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# USING STREPTOCOCCUS MUTANS AS A MARKER ORGANISM IN DENTAL CARIES STUDIES

H. F. Albert Ho B.D.S. Sydney.

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# SUMMARY

Dental caries is caused by bacteria in dental plaque producing acid from carbohydrates in our diet. Streptococcus mutans has been shown to involve in most cases of caries in human studies and in animal studies. The bacterium was first discussed in 1924 by Clarke, rediscovered in the 1960's and studied extensively for the last 15 years.

Clinically, caries is detected as a cavitation on the tooth surface but this stage is preceded by a series of biochemical reactions involving the tooth surface and the plaque-tooth interphases.

Dental plaque has 2 major interphases, an internal one with enamel where cell free salivary components are found and is referred to as the pellicle; an external one in contact with the oral cavity called the plaque-saliva interphase. Bacteria at this interphase have ready access to nutrients for growth. A third interphase is the plaque-gingiva interphase, where the bacteria receive nutritional input from crevicular fluid.

There are various factors affecting this complex and dynamic plaque system such as saliva, bacteria, foodstuffs and crevicular fluid.

Amongst the plaque bacteria, Streptococcus mutans has been studied in great details. It ferments a wide variety of carbohydrates substrates producing a terminal pH of 4.2 - 4.6 and it produces extracellular polysaccharides from sucrose. It is also responsible for the production of dental caries.

The micro-organisms in dental plaque are embedded in an organic matrix which acts as a diffusion limiting membrane retaining high concentrations of potentially harmful products such as lactic acid at particular sites where they initiate caries.

The adherence of Streptococcus mutans and other micro-organisms to tooth surfaces is a pre-requisite for the subsequent formation of dental plaque. Streptococcus mutans increases significantly in attachment to pellicle-coated enamel slabs. It behaves like negatively charged particles in their electrostatic interaction with hydroxylapatite surfaces.

The attachment and accumulation of bacteria is regulated by salivary components. The salivary agglutinating factors are responsible to bind selected

bacterial species.

Sucrose is important for colonization of Streptococcus mutans on teeth and the bacteria could be established much more readily when experimental animals were given sucrose containing diets. In vitro studies have shown active glucan synthesis from sucrose will enhance adherence of Streptococcus mutans to various solid surfaces.

In presence of high molecular weight dextran, strains of Streptococcus mutans will agglutinate homologously; but the bacterium can also agglutinate with other bacterial species. After attachment to tooth surface, Streptococcus mutans will synthesize extracellular polysaccharides such as glucans and fructans from sucrose by enzymatic action of glycosyltranferase and fructosyltransferase. The polysaccharides are insolube in water and will further promote adherence.

The metabolic pathway of sucrose by Streptococcus mutans varies. In the presence of excess sucrose, the major fermentation product is lactate; but if sucrose is limiting significant amount of formate, acetate and ethanol are also produced.

Caries has been induced in animals when streptococcal strains isolated from human mouths were implanted to various animal models, and strains of Streptococcus mutans

almost always induce smooth surface and pit and fissure caries in experimental animals. Sucrose has been shown to be most cariogenic and supports rapid pathogenesis.

Epidemiological surveys have shown dental caries scores correlating with the presence of Streptococcus mutans in the mouth. Cross-sectional as well as longitudinal studies demonstrate cause and effect relationship between Streptococcus mutans and dental caries.

Dental caries can be prevented in theory by eliminating cariogenic bacteria especially Streptococcus mutans from the mouth, by increasing resistance of the teeth and by modifying the diet. As far as elimination of Streptococcus mutans from the mouth is concerned, antibiotics have been used. They are effective but are not practical to use on a public health scale. Topical application of cariostatic agents has yet to show its long term effect.

Other attempts to eliminate Streptococcus mutans from the mouth include inhibition of the bacterium by enzymatic activities, the use of antibodies raised against cellular or extracellular components of the micro-organism and strengthening the host immunological respones. All these methods, however, require further investigations for positive conclusion.

In recent years, dental caries prevention through vaccination against Streptococcus mutans has become a popular theme for various research works. The result is definitely inconclusive especially when cross-reactivity with other organ tissues has been reported.

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# 1. INTRODUCTION

It has been accepted that dental caries is caused by bacteria in dental plaque producing acids from the carbohydrates in our diet. Plaque is a complex ecological system and it is hard to identify any one factor as the sole cause of the disease. Many research and investigations have shown, however, that various micro organisms are essential in the pathogenesis of dental caries; of these micro organisms, the strains characterized as Streptococcus mutans have been demonstrated to involve in some way in the majority of cases of human dental caries. Animal studies also generally reinforce the ralative importance of Streptococcus mutans.

This paper will give an account on the emphasis that Streptococcus mutans is a major etiological agent for dental caries, based on evidence of recent studies. The cariogenic potential of other organisms however, will also be mentioned.

Selected streptococcal species were first demonstrated by Orland (1959) to produce dental caries in germfree rats when fed a high sucrose diet. Also, the involvement of certain penicillin-susceptible bacteria in dental caries was also suggested by the indirect evidence

that antibiotics suppressed experimental dental caries in rodents (McClure & Hewitt 1946). Various investigations have since been carried out to account for the causative relationship between specific oral bacterial species and dental caries. Keys and Fitzgerald (1960) were able to isolate some streptococcal strains from various lesions of rats and hamsters and transmit them to some "caries-resistant" rats and hamsters respectively, producing dental caries. Several streptococcal strains sharing immunological specificity with the cariogenic streptococci derived from rats and hamsters were isolated with a flourescent-antibody technique. The strains also produced severe dental caries in germ free animals (Jablen and Zinner 1966; Zinner et al 1966). Carlsson (1976) indicated that properties of these caroigenic streptococci were similar to those originally isolated from human caries teeth by Clarke in 1924, to which he had given the species name mutans. Thus, Streptococcus mutans was rediscovered after the original observation made about 36 years ago. Extensive research on Streptococcus mutans has been done for the past 15 years and its role in the development of dental caries in animals and humans has been established.

## 2. AETIOLOGICAL FACTORS OF DENTAL CARIES

Dental caries is a complex, multi factorial disease. It occurs across a wide spectrum of the animal kingdom with incidence closely correlated with the dietary intake of fermentable carbohydrates. The etiology of dental caries is directly traceable to the action of certain oral micro organisms which produce organic acids through metabolism of carbohydrate substrates. The acids, in turn, attack the tooth surface, resulting in the dissolution of the organic structure of the enamel. Although caries is noted clinically as a cavitation in the tooth surface, this stage is preceded by a series of reactions at the biochemical level which involve the tooth surfaces and the surrounding medium especially at the plaque-tooth interface. There are correlations in population groups between caries experience and such factors as age, sex, race, geographic location, socio-economic status and family influence. The disease is a major impact on society because its widespread, almost universal occurrence and because of its progressive character, cost and effects on health and personality.

## 2.1 THE FORMATION OF PLAQUE

Dental plaque represents a tenacious bacterial structure formed on tooth surfaces which cannot be washed off with water sprays and which contains large numbers of closely packed micro organisms surrounded by extracellular material of bacterial and salivary origin. Dental plaque has two major interphases, an internal one with enamel where cell free salivary components are found in an intervening cuticle referred as pellicle and an external one in contact with the oral cavity called plaque-saliva interphase. Bacteria at this interphase have ready access to a variety of nutrients essential to bacterial growth. A third interphase could be defined as the plaque-gingiva interphase. Bacteria at this site receive nutritional imput from crevicular fluid and their metabolic end products could affect the gingival tissue in close proximity.

The study of this complex and dynamic ecologic microcosm is made extremely difficult by large variations in bacterial composition due to differences in site, opportunism, time of colonization, nutritional imputs and various other factors. Some of the major factors will be discussed in further details in this section.

## 2.1.1 Saliva

Glandular saliva once enters the oral cavity, will be instantly contaminated with a large variety of micro-organisms, microbial enzymes, sloughed epithelial cells, degenerating polymorphonuclear leukocytes and serous crevicular fluid. This complex mixture is termed the whole saliva.

One of the most important functions of the salivary secretion is to continually provide the constituents of the protein coats for the teeth and oral mucosa. Protein in saliva can either be simple i.e. containing amino acids only or conjugated i.e. containing components other than amino acids. One class of conjugated protein forming an important constituent of saliva is the glycoprotein which has a protein core with side chains comprised of carbohydrate moieties branching off in various lengths. Hay (1967) showed experimentally that salivary glycoproteins absorbed to hydroxylapatite and the presence of bacteria is not essential at this early stage of pellicle formation. Chemically, pellicle appears to be composed of undegraded salivary glycoprotein. Several mechanisms have been suggested for the deposition of salivary glycoproteins on to the enamel surface; these include the effective surface charges, for example, negatively charged terminal sialic acid groups, the concentration of calciumions as increased $Ca^{2+}$ may

precipitate glycoprotein and pH effects as decreased pH favours the adhesion of protein to hydroxylapatite. The pellicles so formed, play a role in the colonization of the tooth/plaque bacteria, acting as a substrate for the growth of certain bacteria. Saxton (1973) studied the initial colonisation of bacteria culturally and by means of a scanning electron microscope. The earliest organisms to be found are predominantly coccal in form. Cultural studies have shown that both gram-positive and gram-negative cocci are present and they may be aerobic or facultative. Streptococci form a prominent proportion of the earliest colonisers, but other organisms, such as Neisseria species, also play a significant role in initial plaque development; and most of these are dreived from saliva.

Several enzymes have been identified in saliva but the biological significance of these is not fully understood. Lysozyme is a hydrolytic enzyme which cleaves the linkage between N-acetylglucosamine and N-acetylmuranic acid. Such linkages occur in the cell wall mucopeptide of bacteria. Some species of bacteria are extremely sensitive to lysis by this enzyme while others are completely resistant. Lysozyme is widely distributed, being found in saliva and other body fluids.
Lactoperoxidase is a haemo-protein enzyme found in saliva, but its contribution to plaque formation has not been defined. There are also in saliva bacterial

agglutinating factors showing some specifity towards particular species such as Streptococcus mutans. These agglutinins appear to be glycoproteins. The presence of trace amounts of any specific salivary agglutinin for a particular organism would cause a dramatic enhancement of aggregation. However, how these salivary components actually behave towards colonisation of the tooth and accumulation of plaque is not fully understood at present.

