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Factors Associated With Root Caries Initiation

David William Banting

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FACTORS ASSOCIATED WITH ROOT CARIES INITIATION

BY

DAVID WILLIAM BANTING

DEPARTMENT OF EPIDEMIOLOGY AND BIOSTATISTICS

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ABSTRACT

Dental caries is a chronic disease which results in the destruction of the mineralized tissues of the teeth. The disease has been known since the beginning of recorded history and continues to be a major cause of tooth loss. It is now widely accepted that dental caries is a demineralization of the calcified tooth tissues caused by acids produced by microorganisms adhering to the tooth surface.

Most of the research related to the etiology of dental caries has focused on coronal caries and children. However, the changing age-structure in the population during the past decade and a dramatic downward shift in the prevalence of coronal caries has diverted the attention of dental researchers toward root caries. As the number of older adults with natural teeth increases, root caries assumes much greater importance since most new dental caries in this group is located on the root surfaces.

Since early work on root caries suggested that the primary etiological agent and pathological process differed from those of coronal caries, this project was designed to provide data relative to the role of specific oral microorganisms in the initiation of root caries. The microorganisms studied were some (but not all) of those previously identified with root and coronal caries

development: Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, Actinomyces naeslundii and Lactobacillus. The null hypothesis was that the presence of these microorganisms is not associated with the occurrence of root caries.

Forty institutionalized adults, considered to be at high risk of root caries, were followed for a two year period. They were grouped according to whether the microorganisms under study were present at baseline on one or more non-carious root surfaces with gingival recession and the incidence of root caries was then recorded. The effect of other variables shown to be associated with root caries, such as age, sex, number of teeth, previous dental caries experience, gingival recession, gingival pocket depth and between-meal snacks were also measured.

Both subjects and tooth root surfaces were used as the units of observation in this study. However, since tooth root surfaces within the same mouth violate the assumption of independent response on which conventional statistical methods rely, an adjustment was used to compensate for the lack of independence of sites within the same mouth.

The incidence of root caries in the forty subjects followed for two years was 0.33 per subject. The presence of Streptococcus mutans was associated with the incidence of root caries in both a statistically ($p=0.008$) and clinically

(odds ratio=10.7) significant way using the subject as the unit of observation. A clinically (odds ratio=2.9) but not statistically (p=0.20) significant association between the presence of Streptococcus mutans and the incidence of root caries was evident using the tooth root as the unit of observation and adjusting for the clustering of teeth within mouths. A positive and possibly linear relationship between the number of tooth roots colonized by Streptococcus mutans in a subject and the risk of root caries was also observed. On the other hand, Streptococcus sanguis was negatively associated with the incidence of root caries suggesting that this microorganism contributes to tooth root health. None of the demographic or periodontal measurements made a statistically significant contribution to the multivariate model.

These data support the hypothesis that the presence of Streptococcus mutans on the roots of teeth is associated with a higher prevalence of dental root caries; but the rate of root caries does not differ among exposed and non-exposed individuals.

The implications of these findings on future directions for research are discussed.

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This thesis has had so many ups and downs and on-again, off-agains that almost everyone I know has in some way contributed. However, there are some people who, whether they wish to be associated with this document or not, were inextricably part of it and should be acknowledged.

I met Richard Ellen in Digby, Nova Scotia at the Association of Canadian Faculties of Dentistry Bienniel meeting in 1974. From that initial meeting the hypothesis of this thesis was developed. A most productive and stimulating working relationship subsequently developed with Rich. He was, of course, responsible for conceptualizing and organizing the microbiological portion of this project. He was also a co-author of several manuscripts.

Both of my Deans, Wes Dunn and Ralph Brooke, have been strongly supportive throughout the years. Without their encouragement and understanding the whole exercise would have been an impossible dream.

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My grandmother, Etta Bethune, had a hand in this as well. When I was young, she impressed upon me that "if a job was worth doing it was worth doing well" and "never leave a job half-done". I have never forgotten these adages.

If it is possible to dedicate a thesis to someone, I would like to dedicate this one to my wife, Judith, and my three children, Jennifer, Melanie and Adam. They have been uncommonly tolerant throughout this whole affair and probably deserve the degree more than I. I have immense love and respect for them and I thank them sincerely.

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CHAPTER 1

GENERAL REVIEW

1.1 INTRODUCTION

Dental caries is an infectious process which results in the destruction of the mineralized tooth tissues. Although uncommon, evidence of dental caries has been found in human skeletal remains dating back to Paleolithic times. The occurrence of dental caries in man increased dramatically during the Neolithic period and has remained at a relatively high level since (Newbrun,1978).

Early civilizations in Japan, India, Egypt, Mesopotamia and Greece believed that dental caries was caused by worms. In fact, caries is a Latin word meaning "rottenness" or "decay". Other theories which have been advanced regarding the etiology of dental caries included humors, internal resorption, acids, parasites, acids and parasites together, proteolysis, proteolysis combined with chelation and a few others (Nikiforuk, 1985). It is presently accepted that dental caries is a chronic disease, it proceeds from the surface of the tooth toward the pulp and the destruction is caused primarily through decalcification of the mineralized tissues.

The caries process depends upon three essential factors: the tooth and its environment, microorganisms and diet. Many secondary factors associated with the host (age, sex, saliva, immune response, oral hygiene), the agent (type, number and virulence of the microorganism) or the substrate (cariogenic potential, presence of fluoride) may affect the rate of progression of the dental caries but without the simultaneous presence of the three primary factors for a sufficient length of time, disease is unlikely.

Although there is unanimity concerning the presence of both decalcification and proteolysis in the root caries process, it has not been conclusively established which is the more significant. Cementum and dentine contain approximately 35 per cent (by volume) organic material, primarily collagen; enamel has about 3 per cent. The lower mineral content of cementum and dentine may not require as intense a challenge for decalcification to occur. Since both decalcification and collagenolysis are highly dependent upon the pH, the local conditions at the root surface are extremely important in determining the process of root caries development (Katz et al, 1987).

Dental caries is often classified according to anatomical location. In ancient man, dental caries was usually found on the root of the tooth; whereas in modern man the crown portion of the tooth is more commonly attacked (Banting and Ellen, 1976). However, root caries has been observed to be

increasing in prevalence in contemporary populations and is expected to assume greater significance as the number of older adults with natural teeth increases (Banting, 1984).

Both coronal and root caries can be active and untreated, arrested without being treated or restored as an outcome of dental treatment. Similarily, they can exhibit low activity (incipient) or high activity (rampant) and may progress to the stage of decalcification (early lesion), cavitation (clinical lesion) or gross destruction of the tooth. Unfortunately, these terms are not mutually exclusive and an incipient carious lesion is considered to be both early and slowly progressive. If the dental caries occurs adjacent to a filling material on either the crown or the root of a tooth, the lesion is referred to as secondary caries.

The prevalence of coronal dental caries in children and young adults has declined dramatically in the past decade in response to extensive efforts over the previous 30 years to prevent the disease through the widespread use of systemic and topical fluorides, mechanical oral hygiene procedures and improved dental health education. This accomplishment will almost certainly result in an increased number of natural teeth being retained by older adults. The extended longevity of people who possess a greater number of retained natural teeth with gingival recession would invite the hypothesis that the prevalence of dental root caries may increase in successive generations of older adults.

Most adults in industrialized countries have coronal caries experience with an average of three untreated, active coronal caries lesions (Beck et al, 1985). Secondary coronal caries was found to be at least as prevalent as primary caries and may be twice as prevalent (Bergman et al, 1982). In addition to continued coronal dental caries activity in adults, carious lesions occurring on the roots of teeth have been identified as a vexing clinical problem which is difficult to diagnose at an early stage. They are difficult to manage clinically and this may be reflected in the higher number of missing teeth observed with advancing age (Banting, 1984). Although root caries affects only about half of the adult population , susceptible adults have an average of 2.5 untreated, active, root caries lesions, most of which occur on previously unaffected surfaces (Beck et al, 1985).

Within an affected individual not all surfaces of all teeth are involved, indicating differential risk both between and within persons. However, caries at a given tooth or surface of a tooth cannot be treated as independent of all other sites within a subject because of common host, agent and dietary factors (Imrey,1986). Conventional statistical techniques, therefore, cannot be directly applied to the analysis of the data (Donner and Banting, 1988).

This study was undertaken to determine the microbial

characteristics of dental plaque associated with the initiation of carious lesions on the roots of teeth and to describe the relationship between the initiation of root caries and the clinical features of the oral tissues adjacent to the lesion. The hypothesis was that dental root caries is associated with exposure to one or a combination of the following oral microorganisms: Streptococcus mutans, Streptococcus sanquis, Actinomyces viscosus, Actinomyces naeslundii and Lactobacillus.

1.2 THE ROLE OF MICROORGANISMS IN CORONAL AND ROOT CARIES

It has been conclusively demonstrated that microorganisms are a prerequisite for the initiation and progression of coronal dental caries; that a single type of microorganism is capable of inducing caries; and that the ability to produce acid as a by-product of metabolism is essential to the process (Nikiforuk, 1985). However, not all acidogenic oral microorganisms induce caries and virulence varies among those that do. Futhermore, there is interaction among different bacterial species and only the microorganisms present at a localized site are considered to be important in the carious process at that site.

Bacterial specificity in the etiology of dental caries has not been convincingly demonstrated, however, Streptococcus mutans is considered to be a major pathogen in coronal

caries. It comprises a much greater proportion of all microorganisms grown on non-selective media when harvested from decayed as opposed to caries-free surfaces of teeth (Loesche et al, 1975). The initiation of dental caries on sound teeth is most often preceded by colonization with elevated levels of Streptococcus mutans (Ikeda et al, 1973). However, the ability of Streptococcus mutans to colonize a site and achieve dominance depends on the immediate environment. Lactobacilli have also been shown to be strongly associated with coronal caries; but are considered to be less cariogenic than Streptococcus mutans, except perhaps in the presence of fluoride (Bowden et al, 1984). The precise roles of each of these microorganisms in the development of a carious lesion are unknown; but it is believed that Streptococcus mutans is more closely associated with incipient caries, whereas lactobacilli tend to be associated with caries progression (Edwardsson, 1986).

Jordan and Keyes (1964) observed that filamentous organisms were associated with carious lesions on the roots of teeth in hamsters fed a high carbohydrate ration. These organisms were later identified as members of the Actinomyces species and human isolates of Actinomyces viscosus and Actinomyces naeslundii were subsequently shown to be capable of inducing root caries in hamsters and gnotobiotic rats (Socransky et al, 1970; Jordan et al, 1972). These animal studies led to the hypothesis that a microflora distinct from that implicated in coronal caries may be involved in the root

caries process.

Jordan and Hammond (1972) were the first to culture samples of microorganisms from human root caries lesions on extracted teeth. Actinomyces viscosus, Actinomyces naeslundii, Actinomyces odontolyticus, Rothia dentocarioso, other unidentified actinomyces-like organisms and Streptococcus mutans were all isolated from the lesions. Sumney and Jordan (1974) cultured surface deposits of plaque and carious debris from teeth freshly extracted because of periodontal disease. The microorganisms isolated included: Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis, enterococci, filamentous organisms of the genus Actinomyces and an anaerobic, diptheroidal organism similar to the genus Arthrobacter. Streptococcus mutans was the predominant organism isolated from the plaque covering the surface of the lesion and the organism resembling the genus Arthrobacter was isolated from the advancing front of the root caries.

Syed et al (1975) obtained plaque samples from active, untreated root caries lesions in patients prior to restorative treatment. Actinomyces viscosus was found to be the dominant bacterial species recovered on anaerobic culture. Actinomyces naeslundii, Streptococcus mutans, Streptococcus sanguis and Veillonella strains were also isolated but as lesser proportions of the total viable counts. When subjects were classified according to the

presence or absence of Streptococcus mutans, Actinomyces viscosus was equally common in both groups, whereas Streptococcus sanquis was only present in the group without Streptococcus mutans. Ellen et al (1985b) were the first to compare the microflora colonizing sound and carious root surfaces. Actinomyces viscosus was the most frequently isolated microorganism and the proportions of the total cultivable anaerobic flora of plaque from intact and carious root surfaces represented by Streptococcus mutans, Lactobacillus, Actinomyces viscosus, Actinomyces naeslundii and Veillonella were similar. Brown et al (1986) also compared the surface microflora of carious and intact root surfaces. No significant differences were seen between carious and intact root surfaces for Streptococcus mutans and Lactobacillus when measured as proportions of the total anaerobic cultivable flora; but intact root surfaces had significantly higher proportions of Actinomyces than did carious root surfaces. Streptococcus mutans made up the largest proportion of the total anaerobic count followed by Actinomyces viscosus and Lactobacillus. Futhermore, Streptococcus mutans represented a significantly higher proportion of the total anaerobic count in initial compared with advanced lesions. Fure et al (1987) compared the proportion of Streptococcus mutans, Lactobacillus, Actinomyces viscosus and Actinomyces naeslundii in subjects with four or more root surfaces affected with caries (group 1) with subjects who were caries-free or had, at the most, one affected root surface (group 2). The

latter subjects also had moderate to severe periodontal disease. The proportions of Streptococcus mutans in group 1 were significantly higher than those of group 2 as were the proportions of Lactobacillus. There was no difference in the proportion of Actinomyces between the groups. Keltjens et al (1987) compared the plaque taken from one to three carious root lesions in periodontal patients on maintenance care with contralateral, intact root surfaces. The root lesions were further classified as soft (active) or hard (arrested). Streptococcus mutans counts on the carious surfaces were significantly higher than those on the sound surfaces and this difference was attributed exclusively to the increased counts on the soft carious surfaces. Streptococcus sanguis and Actinomyces viscosus/naeslundii counts were not significantly different on carious and sound roots.

