CHAPTER I

INTRODUCTION

1. Theories of caries formation

The present theory for dental caries formation accepted by most investigation is referred to as the acid decalcification theory which proposed firstly toward the end of nineteenth century.(1) It stated that the bacterial breakdown of dietary carbohydrate retained in the nonself-cleansing regions of the dentition produced acid which then dissolved the underlying tooth enamel, thus initiating the caries lesion. Under anaerobic conditions most pyruvic acid from the bacterial breakdown of carbohydrate was converted to lactic acid.(2)

2. Bacterial invasion and breakdown of the dental hard tissues

When the enamel was destroyed, microorganisms were able to penetrate the interior of the individual enamel rods and the interod matrix of the enamel. Penetration was usually more extensive in the region of the enamel core than that in the region of the interod matrix. A fewer number of microorganisms were found in the areas of deeper penetration compared to the areas closer to the enamel surface.(3)

Initial invasion of the dentin occurred through the odontoblast fibriles, following with the production of acid to

decalcification and softening of the intertubular dentin. The deepest layer of the active lesion in the dentin was mostly sterile, unless a very late state of caries lesion occurred that the bacteria entered the dental pulp.(3)

3. Microorganisms involved in the caries process

Which microorganisms of those found in the complex oral microflora are the ones responsible for the disease? The unanswered question is still remaining even after many years of research concerning whether the disease is infectious. The microorganisms that have been most intensively studied were the streptococci and lactobacilli.(4)

The predominant cultivable forms of microorganisms found in plaque removed from the enamel surface were 27% facultative streptococci, 23% diphtheroids, 18% anaerobic diphtheroids, 13% peptostreptococci, 6% Veillonella, 4% Bacteroides, 4% fusobacteria, 3% Neisseria and 2% vibrio.(5) Since the lactobacilli were present at a level of less than 0.01% it was evident that they, unlike the streptococci, represented only a minor proportion of the plaque microflora. However, agar replicas of the mouth have demonstrated that the evidence of lactobacilli was much more localized and was highest within fissures, at interproximal embrasures, and at the gingival margins, the areas where caries tended to occur.(6) In the individual with rampant caries, the location of the lactobacilli became more widespread and could be detected even on more easily cleansed areas such

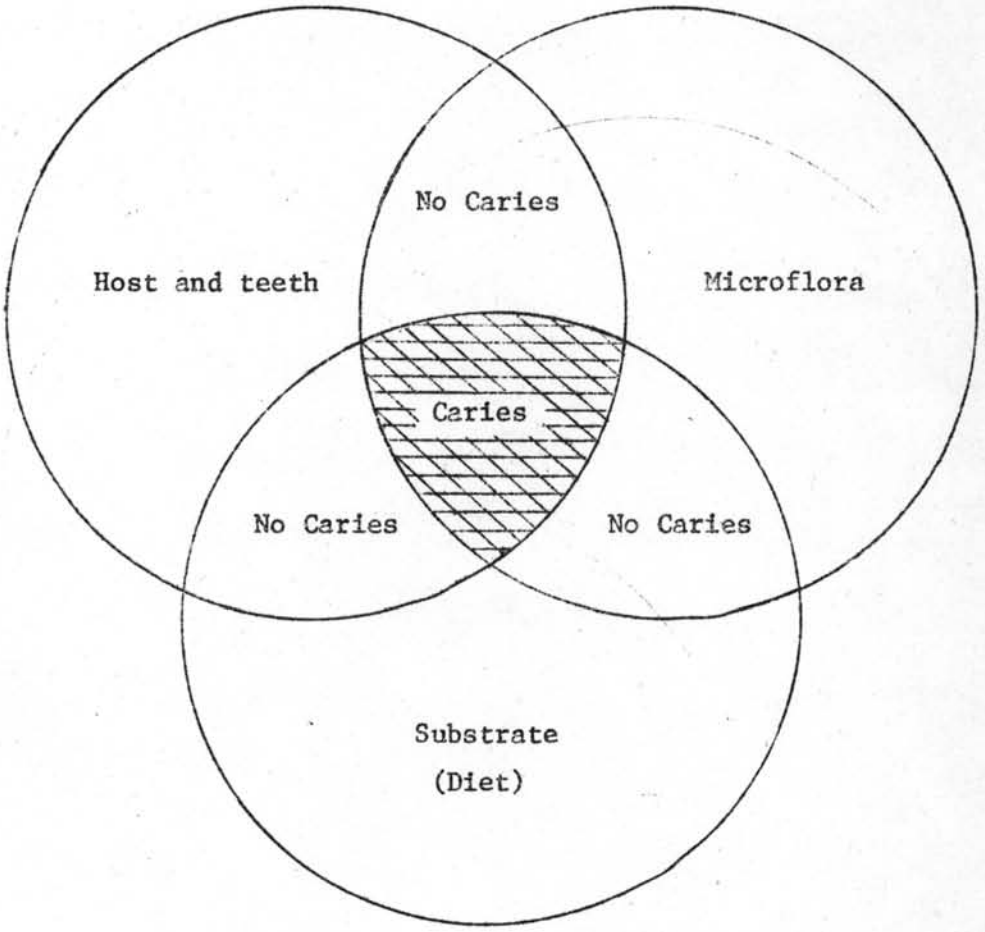
as the palate.[4]