### 2.1.2 Bacteria

As mentioned in the previous section, bacterial colonisation takes place soon after pellicle formation, which in fact, can be considered as the early stage of plaque formation. After the plaque has developed undisturbed for a day or two, it becomes thicker and a great variety of morphological types of bacteria are found: from the early coccal forms to rods and at about the third day, filamentous forms. As well as the shift in morphological types of bacteria seen during early plaque development, there is also a shift towards increasing anaerobiosis as the plaque builds up in mass and complexity. Thus anaerobes such as Fusobacterium Veillonella, Actinomyces, Bacteroides etc. appear in increasing numbers after the initial colonisation by aerobic and facultative species. Some predominant groups of plaque bacteria deserve more detailed discussion.

For various reasons, Streptococci have probably been studied more intensively than any other flora amongst the oral micro flora; and one species in particular, Streptococcus mutans, has received considerable amount of attention in recent years. This species, as said earlier, was first mentioned in 1924 by Clarke and has been extensively studied recently. The name mutans was given because this species characteristically changes from a round coccus shape to a rod shape under certain growth conditions such as low pH. This streptococcus ferments a wide variety of carbohydrate substrates, producing a terminal pH in the regions of 4.2 - 4.6 in broth culture. A particular characteristic of Streptococcus mutans is the production of extracellular polysaccharides of the glucan type from sucrose. There are at least five serologically distinct types of Streptococcus mutans and the serological differentiation is due to the cell wall carbohydrates. This species has been subjected to detailed investigation because of its role in dental caries formation.

Streptococcus salivarius is generally not found in very high numbers in plaque; but can be found as a dominant part of the streptococcal flora of the tongue and other soft tissue and in saliva. Strains of Streptococcus salivarius adhere well to epithelical cells but poorly to hard tissues. Colonies of Streptococcus salivarius are often recognised by their large, mucoid appearance on sucrose agar. This particular morphology is due to the

production of another extracellular polymer fructan from sucrose. Two serotypes have been known to exist. Because of its low cell counts in plaque, Streptoccocus salivarius is not thought to be of great significance in caries. However, some strains have been shown to produce some caries in experimental animals.

Lactobacillus are gram-positive facultative anaerobic rods. Their number in the mouth tend to increase with the amount of caries present and were taken as the most likely causative agent for caries. Their numbers are extremely low on caries free surfaces. Several species of lactobacillus have been isolated from the mouth. The most commonly found variation in plaque are L. casei and L. acidophilus; both of which are homofermentative, but hetero fermentors, producing gas as well as acid from glucose, also occur. These organism produce a low terminal pH in carbohydrate media i.e. they are acidogenic and they can also survive and grow in a low pH environment, i.e. they are aciduric.

Actinomyces are the most common and numerous gram positive rods found in the mouth. They are characteristically anaerobic, although some strains grow quite well aerobically. Microscopically, Actinomyces cells are often plemorphic and may display branching and filamentous forms. The animal pathogen, A. bovis, which causes lumpy jaw in cattle, has never been isolated with

any certainty from human sources. The species found in the human mouth are A. israelli, A. naeslundii, A. viscosus and A. odontolyticus. In addition to their presence in plaque and possible involvement in caries and periodontal diseases, some of the species are the causative agents for actinomycosis.

Despite intrinsic technical difficulties in examining and quantifying the microbial composition of plaque, accurate quantification of the number of bacteria in a given plaque specimen can be done either by direct microscopic or viable counting technique. With microscopic counting method, dental plaque has been shown to contain approximately 2 - 2.5 x $10^8$ bacteria per mg. wet weight. With viable counting method, the number of anaerobes counted in plaque samples range from 4.0 - 7.9 x $10^7$ per mg. wet weight; the ratio of anaerobics to aerobic viable counts also varies from approximately 2:1 to 12:1. Loesche and Green (1972) showed clinical estimates of amount of plaque in a mouth can be correlated with the actual weight of plaque present and microscopic count.

Independent surveys by different author including Handelman and Hess (1966), Loesche and Syed (1973) and Howell et al (1965) reveal that as shown on Table 1, the numerically dominant groups of bacteria are gram-positive rods and filaments which consist largely of Actinomyces

species and the streptococci. Both of these large groups include several different species which may be microaerophilic facultative or strictly anaerobic. Anaerobic gram-negative cocci belonging to the Genus Veillonella and anaerobic gram-negative rods can reach quite high levels but there is considerable variation between the relative number of those organisms reported in different studies.

Table 1. The Microbial flora of Dental Plaque

| Type of Organisms | Percent Viable Count (Range) |
|---|---|
| Streptococci | 17 - 38 |
| Gram-positive rods and filament | 22 - 52 |
| Neisseria | 0 - 2 |
| Veillonella | 1 - 13 |
| Gram-negative anaerobic rods | 0 - 17 |
| Fusobacteria | 0 - 7 |

The micro organisms in dental plaque are embedded in an organic matrix which occupies the space between individual bacterial cells or micro-colonies and accounts for approximately 30% of the total plaque volume. The matrix has a significant effect on the ecology of the plaque and may also be important in caries; by acting as a diffusion-limiting membrane, potentially harmful bacterial products such as lactic acid may be retained in

high concentration at particular sites where they can initiate caries. The same diffusion-limiting effect may also slow down the arrival of buffer from saliva, delaying their neutralising action.

Part of the organic material in the plaque matrix is protein and is derived principally from saliva glycoprotein while the remainder consists of extracellular polysaccharides produced by the bacteria. The bacterial activities contributing to plaque formation and the subsequent causal action in Dental Caries will be discussed in further details, in later sections of this paper, using Streptococcus mutans as the marker organism for discussion, mainly because of the numerous research results so far available. However, for the sake of completeness, it should be remembered that other groups of bacteria, including Neisseria, Actinomyces and Lactobacillus may also contribute similar reactions.

2.1.3 Foodstuff

Dental caries is a food dependent disease. However our knowledge of the effects of various dietary items is insufficient to permit fuller understanding of what makes foods more or less cariogenic and information on the relative destructiveness of the principal classes of foodstuff is lacking.

The existing belief in the dominating importance of sucrose seems to have evolved from casual clinical observation and simple laboratory experiments. Other investigations have shown that factors such as frequency of use and the associated substrates play an important part in determining the cariogenicity of foodstuffs. However, before considering these factors that modify the role of sucrose, the unique importance of sucrose in dental caries should not be overlooked.

Surcrose is the fermentable carbohydrate that appears most frequently in the human diet. The frequent chewing of sugar has been shown to assist the retention of easily identifiable caries-inducing streptococci in human mouths. The formation of a voluminous extracellular polysaccharide from sucrose is a consistent feature of cariogenic streptococci. Numerous studies have shown that attempts to introduce these bacteria into the mouths of hamsters, rats, monkeys and man were more successful when sucrose was

used than when other carbohydrates were included in the diet. Restriction of dietary sucrose reduces an already established population of Streptococcus mutans in man and monkeys. Huxley (1974) showed both the proportion of Streptococcus mutans and the incidence of caries were greatest with sucrose, less with glucose and least with starch. In addition, dental plaque formed in the absence of sucrose is not cariogenic as shown by Littleton et al (1967) that mentally retarded children fed routinely by stomach tube had very little dental caries. Plaque from these children showed very little fall in pH when exposed to sucrose, and hence contained few lactic acid bacteria.

Research to investigate the relative potency in causing caries has been carried out on different carbohydrates and foodstuffs. Carbohydrate of different types was incorporated in a skimmed milk-liver powder diet for experimental animals, sucrose was much more cariogenic than either glucose or raw wheat starch. Fructose had a similar cariogenicity score to glucose. Presumably part of the special effect of sucrose is due to its ability to be converted into bacterial extracellular polysaccharides which form part of the matrix of dental plaque.

The relationship between sugar consumption and the strength of caries is, however, not a direct one. Caries prediction in diet analysis will be a summation of the cariogenicity of the individual foodstuffs that make up

the diet each modified by the frequency of their use. This could be expressed as suggested by Bibby (1978) by an equation:

$$P = \sum fn \times in$$ (f=frequency, i=index of cariogenicity)

### 2.1.4 Crevicular Fluid

Crevicular fluid flow is closely related to gingival inflammation, which in turn, enhances the formation and accumulation of dental plaque. Boedecker (1933) suggested that the crevicular fluid was an inflammatory exudate. While this view is still generally accepted, the clinical significance of gingival crevice fluid flow is subject to some disagreement, and one controversy is whether measurement of the fluid is a reliable indication of gingival imflammation.

There seems to be a general consensus that the flow of crevicular fluid is sufficiently indicative of the gingival inflammatory state that it can be used under clinical conditions to monitor gingival inflammation.

It may also be possible for a clinician for a clinician to identify sub-clinical gingival inflammation by measuring crevicular fluid flow and then analyse the fluid sample for chemical or microbial constituents. The microbial content in the fluid is also responsible for causing root caries in people with gingival recession.

## 2.2 SOME GENERAL FACTORS

Dental caries has been discribed as a 'disease of children' and is seen early in life in especially the Western society. In Britain, U.S.A. and French Polynesia, studies of children aged between 2 and 5 years showed that 57- 80 percent of them already suffer from caries.

Caries in the permanent dentition is found soon after eruption of the first permanent molars where it usually begins in the pits and fissures surface; and DMF scores mount steadily as more permanent teeth erupt. In Western societies, DMF scores continue to rise until around age 24 when they appear to level out. Factors contributing to the decline in caries incidence are likely to be the build up of flouride in the outer layers of enamal and perhaps the change in dietary habits. One caries problem found in older age group is that of root caries, frequently found when gingival recession has led to radicular exposure and where bacterial plaque has accumulated around the exposed roots.