Only two studies have followed a group of subjects and taken repeated samples of plaque or saliva for microbiological analysis. Ellen et al (1985a) reported that the relative risk of a patient developing a carious lesion on a susceptible tooth root was five times greater if both Streptococcus mutans and Lactobacillus were present in the root plaque than when both were absent. High salivary counts of Lactobacillus and Streptococcus mutans were found in patients who had the greatest number of new root caries lesions over an eight year period (Ravald and Hamp, 1986).

Animal models of the role of microorganisms in the caries

process are conclusive. Streptococcus mutans is capable of inducing coronal and root caries in the presence of a carbohydrate source. Actinomyces viscosus is also capable of causing extensive destruction of the bony support of teeth and root caries. Human experimentation, however, has only supported in a general way the findings in animals. The existence of a cause and effect relationship between root caries and an unique microorganism has yet to be demonstrated. Jordan (1986) reviewed the available knowledge relating to the microbiology of root caries and concluded that a wide array of organisms should be considered as causative agents. Streptococcus mutans has not been found to be consistently present in root caries plaque but , when present, may comprise a high proportion of the anaerobic cultivable flora. The presence of Streptococcus mutans and lactobacilli together, increases the risk of root caries. Actinomyces viscosus has been identified as the most numerous organism among the cultivable bacteria isolated from plaque overlying tooth roots but the proportion of the cultivable flora represented by this organism is higher for intact than for carious roots.

1.3 DIETARY FACTORS IN ROOT CARIES

Any consideration of a microbial etiology in root caries must include dietary factors if it is accepted that

decalcification of the calcified components of the tooth is central to the formation of a lesion (Nyvad and Fejerskov, 1982; Hoppenbrouwers et al., 1986). Acidogenic organisms use carbohydrates from the diet for metabolism and in the process produce acids which destroy the inorganic portion of the tooth.

Root caries in people appears to be enhanced by dietary sugars. The frequency of both enamel and cemental caries coincided with the increased daily intake of sugar, especially when taken between meals, in the Vipeholm Dental Caries Study (Gustafsson et al, 1953). This study also demonstrated that caries activity in the younger age groups was much higher than in the older age groups supplied with similar high-sugar diets. In the older age groups, however, the carious lesions occurred most frequently on the tooth roots. Hix and O'Leary (1976) observed that patients with moderate to severe periodontal disease who were affected by root caries had a significantly higher number of fermentable carbohydrate exposures per week than did those without root caries. This relationship was independent of whether or not the patient received periodontal treatment.

Three day food diaries were compiled for 175 elderly subjects and the results were compared with a dental examination for coronal and root caries (Papas et al, 1984). Positive correlations were found between dietary sugars and both coronal and root decay. Associations were also

confirmed between the frequency of snacks and the presence of root caries.

1.4 PREVALENCE AND INCIDENCE OF ROOT CARIES

At least eighteen epidemiologic surveys have been conducted which provide estimates of the prevalence of root caries in contemporary populations. These studies are quite diverse in terms of the populations observed and the number of subjects examined and, therefore, it is difficult to compare them. A composite description of the observed occurrence of root caries in contemporary populations is presented in Table 1. The proportion of people with one or more root caries lesions and/or fillings present at the time of examination ranged from less than 10 per cent to 100 per cent. For healthy, ambulatory, urban adults the occurrence of root caries falls between 20 and 40 per cent but this rate increases dramatically for special population groups such as primitive tribesman, the institutionalized, chronically ill patients, patients with destructive periodontal disease and the elderly. Seven of these studies expressed prevalence as the proportion of "at risk" roots which were decayed or filled. Only tooth roots with gingival recession as a consequence of periodontal disease or periodontal treatment were considered to be "at risk" of root caries. The Root Caries Index (RCI) represents the proportion of susceptible teeth or root surfaces which become carious (Katz, 1980).

The mean prevalence rates expressed in this manner range from 1.2 to 15.0 per cent of teeth and 1.1 to 24.7 per cent of root surfaces of teeth. Approximately 10-20 per cent of teeth and up to 66 per cent of tooth root surfaces exhibit loss of epithelial attachment and are exposed to the oral environment (Katz et al, 1982; Beck et al, 1985; Wallace et al, 1988).

Subjects exhibiting root caries had an average of 3.7 affected surfaces. Primary root caries lesions were found to be almost six times as prevalent as secondary root lesions (Beck et al, 1985). Mandibular molars have been shown to be the most frequently involved teeth with a decreasing susceptibility for premolars and incisors. In the maxillary arch, the anterior teeth have higher prevalence rates than do the posterior teeth (Katz et al,1982, Stamm et al, unpublished). Although these investigators found uniformity in terms of the exposure of surfaces for each tooth type, the widely variable root caries rates suggest that specific, local, intra-oral factors may determine the pattern of root caries attack.

Considerable controversy has arisen regarding the tooth root surfaces most frequently affected by root caries. The evidence suggests that either facial or approximal surfaces are usually affected followed by lingual surfaces (Sumney et al, 1973; Banting et al, 1980). A similar pattern is observed when tooth groupings are considered (Hix and

O'Leary, 1976). Katz et al (1982) compared the relative likelihood of different tooth root surfaces becoming carious by tooth type. The buccal surface of the mandibular molar, for instance, is twice as likely to demonstrate root caries than are the lingual or proximal surfaces of the same tooth. The lingual surface of the maxillary molar is five times more susceptible than its buccal surface. Longitudinal studies of root caries incidence support the observations of the cross-sectional surveys with respect to surface distribution (Banting et al, 1985).

Studies of the incidence rates of root caries are limited to six diverse populations. Furthermore, the methods of reporting incidence were not uniform (Table 2). The Vipeholm Dental Caries Study showed that the incidence of cemental caries in the oldest experimental group (greater than 37 years of age) was 0.51 lesions per person per year. Ravald et al (1986) found an incidence rate of 4.24 per cent after four years and 4.95 per cent after eight years in 31 patients being treated for advanced periodontal disease. Banting et al (1985) followed 45 chronically ill, hospitalized patients for a 34 month period and observed an incidence rate of 1.9 lesions per 100 person-months at risk or 0.25 lesions per person-year. The largest group followed over time was 451 elderly persons living in rural Iowa. A mean of 0.36 new root caries lesions were observed per person per year or 1.8 per 100 susceptible surfaces per year (Hand et al, 1988b).

1.5 CHARACTERISTICS OF PERSON, PLACE AND TIME

Virtually all of the cross-sectional surveys have demonstrated an increased prevalence of root caries with advancing age. Prevalence rates for root caries, including root fillings, range between 3-14 per cent for subjects aged 20-29 years of age, 9-36 per cent for subjects aged 30-39 years of age, 15-47 per cent for subjects 40-49 years of age, 22-64 per cent for subjects 50-59 years of age and 38-70 per cent for subjects over 60 years of age (Table 1). The wide range of observed rates can be partially explained by factors such as fluoridation status, health, institutionalization, culture and definition of root caries. A direct, positive association of root caries prevalence and age is also observed when the prevalence rate is calculated using only teeth with gingival recession in the denominator. (Katz et al, 1982; Wagg, 1984; Katz et al, 1985; Wallace et al, 1988; Stamm et al, unpublished).

Males have higher prevalence rates of root caries than do females for all 10 year age groups from 30 to 60 years and over (Sumney et al, 1973; Vehkalahti et al, 1983). However, when prevalence rates are calculated using only "at risk" surfaces, the sex difference disappears (Katz et al, 1982; Donner and Donald, 1988).

Only two sets of data are available which permit a direct

comparison of the prevalence rate of root caries between countries (Hazen et al, 1972; Vehkalahti et al, 1983). The occurrence of this disease is much lower in Finland than in North America. This may be due to differences in cultural background or, more likely, the criteria used to determine the presence of root caries.

There are no data available which relate occupation and socio-economic status to root caries experience. There are, however, special population groups which exhibit exaggerated prevalence rates of root caries. One common characteristic among these high risk groups is their reduced ability and/or interest in maintaining oral hygiene at a level commonly accepted as being associated with the prevention of progressive periodontal disease.

1.6 PREDICTORS OF ROOT CARIES

Several statistical models have been used to explain the variation in root caries prevalence in different population groups. Banting et al (1980) used stepwise multiple regression to determine the influence of age, sex, number of retained teeth, number of retained roots and decayed and filled coronal surfaces on the prevalence of decayed and filled root surfaces. The only significant partial regression coefficient was related to age and only 9 per cent of the variability of decayed and filled root surfaces

could be explained by those particular variables. A discriminant function analysis was also performed on the same data. The number of retained teeth, decayed and filled coronal surfaces and age had statistically significant ($p<0.05$) discriminant function coefficients and could correctly classify people with root caries experience and those without 83 per cent of the time.

Stepwise multiple regression procedures were also used by Kitamura et al (1986) to test the relative effects of selected predictor variables. The variables with significant partial regression coefficients associated with root caries experience in the populations observed, using the Root Caries Index as the outcome measure, were the number of teeth and the presence of calculus. These two variables accounted for 32 per cent of the variance observed in root caries. The other four variables measured were domicile, oral hygiene, medications causing dry mouth and number of medications taken daily. These four variables explained an additional four per cent of the variation.

The dental component of the Mini-Finland Health Survey examined 5,028 adults aged 30 years and over. A log linear model was used to define the importance of certain demographic and clinical factors relative to the prevalence of root caries (Vehkalahti, 1987). Age, sex, region of domicile, number of teeth and depth of periodontal pockets were defined as the predictor variables. Positive

associations were found between root caries occurrence and age, region and periodontal condition. However, interactions occurred among the variables such that root caries occurrence could not be determined reliably using each of the factors alone.

Burt et al (1986) developed a logistic regression model to examine potentially confounding effects among variables they measured and the presence or absence of root caries in subjects residing in communities with different levels of fluoride in the water supply. The dependent variable was dichotomized into subjects with or without root caries and the model included fluoride level, age, sex, ethnicity, education, number of teeth with recession, presence of plaque, presence of subgingival calculus and loss of periodontal attachment as predictor variables. The fluoride level, age, years of education, mean number of teeth with recession and mean loss of periodontal attachment were statistically significant predictors of root caries ($p<0.05$). Similar results were obtained with a linear regression model using the Root Caries Index as the dependent variable.

Beck et al (1988) employed multivariate analysis to identify potential risk factors for root caries in a cohort of non-institutionalized, older adults. A large number of independent variables gathered through personal interviews and clinical examinations were organized into

physical/medical, social, behavioural, psychological and other dental conditions subsets. Multiple linear regression models were constructed separately for males and females with age and number of teeth with gingival recession forced into the equation. Factors that were not significantly related to root caries prevalence were removed from the model. The regression equation for males explained 33 per cent of the variance in root caries with other dental conditions (number of teeth present and number of teeth with coronal caries) producing the largest partial regression coefficients. The regression model for females accounted for 51 per cent of the variance in root caries. The variables with the largest effects were negative life events and the number of teeth with coronal caries. Regression models were also constructed using a dichotomized dependent variable (presence or absence of root decay). These models explained about the same amount of the variance for males but less of the variance for females and the variables which made the greatest contribution remained unchanged in both equations.

Studies on the incidence of root caries have also attempted to identify predictor variables. Ellen et al (1985) sampled the dental plaque overlying non-carious roots of teeth and compared the isolation frequency and recovery of two microorganisms, Streptococcus mutans and Lactobacillus, with the initiation of root caries over a 34 month period. The presence of both Streptococcus mutans and Lactobacillus together was the best discriminator between subjects who

experienced new root caries and subjects who remained root caries-free. More than 80 per cent of root surfaces with both Streptococcus mutans and Lactobacillus present at the first plaque sampling session were in subjects who subsequently developed root caries. The relative risk of root caries was found to be 0.88 when only Lactobacillus was present, 3.38 when only Streptococcus mutans was present and 4.96 when both were present.

Kohout et al (1987) applied multiple regression procedures to root caries incidence data derived from 447 older adult subjects followed for 18 months. Because sex was found to interact with many other variables, separate sex-specific regression equations were developed. The subject's age, number of root surfaces at risk, prior caries experience, fluoridation history and examiner effects were controlled and three classes of other variables were entered into the equation. For males, the number of pockets, gingival bleeding , physical stress, angina, fingernail and toe problems, social contacts, anxiety and use of smokeless tobacco were all significant ($p<0.05$) effects and explained 60 per cent of the variance of root caries. For females, number of pockets, amount of saliva, physical stress, phlegm production and social participation were significant predictor variables accounting for 40 per cent of the root caries variation.

Root caries development was assessed in 31 patients who had

been treated for periodontal disease eight years earlier (Ravald et al, 1986) . Differences between groups of subjects who developed root caries during that time and those who did not were measured for salivary Lactobacillus counts, salivary Streptococcus mutans counts, plaque scores, salivary secretion rate, salivary buffer rate, oral sugar clearance time, dietary habits and age. Salivary counts of Streptococcus mutans and Lactobacillus, plaque score and dietary habits differed significantly between groups of subjects who had developed no root caries and those who developed root caries on more than 5 per cent of the root surfaces at risk. A positive correlation was found between dental caries scores at the time of treatment and the incidence of new root caries. At the four year examination, only the Lactobacillus counts differed significantly between the two groups (Ravald and Hamp, 1981).

