross-react with three or more antisera specific for acidogenic bacteria. Six of these isolates have been reciprocally tested against the acidogenic bacteria. Some loss of reactivity was noted. The ability of these antisera to inhibit adherence is being measured in a radiolabelled adherence assay. The project is currently being renewed for another two years of effort.

Contract (no intramural research component)	NO1-DE-72409
Period Covered: FY 79	Contract Period: 9/30/77 - 9/29/80

Title of Project: Effect of Strontium, Lithium and Fluorine on the in vitro Formation and Metabolism of Dental Plaque and on Plaque Formation and Development in the Rat

Contractor: Eastman Dental Center
Principal Investigator(s): Martin Curzon
Activity Site: Rochester, New York

P.O.: Albert Kingman (formerly G. Roussos)
Organizational Position: NIDR, NCP, CPR Branch

Relevance to NCP Objectives: Epidemiological studies in the 1930's that revealed a relationship between caries incidence and environmental fluoride after many years of research and testing have led to current wide-spread use of this element for caries prevention. Continuing field studies suggest that caries incidence is related to additional trace elements. Repeatedly these investigations have implicated lithium and strontium in an inverse relationship to caries and pointed to selenium in a caries promoting role. At present in vitro and animal models are needed with which these effects can be confirmed and defined with respect to their strength. High priority certainly should be given to exploring models based on plaque formation and activity and on enamel development and properties. It is reasonable that trace elements would interact in these models so this possibility also should be examined.

Activity: The contract is to search for model systems with which Sr, Li and F effects on caries can be studied. The in vitro models that are being tested are major salivary and microbial phenomena involved in caries etiology. The in vivo models involve rats exposed to the trace elements prenatally or at various periods in tooth development. Various levels and combinations of the trace elements are being screened in the two types of systems.

Results: In each of the models there appears to be definite effects of lithium when tested at low concentration. Maximal caries reduction and weight gains in rats occurred at 20 µg/L. Effects on bacterial aggregation, sorption and extracellular polysaccharide production occurred at lithium concentrations of 100 µg/L.

Contract (no intramural research component)	NO1-DE-72410
Period Covered: FY 79	Contract Period: 9/30/77 - 12/16/78

Title of Project: Application of Fluorescent Antibody Methods to the Clinical Determination of the Etiologic Role of Oral Microorganisms in Human Dental Caries

Contractor: Research Foundation, SUNY
Principal Investigator(s): Ernst Beutner
Activity Site: Buffalo, New York

P.O.: Stanley Robrish (previously G. Roussos)
Organizational Position: NIDR, NCP, CPR Branch

Relevance to NCP Objectives: Though there is direct evidence that S. mutans causes caries in the rat the evidence for the involvement of this microorganism in human caries is limited to cross-sectional epidemiological studies that show correlations in large populations between the level of caries and the micro-organism. This evidence could be made stronger by longitudinal studies of the sequence of the appearance of caries and S. mutans at specific sites on teeth. Such a study has been made technically feasible by the development by NCP of reagents that facilitate enumeration of plaque microorganisms and media and techniques that facilitate sampling and manipulation of minute amounts of plaque.

Activity: The contract is designed to determine the feasibility of and prepare for a longitudinal study to determine the etiologic role of several plaque microorganisms in human caries. Highly specific and sensitive fluorescent antibody reagents have been developed for important plaque microflora, all clinical and laboratory methodologies that would be employed have been tested, and the logistic problems of carrying out the multi-year study have been assessed.

Results: Antisera have been prepared that react positively in the indirect immunofluorescent assay with pure cultures of specific S. sanguis strains.

Contract (no intramural research component)	NO1-DE-72411 CT 0600119
Period Covered: FY 79	Contract Period: 9/30/77 - 9/30/80

Title of Project: Acidulated Phosphate-Fluoride Used Daily as a Tablet or Solution

Contractor: Applied Management Sciences
Principal Investigator(s): Douglas Skinner
Activity Site: Pittsylvania County, Va.

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Though less effective than with full-time use, fluoride supplements given only in school can impart important caries-preventive benefits and allow large numbers of children to be treated with minimal demands on dental manpower equipment and funds. The NCP is actively promoting these programs and needs information on which of the several technics and fluoride formulations is best. Among these are fluoride tablets that are chewed and then swallowed and fluoride solutions that are swished around the mouth and swallowed. Both are available as neutral NaF or as acidulated phosphate fluoride.