Because streptococci are present in the mouth in large numbers and were capable of rapidly converting carbohydrate to acid, most investigators have concluded that streptococci played a dominant role in the formation of the caries lesion. However, streptococci were abundant in both caries-active and caries-inactive individuals.[4] The attempts to relate total streptococcal counts to caries activity have revealed only a slight positive correlation. Also, the streptococci were more widespread and not as localized as the lactobacilli in those regions of the dentition where caries occurred. For these reasons, investigators have turned their attention to the differences in the streptococci in the respective flora, particularly in relation to their ability to form acid, intracellular and extracellular polysaccharide, and plaque. The high numbers of streptococci and the poor correlation with caries activity, in contrast to low numbers of lactobacilli with a high correlation to caries activity were the relationships that have been demonstrated and appeared to be paradoxical. However, a close examination of the studies related to this seeming paradox indicated that these two relationships might not be mutually exclusive. There was the possibility that the streptococci provided much of the acid responsible for the fall in pH in plaque, gingivae, and other sites in the mouth such as the tongue. And that in some locations, particularly the poorly accessible surfaces of the teeth, this acid was sufficient for

lactobacilli to become established and this establishment tended to favor an increase in the total acid produced when dietary carbohydrate was ingested.(4)

Of the streptococci in the mouth, the aciduric streptococci, like lactobacilli, grew in an acidic environment and represented only a minor portion of the total microflora; they included the hemolytic, lactic and enterococcal groups.(7) Of the remaining streptococci organisms, S. mitis, S. salivarius, and more recently S. sanguis and S. mutans have been recieved the most attention in the role of the streptococci in the caries process.(8)

It was important, however, in relating the animal studies to the human conditions which should keep in mind that most human beings harbored many of the microorganisms found to be cariogenic in animals. One might, therefore, consider generally that human beings have been infected and host-dietary factors were primarily responsible for caries absence in man.(9) Since saliva and carbohydrate availability have the marked effects on the microflora, caries in man must be considered that the shift in composition of tooth surface and the grown microflora with its associated acid-base metabolism were more meaningful rather than infection as in the animal studies.



Host-parasitic-dietary relation to the initiation of dental caries. Caries occurred when there was a critical combination of the three variables shown. Prevention consisted of modifying these factorial areas so that the critical combination did not occur (From Keyes, P.H.: Int. Dent. J. 12: 443, 1962).