CHAPTER 2

METHODS

2.1 AIMS

The general aim of this study was to examine the association between the presence of selected oral microorganisms and the incidence of dental root caries. In order to accomplish this, a population at high risk of root caries was selected and followed for a two year period to determine the number and location of new root caries lesions. A variable number of tooth roots were observed in each subject and the dental plaque from these roots was harvested and analyzed throughout the study period. Statistical associations were determined between the presence of the microorganisms and the incidence of root caries taking into account covariates such as age, sex and snacking habits. The analysis used both the subject and the tooth root as the unit of analysis in order to determine whether the presence of the microorganism was associated with the incidence and the rate of root caries.

2.2 STUDY POPULATION

The target population for this study was all residents, 25

years of age and over, of Parkwood Hospital, London, Ontario who had at least one natural tooth remaining. Parkwood Hospital is a continuing care and rehabilitation centre providing a wide spectrum of health services, including dental care, to patients with chronic medical disorders. The capacity of the hospital was 187 beds at that time and the average length of stay was 444 days.

This population was selected because dental examinations, which are conducted as part of routine patient care, indicated a high prevalence of root caries. This clinical impression was confirmed by the results of a prevalence survey which revealed that the average age was 67.9 years and residents had an average of 16.1 remaining teeth. Fifty-three per cent of the residents displayed visible gingival recession and were, therefore, considered to be at risk of root caries. Root caries were found in 83 per cent of the residents (Banting et al, 1980). The high level of root caries experience was a good indication that new lesions would occur within an observation period of two or three years.

The prolonged length of stay of most patients facilitated the repeated measurements required for the study. Since the patients were accustomed to routine dental visits, a high response rate was anticipated with good compliance. Mouth care was similar for all patients because a standardized procedure is performed as part of daily nursing care. The

diet was common for all patients except for food brought to them by friends and relatives.

All patients who had at least one natural tooth remaining with visible gingival recession associated with an intact root surface were identified through the records of the dental department. An informed consent to participate was received from each patient and his/her attending physician. If a patient had several retained natural teeth with intact, supragingival root surfaces, the surfaces to be observed were selected according to a priority list which reflected a descending risk of root caries as determined by clinical experience with chronically ill, hospitalized, older adults.

Because of a limited capacity to process and analyze the microbiological samples, no more than eight surfaces were selected for study in any one subject. Wherever possible, contralateral pairs of surfaces were selected. New subjects meeting the inclusion criteria were added throughout the first year of the study to augment the sample size. Thereafter, no new subjects were added.

2.3 CLINICAL EXAMINATIONS

All examinations of the study subjects took place in the dental clinic of Parkwood Hospital. Clinical examinations were performed upon entry into the study and at 12, 20, 24,

28, 32 and 34 months (Figure 1). The frequency of clinical examinations was increased after the first year to coincide with the tooth root plaque sampling sessions.

The clinical examination consisted of:

(1) subject identification,

(2) soft tissue assessment,

(3) scaling and polishing of teeth, and

(4) caries examination.

The soft tissue assessment included the Gingival Index (Loe and Silness, 1963) and measurements, using a millimeter probe, of the distance of the periodontal attachment from the cemento-enamel junction, the amount of gingival recession measured from the cemento-enamel junction to the crest of the gingiva and the distance from the crest of the gingiva to the apical border of the carious root lesion.

Before the caries examination each subject was given a thorough scaling by an experienced dental hygienist using a Cavitron 1010 and hand instruments. This was followed by a rubber cup prophylaxis using Zircate polishing compound. Decayed, missing (due to caries) and filled coronal and root tooth surfaces were recorded only upon entry into the study. New root caries were recorded as present if a discrete, well-defined and discoloured cavitation existed on the tooth root surface, the explorer entered the cavity easily and displayed some resistance to withdrawal and the lesion was

located either at the cemento-enamel junction or wholly on the root surface. All soft tissue and dental caries examinations were performed by the same examiner.

2.4 ROOT PLAQUE SAMPLING AND PROCESSING

Samples of dental plaque adhering to the tooth root surfaces selected for observation were taken approximately every four months during the study (Figure 1). The root plaque was sampled by the same person using a standard procedure throughout the study. Samples were taken with the edge of a sterile, disposable, long-point blood lancet held by its foil wrapper. Two consecutive sweeps tangent to the midline of the tooth root surface from the gingival margin to the cemento-enamel junction were made on each study surface. The entire lancet was then immediately immersed in 10 millitres of reduced transport fluid (RTF). Each plaque sample was coded and delivered to a microbiology team which dispersed the sample by sonication for 10 seconds under a flow of oxygen-free gas, serially diluted it in RTF and plated it on several media.

2.5 CULTURE MEDIA

Mitis salivarius agar (MSA) was used to cultivate Streptococcus mutans and Streptococcus sanguis. Actinomyces

viscosus and Actinomyces naeslundii were cultivated on a partially-selective medium (CNAC-20) (Ellen and Balcerzak-Raczkowski, 1975) with 150 micrograms of sodium fluoride per millitre. Lactobacilli were also cultivated on a selective agar medium (Rogosa SL) (Rogosa et al, 1951). The total cultivable flora was estimated from growth on modified sucrose blood agar (MM10) (Syed and Loesche, 1972,1973).

All media were purchased in single lots (Difco Laboratories, Detroit, Michigan), prepared in advance of the plaque sampling sessions and incubated for a few days to identify any contamination. Plates with bacterial growth were discarded. Media requiring subsequent anaerobic incubation were stored in an oxygen-free environment until plated with the diluted plaque sample.

MM10 plates were incubated anaerobically for seven days. MSA plates were incubated for two days anaerobically followed by one day in air at room temperature. CNAC-20/NaF plates were incubated for two days anaerobically followed by two days in air with 10 per cent carbon dioxide. The Rogosa SL agar was incubated anaerobically for four days.

One type of culture medium was assigned to each member of the microbiology team for enumeration throughout the study in order to limit observer variation. Enumeration was accomplished by colony morphology on the selective media

only when identification was unequivical. All questionable colonies were subcultured, purified and then identified. Oral streptococci were identified by colony morphology on MSA as Streptococcus mutans, Streptococcus sanquis, Streptococcus salivarius or other. Questionable isolates or isolates chosen for routine verification were identified according to Hardie and Bowden (1976) with the additional criterion of adherence to glass in the presence of sucrose as confirmation of Streptococcus mutans. Actinomyces viscosus and Actinomyces naeslundii colonies cultivated on CNAC-20/NaF medium were identified by a short identification scheme consisting of a combination of a catalase test and serological agglutination with whole cell sera against Actinomyces viscosus (cluster 1) and Actinomyces naeslundii (cluster 5) (Fillery et al, 1978). Gas-liquid chromatography and paper chromatography were used to confirm that strains failing to react serologically were likely neither Actinomyces viscosus nor Actinomyces naeslundii. Lactobacilli were identified to genus level by colony morphology on Rogosa agar and confirmed by Gram stain smears.

2.6 MEASUREMENT OF MICROORGANISMS

The percentage of the total cultivable flora represented by Streptococcus mutans, Streptococcus sanquis, Lactobacillus, Actinomyces viscosus and Actinomyces naeslundii was derived

by dividing the number of colonies formed by the individual genera and species on the selective media by the number of colonies formed by all microorganisms harvested from the same plaque sample on the non-selective MM10 agar. Because of differences in cultivation efficiency of the various media used, the percentages of the total cultivable flora are not comparable between bacterial types.

2.7 RESPONSE VARIABLE

The response variable in this study was the occurrence of root caries. Since from one to eight tooth root surfaces were observed in each subject, a subject could have more than one response. Regardless of the number of tooth roots which experienced caries in a mouth, a subject was classified as either having root caries or not.

2.8 RISK FACTORS

Subjects were characterized according to the presence or absence of five microorganisms which are considered to be associated with the development of root caries. If the dental plaque taken from any one tooth root in a subject contained the particular microorganism (as determined by the techniques described in section 2.4), the subject was classified as being exposed. This definition of exposure is

analagous to that used when stimulated whole saliva is collected to determine the presence of a particular microorganism but is more sensitive in that the microorganisms contained in the plaque must have come from at least one of the tooth roots under observation (Ravald et al, 1986; Jordan et al, 1987; Keltjens et al, 1987).

Normally, subjects are characterized at baseline as being exposed or not exposed. In this case, the microbiological determination used to define exposure was not done until the sixth month following baseline. However, we assumed that what was observed at this time represented the baseline condition, a reasonable assumption since the microorganisms comprising dental plaque are quite stable over the long term and the plaque processed at this session represented mature plaque (undisturbed by professional cleaning for at least 5 months).

2.9 OBSERVATION PERIOD

Although the study lasted 34 months from the time the first subject was entered until the final examination was completed and multiple clinical examinations occurred, we decided to use two fixed observation periods, twelve and twenty-four months, rather than a variable observation period. Fixed observation periods are used more frequently in dental research thus allowing direct comparisons to be

made with other studies. In addition, no new caries occurred after the 24 month examination so to extend the follow-up period longer than that was unnecessary.

2.10 PREDICTOR VARIABLES

Several variables reported to be associated with root caries prevalence or incidence or directly related to the risk variables were also recorded. The subject's age and sex, the number of retained teeth and roots, previous coronal and root caries experience, between-meal snacking patterns, type of diet and medications taken which adversely affect the flow rate of saliva were recorded upon entry into the study. In addition to the periodontal variables mentioned in section 2.2, antibiotic and anti-infective drugs which were prescribed during the month of the plaque sampling session or the previous month and which could affect the microorganisms under study were recorded.

2.11 DATA ANALYSIS

Studies on dental caries have traditionally used the patient as the unit of analysis. Mainland (1963) correctly points out that individual teeth in any mouth are not independent and that treating them as replicate measurements amounts to counting the same thing over again and can produce

misleading results. Imrey (1986) agrees that teeth within the same mouth can not be considered as independent because of host factors influencing all teeth, such as immune response, diet and nutrition, saliva production, composition and distribution, the indiginous microflora , oral hygiene practices, concomitant disease and other factors. Therefore, conventional statistical methods which assume that the observations are independent can not be directly applied to the analysis of individual teeth (or sites) within a mouth.

The outcome measurement in dental caries studies is a carious lesion or a filling which is assumed to have been placed to treat a carious lesion. The number of decayed and filled teeth represents the prevalence or incidence of the disease depending on the whether the outcome is recorded at a certain time or over a period of time. Based on the discussion above, the number of persons with and without dental caries would be the proper way to express information on this outcome. However, expressing dental caries in this way does not take advantage of all of the available information and dental researchers usually express dental caries prevalence as the number of decayed, missing and filled teeth per person. Unfortunately, the number of teeth at risk in each mouth is almost never reported.

Although teeth (or sites) within a mouth are unquestionably affected by host factors which are more similar within a mouth than between mouths, there is also considerable

variation in the local environment surrounding a tooth or a group of teeth that would render that tooth (or site) more or less susceptible to disease or more or less responsive to treatment. The fact that all teeth in a mouth are not equally at risk of dental caries appears to depend on the characteristics and dynamics of the dental plaque attached to the root surfaces, such as the numbers and types of microorganisms, salivary flow rate, plaque pH , and the calcium/phosphorus ratio.

In this study the unit of observation is the tooth or tooth surface because only one surface was observed on each tooth. This is considered to be appropriate because the hypothesis attempts to relate root caries rates to the presence of a specific microorganism. The subject, however, remains the unit of analysis and exposure is defined at that level.

When exposure is defined on a subject basis, and when the unit of analysis is the subject, the standard statistical tests for association are appropriate and the statistical test used is determined by the type of data and whether the observation period is fixed or variable. However, when there are multiple observations within a subject and the unit of observation is the site rather than the subject, standard statistical tests are invalid and procedures for analyzing clustered measurements must be employed (Donald and Donner, 1987).

The first step in the data analysis for this study examines a single risk factor using the subject as the unit of observation and a fixed observation period. Root caries was considered to be present if a subject experienced one or more decayed tooth root surfaces during the observation period specified. The effect of being exposed was measured by cross-tabulating the response variable (root caries) with the risk factor (Streptococcus mutans, Streptococcus sanguis, Actinomyces viscocus, Actinomyces naeslundii and Lactobacillus). Pearson's chi-square statistic was used to determine statistical significance and the odds ratio was used to measure the degree of association between the response and the risk factor (Fleiss, 1981).

The measurement of the effect of being exposed to a single risk factor was then calculated using the tooth root surface (site) as the unit of observation. However, when observations within subjects are correlated as they are in this study, the use of the standard Pearson chi-square statistic is invalid because it is biased. The amount of bias depends on the number of measurements per subject and the correlation among these measurements. When the correlation is positive, the application of the standard chi-square test could lead to spurious statistical significance. Therefore, an adjusted chi-square statistic was employed in this analysis to determine statistical significance (Donald and Donner, 1987; Donner and Banting, 1988). The odds ratio remains a valid measure of the

degree of association between the response and a risk factor in the presence of clustering (Donald and Donner, 1987).

Because this study is a non-randomized comparison, there may be major dissimilarities between the outcome groups regarding the predictor variables. Subjects who experienced one or more root caries during the study were compared with those who remained caries free for each of the predictor variables measured. An imbalance (confounding) was considered to be present if the p-value associated with a statistical test was equal to or less than 0.10. In such instances, multivariate statistical techniques were used to control for these differences.

In the subject-specific analysis, the multivariate technique used to estimate the effect of being exposed adjusting for confounding variables was multiple logistic regression (logit analysis). This approach assumes that the logarithm of the odds of an event is a linear combination of the risk factor (exposure) and predictor variables (Anderson et al, 1980). The coefficient associated with the risk factor is the natural logarithm of the estimated odds ratio associated with exposure. The coefficients associated with the predictor variables provide a measure of the effect of those variables on the log odds of experiencing the outcome. Dividing any of the coefficients by its standard error provides a test statistic similar to the t-statistic which can be used to test the hypothesis that the

coefficient equals zero.