It has been stated that women have a higher caries attack rate than men (Bibby 1970), but it is a difficult claim to substantiate. Most caries epidemiological studies have concentrated on younger people because of the 'disease of childhood' attitudes; because reparative and preventive programmes are aimed at children and because

schools and colleges provide the researcher with convenient groups to study.

It is a fact that teeth erupt earlier in girls than in boys, which is one reason why girls apparently get more caries than boys. Other factors contributing to differences between men and women are oral cleanliness, choice of dentist, attitudes towards dental health and partterns of tooth ions.

Studies in racial comparisons have difficulties even when the different races appears to share the same locality and life style. There is the problem of categorising persons of mixed races, as well as trying to determine just how common are the environmental variable such as diet. It is possible that real differnces in caries experience may exist between races, but even if they do, they are masked by and are of secondary importance to the social and cultural factors in the environment.

Geographic variations do exist in the prevalence and intensity of caries. However, purely geographic factors such as latitude, altitude or mean annual temperature should not in themselves affect the distribution of caries; and if they do have an influence, it is almost certainly an indirect one in that they affect the culture and diet. Most geographic interest at the present centres on relating soil types to caries prevalence, because of

factors in the soil thought to influence caries pattern through diet. Trace elements are thought to influence one way or the other, but nothing as yet has been proved conclusive.

# 3. MICROBIAL FACTORS AFFECTING CARIES POTENTIAL (AS DEMONSTRATED BY STREPTOCOCCUS MUTANS)

## 3.1 ADHERENCE OF STREPTOCOCUS MUTANS

The adherence of Streptococcus mutans and other micro organisms to tooth surfaces and the subsequent formation of dental plaque are of major significance in the development of dental caries. These processes are complex and involve a variety of bacterial and host components. Different aspects of bacterial adherence in the oral caity will be summarised in this section.

3.1.1 Attachment of Streptococcus mutans to Smooth Surfaces.

The attachment of bacteria to the tooth surface is usually preceeded by the formation of an acquired pellicle of salivary origin. The early stages of plaque development on cleaned tooth surface require cell attachment to the pellicle sufficiently firm to resist local cleaning forces such as salivary flow and muscular movements. Streptococcus mutans as well as Streptococcus sanguis and Streptococcus salivarius were found to increase significantly in attachment to pellicle-coated enamel slabs than uncoated slabs (Orstavik, Kraus & Henshaw 1974). Streptococcus mutans has been further found to attach in greater numbers to dextran-coated Hydroxylapatite (Liljemark & Shauer 1977). The invitro affinity of these oral streptococcal species for pellicle-coated surfaces appears to correlate with the proportions of that species found in vivo, despite the fact that Streptococcus mutans appears not to play a key role in the initial stages of tooth colonization. Rolla (1976) proposed a model suggesting that cells of Streptococcus mutans and Streptococcus sanguis behave like negatively charged particles in their electrostatic interaction with Hydroxylapatite surface in vitro. He demonstrated that calcium and protamine phosphate significantly increase uptake of bacteria, but fluoride, phosphate and even saliva decreased the uptake of the cells. The acidic proteins in

saliva are selectively bound by Hydroxylapatite, and the pellicle so formed results in a reduction of the cationic nature of the surface and reduces the binding of the negatively charged bacterial cells. It also appears that a large number of hydroxyl groups of sucrose-grown Streptococcus mutans and Streptococcus sanguis cells preferentially form hydrogen bonds with the pellicle proteins.

### 3.1.2 Interaction of Salivary Components with Streptococcus Cells.

Interaction of salivary components with bacterial cells seems to be significant in regulating the attachment and accumulation of different bacterial species involved in plaque formation. Gibbons and Spinell (1970) suggested that saliva possessed the ability to agglutinate many plaque bacteria and the salivary agglutinating factor was reported to be a high molecular weight glycoprotein which is heat stable and $Ca^{2+}$ dependent and which occurs optimally between pH 5 to 7.5. Different agglutinating factors have been found for Streptococcus mutans, Streptococcus mitior and Streptococcus sanguis (Gibbons & van Houte 1975). When saliva is pre-treated with wheat germ agglutin, the saliva does not induce the agglutination of Streptococcus mutans strain. The salivary agglutinating factors may need specific determinants to bind selected bacterial species. For example, the salivary agglutinating factor responsible for binding Streptococcus sanguis was destroyed by neuraminidase or protease treatment, while glucoprotein aggultinated Streptococcus mutans and Streptococcus sanguis (Levine et al 1978). Poulsen et al (1978) also suggested that salivary lysosyme may participate in the agglutination of some Streptococcus mutans strains. Preincubation of various bacteria and saliva reduced the attachment of bacteria to hydroxylapatite surface (Clark

et al 1978), suggesting that the agglutinating factor free in saliva competitively inhibits the interaction between salivary coated hydroxylapatite and those surface components of the bacterial cells which contain bound salivary glycoproteins. Gibbons and Qureshi (1976)(1978) found that strains of Streptococcus mutans and other oral bacteria bind the blood group-reactive mucin of saliva after exposure to whole saliva or partially purified mucin preparations. Blood group-reactive mucins are present in the acquired pellicle on the tooth surface which may serve as receptor molecules involved in the attachment of bacteria to teeth, suggesting that a lectin receptor type mechanism is involved (Gibbons 1979).

### 3.1.3 Sucrose Dependent in vivo Adherence of Streptococcus mutans

Sucrose is important in the colonization of Streptococcus mutans on teeth. In earlier studies using hamsters and rats, it was found that Streptococcus mutans could be established much more readily when the animals were given sucrose containing diets (Edwardsson & Krasse 1967, Krasse 1965). It was also found that Streptococcus mutans could implant in the human oral cavity after inoculation with a pure culture and the frequent chewing of sucrose gum enhance the implantation (Edwardsson & Krasse 1967). More recently, Van Houte et al (1976) found that colonization of Streptococcus mutans occurred in rats fed diets with a sucrose content from 56% to as low as 1%, in which the lowest effective inoculum was $10^7$ CFU by a single oral administration. More frequent inoculations were needed to establish the organims on a high sucrose diet. When inoculated with less than $10^7$ CFU, however, the cells were gradually eliminated from the teeth. It should be noted that streptomycin resistant mutants of Streptococcus mutans frequently colonize less effectively than parent strains.

Preformed dextran/glucan, whether associated with Streptococcus mutans cells or with the tooth surface, does not permit the degree of cell attachment as occurred in the presence of sucrose (Van Houte & Upeslacis 1976). It

is apparent that de novo glucan synthesis leads to a stronger adherence to the tooth surface than that which occurs in the presence of dextran/glucan precoated on either tooth surface or bacterial cell surface, although sucrose may not be indispensable in the initial attachment of Streptococcus mutans in the oral cavity. It is based on the same understanding that high numbers of Streptococcus mutans may be detected in the mouth of children with sucrase - isomaltase deficiency, consuming a diet with an extremely low sucrose content (Van Houte & Duchin 1975).

In Macaca irus monkeys fed by stomach tube and provided with oral supplements, the colonization of Streptococcus mutans was dependent upon sucrose from the drinking water. Withdrawal of the sucrose resulted in complete absence of detectable Streptococcus mutans on the teeth, although the salivary counts of Streptococcus mutans remained unchanged (Kilian & Rolla 1976).

### 3.1.4 Sucrose Dependent in vitro Adherence

Active glucan synthesis from sucrose has been found to enhance the adherence of Streptococcus mutans to various solid surfaces (Gibbons & Van Houte 1975, McCabe et al 1967). Synthesis of the glucan is initiated by the emzymatic action of cell-free or cell-bound glycosyltransferase. It contrast mutants of Streptococcus mutans lacking the ability to synthesise water-insoluble adherent glucans, do not adhere to solid surfaces (De Stoppelaar et al 1971). When incubated with sucrose and exogenous glycosyltransferase, heat treated Streptococcus mutans cells not having cell-bound glycosyltransferase, adhered to a glass surface. In absence of de novo glucan synthesis, no adherence occurred. The adherence of Streptococcus mutans in the presence of sucrose depends primarily upon the specific binding of extracellular glycosyltransferase synthesised by the bacteria; while the non-adherent property of other bacterial species is due to their inability to bind glycosyltransferase to the cell surface. However, a variety of bacterial species can produce non-specific adherence when incubated with sucrose and glycosyltransferase by a non-specific mapping mechanism and may contribute to the development of dental plaque.

Glucan on the surface of Streptococcus mutans appears to function as a binding site for glycosyltransferase as

antiserum against glucan synthesis blocked binding of glycosyltransferase and subsequent adherence (Hamada & Torii 1978). Other complex polysaccharides may also mediate the process..

When Streptococcus mutans is grown in sucrose-free complex medium, the organisms do not have enough cell-bound glycosyltransferase to produce significant adherence to solid surfaces even when sucrose is added. On the other hand, cells grown in complex media containing sucrose have strong cell-associated glycosyltransferase activity and produce marked adherence to solid surfaces in the presence of exogenous sucrose (Hamada & Torii 1978).

### 3.1.5 Bacterial Aggregation

In addition to cell-to-surface adherence described above, bacterial aggregation or cell-to-cell adherence is of importance for dental plaque formation. Surface compnents which affect the aggregation of bacterial cells are therefore functionally critical for adhesion among bacteria in dental plaque.

Many strains of Streptococcus mutans agglutinate homologously upon addition of high molecular weight dextran (Gibbons& Fitzgerald 1969). Certain strains of Streptococcus mutans are also reported to form aggregates with other bacterial cells such as Nocardia and Neisseria i.e. heterologus cell-cell adherence (Gibbons& Nygaard 1970). The same authors also showed strains of A. naeslundii and A. viscosus formed aggregates more often with strains of Streptococcus sanguis and Streptococcus mitior than with strains of Streptococcus mutans. However, when Streptococcus mutans cells are coated with high-molecular-weight dextran or grown in presence of sucrose, they form visible aggregates with A. viscosus (Bourgeau & McBride 1976).