Activity: The contractor is carrying out a three-year longitudinal clinical trial to determine the effect on dental caries of neutral sodium fluoride (NaF) and acidulated phosphate fluoride (APF) when each is used as a tablet or as a solution. A population of approximately 1600 school children, 12-14 years of age, was assembled and divided into five groups. One group receives a placebo tablet. The other groups receive 1 mg F per day as NaF or APF either in a tablet that is chewed and swallowed or solution that is swished and swallowed. Caries experience is recorded on NCP data forms and submitted to NCP Biometry Section for processing.

Results: First year data have been collected and are being analyzed. Results not available.

Contract (no intramural research component)	NO1-DE- 82416 CT 0600127
Period Covered: FY 79	Contract Period: 9/30/78 - 9/29/81

Title of Project: Effect of Mouthrinsing with Stannous Fluoride or Sodium Fluoride

Contractor: University of Texas Health Science Center at Houston
Principal Investigator(s): Lawrence Friedman
Activity Site: Houston, TX

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Daily mouthrinsing with dilute solutions of either sodium fluoride or stannous fluoride has been found effective against caries. Stannous fluoride has been reported to have an unattractive taste, to stain teeth and to be unstable in solution. On the other hand it has been reported to remove plaque from teeth. If use of a stannous fluoride mouthrinse can produce meaningful reductions in plaque and gingivitis scores with minimal tooth staining and is equivalent to sodium fluoride in cariostatic properties it may be the preferred agent in regimens that involve daily use.

Activity: The clinical trial involves approximately 500 students, 11-13 years of age, living in Houston, Texas, a low-fluoride area. The students rinse daily during three school years with a dilute solution of either stannous or sodium fluoride. Caries, dental plaque, gingivitis and extrinsic staining are evaluated at baseline and at appropriate intervals. Data is recorded on NIH forms and processed by the NCP Biometry Section.

Results: First year findings are being analyzed. Results not available.

Contract (no intramural research component)	NO1-DE-82417 CT 0600113
Period Covered: FY 79	Contract Period: 9/30/78 - 9/29/81

Title of Project: Effect of Mouthrinsing with Stannous Fluoride or Sodium Fluoride

Contractor: Eastman Dental Center
Principal Investigator(s): Dennis Leverett and William McHugh
Activity Site: Seneca and Tompkins Counties, NY

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Daily mouthrinsing with dilute solutions of either sodium fluoride or stannous fluoride has been found effective against caries. Stannous fluoride has been reported to have an unattractive taste, to stain teeth and to be unstable in solution. On the other hand it has been reported to remove plaque from teeth. If use of a stannous fluoride mouthrinse can produce meaningful reductions in plaque and gingivitis scopes with minimal tooth staining and is equivalent to soduim fluoride in cariostatic properties it may be the preferred agent in regimens that involve daily use.

Activity: The clinical trial involves approximately 500 students, 11-14 years of age, living in Seneca and Tompkins Counties, NY, a low-fluoride area. The students rinse daily during three school years with a dilute solution of either stannous or sodium fluoride. Caries, dental plaque, gingivitis and extrinsic staining are evaluated at baseline and at appropriate intervals. Data is recorded on NIH forms and processed by the NCP Biometry Section.

Results: First year findings are being analyzed. Results not available.

Contract (no intramural research component)	NO1-DE-92419 CT 0600129
Period Covered: FY 79	Contract Period: 1/15/79 - 6/30/82

Title of Project: Effect of Prior Toothcleaning upon the Efficacy of Semi-Annual Topical Fluoride Treatment

Contractor: Research Foundation of SUNY
Principal Investigator(s): Louis Ripa and Gary Leske
Activity Site: Stony Brook, New York

P.O.: Philip Swango
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: Several clinical studies have clearly shown that appreciable caries prevention can be obtained by treating the teeth semi-annually with an APF gel containing approximately 1% fluoride. Customarily topical treatment is preceded by a thorough prophylactic cleaning of the teeth surfaces. It has been suggested, however, that tooth cleaning may not be necessary or indeed be detrimental--that plaque would hold fluoride in proximity to the enamal and serve to increase effective treatment time. The value of prior prophylaxis should be determined so that the Program can properly advise on this technique.