Risk factors and predictor variables were entered into a multivariate model if the p-value associated with the univariate test statistic of group differences for the factor or variable was approximately 0.10 or less. Interaction terms were generated only if the first order coefficients associated with the risk factor or predictor variables were statistically significant.

The risk factor was first entered into the model alone to verify that the odds ratio was identical to that computed using univariate analysis. Other potentially confounding variables were then added. The appropriateness of the resulting model was tested using a goodness-of-fit chi-square statistic. High p-values indicate that the logistic model fits the data.

For this study, a p-value of less than 0.05 was considered to be statistically significant. Since the chi-square value could be biased upwards if the expected cell frequencies were small, the Fisher exact test was used to determine probability using the subject-specific analysis (Zar, 1984). An odds ratio of 1.5 or greater was considered to be clinically significant.

CHAPTER 3

RESULTS

3.1 ENTRY AND LOSS OF STUDY SUBJECTS

Figure 1 illustrates chronologically when subjects entered and were lost from the study. Forty-five subjects entered the study in August of the first year. During the next six months, seven new subjects were added and, at the 12 month point, four more.

Nine subjects died before a second clinical examination was conducted and, therefore , only baseline information is available for these people. One subject was examined at all clinical examinations but would not provide a root plaque sample and one subject joined the study late in the first year but died soon after the second examination resulting in an observation period of only three months. These eleven subjects were considered to be drop-outs and are not included in the results because the information they supply is minimal. The drop-out rate was 20 per cent.

Forty-five subjects contributing 150 tooth root surfaces were observed for 12 months. Four subjects were followed for only 12 months and one subject died following the 12 month examination but before the 24 month examination. These

five subjects were lost to follow-up and, therefore, not included in the 24 month observation period. Forty subjects contributing 135 tooth root surfaces were observed for 24 months.

Study subjects contributed from one to eight root surfaces for observation with a mean of 3.3 root surfaces. These surfaces were all caries-free at the baseline clinical examination and were considered to be susceptible to attack by dental caries.

3.2 INCIDENCE OF ROOT CARIES

Twelve of the 45 subjects observed for 12 months experienced root caries. Five subjects had two new lesions and seven subjects had one each. Thirteen of the 40 subjects observed for 24 months experienced root caries. One subject had three new lesions, seven subjects had two and five subjects developed one each. The incidence rate of root caries in subjects was 0.27 after 12 months and 0.33 over 24 months. The root caries incidence rate for surfaces after 12 and 24 months was 0.11 and 0.16 respectively (Table 3). Root caries occurred most frequently on the proximal (mesial and distal) surfaces.

3.3 EXPOSURE

The isolation frequency and mean proportion of the total number of colony forming units (CFU) are presented in Table 4 for each of the microoganisms under study. Although these microorganisms could be isolated in 22 to 93 per cent of the subjects using the selective media, they represented only a small proportion of all microorganisms cultivated from the plaque samples on a non-selective medium. Actinomyces viscosus comprised the largest mean proportion of CFU at 0.08. For the 45 subjects and the 150 tooth root surfaces the isolation frequencies and mean proportions of the total CFU were remarkably similar with the values for the surfaces being just slightly larger than those for the subjects.

3.4 UNIVARIATE EFFECT OF EXPOSURE TO A RISK FACTOR USING THE SUBJECT AS THE UNIT OF OBSERVATION

Tables 5 and 6 show the association of the five microorganisms with the incidence of root caries using the subject as the unit of observation and observation periods of 12 and 24 months. This represents the conventional method of univariate analysis using dichotomous measures.

Fifty per cent of subjects in whom Streptococcus mutans was isolated developed one or more root caries by the twelve

month examination compared with 20 per cent of subjects who did not have the microorganism present in their tooth root plaque (Table 5). This difference was clinically significant with an odds ratio of 4.0. After 24 months, the proportion of subjects with one or more root caries was 75 per cent and 22 per cent for the subjects with and without Streptococcus mutans respectively (Table 6). The risk (incidence) attributable to Streptococcus mutans was 0.53.

There were no statistically or clinically significant associations detected between Streptococcus sanquis, Actinomyces viscosus or Actinomyces naeslundii and the occurrence of root caries after either 12 or 24 months (Tables 5 and 6).

Forty-two per cent of subjects with Lactobacillus present experienced root caries after 24 months compared with 29 per cent of subjects without this microorganism. Although this difference was not statistically significant, the degree of association was moderately strong (Table 6).

3.5 UNIVARIATE EFFECT OF EXPOSURE TO A RISK FACTOR USING THE SITE AS THE UNIT OF OBSERVATION

The test of a statistical association between the risk factors and the response variable was recalculated using the tooth as the unit of observation and taking into account the

fact that teeth within the same mouth were likely to be more similar than teeth from different mouths (Tables 7 and 8).

For all of the microorganisms and for both subjects with and without the microorganism, the proportion of root surfaces with caries was lower than the proportion of subjects with root caries. The likelihood that sites within the same subject would respond similarily is measured by the intraclass correlation coefficient which was found to range from 0.38 to 0.53.

The results using the site as the unit of observation were similar to those using the subject as the unit of observation. The degree of association between the presence of Streptococcus mutans and root caries after 12 months was clinically important but this association was not statistically different from 1.0. The presence of Streptococcus sanguis was found to be negatively associated with root caries and this association was statistically significant (Table 7). After 24 months, a statistically significant association was only observed between the presence of Streptococcus sanguis and root caries incidence although the odds ratio associated with Streptococcus mutans was clinically significant (Table 8).

The values of the Pearson and adjusted chi-square statistics and their associated p-values are included in Tables 7 and 8 in order to illustrate the effect of the adjustment for the

lack of independence of sites within the same subject. After 12 months, the association between the presence of Streptococcus sanquis and root caries incidence was statistically significant for both chi-square values but the statistically significant association between Actinomyces naeslundii and root caries disappeared when the adjusted chi-square was used (Table 7). A similar situation existed after 24 months of observation for this microorganism and for Streptococcus mutans (Table 8). The use of the Pearson chi-square however, is invalid when there are multiple sites observed within the same mouth.

3.6 PREDICTOR VARIABLES

Appendix A displays the frequency distributions and descriptive statistics for the response variables, risk factors and predictor variables measured in this study. Whenever appropriate, separate summaries are presented for surfaces and subjects. When repeated measurements were made on the same subject the values relating to subjects represent the average of all measurements. Unless otherwise specified, the variables were measured upon entry of the subject into the study.

Three continuous predictor variables, age, decayed and filled coronal surfaces and decayed and filled root surfaces, were reorganized into dichotomous categories and

five discrete predictor variables, number of between-meal snacks/day and week, type of diet, medications affecting saliva and antimicrobial drugs, were dichotomized to provide fewer categories. These changes are presented in Appendix B.

The characteristics defined by quantitative predictor variables of subjects who did and did not develop root caries after 12 and 24 months are presented in Tables 9 and 10 respectively. At 12 months, none of the variables was found to differ in any meaningful way between the groups. At 24 months, the number of decayed and filled coronal surfaces, gingival pocket depth and gingival recession had p-values close to 0.10 (Table 10). These variables are potential confounders which may exaggerate the effect of exposure to a risk factor because of their association with root caries.

The characteristics defined by qualitative predictor variables of subjects who developed root caries after 12 and 24 months are presented in Tables 11 and 12 respectively. After 12 months, one or more medications which affect the flow rate of saliva were taken by one third of the subjects with new root caries (Table 11). This was the only qualitative predictor variable found to be significant among the subjects observed for twelve months. After 24 months, 45 per cent of subjects with new root caries consumed one or more between-meal snacks each week (Table

12). This difference was statistically significant. These predictor variables are, therefore, also considered to be potential confounders of the association between the risk factor and the outcome.

3.7 MULTIVARIATE EFFECT OF A RISK FACTOR USING THE SUBJECT AS THE UNIT OF OBSERVATION

For risk factors found to have a statistically significant association with the outcome variable as ascertained by univariate analysis, the degree of association was verified by entering each risk factor as the only independent variable in a multiple logistic model. For Streptococcus mutans at 12 and 24 months, the exponents of the logistic coefficients were precisely equivalent to the odds ratios computed using univariate statistics.

The results of multiple logistic analysis using all predictor variables that satisfied the selection criteria outlined in the methods section are presented in Tables 13 and 14. After 12 months, the odds ratio for root caries associated with the presence of Streptococcus mutans decreased from 4.0 to 2.7 when only subjects who were on medications which adversely affected the salivary flow rate were considered (Table 13). No subjects developed root caries who were not taking such medications. The logistic coefficient for Streptococcus mutans was not statistically

significant for those subjects.

After 24 months, the multiple logistic model contained four predictor variables in addition to the risk factor, Streptococcus mutans. The odds ratio of the association of root caries and the presence of Streptococcus mutans increased from 9.3 to 22.1 when these predictor variables were considered (Table 14). The logistic coefficients for Streptococcus mutans, the number of decayed and filled coronal surfaces, one or more between-meal snacks each week and mean gingival pocket depth were all positive indicating that the chances of getting root caries increases with higher values of these variables. The effect of the mean gingival recession score is in the opposite direction. Only the effect of Streptococcus mutans was statistically significant. Expanding the logistic model to include a second, clinically significant risk factor, Lactobacillus, resulted in an interaction between Streptococcus mutans and Lactobacillus. Separate logistic models were then created to allow for the presence or absence of Lactobacillus. For subjects without Lactobacillus, the odds ratio associated with the presence of Streptococcus mutans decreased to 6.3. However, for subjects with Lactobacillus present the odds ratio increased to 24.0.

3.8 SITES AND EXPOSURE

An association between the presence of a risk factor and an outcome does not provide any information with regard to whether all sites within the same subject are at risk and whether there is a relationship between the number of sites with the risk factor present and the occurrence of the outcome in a subject.

In this study, the maximum number of root surfaces with Streptococcus mutans present in any subject was two. After 24 months, all of the subjects in this category developed root caries. Sixty per cent of subjects with one infected root and 22 per cent with no infected roots developed caries. The proportion of subjects experiencing root caries increased with an increasing number of root surfaces colonized by the microorganism. A chi-square test for trend produced a statistically significant result ($p=0.05$) (Fleiss, 1981). A test of the hypothesis of a linear association revealed that there was no convincing evidence against the presence of a linear relationship (Table 15).

3.9 SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUE

Since plaque samples were taken on several successive occasions from the same tooth root is was possible to

determine the probability that a tooth root, once colonized by a particular microorganism, would continue to remain colonized. Table 16 shows the distribution of four successive plaque samplings taken over a nine month period for Streptococcus mutans. Only those subjects for whom four plaque samplings were taken are included.

Table 17 presents the sensitivity, specificity and positive predictive value calculations for the microbiological test used to determine exposure to Streptococcus mutans for subjects at 24 months. The positive predictive value of the MSA culture system (i.e. the probability of root caries if Streptococcus mutans were cultured) using a single plaque sample was 0.75. Repeating the test and redefining exposure as two consecutive positive readings can affect the properties of a test (Fleiss, 1981). When this was done, the sensitivity was reduced to .38, the specificity was increased to .96 and the positive predictive value of the procedure was increased to .83. The likelihood ratio for a single positive culture of Streptococcus mutans test was 6.6 and the odds that a positive test would be expected in a subject with root caries increased to 9.5 when two consecutive positive Streptococcus mutans cultures were present.

CHAPTER 4

DISCUSSION

4.1 THE HYPOTHESIS

Within high risk subjects, the number of tooth roots exposed to the risk factor may represent only a portion of all teeth present. Some roots may be at higher risk than others and the number of tooth roots at risk varies widely across subjects. Furthermore, teeth are not biologically independent. The hypothesis recognizes these possibilities and proposes to determine firstly whether the proportion of subjects with at least one root caries is the same in both exposure groups and then whether the exposed and non-exposed subjects differ in the proportion of sites which proceed to a diseased state.

4.2 STUDY DESIGN

The objective of this project was to determine whether certain microorganisms are associated with the incidence of dental root caries. It was designed as a comparative, prospective (follow-up) study in which members of a high-risk group were characterized by the presence or absence of

the microorganism and then followed over time to determine what proportion of subjects in each of the two groups developed the disease (Fleiss, 1981).

At the time it was begun (1976), this was the first study to investigate the relationship between root caries and several oral microorganisms (Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, Actinomyces naeslundii and Lactobacillus) which have been shown to be associated with the disease in cross-sectional studies. One other prospective investigation was in progress at that time but it was limited to only one microorganism, Lactobacillus (Ravald and Hamp, 1981). In the second four year period of that study, Streptococcus mutans was cultured from tooth roots (Ravald et al, 1986). This study, therefore, is one of only two follow-up studies of the association of root caries incidence with the presence of specific oral microorganisms.

Characteristics of both the subject (age, sex, previous caries experience, diet and salivary flow rate) and the tooth root (gingival recession, gingival pocket depth and gingival inflammation) have been identified as contributing to the root caries process. Interpreting the role that these factors play in addition to the primary etiological agent was a secondary objective of this study.

The original study design specified that a cohort of

subjects would enter the study initially and be re-examined at 12 month intervals thereafter. The plaque sampling sessions were scheduled to take place three months following the initial clinical examination (in order to allow the oral flora to return to normal) and approximately every four months thereafter. Unfortunately, there were processing problems encountered at the microbiology laboratory and the results of the first plaque sampling session could not be used. The next plaque sampling session was held three months later and represents the best estimate of the microbiological situation at the time of entry into the study.