Streptococcus mutans has also been shown to form heterologous cell-cell aggregation with Candida albicans. Artificial plaque formation by a Streptococcus mutans strain is augmented when a C. albicans strain is

inoculated with the Streptococcus mutans (Miller & Kleinman 1974).

It appears that the adherence of Streptococcus mutans and other oral species to pellicle-covered teeth occurs in several steps. The initial attachment of single cells, chains of cells or aggregated cells may involve divalent ions and the negative charges on the bacterial cell and the tooth pellicle. The initial complex may also be formed between a glycoprotein in the pellicle and a polysaccharide on the bacteria. The second phase of the process would depend largely on the multiplication of Streptococcus mutans and glucan synthesis. The maturation of the plaque, containing various gram-positive and some gram-negative species, would be mediated by the synthesis of glucan by Streptococcus mutans. Glucan would ensure the stability of the plaque. Bacterial species which enter the developing plaque after the phase of initial attachment may do so by random contact with the adhesive glucan of the plaque (Hamada et al 1978).

## 3.2 POLYMER SYNTHESIS.

Polymer synthesis is important in the activities of Streptococcus mutans because polymers are involved in the adherence of the bacteria to hard surfaces. The main function of the polymers is to act as an insoluble extracellular matrix which irreversibly binds the organisms together and to the tooth surface.

### 3.2.1. Extracellular Polysaccharides.

Streptococcus mutans synthesizes extracellular polysaccharides, namely, glucans and fructans from sucrose by the enzymatic action of glycosyltransferase and fructosyltransferase. These polysaccharides, especially glucans are considered to be critically important in dental plaque formation and hence in the subsequent dental caries formation. They are water insoluble and possess a marked ability to promote adherence when synthesis de novo on various solid surfaces.

#### 3.2.1.1 Glucans.

In general, polymers of glucose of all types are called glucans, including starch, glycogen and cellulose. All bacterial glucans will contain $\alpha$(1-6) and $\alpha$(1-3) bonds and they can be water soluble or water insoluble, depending on the amount of different bonding. Water soluble glucans from Streptococcus mutans has been reported to consist of an $\alpha$(1-6) linked linear glucose polymer with an $\alpha$(1-3) branch linkage. However, structurally different water soluble and water insoluble fractions of glucan have been separated by an experimental process involving alkaline extraction and ethanol precipitation. The water insoluble fractions were found

to possess more $\alpha$(1-3) linkage (Nisizawa et al 1976), and more resistant to the enzymatic action of $\alpha$(1-6) glucanase. Insoluble glucans can be more conveniently obtained by incubating cell-free glycosyltransferase and sucrose. The glucan is obtained by centrifugation and then washed extensively with water and lyophilized. The glucan thus obtained was shown to contain a markedly high proportion (up to 90%) of $\alpha$(1-3) linkage in the water-insoluble fraction. The large proportion of $\alpha$(1-3) linkage found in the insoluble glucan explains the insoluble nature of this polymer.

3.2.1.2 Fructans.

Streptococcus mutans synthesizes fructans or polymers of fructose in addition to glucans from sucrose extracellularly. It forms a significantly secondary component of the polysaccharide. It is utilised quite rapidly by plaque bacteria and is a substrate for acid production after sucrose has been cleared from the mouth. A fructosyltransferase to form the $\beta$ (2-6) linkage for fructans has been purified from Streptococcus mutans (Carlsson 1970). Different polymeric variations of fructans structure may be formed by different strains. Some strains of Streptococcus mutans form virtually no fructans from sucrose while other strains may produce fructans as 30% of total extracellular polysaccharides.

3.2.2. Intracellular Polysaccharides.

Many plaque bacteria can synthesize intracellular iodine-staining polysaccharides from various sugars in low concentration. Streptococcus mutans produces a storage of intracellular polysaccharide which may contribute to the pathogenicity of Streptococcus mutans (Berman & Gibbons 1966). Stored intracellular polysaccharides may be the source of acid when exogenous sugar is not sufficient or is absent. However, strains of Streptococcus mutans producing little or less intracellular polysaccharide can produce marked dental caries in experimental animals (Guggenheim 1968). Therefore intracellular polysaccharides appear not to be a pre requisite for cariogenicity of Streptococcus mutans.

Intracellular polysaccharides is a glycogen-like glucan with $\alpha$(1-4) and $\alpha$(1-6) linkages. Its metabolism appears to be influenced mainly by the pH of the external environment. Streptococcus mutans will produce ethanol and acetic acid in addition to lactic acid from intracellular polysaccharides under the limitation of exogenous glucose, whereas only lactic acid is formed in the presence of excess glucose (Huis in't Veld & Backer Dirks 1878).

In the deep region of plaque, the cell walls of gram positive coccal bacteria become thickened and the majority of the cells contain scattered intracellular

polysaccharides granules in the cytoplasm. On the other hand, cells located in the superficial portion of the plaque possess normal cell wall morphology and fewer intracellular polysaccharides granules (Van Houte & Saxton 1971).

Intracellular polysaccharides synthesis may be influenced by various cultural conditions as rifampin treatment of Streptococcus mutans cultures results in accumulation of intracellular polysaccharides and thickening of the cell walls accompanying inhibition of ribonucleic acid synthesis, whereas tetracycline treatment causes cell wall thickening accompanying inhibition of protein synthesis but little accumulation of intracellular polysaccharides.

3.2.3 Glycosyltransferase.

The distribution of glycosyltransferase in broth culture of Streptococcus mutans is strongly influenced by various factors. In many cases, almost all of the glycosyltransferase activities are found extracellularly in sucrose free media, although the occurrence of significant cell-associated glycosyltransferase activity in addition to cell-free glycosyltransferase activity has been reported. The presence of or the addition to culture media of sucrose results in the synthesis of cell-associated glycosyltransferase.

McCabe and Smith (1973) consider that glycosyltransferase is reversibly bound to the insoluble glucan during the synthesis of the glucan by sucrose-grown Streptococcus mutans cells. The enzyme then becomes irreversibly bound, and is finally inactivated as insoluble glucan accumulates.

The addition of soluble dextran stimulates the reaction of glycosyltransferase with sucrose because dextran acts as a primer molecule, where newly synthesised glucan units are added.

The addition of increasing amounts of soluble dextran will cause a decrease in the synthesis of insoluble glucan and an increase in the synthesis of soluble glucan (Robyt & Corrigan 1977).

The water soluble glucan synthesis by Streptococcus mutans glysocyltransferase has also been found to increase by the addition of a phosphoglyceride namely lysophosphatidylcholine (Harlander & Schachtele 1978). The activity of glycosyltransferase is also enhanced by the presence of phospholipids normally detected in human oral fluid e.g. saliva from various glands, gingival crevicular fluid and serum (Schachtele et al 1978). Many other non-ionic surfactants promote the activity of glysocyltransferase, whereas anionic and cationic surfactants inhibit this activity; and lower concentrations of ampholytic surfactants activate glysocyltransferase activity.

3.2.4 Invertase.

Invertase is a sucrase that catalyzes the hydrolysis of the glucosidic linkage of sucrose, which results in the release of an equimolar ratio of glucose and fructose.

The presence of this intracellular sucrose was first suggested by Gibbons (1972). The intracellular location of the invertase implies the presence of a sucrose permease system, but little is known about the sucrose transport mechanism of Streptococcus mutans.

Extracellular invertase also exists (Fukui et al 1974).

The physiological role of Streptococcus mutans invertase is not fully understood. However, a large portion of available sucrose is hydrolysed by this enzyme while the remainder is converted to the synthesis of glucan or fructan via glycosyltranferase or fructosyltransferase.

### 3.2.5 α(1-6) Glucanase

α(1-6) glucanase is synthisized constitutively by some strains of Streptococcus mutans as well as certain other bacterial species in dental plaque (Dewar & Walker 1975).

The biochemical property of the enzyme is similar to that of mold dextranases in general, and most glucans produced by growing Streptococcus mutans cells could be synthesized under the influence of intrinsic or contaminating α(1-6) glucanase. Structural heterogenity of the Streptococcus mutans glucans may be a result of the combined enzymatic action of glycosyltransferase and endoglucanases activities. Various quantities of the enzymes may significantly affect the chemical and physical properties of glucans synthesized by Streptococcus mutans.

## 3.3 SUGAR METABOLISM.

The metabolic pathway of sucrose by Streptococcus mutans varies depending on environmental factors. The major fermentation product of Streptococcus mutans is lactate, especially when the organism is grown in the presence of excess glucose whereas Streptococcus mutans produce significant amounts of formate acetate and ethanol in addition to lactate when glucose is limiting (Carlsson & Griffith 1974).

Sucrose has also been shown to serve as an energy source during growth of Streptococcus mutans in addition to its role as the substrate for extracellular glucan synthesis. Most of the glucose in sucrose is converted into lactic acid. Only a small portion of sucrose is diverted to extracellular polysaccharide synthesis (Tanzer 1972). Streptococcus mutans is also known to utilise sucrose at a significantly faster rate than other bacterial such as Streptococcus sanguis and Streptococcus mitis (Onose & Sandham 1977). Streptococcus mutans produces significant amounts of intracellular polysaccharide from sucrose, which can be converted into lactic acid after prolonged incubation; the organism can also produce mannitol when high levels of sucrose of glucose are present (Loesche & Korman 1976). Comparison of metabolic activities of cariogenic and non-cariogenic plaques indicates that Streptococcus mutans is metabolically dominant in plaque closely associated with

the carious lesion, and Streptococcus mutans is more aciduric than other oral Streptococcal species (Donoghue & Tyler 1975). In the presence of sucrose, Streptococcus mutans grows in the same expoential rate as it does on glucose and Streptococcus mutans transports glucose into cells via a memebrane-associated PEP-dependent phosphotransferase system (Schachtele 1975). Sucrose and lactose are similarly transported in Streptococcus mutans by this system.