4. Microorganisms under studies

4.1 Streptococcus mutans

S. mutans was isolated from human carious lesion by Clarke in 1924.[10] His description was as follows.

"S. mutans was isolated from 36 of the 50 teeth. Acid is very rapidly produced, the medium, originally pH 7, giving a reaction of pH 4.2 in about 24 hrs. All the strains isolated ferment glucose, lactose, raffinose, mannite (mannitol), inulin, and salicin with production of acid. There is usually neither haemolysis nor discoloration on blood agar. The fact that the colonies of S. mutans adhere closely to the surface of the teeth appears to be of great importance."

The occurrence of S. mutans in human carious lesions was confirmed.[11, 12, 13, 14, 15] Extensive taxonomic studies revealed that these organisms formed a fairly homogenous group of nonmotile, catalase-negative, gram-positive streptococci.[16, 17, 18, 19] A number of investigators have also revealed an association between the occurrence of S. mutans and the development of caries. Collected data from many references[16, 19, 34, 37, 42, 45, 46, 47 and 48] are tabulated on page 9 showing most streptococcal strains that ferment mannitol and sorbitol in addition to various other sugars, and synthesize adherent water soluble glucan from sucrose, are considered to be S. mutans. They do not usually deaminate arginine to produce ammonia. S. mutans is mostly α or γ-hemolytic on

sheep blood agar, but a few β-hemolytic strains have been reported.[20] A further characterization of these β-hemolytic strains is needed before they could be identified as S. mutans. S. mutans has been subclassified into several types based on immunological, biological and genetic properties. These properties will be discussed latter in detail.

The natural habitat of S. mutans is the human mouth. The organism can be isolated frequently from feces of human[21, 22, 23] and rats.[24, 25] Although S. mutans appears not to be widely distributed in wild animals, Dent et al.[26] isolated S. mutans from the Patas monkey and Indian fruit bat of 18 animal species examined. Coykendall et al.[27] and Lehner et al.[28] also isolated S. mutans from wild rats inhabiting sugar cane fields and from Rhesus monkeys. It has also been isolated from experimental rats and hamsters.[29]

4.2 Streptococcus sanguis

The species name was given by White and Niven to the α-hemolytic streptococci, isolated from the blood of patients with subacute endocarditis, that split arginine and esculin and produced glucan from sucrose. They produced hydrogen peroxide when grown aerobically.[30] Carlsson demonstrated that the main habitat of S. sanguis in humans was the oral cavity, especially in plaque.[31] Low levels of S. sanguis were reported in human feces.[32]

On mitis salivarius (MS) agar, S. sanguis produces small zoogleic colonies with a firm consistency which were embeded in the medium and which deform the surrounding agar. Many S. sanguis strains produced spreading zones typical of twitching motility on blood agar plate.(33) Strain with a similar colonial morphology which did not hydrolyses arginine and esculin but synthesized glucan were considered to be another type of S. sanguis.(16) Although other investigators considered the latter strains as S. mitior.(34, 35, 36) They were included within the species sanguis for convenience and were separated into biotypes A and B as shown on page 9.(37, 38)

Serological studies on S. sanguis strains demonstrated the presence of at least three(39) or four(38) types. The close relationship of S. snaguis to group H streptococci has been suspected for many years, but still remained to be defined.((37, 40, 41, 42, 43, 44) In spite of the complexity of its antigenic structure, S. sanguis was not difficult to identify because of the unique physiological properties and colonial morphology on sucrose agar.