In order to supplement the sample size, subjects were added to the study throughout the first 12 months. Although it was feasible to conduct a clinical examination at any time, the plaque sampling sessions could only be scheduled at specific intervals because of the time required to process and enumerate the plaque samples and the effort required to gather both equipment and manpower together to set up the remote laboratory. Therefore, the time period between the initial clinical examination and the first plaque sampling session ranged from two to six months.

A critical assumption in this study is that the microorganisms cultivated from the first plaque sample after the initial clinical examination were representative of the microorganisms present at the time of the examination. The

validity of this assumption for one of the microorganisms, Streptococcus mutans, was tested by analysing four successive plaque samples. The majority (66 per cent) of the surfaces had the same reading on all four occasions and 76 per cent had the same reading on three of the four occasions. The assumption was considered to be valid based on these findings.

4.3 THE ASSESSMENT OF RISK

It is now universally accepted that a tooth root must have direct contact with the oral environment in order to be at risk of the dental caries process. For practical purposes this means that saliva and dental plaque must be able to adhere directly to the surface of the root. From an epidemiological standpoint, any tooth which displays gingival recession to a point where the crest of the gingival tissue is apical to the cemento-enamel junction is deemed to be at risk (Katz,1980). This is a functional definition which ignores the possibility that a tooth root is at risk before the recession proceeds to the point where the cemento-enamel junction can be clinically visualized.

Exposure was defined in this study as the presence of a specific microorganism on one or more tooth roots within a subject. This definition was chosen because sites within a mouth can not be treated independently and because

screening and diagnostic tests and preventive procedures are performed on subjects rather than individual teeth.

Once a subject is considered to be at risk of root caries, the incidence rate increases depending on the number of tooth roots exposed to the risk factor. The incidence rate of root caries in the population observed in this study was 0.22 after 24 months when Streptococcus mutans was not present. This would represent the "background" incidence for roots with gingival recession and it is influenced by the large number of false-negative determinations of exposure. When Streptococcus mutans was present in the plaque taken from at least one tooth root in a subject, the incidence rate of root caries increased to 0.60 and when the microorganism was present on two roots, the rate was 1.00. The possibility of a linear relationship between the presence of the risk factor on more than one root in the same subject and the outcome provides added weight to the likelihood that the risk factor measured caused the disease.

If a simple microbiological test could be developed which could be economically applied to every tooth root with recession, the proportion of tooth roots colonized by a particular microorganism considered to be the risk factor might be a more useful diagnostic measure of exposure. Intuitively, at least, when proportionately more root surfaces are colonized by a particular microorganism

considered to be essential for the disease, the subject is at higher risk.

A further consideration regarding exposure is whether a tooth root, once exposed, continues to remain exposed. The results of this study indicate that this is likely to be the case.

There may be other important implications as well. When selecting high risk populations for intervention studies, the presence of Streptococcus mutans on at least one root surface with gingival recession would contribute to a more efficient study. Conversely, inclusion of a large proportion of subjects with non-infected tooth roots could create a swamping effect requiring a much larger sample size.

4.4 MEASUREMENT ERROR

The single plaque sample used to define exposure resulted in some misclassification (false-positive and false-negative) errors which complicate the determination of exposure in an uncontrolled follow-up study (Imrey, 1986; O'Neill,1986). Since the incidence of root caries in this study was relatively high, the ability of the test to predict disease was quite respectable. With a lower rate of root caries the positive predictive value would also be reduced (Fletcher et

al, 1982). Multiple testing may not be feasible for mass screening purposes; however, repeating the plaque samples and redefining exposure as two consecutive positive readings reduced the rate of false-positives and is recommended for future research studies on the etiology of root caries.

Appendix C illustrates the effect of changing the definition of exposure. Requiring two positive cultures in order to consider Streptococcus mutans as being present resulted in an adjusted chi-square statistic of 1.9 with a p-value of 0.16 at 24 months. The associated odds ratio was 3.21. Redefining exposure to reduce the false-positive rate, therefore, resulted in a modest gain in the strength of the statistical association.

One explanation for the results observed when the subject was the unit of observation differing (in terms of statistical significance) from those observed when the site was the unit of observation may be missclassification of subjects by exposure category. The false-negative rate was high (0.54). Therefore, many subjects who were classified as not being exposed actually acquired root caries. This resulted in a high incidence of the disease in unexposed subjects. When the site was used as the unit of observation, this may have resulted in a difference too small to detect with the sample size employed.

4.5 STATISTICAL APPROACH

There are two levels of experimental unit used in this study: the subject and the tooth root(s) in that subject. Since teeth within the same mouth are influenced largely by the same host factors, salivary production, composition and distribution, dietary and oral hygiene habits, oral microflora, immune response and disease states, they will tend to demonstrate similar behaviour (Imrey, 1986). Associated sites within a mouth do not present any analytical problem when the subject is the unit of observation. Usually, sites within subjects are summarized using measures such as the presence or absence of an outcome or the total number or the average number of sites in each subject exhibiting the outcome. This approach is indisputably valid and is most commonly employed.

However, when the site is chosen as the unit of observation, ignoring the lack of biological and, therefore, statistical independence of sites within the same mouth underestimates the statistical significance (p-value) of a treatment effect estimator (Blomqvist, 1985; Laster, 1985). The amount of underestimation depends on the number of sites per subject and the correlation among those sites. The formula:

$$1-(1/\sqrt{1+(k-1)\,rho}) \times 100$$

estimates the per cent of underestimation where k is the average number of sites in a mouth and rho is the intraclass

correlation coefficient. The intraclass correlation coefficient may be estimated from an analysis of variance table by:

$$rho = (MSB-MSW)/((MSB+(m-1)MSW))$$

where MSB is the mean square between clusters, MSW is the mean square within clusters and m is the mean cluster size (Donald and Donner, 1987). Increasing the number of sites in a subject and/or the existence of a high correlation among sites enhances the error considerably. When rho = 1 the degrees of freedom are equal to the number of sites whereas when rho = 0 the degrees of freedom corresponds to the number of subjects. In this study the intraclass correlation coefficients ranged from 0.38 to 0.53 and the mean number of sites per subject was 3.3. The amount of underestimation of the p-values associated with the test statistic would, therefore, be approximately 27 to 33 per cent if the clustering effect of sites within a mouth is not considered.

One might expect the true p-value of the univariate statistical test to lie somewhere between the value determined using the subject and that using the site within the subject as the unit of observation. This reasoning, however, assumes a mathematical connection between the two p-values which were derived in quite different ways. Actually there is no known systematic relationship between the p-values produced using the subject as the unit of observation and those produced using the site. Two different units of observation are being employed in an attempt to

test two completely different hypotheses. For the subject, the hypothesis states that the proportion of subjects with at least one root cavity is the same in both exposure groups whereas for the site, the hypothesis states that the root caries rate is the same in both exposure groups.

The adjusted chi-square procedure is not necessarily appropriate in all situations where the outcome variable is dichotomous and there are repeated observations. A Monte Carlo simulation study revealed that the adjusted chi-square test provides valid significance levels (within +.02 or -.02 of the true p-value) over a wide range of parameter values provided the number of subjects per group is greater than ten and the ratio of the number of subjects per group to the average number of sites per subject exceeds 2 (Donner and Banting, 1988). In this study, the minimum number of subjects per group was eight and the ratio of subjects to the average number of sites per subject was 3.0 and 10.6 for the exposed and non-exposed groups respectively. The p-values derived from the adjusted chi-square statistic should, therefore, be valid.

4.6 THE UNIT OF OBSERVATION

The odds ratio measures the degree of association between two variables and is a function of the cell proportions in a 2x2 table. When the site was used as the unit of

observation the odds ratios were generally lower than when the subject was used.

The chi-square statistic measures the statistical significance of an association between two variables and is not only a function of cell proportions but also the total number of units. A larger sample size, assuming the same joint proportions, would result in a larger chi-square value when the subject is the unit of analysis and increases the statistical power. A similar effect would be expected when the site is the unit of observation. Although increasing the number of sites will provide more information, the gain in power depends on the value of rho, the intraclass correlation coefficient. The adjusted chi-square statistic is not as responsive to increases in the number of sites as the Pearson chi-square would be to the number of subjects unless the intraclass correlation coefficient approaches zero.

4.7 THE OBSERVATION PERIOD

Fixed observation periods of 12 and 24 months were used as a matter of convention and convenience. However, the data could have been analyzed using a life table approach which is frequently employed when several four-fold tables are used. The Mantel-Haenszel procedure estimates a common odds ratio over all four-fold tables and tests the overall degree

of association between the risk factor and the outcome (Fleiss, 1981). Appendix D presents the results of the Mantel-Haenszel procedure applied to Streptococcus mutans. For subjects, there was a statistically significant overall association with an estimated common odds ratio of 5.87. For sites, an adjustment similar to the type used for the chi-square (Donner and Banting, in press) was used. The adjusted Mantel-Haenszel chi-square was not able to detect an overall association and estimated the common odds ratio to be 2.91. This result was essentially the same as that found using the 24 month fixed observation period.

4.8 INCIDENCE OF ROOT CARIES

It is interesting to note that the incidence rate of 0.33 root lesions per person over a 24 month period found in this study among a high risk population is almost identical to the incidence rate reported for a comparatively healthy, ambulatory group of older adults followed for 36 months (Hand et al, 1988b). The use of different diagnostic criteria for root caries, the different number of examiners employed and the interpretation of why restorations were placed may explain why the subjects in this study did not have higher incidence rates than did a non-institutionalized population of approximately the same age. Furthermore, the subjects in this study received routine dental care throughout the 24 month observation period and were given a

thorough scaling and professional prophylaxis after each clinical examination. This unusually high level of dental care could, and likely did, interfere with the repopulation of the root surfaces by the microorganisms resulting in lower rates of root caries.

4.9 THE ROLE OF MICROORGANISMS IN ROOT CARIES INCIDENCE

The association between Streptococcus mutans and root caries found in this study supports previous reports (Sumney and Jordan, 1974; Syed et al, 1975; Ravald et al,1986; Emilson et al, 1987; Fure et al,1987 and Keltjens et al, 1987) which used the subject as the unit of observation. Reports which used the site as the unit of analysis without allowing for the lack of independence of teeth within mouths are clearly statistically invalid (Billings et al,1985; Brown et al, 1986 and Keltjens et al, 1987) and the data should be reanalyzed using the adjusted chi-square or a summary outcome measure weighted by the number of teeth per subject (Imrey, 1986). On the subject level, the presence of Streptococcus mutans on the tooth root is undeniably associated with root caries and is quite likely to be an important causative agent.

The role of Lactobacillus in the root caries process is unclear although detection in high numbers is considered to be indicative of favourable conditions for root caries. The

lack of a statistically significant association found in this study after 24 months was most likely the result of an inadequate sample size. A statistically significant association was found by Ravald et al (1986) and Fure et al (1987) for this microorganism with the incidence of root caries. Lactobacillus is not considered to be a primary causative agent because it is slow growing and produces relatively weak acid through metabolism but its presence is indicative of suitable local conditions for root caries to occur.

Higher proportions of Streptococcus sanguis, Actinomyces viscosus and Actinomyces naeslundii were found on the sound compared with decayed root surfaces. The difference for Streptococcus sanguis was statistically significant after both 12 and 24 months using the tooth root as the unit of observation but there was no difference using the subject as the unit of observation. Keltjens et al (1987), Fure et al (1987) and Brown et al (1986) also found no statistically significant difference in the number of these microorganisms colonizing sound and either hard or soft carious surfaces using the subject as the unit of observation. When the tooth surface was used as the unit of observation, a significant difference was observed by Keltjens et al (1987) and Emilson et al (1987) for Streptococcus sanguis and by Brown et al (1986) for Actinomyces viscosus. Unfortunately the p-values found in these studies were biased downward because the effect of clustering was not controlled. It is unlikely

that these microorganisms are directly involved in root caries initiation and Streptococcus sanguis may play a protective role.

Two important questions remain unanswered with regard to Streptococcus mutans as the primary causative agent in root caries. Why did subjects not infected with the microorganism get root caries? After 24 months, seven (22 per cent) subjects who were not exposed to the microorganism experienced root caries. Repeating the test and redefining exposure as two consecutive positive results resulted in about the same false-negative rate. Ellen et al (1980) reported that the plating efficiency for Streptococcus mutans can be enhanced using a more selective plating medium (mitis salivarius agar with sucrose and bacitracin) and that the use of a selective broth (M-broth) resulted in even higher rates of recovery. It is possible, therefore, that Streptococcus mutans was present in some or all of the seven subjects identified by the differential plating medium used in this study (mitis salivarius agar) as not being exposed.

The second question relates to the magnitude of an infective dose of the microorganism. Although other studies, as well as this study, counted the number of Streptococcus mutans colony forming units in a millilitre of plaque serially diluted in reduced transport fluid, the minimun number associated with root caries was not determined. Comparing the counts of these microorganisms on the selective medium

is hazardous because the weight and volume of the plaque samples vary despite attempts to standardize the collection and enumeration process.

4.10 PREDICTOR VARIABLES

The multiple logistic regression models using the subject as the unit of observation did not identify any of the predictor variables measured in this study as being important determinants of root caries. Beck (1988) was able to classify correctly 77 per cent of men who experienced one or more root caries after 18 months using the following predictor variables: 23 or more teeth, 9 or fewer teeth, number of surfaces with recession, number of periodontal pockets greater than three millimeters, number of teeth with root fillings and six other socio-medical variables. Dental predictors accounted for 39 per cent of the variance and the complete eleven variable equation accounted for 48 per cent of the variance. Unfortunately, the presence of oral microorganisms was not measured and a direct comparison of the two studies can not be made.