## 3.4 CARIOGENICITY IN EXPERIMENTAL ANIMALS

Until 1954, there was no conclusive evidence that bacteria were essential for the initiation of caries. In that year, Orland and co-workers demonstrated that completely germ-free rats of a caries susceptible line failed to develop caries even when fed a cariogenic, high sucrose diet. By implanting bacteria into the mouths of these germ-free animals, caries was produced (Orland et al 1954).

Unfortunately, the precise identity of the strains of bacteria used in these pioneer experiments is not known, except in general descriptive terms such as 'an enterococcus' and 'a proteolytic rod', and the original strains are no longer available for further study. However, these experiments established a basic method by which dental caries can be studied in an animal model system. Such experimental systems have subsequently been widely adopted in dental caries research for studying the microbial aetiology of the disease. They can also be used for estimating the caries-enhancing properties of various dietary substrates and for testing potential cariostatic or preventive measures.

### 3.4.1 Caries Induction in Animals

In the earlier stage of caries research, it was thought that there might be a specificity between the caries-inducing streptococci and the host animal species. However, Zinner et al (1966) demonstrated that human strains of Streptococcus mutans which reacted with the antiserum against the hamster strains of Streptococcus mutans, could produce extensive caries in hamster. Since then, many streptococcal strains isolated from the human mouth have been shown to be cariogenic in various animal model systems. Most of the cariogenic strains belong to the species Streptococcus mutans. However, organisms other than Streptococcus mutans can occassionally induce variable levels of caries in animals.

Dental caries have been induced in various kinds of animals, including monkeys, mice, rats and hamsters. Although much useful information has been gained from studies on caries in rodents, there are obviously fundamental differences between the situation in the rodent mouth and that of man. Primates are in many ways preferable to rodents for experimental caries studies, since their dentition and the pattern of caries attack is similar to that of man. In addition, it is possible to maintain primates on a diet which closely resembles that consumed by humans, and the microbial flora of the mouth of monkeys is also quite similar to that of man. Caries research on monkeys, however, has been limited, largely

due to the high cost of maintaining a colony of animals and also because of the considerable technical problems involved.

Strains of Streptococcus mutans almost always induce smooth surface and pit and fissure caries in animals (Krasse & Carlsson 1970), with variations frequently observed in the pattern and severity of the induced carious lesions in experimental animals. In general, young animals are more susceptible to a caries attack (Navia & Lopez 1977).

Dietary factors critically influence the commission and pathogenic potential of inoculated Streptococcus mutans by affecting the implantation, colonization and metabolic activities of the bacteria. Sucrose has been demonstrated to be most cariogenic and supports most rapidly progressive pathogenesis, although other sugars, such as maltose, lactose and fructose, also support the induction of dental caries in animals to some extent.

### 3.4.2. Non-cariogenic and Supercariogenic Mutants of Streptococcus mutans.

For a particular bacteria causing an infectious disease, a special vivulence factor in its pathogenesis can be indentified if mutants of the same bacteria, lacking one or more characteristics possibly responsible for pathogenic processes, can be obtained and used to analyse the mechanism of the pathogenesis.

In various research works reported, the presence of a single mutation has not been established; more than one mutation are normally assumed and a mutation if present, may only be indirectly related to different characters being considered.

A mutant of Streptococcus mutans was isolated by De Stoppelaar et al (1971) which failed to synthesize cell-bound glucan in presence of sucrose. The inability to synthesize insoluble glucose of an adherent nature was accompanied by a significant reduction of cariogenic potential in experimental animals. The mutant also showed a diametic loss of viability due to acid reduction from either glucose or sucrose.

Mutants were also isolated by Freedman and Tanzer (1974) which differed from each other in colonial morphology on monosaccharide agar. The mutants were found to lose the ability to adhere to a wire surface but

retain the ability to agglutinate and form macroscopically visible clumps in the presence of sucrose or exogenous glucans. The mutants were also found to produce increased amounts of water-soluble extracellular glucans and they failed to form plaque on the smooth surface of the teeth. These results indicate that surface-associated glucan synthesis by Streptococcus mutans apparently contributes to the local environment and promotes the pathogenic potential of Streptococcus mutans on smooth tooth surfaces. This is probably due to a barrier effect of the glucan layer to the diffusion of metabolically excreted lactic acid, which has been considered to be critical in the demineralization of the teeth (Tanzer et al 1974). It also indicates that cell-to-surface adherence via insoluble glucan synthesis from sucrose is a more important factor than cell-to-cell agglutination induced by glucan in the pathogenesis of dental caries.

Mutants producing elevated levels of glycosyltransferase have been isolated by Schachtele et al (1975). These mutants demonstrate increased ability to adhere to glass surface and produce more carious lesions than the parent strains, showing a clear correlation between cariogenicity, in vitro adherence and insoluble glucan synthesis in Streptococcus mutans.

Mutants that synthesize or degrade less intracellular polysaccharides have been isolated from strains of Streptococcus mutans which are strong

producers of intracellular polysaccharides. These mutants had diminished virulence both on smooth tooth surfaces and in fissures. The loss of cariogenicity of these mutants is attributed to diminished ability to produce acid from endogenous intracellular polysaccharide storage in the absence of exogenous carbohydrates. (Tanzer et al 1976).

## 3.5 STREPTOCOCCUS MUTANS AND DENTAL CARIES IN HUMANS.

Following the observations made upon the ability of Streptococcus mutans to produce dental caries in experimental animals, investigators have looked for an association between this species and caries in humans, and a number of reports have appeared on epidemiological surveys in which dental caries scores have been correlated with the presence of Streptococcus mutans in the mouth.

3.5.1 Effect of Sucrose on The Proportion of Streptococcus mutans.

It is well known that dietary carbohydrates and infection with Streptococcus mutans are essential factors in the development of dental caries. Among dietary carbohydrates, sucrose is considered to be directly related to dental caries. Recent studies with gnotobiotic rats by Micholek et al (1977), however, have revealed that as little as 0.1% sucrose in the diet can significantly promote the development of dental caries by Streptococcus mutans, indicating that the consumption of artificially high levels of sucrose is not necessary for the induction of dental caries.

Several studies on the effect of dietary sucrose on streptococcal composition in plaque flora have been carried out with human subjects. Plaque formation was heavier during high sucrose diet periods than in glucose diet periods. De Stoppelaar et al (1970) showed when six subjects were instructed to abstain from any dietary carbohydrates for 17 days, the Streptococcus mutans count decreased to an undetectable level while the percentage of Streptococcus sanguis increased. A low sucrose diet, however, did not completely eliminate Streptococcus mutans from the oral flora as shown by Staat et al (1975).

### 3.5.2 Epidemiological Relationship between Streptococcus mutans and Caries Development..

Despite strains of Streptococcus mutans isolated from human have been shown to be cariogenic in experimental animals, these results cannot indicate the bacteria actually are cariogenic in humans. To clarify the etiological role of Streptococcus mutans in caries development in humans, one has to depend on epidemiological studies which relate the microbes of the carious lesion or dental plaque to the initiation of caries at the tooth site. Some epidemiological studies will be discussed in this section.

Littleton et al (1970) were able to isolate Streptococcus mutans from all carious lesions of children aged 13 to 14 years, whereas only 23% of the samples from sound tooth surfaces of the same group of children contained the bacterium. Englander and Jordan (1972) found similar results in deciduous dentition while Hoerman et al (1972) noted similar results in 17 to 22 years old groups.

After an extensive study by Loesche et al (1975), it came to a conclusion that there is a strong association between levels of Streptococcus mutans in single occlusal fissures and dental caries. Seventy-one (71) percent of the carious fissures retained Streptococcus mutans, accounting for more than 10% of the viable count, whereas

70% of the fissures free from caries had no detectable levels of Streptococcus mutans. Furthermore, it has been shown that aciduric bacteria such as Lactobacillus are detected in significant quantities in the dentinal carious lesion as the decay progresses (Loesche & Syed 1973).

In a later study, Duchin and Van Houte (1978) demonstrated that the proportion of Streptococcus mutans in samples from early carious lesions (white spots) of smooth tooth surfaces was significantly higher than that from the adjacent sound surface. In these early lesions, no significant numbers of lactobacilli were found.

However, in the case of a chronic disease such as dental caries, cross-sectional studies alone, will not give a convincing explanation to the etiological involvement of a bacterium in the oral flora, Longitudinal studies, demonstrating cause-and-effect relationship have been reported. Ikeda et al (1973) followed the distribution of Streptococcus mutans on the tooth surfaces over a period of 18 months. The development of caries was frequently preceded by colonization with elevated levels of Streptococcus mutans. Keene and Shklair (1974) found similar results in another study. In longitudinal studies, however, complex factors such as sampling sites, methods of cultivation, fluoride content, eating habits of the subjects, sucrose intake and possible immunity in the oral cavity cannot be standardized and careful consideration must be given when interpreting the

results. Llory et al (1972) observed a significant increase in Streptococcus mutans in saliva and dental plaque in patients who have received radiation therapy of the major salivary gland. A close relationship is established among rampant caries, xerostomia due to degeneration of salivary glands and an increase in Streptococcus mutans.

In another survey Masuda et al (1979)found that over a period of 30 months, Streptococcus mutans was isolated from all 12 of the infants who developed caries out of a total sample of 22; and as the number of erupted teeth increases, there is a gradual increase in the prevalance of Streptococcus mutans.

## 4. PREVENTION OF CARIES CAUSED BY STREPTOCOCCUS MUTANS.

Dental caries, in theory, can be prevented by eliminating cariogenic bacteria especially Streptococcus mutans from the mouth, by increasing the resistance of teeth and by modifying the diet.

In this section, the various methods to eliminate Streptococcus mutans as a preventive measure for dental caries will be discussed.