Generalized key characteristics for indentifying the predominant streptococcal species.[a]

Organism	Fermentation						Hydrolysis		Polysaccharide from sucrose	Peroxide	Hemolysis on sheep blood agar plate
	Mannitol	Sorbitol	Melibiose	Raffunose	Esculin	Inulin	Arginine	Esculin			
S. mutans[b]											
a	+	+	+	+	+	+	-	+	Glucan fructan	+	β
b	+	+	+	+	+	+	+	+	Glucan fructan	-	γ
c/e/f	+	+	+	+	+	+	-	+	Glucan fructan	-	γ
d/g	+	±	-	-	-	+	-	+	Glucan fructan	+	β
S. sanguis[c]											
A	-	-	-	+	+	+	+	+	Glucan	+	α
B	-	-	-	-	-	-	-	-	Glucan	+	α

[a] Collected data from references (16, 19, 34, 37, 42, 45, 46, 47 and 48)

[b] Serotypes according to Bratthall (49) and Perch et al. (46)

[c] Biotypes according to Torii. (38)

4.3 Polymer Synthesis by S. mutans

4.3.1 Extracellular Polysaccharides

S. mutans synthesized extracellular polysaccharides, namely, glucans and fructans, from sucrose by the enzymatic action of glucosyltransferase (G Tase; En yme classification (EC.) 2.4.1.5) and frucosyltransferase (F Tase; EC. 2.4.1.10). These polysaccharides especially glucans, were considered to be critically important in dental plaque formation and hence in the pathogenesis of dental caries, because they were water insoluble and possed a marked ability to promote adherence when synthesized de novo various solid surfaces.

4.3.2 Intracellular polysaccharides

Many plaque bacteria could synthesize intracellular iodine-staining polysaccharide (IPS) from high concentrations of various sugars. Most S. mutans strains produced a storage IPS which might co atribute to the pathogenicity of S. mutans.[49, 50, 51, 52] Stored IPS might be the source of acid when exogenous sugar was not sufficient or is absent.

4.4 Sugar metabolism by S. mutans

S. mutans has been reported to be a homofermentative lactic acid bacterium.[53, 54, 55] However, the metabolic pathway of glucose by S. mutans varied, depending on environmental factors. The major fermentation product of S. mutans

was lactate, especially when the organism was grown in the presence of excess glucose, whereas S. mutans produced significant amounts of formate, acetate and ethanol in addition to lactate when glucose was limiting.[56] An in vivo study supported the latter finding.[57]

Sucose has also been shown to serve as the energy source during growth of S. mutans in addition to its role as the substrate for extracellular glucan synthesis. Most of the glucosyls of sucrose were converted into lactic acid. Only a small portion of sucrose was diverted to extracellular polysaccharide synthesis.[58, 59, 60]

Furthermore, S. mutnas was known to utilize sucrose at a significantly faster rate than other oral bacteria such as S. sanguis, S. mitis and Actinomyces viscosus.[61, 62] S. mutans produced significant amount of intracellular polysaccharide from sucrose, which could be converted to lactic acid after prolonged incubation.[63] The organism also produced mannitol when high levels of sucrose or glucose were present.[64] Comparision of metabolic activities of "cariogenic" and "noncariogenic" plaques indicated that S. mutans was metabolically dominant in plaques closely associated with the carious lesion.[61] S. mutans was more aciduric than other oral streptococcal species.[65]

In the presence of sucrose, S. mutans grew at the same exponential rate as it did in glucose.[66] A previous finding that the growth of S. mutans was linear in sucrose culture[67]

was attributed to an optical artifact based on the formation of visible cell aggregates.[66]

S. mutans transported glucose into cells via a membrane-associated phosphoenol pyruvate (PEP)-dependent phosphotransferase system.[68, 69, 70, 71] Sucrose and lactose was similarly transported in S. mutans by this system.[72, 73, 74]

4.5 Adherence of S. mutans

The adherence of S. mutans and other oral bacteria to tooth surfaces and the formation of dental plaque were of major significance in the development of dental caries. These processes were complex and involved a variety of bacterial and host components. Various aspects of bacterial adherence in the oral cavity have been extensively reviewed.[75, 76, 77, 78, 79, 80]

4.6 Initial attachment of S. mutans to smooth surfaces

Bacterial attachment to the tooth surface was usually preceded by the formation of an acquired pellicle of salivary origin. The initial stages of plaque development on cleaned tooth surfaces required cell attachment to the pellicle sufficiently firm to resist local cleansing forces of salivary flow and muscular movements. The attachment might involve specific interaction of pellicle components with selected bacterial species.