After 8 years of follow-up, Ravald et al (1986) found that the only predictor variable other than Streptococcus mutans and Lactobacillus which demonstrated a statistically significant difference between subjects with and without new root caries was the dietary habit index which was similar to

the number of between-meal snacks per week variable used in this study; but the latter variable was not significant.

There is no consensus at the present time regarding the predictor variables influencing root caries incidence. Age and sex, which are frequently implicated in prevalence studies, would not appear to be as relevant when incidence is considered. Pocket depth or the presence of a periodontal pocket of 3 millimeters or more is the only predictor variable which has been shown to play a significant role in the root caries process (Beck, 1988).

4.11 FUTURE DIRECTIONS

There are many possibilities for future research which are suggested by this study. The most obvious one is a replication of this study on a high risk population using a larger sample. High risk subjects would be those with at least one tooth root colonized by Streptococcus mutans. The definition of exposure might be altered to include at least two positive readings from successive plaque samples thus lowering the rate of occurrence of false-positives. The rate of false-negative readings could be further minimized by using the more selective mitis salivarius agar plates with sucrose and bacitracin and/or the M-broth.

It would be useful to confirm the finding that the risk of

root caries increases with the number of root surfaces infected with Streptococcus mutans and determine whether there is a relationship between a salivary Streptococcus mutans count and the total count from dental plaque taken from all tooth root surfaces with gingival recession. The confirmation of a dose-response relative to the number of tooth roots which are colonized by Streptococcus mutans and the identification of a correlation between salivary and tooth root plaque counts would allow the determination of exposure to be made more simply from a saliva sample and provide a means of ranking subjects based on risk. Alternatively, a hierarchy of exposure categories could be developed based on the number or proportion of root surfaces colonized by the microorganism and the hypothesis would test the likelihood of an outcome associated with the degree of exposure.

The adjusted chi-square analysis used in this study represents an improvement over the conventional Pearson chi-square statistic for intergroup comparisons of dichotomous outcomes by estimating an average variance inflation factor for each group and using it to reduce the value of the chi-square. The probability model giving rise to the adjusted chi-square statistic assumes that the correlation between the responses on any two sites within the same mouth is constant. The intraclass correlation coefficient measures concordance among sites but there may be considerable variation within a mouth especially when the

number of sites is large. More elaborate models are required if it can be demonstrated that the within-mouth correlation of responses between pairs of sites is variable. Imrey (1986) suggests using probability models based on "contagious" distributions or correlation structures based on spatial proximity. A similar type of cluster sampling adjustment also needs to be developed for larger contingency tables and inter-group comparisons of continuous outcome data. Imrey (1986) has also made several suggestions regarding the analysis of continuous data when there is lack of independence.

The sample size requirements for future studies involving the site as the unit of observation deserves the final comment. The required sample size for each group can be estimated using the traditional sample size formula for comparing two proportions and multiplying the result by 1+(k-1)rho where k equals the average number of observations per subject and rho is the intraclass correlation coefficient.

CHAPTER 5

CONCLUSIONS

Based on the results of this study the following conclusions can be made:

1. the incidence rate of root caries in forty-five subjects after 12 months was .27 per subject,

2. the incidence rate of root caries in forty subjects after 24 months was .33 per subject,

3. the risk (incidence) attributed to the presence of Streptococcus mutans after 24 months was 0.53 using the subject as the unit of observation,

4. a statistically significant, but inverse association between the presence of Streptococcus sanguis and the incidence of root caries was evident after 12 and 24 months of follow-up using the tooth root as the unit of observation and adjusting for the lack of independence of teeth within the same mouth,

5. none of the predictor variables used in this study, including the periodontal measurements made a statistically significant contribution to the

multivariate logistic regression model although weekly snacking patterns showed a strong association with root caries incidence,

6. the sensitivity and specificity of a single positive culture on MSA to determine the presence or absence of Streptococcus mutans were 0.46 and 0.93 respectively and the positive predictive value of the test was 0.75, and

7. there is a positive, and possibly a linear, relationship between the number of tooth roots colonized by Streptococcus mutans in a subject and the probability of root caries.

The data support the hypothesis that a greater proportion of subjects with root caries is associated with exposure to Streptococcus mutans but the rate of root caries was not found to differ among exposed and non-exposed individuals. However, the proportion of roots which became decayed was significantly lower in subjects with Streptococcus sanguis present on one or more tooth root surfaces.

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TABLE 1 - PREVALENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	AGE (YEARS)	% PERSONS WITH AT LEAST ONE DECAYED OR FILLED ROOT SURFACE	ROOT CARIES INDEX	MEAN NUMBER LESIONS** PER PERSON
Hazen et al, 1972	Insurance company employees, USA	500	20-29 30-39 40-49 50-59 60+	14 26 46 60 70	n/a	n/a
Sumney et al, 1973	Coast Guard base residents USA	172	30-39 40-49 50-59	36 47 58	n/a	0.9 1.0 1.7
	Veterans' hospital patients and staff, USA	135	30-39 40-49 50-59	27 47 64	n/a	1.4 1.4 1.1
Schamschula et al, 1974	Tribesmen, New Guinea	222	18-29 30-39 40+	n/a	n/a	1.7 6.4 6.4
El-Hadary et al, 1975	Dental school patients	220	30-39 40-49 50-59	35 46 55	n/a	n/a
Hix and O'Leary, 1976	Treated periodontal patients	120	39 or less 40-49 50-59 60+	39 43 51 42	n/a	n/a
Lohse et al, 1977	Military Personnel, USA	281	20-29 30-39 40-49 50-59 60+	4 11 28 22 38	n/a	n/a

cont'd

TABLE 1 - PREVALENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	AGE (YEARS)	% PERSONS WITH AT LEAST ONE DECAYED OR FILLED ROOT SURFACE	ROOT CARIES INDEX	MEAN NUMBER LESIONS PER PERSON
Banting et al, 1980	Chronic hospital patients Canada	59	36-89	83	n/a	7.6
Katz et al, 1982	Insurance company employees USA †	473	20-29	42	1.1*	0.2
			30-39		4.7	0.6
			40-49		13.0	1.9
			50-59		22.0	3.0
			60-64		17.2	3.4
Stamm et al, (unpublished)	Non-fluoridated community, Canada	465	20-29	9	n/a	n/a
			30-39	32		
			40-49	40		
			50-59	52		
			60+	67		
	Naturally fluoridated community, Canada	502	20-29	3	n/a	n/a
			30-39	15		
			40-49	25		
			50-59	37		
			60+	48		
Vehkalahti et al, 1983	General population, Finland	5028	30-39	9	n/a	n/a
			40-49	15		
			50-59	22		
			60-69	24		
			70+	30		
Fejerskov et al, 1985	Dental school patients, Denmark	91	65+	100	n/a	n/a
Katz et al, 1985	Private dental patients, USA	3361	20-79	-	15.0*	n/a

cont'd

TABLE 1 - PREVALENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	AGE (YEARS)	% PERSONS WITH AT LEAST ONE DECAYED OR FILLED ROOT SURFACE	ROOT CARIES INDEX	MEAN NUMBER LESIONS PER PERSON
Beck et al, 1985	Rural residents, USA	520	65-69 70-74 75-79 80+	63	n/a	0.6
Forgay et al, 1986	Senior citizen volunteers, Canada	141	52-90	43	15.2	n/a
Burt et al, 1986	Fluoridated community (0.7 mg/L), USA	151	27-40 41-50 51-65	11.5 34.8 55.6	6.7	0.15 1.35 1.61
	Fluoridated community (3.6 mg/L) USA	164	27-40 41-50 51-65	3.7 5.4 15.2	1.2	0.04 0.05 0.17
Kitamura et al, 1986	Community residents, USA	24	55-95	n/a	17.7*	n/a
	Nursing home residents USA	23				
Gustavaen et al, 1988	Adult dental patients, Norway	2839	20-29 - - - - 70+	n/a	12.1* 19.3 18.3 23.6 25.5 24.7	1.2*** 3.9 4.9 8.8 9.4 8.1
Wallace et al, 1988	Community residents, USA	603	60-64 65-69 70-74 75-79 80+	70	7.3 9.0 9.1 7.8 6.8	n/a

cont'd

TABLE 1 - PREVALENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	AGE (YEARS)	% PERSONS WITH AT LEAST ONE DECAYED OR FILLED ROOT SURFACE	ROOT CARIES INDEX	MEAN NUMBER LESIONS PER PERSON
U.S. Department of Health and Human Resources, 1987	Employed adults, U.S.A.	20,818	18,19	7		0.1
			20--24	6		0.2
			25-29	9		0.3
			30-34	14		0.4
			35-39	18		0.3
			40-44	25	n/a	0.5
			45-49	33		0.6
			50-54	42		0.6
			55-59	43		0.6
			60-64	54		1.0
	Senior adults, U.S.A.	5,686	65-69	64		1.3
			70-74	65	n/a	1.5
			75-79	71		1.5
			80+	63		1.8

n/a not available
* surfaces
** each decayed surface is counted as a lesion
† This is the same population as observed by Hazen et al.
*** df surfaces per person

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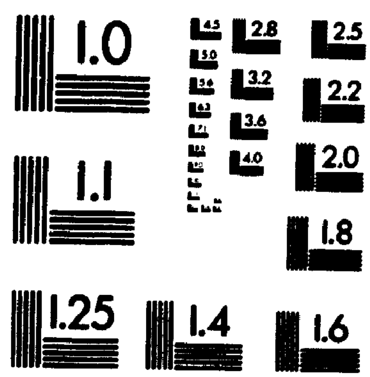


TABLE 2 - INCIDENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	STUDY DURATION	AGE (YEARS)	PROPORTION OF SUBJECTS WITH NEW ROOT CARIES	INCIDENCE RATE(S)
Gustaffson et al, 1953	Mentally deficient, institution-alized	436	5 years	37+	n/a	0.51/ person/ period
Ravald and Hamp, 1981	Patients treated for advanced periodontal disease, Sweden	31	4 years	42-81		4.24% of surfaces at risk
Banting et al, 1985	Hospitalized, chronically-ill patients, Canada	45	34 months	36-89	36%	a) 1.9/100 person-months b) 6.3/1000 surface-months c) 0.25/ person-year
Ravald et al, 1986	Patients treated for advanced periodontal disease, Sweden	31	8 years	42-81	74%	4.95% of surfaces at risk 1980-84
Emilson et al, 1988	Periodontal patients, Sweden	35	3 years	33-74	69%	4.5% of surfaces at risk

TABLE 2 - INCIDENCE OF ROOT CARIES IN CONTEMPORARY ADULT POPULATIONS

INVESTIGATOR AND YEAR	POPULATION OBSERVED	NUMBER OF SUBJECTS	STUDY DURATION	AGE (YEARS)	PROPORTION OF SUBJECTS WITH NEW ROOT CARIES	INCIDENCE RATE(S)
Hand et al, 1988a	Rural residents, USA	451	18 months	65+	44%	a) 2.6/100 surfaces /year b) .57/ person-year
Hand et al, 1988b	Rural residents, USA	338	36 months	65+	44%	a) 1.8/100 surfaces /year b) .36/ person-year
Ripa et al, 1988	Employees, placebo rinse, USA	350	36 months	18-65		0.12/person-year
	Employees, fluoride rinse, USA	381	36 months		n/a	0.14/person-year

TABLE 3 - NUMBER AND PROPORTION OF SUBJECTS AND SURFACES WITH NEW ROOT CARIES, 12 AND 24 MONTHS

NUMBER (PROPORTION) WITH ROOT CARIES		
	12 MONTHS	24 MONTHS
SUBJECTS	12 (0.27)	13 (0.33)
SURFACES	17 (0.11)	22 (0.16)

TABLE 4 - ISOLATION FREQUENCIES AND MEAN PROPORTION OF THE TOTAL CULTIVABLE FLORA OF MICROORGANISMS

MICROORGANISM	PROPORTION PRESENT		MEAN PROPORTION OF TOTAL CFU	
	SUBJECT	SURFACE	SUBJECT	SURFACE
STREPTOCOCCUS MUTANS	0.22	0.25	<0.01 (0.00)*	<0.01 (0.00)
STREPTOCOCCUS SANGUIS	0.78	0.85	<0.01 (0.04)	<0.01 (0.04)
ACTINOMYCES VISCOSUS	0.93	0.96	0.08 (0.08)	0.08 (0.12)
ACTINOMYCES NAESLUNDII	0.40	0.45	0.01 (0.05)	0.02 (0.04)
LACTOBACILLUS	0.31	0.41	0.00 (0.01)	0.00 (0.00)

* standard deviation of the mean

TABLE 5 - INCIDENCE OF ROOT CARIES ASSOCIATED WITH THE PRESENCE OF SELECTED MICROORGANISMS, 12 MONTHS, USING THE SUBJECT AS THE UNIT OF OBSERVATION

MICROORGANISM	PRESENCE OR ABSENCE	NUMBER OF SUBJECTS	NUMBER (PROPORTION) OF SUBJECTS WITH ONE OR MORE ROOT CARIES	PEARSON CHI-SQUARE (1df)	FISHER EXACT p-VALUE	ODDS RATIO
STREPTOCOCCUS MUTANS	PRESENT	10	5 (0.50)	3.58	0.10	4.00
	ABSENT	35	7 (0.20)			
STREPTOCOCCUS SANGUIS	PRESENT	35	8 (0.23)	1.17	0.42	0.44
	ABSENT	10	4 (0.40)			
ACTINOMYCES VISCOSUS	PRESENT	42	11 (0.26)	0.07	1.00	0.71
	ABSENT	3	1 (0.33)			
ACTINOMYCES NAESLUNDII	PRESENT	17	2 (0.12)	2.08	0.27	0.30
	ABSENT	26	8 (0.31)			
LACTOBACILLUS	PRESENT	14	4 (0.29)	0.04	1.00	1.15
	ABSENT	31	8 (0.26)			