## 4.1 SUPPRESSION OF STREPTOCOCCUS MUTANS BY ANTIMICROBIAL AGENTS.

The effectiveness to prevent experimentally induced dental caries by the use of penicillin has been noted since the pioneering work of McClure and Hewitt when they suspected Lactobacillus acidophilus as the causative agent. Since then, many investigations have shown that most antibiotics with antimicrobial activity against gram-positive bacteria will depress the development of dental caries induced in experimental animals. In addition, Handelman et al (1966) observed that young human patients aged 6 to 19 years who had received long term administration of penicillin and/or tetracycline for treatment of various other chronic infectious diseases, developed about two-thirds fewer caries than did control subjects. The above observation is supported by another finding that the presence of very low concentrations of both penicillin G and sulfadiazine markedly inhibits in vitro plaque formation by Streptococcus mutans (Wild & Sandham 1976). These same authors, however, reported that long term therapy with penicillin and sulfadiazine did not cause a significant reduction in the proportions of Streptococcus mutans or lactobacilli, although the organisms isolated from the patients demontrated high susceptibility to penicillin. So penicillin-resistant strains of Streptococcus mutans have been described.

In vitro tests have shown that Streptococcus mutans is highly susceptible to penicillin, ampicillin, erythromycin, cephalothin, methicillin and some other antibiotics. But in spite of the in vitro effectiveness of antibiotics, it is not practical to use them for caries control. Several investigations suggested that certain antimicrobial agents may be used in a short-term basis to suppress Streptococcus mutans. De Paola et al (1974) noted the temporary suppression of Streptococcus mutans in human through topical application of vancomycin; Loesche et al (1977) observed the effect of short term treatment with kanamycin on patients with rampant caries.

The use of antibiotics for control of Streptococcus mutans has proved to be a valuable research tool, but it is dubious whether it is the correct approach to caries prevention on a public health scale. There are various theoretical objections to the widespread, long-term use of antibiotics for this purpose, including selection of antibiotic resistant strains of bacteria; suppression of normal flora and overgorwth by less desirable species and allergic or other adverse reactions to the drug.

Caulfield and Gibbons (1979) described about the topical application of iodine leading to a suppression of Streptococcus mutans in human mouths.

Chlorhexidine is another disinfectant which has been in general use for many years and is known to be active against a wide range of gram-positive and gram-negative bacteria, as well as some yeasts. It is usually used in the form of chlorhexidine gluconate. Loe and Schiott (1970) demonstrated that chlorhexidine gluconate can inhibit dental plaque formation, and substitution of mechanical tooth cleaning by mouth rinsing or topical application of chlorhexidine can prevent plaque accumulation and the development of gingivitis. Schiott and Loe (1972) also showed a reduction in the number of Streptococcus mutans isolated after the use of chlorhexidine mouthwash for 6 months. The possibility of combining chlorhexidine with some other known cariostatic agent, such as fluoride, is currently being explored and short term use of a fluoride containing chlorhexidine-gel can cause alterations in the plaque microflora including a marked reduction in Streptococcus mutans levels. The long term beneficial effect of chlorhexidine as a cariostatic agent remains to be demonstrated.

Fluoride is well known for its ability to inhibit a variety of enzymes, including some of those involved in acid production by bacteria. When a strain of Streptococcus mutans was grown under glucose-limited conditions, at a fixed pH of 6.5, it was observed that 15 p.p.m. of fluoride ion could prevent acid production when the organisms were growing slowly (Hunter et al 1973).

Topical application of fluoride can also influence the streptococcal population within plaque, especially the relative number of Streptococcus mutans (Woods 1971). The practical significance of the antimicrobial or anti-enzymic properties of the fluoride ions in relation to the overall caries reducing effect of this element, however, is not clear at present.

## 4.2 INHIBITION OF ADHERENCE OF STREPTOCOCCUS MUTANS BY GLUCAN-HYDROLYSING ENZYMES.

The synthesis of insoluble adherent glucan from sucrose by Streptococcus mutans is a prerequisite for the induction of dental caries in experimental animals. In vitro studies indicate that $\alpha$(1-6) glucanases have limited ability to degrade the extra-cellular glucans produced by Streptococcus mutans (Newbrun 1972). Fitzgerald also showed $\alpha$(1-6) glucanase effectively prevented plaque formation and caries induction by Streptococcus mutans strains in hamsters.

Another glucanase, the $\alpha$(1-3) glucanase or so called "mutanase" has been isolated from a strain of Trichoderma harzianum and was found to inhibit caries induction. It also impaired the colonization of Streptococcus mutans in plaque and inhibited the formation of dental plaque and gingivitis in humans. However, in vivo effects of these $\alpha$(1-3) glucanase have not been examined.

## 4.3 IN VITRO EFFECT OF ANTISERA AGAINST STREPTOCOCCUS MUTANS.

Antibodies raised against various cellular and extracellular components of Streptococcus mutans have been shown to exert a variety of effects on the biological activities of Streptococcus mutans. Antisera against whole cells of Streptococcus mutans markedly inhibit the adherence of homologous or immunologically related cells of Streptococcus mutans to smooth surfaces. The adherence inhibition was found by Olson et al (1972) to depend on IgG antibody. The antibody is specific for certain sites for strains of Streptococcus mutans and was reported to inhibit the binding of glycosyltransferase and subsequent adherence of Streptococcus mutans cells.

Some antisera against whole cells of Streptococcus mutans significantly inhibit the enzymatic activity of extracellular glycosyltransferase and hence the subsequent adherence to smooth surfaces.

Information on the penetration of antibody into the plaque is limited. In vitro Streptococcus mutans plaque was found to contain the specific antibody at the plaque surface as shown by immuno-fluorescence. Additional studies in this field, however, are needed.

## 4.4 IMMUNOLOGICAL RESPONSES OF HOST TO STREPTOCOCCUS MUTANS

The concept that dental caries is an infectious disease caused by specific pathogen such as Streptococcus mutans has led research workers to explore the possibility that the disease can be prevented by immunization. Studies in human populations have indicated that there may be differences in antibody level between subjects with different amount of of caries. Various antibodies reacting with Streptococcus mutans have been detected in serum, saliva and colostrum by several investigators using different methods. Serum antibodies play a protective role in many infectious diseases and patients with immunoglobulin dysfunction have been found to have a greater susceptibility to dental caries. Challacombe (1974) reported a correlation between low caries experience and high titres of serum IgG and IgM antibodies against antigens of Streptococcus mutans. The specific basis of attempting to control dental caries immunologically may be stated simply as:-

(1) The disease is a specific infection caused by Streptococcus mutans,

(2) An artificially induced immune response to Streptococcus mutans antigen may protect against caries,

(3) Protective antibodies may reach caries

susceptible sites either via saliva i.e. local IgA response or crevicular fluid i.e. systemic IgG and IgM response.

Many oral bacterial species have been found to react with salivary antibody and significant levels of agglutinins specific for Streptococcus mutans were detected in normal human colostrum and saliva. A positive correlation between increased caries incidence and decreased levels of salivary IgA in humans has been reported by different groups of investigators including Brown et al (1978) and Challacombe (1976).

## 4.5 POSSIBLE VACCINATION WITH STREPTOCOCCUS MUTANS ANTIGENS.

Immunization with Streptococcus mutans antigens is an attractive concept for the control of dental caries. Two different hypothesis have been proposed for the mechanisms of immunological control against dental caries. The British groups proposed that serum IgG antibodies are mainly responsible for protective effect whereas the American workers suggest secretory IgA in saliva inhibits adherence of Streptococcus mutans to tooth surfaces. However, these two mechanisms are not necessarily mutually exclusive.

Bowen (1969) showed in a preliminary study that three monkeys vaccinated with whole cells of a serotype Streptococcus mutans developed significantly fewer carious lesions than control animals. Bowen et al (1975) further demonstrated that whole cell or broken cell vaccines conferred significant protection in monkeys especially when the immunogen was administered by intraoral submucosal injection. More recently, protection was demonstrated against dental caries in rhesus monkeys infused passively by the intravenous route with antibodies of IgG class. Intact molecules of IgG, IgA and IgM have been shown to pass from plasma to the oral cavity via crevicular fluid, and therefore can contribute to local defence mechanisms.

The primary importance of local immunity due to secretory IgA antibodies has also been reported. Taubman and Smith (1974) demonstrated that local immunization with formalinized whole cells of Streptococcus mutans resulted in an enhanced salivary IgA response and reduce caries development in both conventional and gnotobiotic rats. It was also found that similar immunization using cell-free glycosyltransferase preparations resulted in the presence of antibodies in saliva of rodents and monkeys. Reduction in carious lesions were greater on smooth surfaces of teeth than on occlusal surfaces probably due to interference with adherence of Streptococcus mutans (Krasse & Jordan 1977). In these cases, salivary antibody is the most likely protective principles in the rodents. Local immunization with whole cells of Streptococcus mutans stimulates a specific salivary IgA response which is protective against caries induction by Streptococcus mutans infection.

It should be noted however, that immunological cross-reactions may occur between human heart tissues and certain components of Streptococcus mutans strains and at the present time, there is no evidence of adequate clinical trials on humans having been held. The statement made by the National Health and Medical Research council at its 90th Session in October, 1980, regarding the introduction of vaccines for the control of dental caries in humans still holds, namely 'The Council noted that

extensive research is occurring in many countries on the development of vaccines against dental caries. However, it was recognised that the current states of research is such that the efficacy, and safety of those products in man have not yet been established.

# 5. DISCUSSION

Streptococcus mutans is considered to be the primary cariogenic bacterium in both humans and animals; and its in vivo and in vitro activities have been discussed in some detail in this paper. Other bacteria found in actively progressive carious lesions are considered to be secondary invaders, probably commensal with Streptococcus mutans with regard to their physiological activities.

Virulence factors of Streptococcus mutans responsible for its cariogenicity include the ability to adhere to smooth surfaces; the ability to synthesize polymers and the ability to metabolise carbohydrates especially sugars.