Activity: A 3-year longitudinal clinical trial with school children is being carried out to determine whether cleaning the teeth prior to semi-annual professionally applied topical fluoride prevents significantly more caries than topical treatment without prior cleaning. An appropriate study population of approximately 1500 school children, 11-13 years of age has been assembled and divided into 3 groups. Children in all groups receive periodic instruction in the proper dental hygiene and receive a semi-annual professional topical application of acidulated phosphate fluoride gel. Children in one group do not clean their teeth prior to the topical application. Those in the other groups either brush and floss their own teeth or receive a thorough dental prophylaxis prior to the topical treatment. Caries experience is recorded on NIH data forms and submitted to NCP Biometry Section.

Results: The clinical trial is underway. First year results will be available approximately October, 1980.

Contract (no intramural research component)	NO1-DE-92421
Period Covered: FY 79	Contract Period: 4/15/79 - 10/14/80

Title of Project: National Dental Caries Prevalence Survey

Contractor: Westat, Inc., Rockville, MD
Principal Investigator(s): Thomas McKenna
Activity Site: Nationwide

P.O.: Ann Miller and Janet Brunelle, co-PO
Organizational Position: NIDR, NCP CRG&C and CP&R Branches

Relevance to NCP Objectives: Information on caries prevalence in children of different ages and in different areas of the U.S. would identify the populations at greatest risk, aid in deciding priority to be given to alternative caries preventive agents and delivery programs, and provide a baseline with which to measure changes in prevalence of the disease. If indications of increased or unchanged prevalence are found despite introduction of techniques that increase resistance of the host it would suggest that more attention should be given to caries promoting factors such as frequent consumption of sugary snacks.

Activity: Plan and conduct a base-line survey of the prevalence of caries among school aged children. The prevalence data will be for each of seven regions of the continental United States, be for single years of age by region, and be of sufficient precision to allow detection of a 15% change over time by single age and region.

PHASE I - Develop: (1) a sample survey, (2) plans for moving the examining team between sites and (3) plans for quality control.

PHASE II - Calibrate and standardize the examining teams and test the procedures.

PHASE III - Conduct all examinations in 1979-80 school year. Measure and record caries, gingivitis, dental treatment need and water F content.

Deliver results to NCP for processing by the Biometry section.

Results: Phases I and II are completed. Results of Phase III will be available in August-September of 1980.

Contract (no intramural research component)	Y01-DE-60020
Period Covered: FY 79	Contract Period: 6/30/75 - 2/28/79

Title of Project: Community Caries Prevention Demonstration Project

Contractor: PHS Region IX San Francisco
Principal Investigator(s): Reginald Louie
Activity Site: Guam

P.O.: Ralph Frew
Organizational Position: NIDR, NCP, CRG&C Branch

Relevance to NCP Objectives: As caries preventive technics pass final stages of testing and are determined by NCP staff to be ready for use, a practical way of implementing wide-scale acceptance is to establish geographically distributed, highly visible and accessible, community-run models of the preventive programs. These models demonstrate cost/effectiveness and ease-of-use characteristics, are convenient for other communities to copy, and provide widespread information on advantages of the technic. In addition the models provide essential data to the Program on the utility of the technic under field conditions.

School-based weekly mouthrinsing with a dilute solution of neutral sodium fluoride has been found highly valuable for populations not having the benefit of water fluoridation. A program of nationally distributed community-run projects would promote the widespread use of this technique.

Activity: Commencing in September, 1975, establish and monitor a voluntary school-based preventive program for grades K-6(8) (approximately 5,000 children) in a non-fluoridated community utilizing a 0.2 percent neutral sodium fluoride mouthrinse weekly. In addition provide caries prevalence data, cost data and participation statistics, and develop and annually update a plan for the continuation of the school-based program for a 3-year period after termination of federal support.

The project coordinator and rinse dispensers in the school are employees of the Government of Guam. Volunteer help is also provided by mothers of students.

Results: The average participation for all sites at the end of three school years for grades K-8 was 83% with greatest participation in the lower grades (K-5). In all studies the average number of decayed, missing, and filled tooth surfaces decreased from their initial levels. Children in grades K-3 receive the greatest protection. The costs of supplies, equipment, and personnel ranged from 40¢ to $9.52 per child per year for the first three school years. Differences in types of personnel accounted for the major cost differences. The procedure is highly cost-effective in a variety of community settings. The geographic areas with higher DMFS levels had the greater cost-benefit ratio.

DATE DUE

GAYLORD			PRINTED IN U.S.A.