Orstavik et al. found a significant increase in attachment of S. mutans, S. sanguis and S. salivarious to

pellicle-coated enamel slabs when compared to an uncoated slab. The in vitro adherence of S. sanguis was significantly greater than that of S. salivarius, and both species adhered in greater numbers than did S. mutans.(81)

4.7 Sucrose-dependent in vivo adherence of S. mutans

Sucrose has been reported to markedly facilitate the colonization of S. mutans on teeth. In early studies using hamsters and rats, it was found that S. mutans could be established far more easily when the animals were given sucrose-containing diets.(82, 83, 84, 85) Moreover S. mutans could implant in the human oral cavity after inoculation with pure culture, and the frequent chewing of sucrose gum enhanced the implantation.(82)

5. Cariogenicity of S. mutans in experimental animals

Caries induction in animals

After the epoch-making experiments by Orland and his co-workers(86) in which germfree rats were used, Fitzgeral and Keyes demonstrated in 1960 that certain streptococcal strains isolated from carious lesions of rats and hamsters could produce caries in gnotobiotic rats and "caries-inactive" hamsters.((87, 88, 89) These strains were not termed S. mutans. The "caries-inactive" hamsters have been found to be free from indigenous microflora which could induce dental caries when a caries-inducing high-sucrose diet was fed. Once S. mutans was established in the mouth of the animal, caries activity was transmitted from

parent to off spring.[86, 89]

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.[90] demonstrated that human strains of S. mutans, which reacted with the antiserum against the hamsters strains of S. mutans, could produce extensive caries in hamsters. Since then, many streptococcal strains isolated from the human mouth have been shown to be cariogenic in various animal model systems.[12, 14, 17, 19, 83, 91, 92, 93, 94, 95] Most of the cariogenic strains belong to the species S. mutans. However, organisms other than S. mutans can occasionally induce variable levels of caries in animals.[96]

Dental caries have been induced in various kinds of animals, including monkeys,[97] gerbils,[98] mice,[93, 99] rats and hamsters. The transmission of S. mutans from hamsters to mice and caries induction in mice have also been demonstrated.[99]

Strains of S. mutans regardless of their serotypes, almost always induce smooth surface and pit-and fissure caries in animals.[93, 94, 100] Strain of serotypes a, d, and g S. mutans tended to produce smooth-surface caries preferentially in rats.[93, 100] However, variations were frequently observed in the pattern and severity of the induced carious lesions in experimental animals.[101] In gereral, young animals were more susceptible to a caries attack.[102, 103, 104, 105]

Dietary factors critically influenced the composition and pathogenic potential of inoculated S. mutans by affecting the implantation, colonization, and metabolic activities of the bacterium. Sucrose has been demonstrated to be most cariogenic and supported the most rapidly progressive pathogenesis. Although other sugars, such as maltose, lactose, and fructose, also supported the induction of dental caries in animals to some extent.[106, 107, 108]

6. S. mutans and dental caries in humans

Effect of sucrose on the proportion of S. mutans

The famous Vipeholm study provided strong support for a close relationship between sucrose intake and human caries prevalence.[109] Recent studies with gnotobiotic rats have revealed that as little as 0.1% sucrose in the diet could significantly promote the development of dental caries by S. mutans 6715.[110] It was wellknown that dietary carbohydrated and infection with S. mutans were essential factors in the development of dental caries.[111, 112] Among dietary carbohydrates, sucrose was considered to be directly related to dental caries.[106, 107, 113] Several studies on the effect of dietary sucrose on streptococcal composition in plaque flora have been carried out with human subjects. Carlsson and Egelberg reported that plaque formation was heavier during high-sucrose diet period than in glucose diet periods.[114] When six subjects were instructed to obtain from any dietary carbohydrates for 17 days, the S. mutans count decreased to an undetectable level

while the percentage of S. sanguis increased.[115] Such an inverse relationship between S. mutans and S. sanguis population was observed in other investigations.[116, 117] Other nutritional interactions between S. mutans and S. sanguis might be important for the ecology of these organisms in the oral flora.[118]