The several different mechanisms thought to play a significant part in the initial colonization of tooth surfaces by Streptococcus mutans and the subsequent development into dental plaque include:

1. Adherence of bacteria to pellicle and/or exposed enamel surface;

2. Adhesion between bacteria either of the same or different species;

3. Growth of bacteria, either from small cracks or defects in the

enamel surfaces or from the cells
which have initially become
attached to the tooth.

The various adhesive mechanisms that exist in plaque serve to maintain the integrity of the material and prevent its removal by normal physiological process and gentle forces such as mouth rinsing.

Streptococcus mutans has its own characteristic surface polymers, which vary in chemical and antigenic properties and may carry different charges. The extracellular polysaccharides may play an important role in the initial colonization of the teeth and may contribute towards the inter-microbial plaque matrix. Extracellular polysaccharides may also be responsible for retention of bacteria in addition to the initial adherence to plaque. Streptococcus mutans has a series of enzymes which ensure a continuous generation of the polymers.

Dental caries is a food-dependent disease. Sucrose is the fermentable carbohydrate that appears most frequently in human diet and a positive close correlation has been established between proportions of Streptococcus mutans and the incidence of dental caries in the presence of sucrose. Sucrose is also important for Streptococcus mutans colonization on tooth surfaces. In vivo and in vitro studies both support this view.

The essential cariogenicity of Streptococcus mutans lies in its ability to produce acid rapidly from carbohydrates, that is, Streptococcus mutans is acidogenic; and its ability to survive under acid conditions, that is, they are aciduric. Acids produced at or near the tooth surface by bacterial fermentation of dietary carbohydrates and these acids dissolve the apatite crystals which make up some 95 percent of the mass of enamel. Washing away of the acids is reduced by the presence of dental plaque which also serves to hold the products of dissolution close to the tooth surfaces.

In general, increase in types and frequency of carbohydrate ingestion lowers intra oral pH value, and increases in dietary refined carbohydrate levels will increase caries incidence.

An association between Streptococcus mutans and caries in humans has been established by epidemiological surveys, in which dental caries scores are correlated with the presence of Streptococcus mutans in the mouth. Cross sectional and longitudinal studies demonstrate cause and effect relationship between Streptococcus mutans and dental caries.

The fact that Streptococcus mutans is a major causal bacterium for dental caries has led to the concept that elimination of the bacterium will prevent the spread of

the disease. And various such preventive methods have been discussed.

The use of antibiotics has been shown to be effective in the suppression of bacterial growth, but it is doubtful that unrestricted use of antibiotics is a good caries prevention measure on a public health scale. Besides, antibiotic resistant strains have been developed, further discrediting the practicality of long term use of the medicine.

Topical applications of iodine and disinfectants such as chlorhexidine and fluoride have led to a reduction in the number of Streptococcus mutans isolated. The long term effects to establish them as cariostatic agents however, remain to be demonstrated.

As adherence of Streptococcus mutans is a pre requisite for subsequent caries induction, interference with the adherence will undoubtedly affect bacterial colonization. This can be done by either the glucan hydrolysing enzymes which may degrade extracellular polymers produced by Streptococcus mutans or by antisera raised against cellular or extracellular components of Streptococcus mutans, which will effectively inhibit adherence. But further investigations are needed in this field.

Vaccination against dental caries was first suggested in late 1960's wherein immunization had been achieved with whole cells and with broken cells. For the past one and a half decades, extensive research has been carried out in many countries. Investigations from the Royal College of Surgeons of England have demonstrated a vaccine produced from the cell walls of Streptococcus mutans, strain Ingbritt, is an effective agent when used in the control of dental caries in monkeys. The animal model of the monkey Macaca fascicularis has been chosen because of it simulates conditions found in human situations. An immunization programme was instituted with crude vaccine in a small number of animals. Booster injections were given in intervals and virtually eliminated dental caries in the permanent dentition. The numbers of cariogenic Streptococcus mutans were found to be very much reduced in the mouths of the immunized animals. However, translation of this therapeutic procedures to human purposes is neither straightforward nor simple. Cross reactivity with heart tissues as well as reactions in ventricular muscle, in the kidney, liver and brain have been reported when pure antigens or protein component serologically indistinguishable from the pure antigens were injected into experimental animals.

In this field of research, the situation can best be summed up by Professor Bertram Cohen of the Nuffield Faculty of Dental Science in the Royal College of Surgeons

of England, who stated 'Successful though this preventive measure has proved to be, there is a formidable literature devoted to the incidence of untoward side-effects. It goes without saying that attention directed against such dangers must be especially alert when the disease to be prevented does not directly threaten life. Moreover, the known association between streptococci and lesions of the heart and kidney calls for special care in introducing components of these organisms into the human situation.' To this must be added the fact that this warning is applicalbe to whatever the source of the antigenic material, whether it be special cultures of Streptococcus mutans or from the patients' oral streptococci.

# 6. CONCLUSION

Laboratory, animal and clinical studies in recent years have produced a better perspective on the role that particular organisms may play in dental caries. Many studies have shown that Streptococcus mutans does have an exclusive role and its participation as the causal bacterium for dental caries is inevitable. However, it has to be remembered that a more balanced approach and a greater degree of flexibility both in the planning of experiments and the development of rational means of disease control will be required in order that other bacteria which may also be essential in causing the disease are not omitted in the course of investigation.

When a practitioner explains a clinical situation to a patient, it may be advisable to build a story simply around Streptococcus mutans, this marker organism, and sucrose alone on the caries aspect. However, the dentist, as a professional person, should be aware of other microbial participants and be readily receptive to new knowledge.

## List of References

BERMAN KS & GIBBONS RJ (1966).

Iodophilic polysaccharide synthesis by human and rodent oral flora.
Arch Oral Biol 11:533-542.

BIBBY BG (1970).

Inference from natural occurring variations in caries prevalence.
J Dent Res 49:1194-1200,

BIBBY BG (1975).

The cariogenicity of snack foods and confections.
JADA 90:121-132.

BIBBY BG (1978).

Cariogenicity and caries prediction: Proceedings methods of caries prediction.
Microbiology Abst P.65-71.

BOURGEAU G & McBRIDE BC (1976).

Dextran-mediated interbacterial aggregation between dextran-synthesizing streptococci and Actinomyces viscosus.
Infect Immun 13:1228-1234.

BOWEN WH (1969).

A vaccine against dental caries. A pilot experiment in monkeys (Macaca irus).
Br Dent J 126:159-160.

BROWN LR, DREIZENS S, DALY TE, DRANE JB,

HAMDLER S, RIGGAN LJ & JOHNSTON DA (1978).

Interrelation of oral micro-organisms, immunoglobulins and dental caries following radiotherapy.
J Dent Res 57:882-893.

CARLSSON J. (1967).

A medium for isolation of Streptoccucus mutans.
Arch Oral Biol 12:1657-1658

CARLSSON J (1970).

A levansucrase from Streptococcus mutans.
Caries Res 4:97-113.

CARLSSON J & GRIFFITH CJ (1974).

Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of streptococci.
Arch Oral Biol 19:1105-1109.

CAUFIELD PW & GIBBONS RJ (1979).

Suppression of Streptococcus mutans in the mouths of humans by a dental prophylaxis and topically applied iodine.
J Dent Res 58:1317-1326.

CHALLACOMBE SJ (1976).

Immunoglobulins in parotid saliva and serum in relation to dental caries in man.
Caries Res 10:165-177.

CLARK WB, BAMMANN LL & GIBBONS RJ (1978).

Comparative estimates of bacterial affinites and adsorption sites on hydroxyapatite surfaces.
Infect Immun 21:681-684.

CLARKE JK (1924).

On the bacterial factor in the aetiology of dental caries.
Br J Exp Pathol 5:141-147.

DePAOLA PE, JORDON HV & BERG J (1974).

Temporary suppression of Streptococcus mutans in humans through topical application of vancomycin.
J Dent Res 53:108-114.

DeSTOPPELAAR JD, KONIG KG, PLASSCHAERT AJM & van der HOEVEN JS (1971).

Decreased cariogenicity of a mutant of Streptococcus mutans.
Arch Oral Biol 16:971-975.

DeSTOPPELAAR JD, van HOUTE J & DIRKS OB (1970).

The effect of carbohydrate restriction on the presence of Streptococcus mutans, Streptococcus sanguis and iodophilic polysaccharide-producing bacteria in human dental plaque.
Caries Res 4:114-123.

DEWAR MD & WALKER GJ (1975).

Metabolism of the polysaccharides of human plaque.
Caries Res 9:21-35.

DONOGHUE HD & TYLER JE (1975).

Antagonisms amongst streptococci isolated from the human oral cavity.
Arch Oral Biol 20:381-387.

DUCHIN S & van HOUTE J (1978).

Colonization of teeth in humans by Streptococcus mutans as related to its concentration in saliva and host age.
Infect Immun 20:120-125.

EDWARDSSON S & KRASSE B (1967).

Human streptococci and caries in hamsters fed diets with sucrose or glucose.
Arch Oral Biol 12:1015-1016.

ENGLANDER HR & JORDAN HV (1972).

Relation between Streptococcus mutans and smooth surface caries in the deciduous dentition.
J Dent Res 51:1505.

FITZGERALD RJ & KEYES PH (1960).

Demonstration of the etiologic role of streptococci in experimental caries in the hamster.
J A D A 61:9-19.

FITZGERALD RJ, SPINELL DM & STOUDT TE (1968).

Enzymatic removal of artificial plaques.
Arch Oral Biol 13:125-12

FREEDMAN ML & TANZER JM (1974).

Dissociation of plaque formation from glucan induced agglutination in mutants of Streptococcus mutans.
Infect Immun 10:189-196.

FUKUI K, FUKUI Y & MORIYAMA T (1974).

Purification and properties of dextransucrase and invertase from Streptococcus mutans.
J Bacteriol 118:796-804.

GIBBONS RJ (1972).