TABLE 6 - INCIDENCE OF ROOT CARIES ASSOCIATED WITH THE PRESENCE OF SELECTED MICROORGANISMS, 24 MONTHS, USING THE SUBJECT AS THE UNIT OF OBSERVATION

MICROORGANISM	PRESENCE OR ABSENCE	NUMBER OF SUBJECTS	NUMBER (PROPORTION) OF SUBJECTS WITH ONE OR MORE ROOT CARIES	PEARSON CHI-SQUARE (1df)	FISHER EXACT p-VALUE	ODDS RATIO
STREPTOCOCCUS MUTANS	PRESENT	8	6 (0.75)	8.23	0.008	10.71
	ABSENT	32	7 (0.22)			
STREPTOCOCCUS SANGUIS	PRESENT	31	9 (0.29)	0.76	0.44	0.51
	ABSENT	9	4 (0.44)			
ACTINOMYCES VISCOSUS	PRESENT	37	12 (0.32)	0.001	1.00	1.44
	ABSENT	3	1 (0.33)			
ACTINOMYCES NAESLUNDII	PRESENT	15	2 (0.13)	2.94	0.15	0.24
	ABSENT	23	9 (0.39)			
LACTOBACILLUS	PRESENT	12	5 (0.42)	0.66	0.48	1.79
	ABSENT	28	8 (0.29)			

TABLE 7 - INCIDENCE OF ROOT CARIES ASSOCIATED WITH THE PRESENCE OF SELECTED MICROORGANISMS, 12 MONTHS, USING THE SITE AS THE UNIT OF OBSERVATION

MICROORGANISM	PRESENCE OR ABSENCE	NUMBER OF SITES	NUMBER (PROPORTION) OF SITES WITH ROOT CARIES	INTRACLASS CORRELATION COEFFICIENT	PEARSON CHI-SQUARE (1df)	ADJUSTED CHI-SQUARE (1df)	ODDS RATIO
STREPTOCOCCUS MUTANS	PRESENT	37	6 (0.16)	0.48	1.17	0.43	1.79
	ABSENT	113	11 (0.10)		(p=.28)	(p=.51)	
STREPTOCOCCUS SANGUIS	PRESENT	128	10 (0.08)	0.44	10.77	6.11	0.18
	ABSENT	22	7 (0.32)		(p=.001)	(p=.013)	
ACTINOMYCES VISCOSUS	PRESENT	144	16 (0.11)	0.48	0.18	0.12	0.63
	ABSENT	6	1 (0.17)		(p=.67)	(p=.73)	
ACTINOMYCES NAESLUNDII	PRESENT	66	3 (0.05)	0.38	3.45	1.59	0.30
	ABSENT	81	11 (0.17)		(p=.06)	(p=.21)	
LACTOBACILLUS	PRESENT	61	5 (0.08)	0.48	1.01	0.40	0.57
	ABSENT	89	12 (0.13)		(p=.31)	(p=.53)	

TABLE 8 - INCIDENCE OF ROOT CARIES ASSOCIATED WITH THE PRESENCE OF SELECTED MICROORGANISMS, 24 MONTHS, USING THE SITE AS THE UNIT OF OBSERVATION

MICROORGANISM	PRESENCE OR ABSENCE	NUMBER OF SUBJECTS	NUMBER OF SITES	NUMBER (PROPORTION) OF SITES WITH ROOT CARIES	INTRACLASS CORRELATION COEFFICIENT	PEARSON CHI-SQUARE (1df)	ADJUSTED CHI-SQUARE (1df)	ODDS RATIO
STREPTOCOCCUS MUTANS	PRESENT	8	31	9 (0.29)	0.51	4.79	1.63	2.86
	ABSENT	32	104	13 (0.13)		(p=.03)	(p=.20)	
STREPTOCOCCUS SANGUIS	PRESENT	31	116	15 (0.13)	0.50	6.84	3.74	0.23
	ABSENT	9	19	7 (0.37)		(p=.009)	(p=.05)	
ACTINOMYCES VISCOSUS	PRESENT	37	129	20 (0.16)	0.52	1.34	0.86	0.37
	ABSENT	3	6	2 (0.33)		(p=.25)	(p=.35)	
ACTINOMYCES NAESLUNDII	PRESENT	15	58	4 (0.07)	0.45	4.72	1.93	0.29
	ABSENT	23	74	15 (0.20)		(p=.03)	(p=.16)	
LACTOBACILLUS	PRESENT	12	53	8 (0.15)	0.53	0.09	0.40	0.86
	ABSENT	28	82	14 (0.17)		(p=.76)	(p=.86)	

TABLE 9 MEAN VALUES FOR QUANTITATIVE PREDICTOR VARIABLES FOR SUBJECTS WITH AND WITHOUT NEW ROOT CARIES, 12 MONTHS

VARIABLE	SUBJECTS WITH ROOT CARIES (N=12)	SUBJECTS WITHOUT ROOT CARIES (N=33)	T-STATISTIC	D.F.	P-VALUE
AGE	67.6 (18.4)*	69.0 (13.5)	.25	15.5	.81
NUMBER OF TEETH	18.1 (9.0)	17.1 (5.9)	.36	14.6	.73
NUMBER OF RETAINED ROOTS	0.5 (1.7)	0.4 (1.0)	.26	13.5	.80
NUMBER OF DECAYED AND FILLED CORONAL SURFACES	12.6 (10.2)	17.2 (11.3)	1.31	21.4	.21
NUMBER OF DECAYED AND FILLED ROOT SURFACES	7.3 (9.2)	5.4 (6.3)	.69	14.9	.51
GINGIVAL POCKET DEPTH (MM)	3.3 (1.3)	2.6 (1.0)	1.49	15.3	.16
GINGIVAL RECESSION (MM)	2.1 (0.9)	1.7 (0.7)	1.43	16.1	.17
% GINGIVITIS SCORES >2	0.6 (0.6)	0.4 (0.5)	1.11	18.1	.28

* standard deviation of the mean

TABLE 10 MEAN VALUES FOR QUANTITATIVE PREDICTOR VARIABLES FOR SUBJECTS WITH AND WITHOUT NEW ROOT CARIES, 24 MONTHS

VARIABLE	SUBJECTS WITH ROOT CARIES (N=13)	SUBJECTS WITHOUT ROOT CARIES (N=27)	T-STATISTIC	D.F.	P-VALUE
AGE	69.1 (18.3)*	68.0 (13.7)	0.19	18.7	.85
NUMBER OF TEETH	17.4 (9.1)	17.7 (5.8)	0.10	16.9	.92
NUMBER OF RETAINED ROOTS	0.5 (1.7)	0.4 (0.4)	0.11	16.7	.92
NUMBER OF DECAYED AND FILLED CORONAL SURFACES	11.0 (9.0)	18.9 (10.8)	2.44	28.3	.02
NUMBER OF DECAYED AND FILLED ROOT SURFACES	7.4 (9.2)	5.7 (6.5)	0.59	18.0	.56
GINGIVAL POCKET DEPTH (MM)	3.3 (1.4)	2.6 (0.9)	1.66	17.2	.12
GINGIVAL RECESSION (MM)	2.2 (1.1)	1.7 (0.6)	1.70	15.8	.11
% GINGIVITIS SCORES >2	0.5 (0.6)	0.4 (0.5)	0.99	22.2	.33

* standard deviation of the mean

TABLE 11 CHARACTERISTICS OF SUBJECTS WITH NEW ROOT CARIES, QUALITATIVE PREDICTOR VARIABLES, 12 MONTHS

CHARACTERISTICS	PERCENT WITH ROOT CARIES	FISHER EXACT TEST p-value
SEX		
MALE	38.5	
FEMALE	21.9	.29
PREVIOUS CORONAL CARIES EXPERIENCE		
YES	27.9	
NO	0.0	1.00
PREVIOUS ROOT CARIES EXPERIENCE		
YES	29.0	
NO	14.3	1.00
NUMBER OF BETWEEN-MEAL SNACKS/DAY		
NONE	20.0	
ONE OR MORE	25.0	.72
NUMBER OF BETWEEN-MEAL SNACKS/WEEK		
NONE	11.1	
ONE OR MORE	30.4	.25
TYPE OF DIET		
REGULAR	11.6	
OTHER	28.0	.27
MEDICATIONS AFFECTING SALIVA		
NONE	0.0	
ONE OR MORE	33.3	.04
ANTIMICROBIAL DRUG		
YES	25.7	
NO	20.0	1.00

TABLE 12 CHARACTERISTICS OF SUBJECTS WITH NEW ROOT CARIES, QUALITATIVE PREDICTOR VARIABLES, 24 MONTHS

CHARACTERISTICS	PERCENT WITH ROOT CARIES	FISHER EXACT TEST p-value
SEX		
MALE	33.3	
FEMALE	32.1	1.00
PREVIOUS CORONAL CARIES EXPERIENCE		
YES	30.8	
NO	100.0	.33
PREVIOUS ROOT CARIES EXPERIENCE		
YES	100.0	
NO	30.8	.33
NUMBER OF BETWEEN-MEAL SNACKS/DAY		
NONE	20.8	
ONE OR MORE	46.2	.14
NUMBER OF BETWEEN-MEAL SNACKS/WEEK		
NONE	11.8	
ONE OR MORE	45.0	.04
TYPE OF DIET		
REGULAR	14.3	
OTHER	37.5	.16
MEDICATIONS AFFECTING SALIVA		
NONE	8.3	
ONE OR MORE	33.3	.18
ANTIMICROBIAL DRUG		
YES	32.3	
NO	20.0	1.00

TABLE 13 MULTIPLE LOGISTIC REGRESSION MODEL FOR THE INCIDENCE OF ROOT CARIES, 12 MONTHS, USING THE SUBJECT AS THE UNIT OF OBSERVATION FOR SUBJECTS TAKING MEDICATIONS AFFECTING THE SALIVARY FLOW RATE.

FACTOR	MEASUREMENT	LOGISTIC COEFFICIENT	STANDARD ERROR	Z STATISTIC
CONSTANT		0.51	1.00	0.51E-17
STREPTOCOCCUS MUTANS	1 = ABSENT 2 = PRESENT	0.98	1.21	0.81

NUMBER OF SUBJECTS = 15

LOG LIKELIHOOD RATIO = -9.22

GOODNESS OF FIT X^2 = 0.66 d.f. = 1 p = 0.42

ESTIMATED ODDS RATIO FOR RISK FACTOR = 2.67

TABLE 14 MULTIPLE LOGISTIC REGRESSION MODEL FOR THE INCIDENCE OF ROOT CARIES, 24 MONTHS, USING THE SUBJECT AS THE UNIT OF OBSERVATION

FACTOR	MEASUREMENT	LOGISTIC COEFFICIENT	STANDARD ERROR	Z STATISTIC
CONSTANT		-2.72	2.35	-1.15
STREPTOCOCCUS MUTANS	1 = ABSENT 2 = PRESENT	3.10	1.36	2.27
NUMBER OF DECAYED AND FILLED CORONAL SURFACES		0.05	0.05	0.86
NUMBER OF BETWEEN MEAL SNACKS/WEEK	1 = NONE 2 = ONE OR MORE	2.23	1.18	1.90
GINGIVAL POCKET DEPTH	M.M.	1.06	0.83	1.27
GINGIVAL RECESSION	M.M.	-1.79	1.31	-1.37

NUMBER OF SUBJECTS = 37

LOG LIKELIHOOD RATIO = -14.21

GOODNESS OF FIT X^2 = 16.62 d.f. = 5 p = .005

ESTIMATED ODDS RATIO FOR RISK FACTOR = 22.09

TABLE 15 - NUMBER AND PROPORTION OF SUBJECTS WITH NEW ROOT CARIES, 24 MONTHS, BY NUMBER OF SURFACES WITH STREPTOCOCCUS MUTANS PRESENT

NUMBER OF SURFACES WITH STREPTOCOCCUS MUTANS	NUMBER OF SUBJECTS	NUMBER AND PROPORTION WITH ROOT CARIES
0	32	7 (0.22)
1	5	3 (0.60)
2	3	3 (1.00)