Contrary to an earlier study,[114] it was reported that high-sucrose diets had no significant effect on total plaque accumulation, although total viable microbial density and populations of S. mutans and lactobacilli increased.[117] A low-sucrose diet did not completely eliminate S. mutans from the oral flora as was shown in a study with monkeys.[117, 119]

7. Epidemiological relationship between S. mutans and caries development

Many strains of S. mutans isolated from human have been demonstrated to be cariogenic in experimental animals as described before. However, these results did not necessarily apply to human dental caries. To clarify the etiological role of S. mutans in caries development in humans, one should depend on epidemiological studies which relate the microbes of the carious lesion or dental plaque to the initiation of caries at the tooth site. The rationale for the hypothesis that S. mutans is strongly associated with human caries has been supported by the following epidemiological studies. S. mutans was isolated from all carious lesions, where as only 23% of samples from

sound tooth surfaces of children (13 to 14 years old) contained the bacterium.[120] Similar tendencies were also found in younger and older (17 to 22 years old) subjects.[121, 122, 123, 124]

In an extensive study, it was concluded that there was a strong association between percentage levels of S. mutans in single occlusal fissures and dental caries. Seventy-one percent of the carious fissures retained S. mutans, accounting for more than 10% or the viable count, wherease 70% of the fissures free from caries had no detectable levels of S. mutans.[125] Furthermore, it has been shown that aciduric bacteria such as Lactobacillus were detected in significant quantities in the dentinal carious lesion as the decay progresses.[124, 126, 127]

More recently, it was demonstrated that the proportion of S. mutans in samples from early carious lesions (white spots) of smooth tooth surfaces was significantly higher than that from the adjacent sound surface. No significant number of lactobacilli was found in the early lesions.[128]

However, the etiological involvement of a bacterium in the oral flora could not be fully attributed by cross-sectional studies in the case of chronic disease such as dental caries. To overcome the problem, several longitudinal studies that demonstrated cause-and-effect relationships have been reported. The distribution of S. mutans on the tooth surfaces was followed over a period of 18 months. The development of caries was more frequently preceded by colonization with elevated levels of

S. mutans.[129] Subsequently, other investigations have led to similar findings.[130, 131, 132]

On the other hand, no significant relationship between S. mutans and the initiation of dental caries in Denish pre-school and British school children was found.[133, 134] The variable results might be attributed to complex factors such as sampling sites, method of cultivation, fluoride content, eating habits of the subjects, sucrose intake, and possible immunity in the oral cavity.

It is of interest to note here that a significant increase in S. mutans in saliva and dental plaque was observed in patients who have received radiation therapy of the major salivary gland. A close relationship was established among rampant caries, xerostomia due to degeneration of salivary glands, and an increase in S. mutans.[135]

In a survey of 22 infants over a period of 30 months, no clearcut association between the development of caries and previous detection S. mutans was reported.[15] However, S. mutans isolated from all of the 12 infants who developed caries, during the test period, changes in the distribution of serotypes were occasionally noted. Serotype d/g strains had a tendency to give rise to smooth-surface caries, serotype c strains were always present.[15]

In this context, as the number of erupted teeth increased, there was a gradual increase in the prevalence of S. mutans. Edentoulous newborns or aged person did not harbor

significant quantity of S. mutans.[15, 136, 137, 138] It appeared that S. mutans was most likely transmitted intrafamiliarly.[15, 139, 140, 141]

8. Perpose of study

The objective of this project is the attempt to determine the amount lactic acid produced from oral microflora such as S. mutans and S. sanguis by formentation of some sugars at various concentrations and pH both in aerobic and anaerobic conditions.