Presence of an invertase-like enzyme and a sucrose permeation system of Streptococcus mutans.
Caries Res 6:122-131.

GIBBONS RJ & FITZGERALD RJ (1969).

Dextran-induced agglutination of Streptococcus mutans and its potential role in the formation of microbial dental plaques.
J Bacteriol 98:341-346.

GIBBONS RJ & van HOUTE J (1973).

On the formation of dental plaques.
J Periodontol 44:347-360.

GUGGENHEIM B (1968).

Streptococci of dental plaque.
Caries Res 2:147-163.

HAMADA S, TAI S & SLADE HD (1978).

Binding of glycosyltransferase and glucan synthesis by Streptococcus mutans and other bacteria.
Infect Immun 21:213-220.

HAMADA S & SLADE HD (1980).

Biology, Immunology and Cariogenicity of Streptococcus mutans.
Microbiol Rev 44:331-384.

HANDELMAN SL & HESS C (1966).

Bacterial populations of selected tooth surface sites.
J Dent Res 48:67-70.

HARLANDER SK & SCHACHTELE CF(1978).

Streptococcus mutans dextransucrase: stimulation of glucan formation by phosphoglycerides.
Infect Immun 19:450-456.

HAY DJ (1967).

The adsorption of salivary proteins by hydroxyapatite and enamel.
Arch Oral Biol 12:937-946.

HOERMAN KC, KENNE HJ, SHKLAIR IL & BURNEISTER JA (1972).

The association of Streptococcus mutans with early lesions in human teeth.
J A D A 85:1349-1352.

HOWELL A, RIZZO A & PAUL F (1965).

Cultivable bacteria in developing and mature dental calculus.
Arch Oral Biol 10:307-313.

HUIS in't VELD JHJ & BACKER DIRKS O (1978).

Intracellular polysaccharide metabolism in Streptococcus mutans.
Caries Res 12:243-249.

HUNTER JR, BAIRD JK & ELLWOOD DC (1973).

Effect of fluoride on the transport of sugars into chemostatic grown Streptococcus mutans.
J Dent Res 52:954.

HUXLEY HG (1974).

The effect of dietary carbohydrate upon the colonization of plaque by Streptococcus mutans in rats.
Arch Oral Biol 19:941-946.

IKEDA T, SANDHAM HJ & BRADLEY JR EL (1973).

Changes in Streptococcus mutans and lactobacilli in plaque in relation to the initiation of dental caries in Negro children.
Arch Oral Biol 18:555-566.

JABLON JM & ZINNER DD (1966).

Differentiation of cariogenic streptococci by fluorescent antibody.
J Bacteriol 92:1590-1596.

KEENE HJ & SHKLAIR IL (1974).

Relationship of Streptococcus mutans carrier status to the development of carious lesions in initially caries free recruits.
J Dent Res 53:1295-1296.

KEYES PH (1960).

The infectious and transmissible nature of experimental dental caries.
Arch Oral Biol 1:304-320.

KILIAN M & ROLLA G (1976).

Initial colonization of teeth in monkeys as related to diet.
Infect Immun 14:1022-1027.

KRASSE B (1965).

The effect of diet on the implantation of caries-inducing streptococci in hamsters.
Arch Oral Biol 10:215-221.

KRASSE B (1965).

The effect of caries-inducing streptococcus in hamsters fed diet with sucrose or glucose.
Arch Oral Biol 10:223-226.

KRASSE B & CARLSSON J (1970).

Various types of streptococci and experimental caries in hamsters.
Arch Oral Biol 15-25-32.

KRASSE B & JORDON HV (1977).

Effect of orally applied vaccines on oral colonization by Streptococcus mutans in rodents.
Arch Oral Biol 22:479-484.

LILJEMARK WF & SCHAUER SV (1975).

Studies on the bacterial components bind Streptococcus sanguis and Streptococcus mutans to hydroxyapatite.
Arch Oral Biol 4:609-615.

LITTLETON NW, KAKEHASHI S & FITZGERALD RJ (1970).

Recovery of specific caries-inducing streptococci from carious lesions in the teeth of children.
Arch Oral Biol 15-461-463.

LITTLETON RW, CARTER CH & KELLY RT (1967).

Studies of Oral Health in persons nourished by stomach tube.
J A D A 74:119-123.

LLORY H, DAMMRON A, GIOANNI M & FRANK RM (1972).

Some population changes in oral anaerobic micro-organism, Streptococcus mutans and yeast following irradiation of the Salivary glands.
Caries Res 6:298-331.

LOE H & SCHIOTT CR (1970).

The effect of mouth rinsing and topical application of chlorhexidine on the development of dental plaque and gingivitis in man.
J Periodont Res 5:79-83.

LOESCHE WJ, BRADLEY DR & WOODFOLK MT (1977).

Reduction of dental decay in rampant caries in individuals following short term kanamycin treatment.
J Dent Res 56:254-265.

LOESCHE WJ & GREEN E (1972).

Comparison of various plaque parameters in individuals with poor oral hygiene.
J Periodont Res 7:173-179.

LOESCHE WJ & KORMAN KS (1976).

Production of mannitol by Streptococcus mutans.
Arch Oral Biol 21:551-553.

LOESCHE WJ, MURRAY RJ & MELLBERG JR (1973).

The effect of topical acidulated fluoride on percentage of Streptococcus mutans and Streptococcus sanguis in interproximal plaque samples.
Caries Res 7:283-296.

LOESCHE WJ & SYED SA (1973).

The predominant cultivable flora of carious plaque and carious dentine.
Caries Res 7:201-216.

MASUDA N, TSUTSUMI N SOBUE S & HAMADA S (1979).

Longitudinal survey of the distribution of various serotypes of Streptococcus mutans in infants.
J Clin Microbiol 10:497-502.

McCABE RM, KEYES PH & HOWELL A (1967).

An in vitro method for assessing the plaque forming ability of oral bacteria.
Arch Oral Biol 12:1653-1656.

McCLURE FF & HEWITT WL (1946).

The relation of penicillin to induced rat dental caries and oral L. acidophilus.
J Dent Res 25:441-443.

MILLER CH & KLEINMAN JL (1974).

Effect of microbial interactions on in vivo plaque formation by Streptococcus mutans.
J Dent Res 53:427-434.

NAVIA JM & LOPEZ H (1977).

Sources of variability in rat caries studies: weaning age and diet fed during tooth eruption.
J Dent Res 56:222-227.

NEWBRUN E (1972).

Extracellular polysaccharides synthesized by glycosyltransferase of oral streptococci. Composition and susceptibity to hydrolysis.
Caries Res 6:132-147.

NISIZAWA T, IMAI S, AKADA H, HINOIDE M & ARAYA S (1976).

Extracellular glucans produced by oral streptococci.
Arch Oral Biol 21:207-213.

OLSON GA, BLEIWEIS AS & SMALL JR PA (1972).

Adherence inhibition of Streptococcus mutans: an assay reflecting a possible role of antibody in dental caries prophylaxis.
Infect Immun 5:419-427.

ONOSE H & SANDHAM HJ (1978).

pH changes during culture of human dental plaque Streptococcus on mitis-salivarius agar.
Arch Oral Biol 21:291-296.

ORSTAVIK D, KRAUS FW & HENSHAW LC (1974).

In vitro attachment of streptococci to the tooth surface.
Infect Immun 9:794-880.

SAXTON CA (1973).

Scanning electron microscopic study of the information of dental plaque.
Caries Res 7:102-119.

SCHACHTELE CF (1975).

Glucose transport in Streptococcus mutans: preparation of cytoplasmic membranes and characteristics of phosphotransferase activity.
J Dent Res 54:330-338.

SCHACHTELE CF, GERMAINE GR & HARLANDER SK (1975).

Production of elevated levels of dextransucrase by a mutant of Streptococcus mutans.
Infect Immun 12:934-937.

SCHACHTELE CF, HARLANDER SK, BRACKE JW, OSTRUM LC,

MALTAIS JB & BILLINGS, RJ (1978).

Streptococcus mutans dextransucrase: stimulation by phospholipid from human sera and oral fluids.
Infect Immun 22:714-720.

SCHIOTT CR & LOE H (1972).

The sensitivity of oral streptococci of chlorhexidine.
J Periodont Res 5:79-83.

TANZER JM (1972).

Studies on the fate of glycosyl moiety of sucrose metabolized by Streptococcus mutans.
J Dent Res 51:415-423.

TANZER JM, FREEDMAN ML, FITZGERALD RJ & LARSON RH (1974).

Diminished virulence of glucan synthesis-defective mutants of Streptococcus mutans.
Infect Immun 10:197-203.

TAUBMAN MA & SMITH DJ (1974).

Effects of local immunization of Streptococcus mutans of salivary immunoglobulin A antibody and experimental dental caries in rats.
Infect Immun 9:1079-1091.

VAN HOUTE J & DUCHIN S (1975).

Streptococcus mutans in the mouths of children with congenital Sucrase deficiency.
Arch Oral Biol 20:771-773.

VAN HOUTE J & SAXTON CA (1971).

Cell wall thickening and intracellular polysaccharide in micro-organisms of dental plaque.
Caries Res 5:30-43.

VAN HOUTE J & UPESLACIS VN (1976).

Studies of the mechanism of sucrose-associated colonization of Streptococcus mutans on teeth of conventional rats.
J Dent Res 55:216-222.

VAN HOUTE J UPESLACIS VN, JORDAN HV, SKOBE Z &

GREEN DB (1976).

Role of sucrose in colonization of Streptococcus mutans in conventional Spraque-Dawley rats.
J Dent Res 55:202-215.

WOODS R (1971).

The short term effect of topical fluoride application on the concentration of Streptococcus mutans in dental plaque.
A D J 16:152-155.

ZINNER DD, JABLON JM, ARAN AP & SASLAW MS (1966).

Comparative pathogenicity of streptococci of human origin in hamster caries.
Arch Oral Biol 11:1419-1420.

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