OVERALL PEARSON $X^2 = 4.83$ d.f. = 2 p = .09

SLOPE $X^2 = 3.95$ d.f. = 1 p = .05

LINEAR $X^2 = 0.92$ d.f. = 1 p = .34

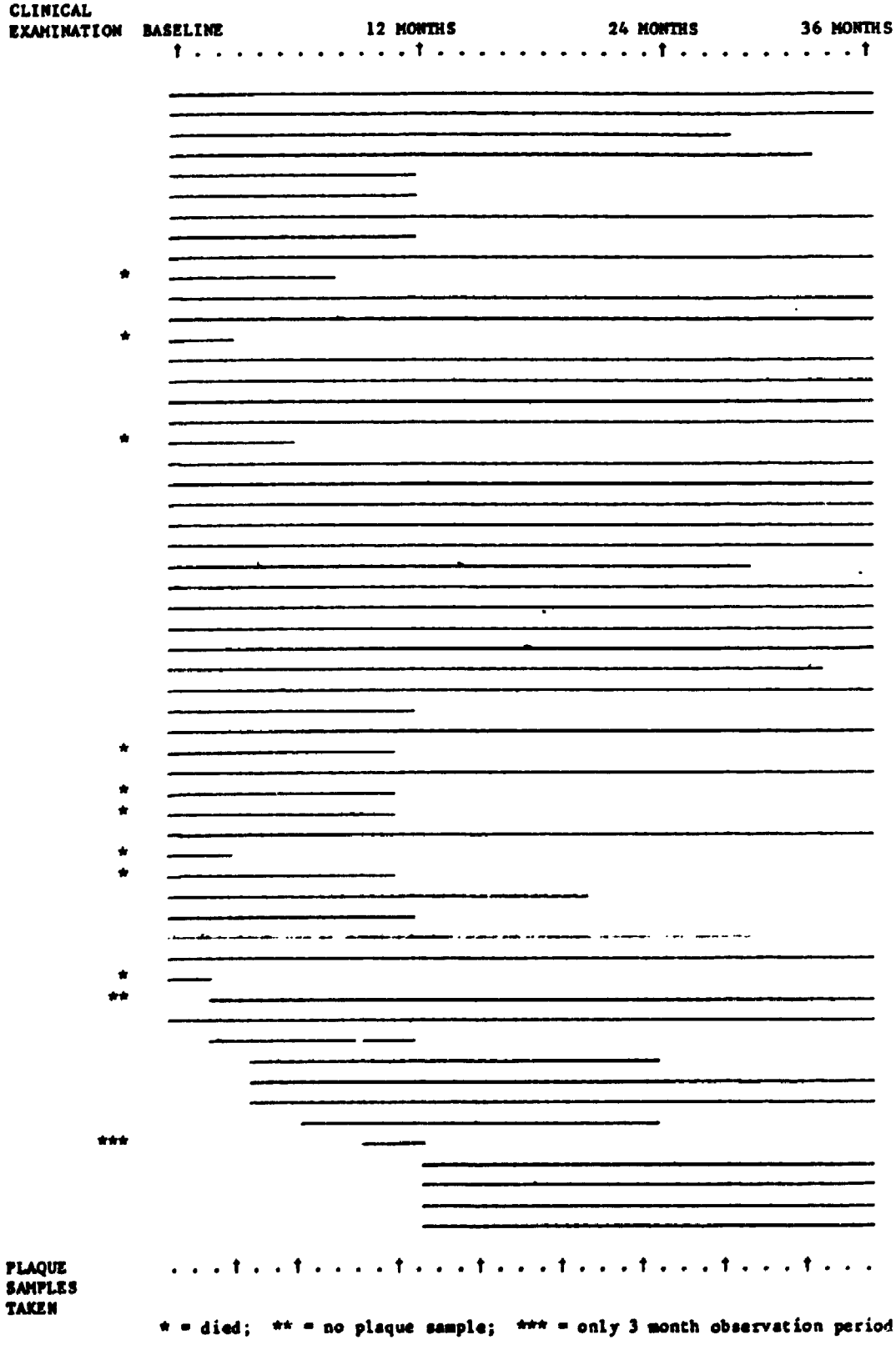
TABLE 16 - DISTRIBUTION OF THE PRESENCE OF STREPTOCOCCUS MUTANS ON TOOTH ROOTS ON FOUR SUCCESSIVE PLAQUE SAMPLING SESSIONS

NUMBER OF TIMES STREPTOCOCCUS MUTANS WAS DETECTED	NUMBER AND PERCENT OF ROOT SURFACES	
0	67	(59.29)
1	21	(18.58)
2	8	(7.08)
3	11	(9.73)
4	6	(5.31)
	113	100.00

TABLE 17 - SENSITIVITY, SPECIFICITY AND POSITIVE PREDICTIVE VALUES FOR MEASURING EXPOSURE TO *STREPTOCOCCUS MUTANS*, 24 MONTHS

DEFINITION	SENSITIVITY	SPECIFICITY	POSITIVE PREDICTIVE VALUE
PRESENT IN SINGLE PLAQUE SAMPLE	0.46	0.93	0.75
PRESENT IN TWO CONSECUTIVE PLAQUE SAMPLES	0.38	0.96	0.83

FIGURE 1 - ENTRY AND LOSS TO FOLLOW-UP OF SUBJECTS RELATIVE TO CLINICAL EXAMINATIONS AND PLAQUE SAMPLING SESSIONS



* = died; ** = no plaque sample; *** = only 3 month observation period

APPENDIX A

FREQUENCY DISTRIBUTIONS AND DESCRIPTIVE STATISTICS FOR ALL STUDY VARIABLES BY SUBJECT AND TOOTH ROOT SURFACE

A. RESPONSE VARIABLE	SURFACES	SUBJECTS
ROOT CARIES (12 MONTHS)		
PRESENT	17	12
ABSENT	133	33
ROOT CARIES (24 MONTHS)		
PRESENT	22	13
ABSENT	113	27

B. TREATMENT VARIABLES		
STREPTOCOCCUS MUTANS (6 MONTHS)		
MEAN	0.001	0.001
MEDIAN	0.000	0.000
STANDARD DEVIATION	0.003	0.002
MAXIMUM	0.029	0.009
MINIMUM	0.000	0.000
N	150	45
STREPTOCOCCUS SANGUIS (6 MONTHS)		
MEAN	0.010	0.012
MEDIAN	0.001	0.002
STANDARD DEVIATION	0.036	0.040
MAXIMUM	0.356	0.258
MINIMUM	0.000	0.000
N	150	45

Appendix A.2

	SURFACES	SUBJECTS
ACTINOMYCES VISCOSUS (6 MONTHS)		
MEAN	0.078	0.077
MEDIAN	0.024	0.057
STANDARD DEVIATION	0.124	0.077
MAXIMUM	0.759	0.310
MINIMUM	0.000	0.000
N	150	45
ACTINOMYCES NAESLUNDII (6 MONTHS)		
MEAN	0.015	0.014
MEDIAN	0.000	0.000
STANDARD DEVIATION	0.049	0.038
MAXIMUM	0.410	0.217
MINIMUM	0.000	0.000
N	147	43
LACTOBACILLI (6 MONTHS)		
MEAN	0.003	0.002
MEDIAN	0.000	0.000
STANDARD DEVIATION	0.011	0.005
MAXIMUM	0.105	0.019
MINIMUM	0.000	0.000
N	150	45

Appendix A.3

		SURFACES	SUBJECTS
C.	PREDICTOR VARIABLES		
	AGE		
	MEAN		68.6
	MEDIAN		71.0
	STANDARD DEVIATION		14.8
	MAXIMUM		91.0
	MINIMUM		25.0
	N		45
	NUMBER OF TEETH		
	MEAN		17.4
	MEDIAN		19.0
	STANDARD DEVIATION		6.7
	MAXIMUM		30.0
	MINIMUM		2.0
	N		45
	NUMBER OF RETAINED ROOTS		
	MEAN		0.4
	MEDIAN		0.0
	STANDARD DEVIATION		1.2
	MAXIMUM		6.0
	MINIMUM		0.0
	N		45
	DECAYED AND FILLED CORONAL SURFACES		
	MEAN		16.0
	MEDIAN		15.0
	STANDARD DEVIATION		11.1
	MAXIMUM		43.0
	MINIMUM		0.0
	N		45

Appendix A.4

	SURFACES	SUBJECTS
DECAYED AND FILLED ROOT SURFACES		
MEAN		5.9
MEDIAN		3.0
STANDARD DEVIATION		7.1
MAXIMUM		34.0
MINIMUM		0.0
N		45
SEX		
MALE		13
FEMALE		22
NUMBER OF BETWEEN MEAL SNACKS/DAY		
NONE		25
ONE		11
MORE THAN ONE		5
MISSING		4
NUMBER OF BETWEEN MEAL SNACKS/WEEK		
NONE		18
1-3		4
4-7		9
MORE THAN 7		10
MISSING		4
TYPE OF DIET		
REGULAR		17
DIABETIC		4
REDUCING		5
LOW FAT		3
OTHER		13
MISSING		3
MEDICATIONS AFFECTING SALIVA		
NONE		15
ANTIPARKINSON		7
TRANQUILIZER		3
OTHER OR COMBINATION		5
MISSING		15

Appendix A.5

	SURFACES	SUBJECTS
TOOTH SURFACE		
MESIAL	53	
DISTAL	17	
FACIAL	70	
LINGUAL	10	
GINGIVAL INFLAMMATION (12 MONTHS)		
NONE	13	
MILD	84	
BLEEDING ON PROBING	53	
SEVERE	0	
PERIODONTAL POCKET DEPTH (mm)		
MEAN	2.913	2.816
MEDIAN	3.000	2.750
STANDARD DEVIATION	1.451	1.097
MAXIMUM	9.000	6.000
MINIMUM	0.000	1.000
N	150	45
GINGIVAL RECESSION (mm)		
MEAN	1.860	1.813
MEDIAN	2.000	1.667
STANDARD DEVIATION	1.141	1.792
MAXIMUM	7.000	4.167
MINIMUM	0.000	0.800
N	150	45
ANTIMICROBIAL DRUG (6 MONTHS)		
NONE		34
AMPICILLIN, PENICILLIN G, PENICILLIN V, 'RYROMYCIN		3
BACTRIM		1
GANTRICIN		0
KANTREX		0
NEGRAM		1
SEPTRA		0
TETRACYN		0
AMOXIL		0
MISSING		6

APPENDIX B

FREQUENCY DISTRIBUTIONS FOR QUALITATIVE AND QUANTITATIVE VARIABLES REORGANIZED INTO DICHOTOMOUS CATEGORIES BY SUBJECT

A. TREATMENT VARIABLES	NUMBER (%)
STREPTOCOCCUS MUTANS (6 MONTHS)	
PRESENT	10 (22.2)
ABSENT	35
STREPTOCOCCUS SANGUIS (6 MONTHS)	
PRESENT	35 (77.8)
ABSENT	10
ACTINOMYCES VISCOSUS (6 MONTHS)	
PRESENT	42 (93.3)
ABSENT	3
ACTINOMYCES NAESLUNDII (6 MONTHS)	
PRESENT	17 (39.5)
ABSENT	26
LACTOBACILLI (6 MONTHS)	
PRESENT	14 (31.1)
ABSENT	31

Appendix B.2

	NUMBER (%)
B. PREDICTOR VARIABLES	
AGE	
LESS THAN 65 YEARS	14 (31.1)
65 YEARS AND OVER	31
SEX	
MALE	13 (28.9)
FEMALE	32
PREVIOUS CORONAL CARIES EXPERIENCE	
YES	43 (95.6)
NO	2
PREVIOUS ROOT CARIES EXPERIENCE	
YES	38 (84.4)
NO	7
NUMBER OF BETWEEN-MEAL SNACKS/DAY	
NONE	25 (61.0)
ONE OR MORE	16
NUMBER OF BETWEEN-MEAL SNACKS/WEEK	
NONE	18 (43.9)
ONE OR MORE	23
TYPE OF DIET	
REGULAR	17 (40.5)
OTHER	25
MEDICATIONS AFFECTING SALIVA	
NONE	15 (50.0)
ONE OR MORE	15
ANTIMICROBIAL DRUG (6 MONTHS)	
YES	5 (12.8)
NO	34

APPENDIX C - EFFECT OF CHANGING THE DEFINITION OF EXPOSURE, STREPTOCOCCUS MUTANS, 24 MONTHS

A. SINGLE POSITIVE PLAQUE SAMPLE

STREPTOCOCCUS MUTANS	ROOT CARIES	NO ROOT CARIES
PRESENT	9 (0.29)	22
ABSENT	13 (0.13)	91

ADJUSTED CHI-SQUARE = 1.63 d.f. = 1 p = .20

ODDS RATIO = 2.86

B. TWO SUCCESSIVE POSITIVE PLAQUE SAMPLES

STREPTOCOCCUS MUTANS	ROOT CARIES	NO ROOT CARIES
PRESENT	8 (0.32)	17
ABSENT	16 (0.13)	109

ADJUSTED CHI-SQUARE = 1.92 d.f. = 1 p = .16

ODDS RATIO = 3.21

APPENDIX D - LIFE TABLE ANALYSIS, STREPTOCOCCUS MUTANS, 12 AND 24 MONTHS

A. SUBJECTS

12 MONTHS	ROOT CARIES	NO ROOT CARIES	TOTAL
STREPTOCOCCUS MUTANS			
PRESENT	5 (0.50)	5	10
ABSENT	7 (0.20)	28	35
TOTAL	12	33	45

24 MONTHS	ROOT CARIES	NO ROOT CARIES	TOTAL
STREPTOCOCCUS MUTANS			
PRESENT	2 (0.50)	2	4
ABSENT	0 (0.00)	25	25
TOTAL	2	27	29*

M-H X^2 = 7.51 d.f. = 1 p = .003

COMMON ODDS RATIO = 5.87

* 12 subjects who reached their end point and 4 subjects who were administrative losses in the first 12 months did not enter the second 12 month period of observation.

B. SURFACES

12 MONTHS	ROOT CARIES	NO ROOT CARIES	TOTAL
STREPTOCOCCUS MUTANS			
PRESENT	6 (0.16)	31	37
ABSENT	11 (0.10)	102	113
TOTAL	17	133	150

24 MONTHS	ROOT CARIES	NO ROOT CARIES	TOTAL
STREPTOCOCCUS MUTANS			
PRESENT	5 (0.17)	24	29
ABSENT	2 (0.02)	88	90
TOTAL	7	112	119

INTRACLASS CORRELATION COEFFICIENT = .47

UNADJUSTED M-HX^2 = 6.43 d.f. = 1 p = .01

ADJUSTED M-HX^2 = 1.67 d.f. = 1 p = .11

COMMON ODDS RATIO = 2.91