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Role of Micro-organisms in Caries Etiology

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Abstract. The microbial etiology of dental caries is discussed in terms of the dynamic relationship among the dental plaque microbiota, dietary carbohydrate, saliva, and the pH-lowering and cariogenic potential of dental plaque. The evidence supports a concept of caries as a dietary carbohydrate-modified bacterial infectious disease. Its key feature is a dietary carbohydrate-induced enrichment of the plaque microbiota with organisms such as the mutans streptococci and lactobacilli which causes an increase of plaque's pH-lowering and cariogenic potential. The shift in the plaque proportions of these organisms appears to be related to their relatively high acid tolerance.

A large body of evidence also supports a major effect of saliva on caries development. Integration of salivary effects with the concept of caries as a dietary carbohydrate-modified bacterial infectious disease suggests a broader concept whichincludes a major role of saliva in the regulation of the exposure of tooth surfaces to carbohydrate and of plaque acidity and, hence, the microbial composition and the pH-lowering and cariogenic potential of dental plaque. It is proposed that caries occurs preferentially in dentition sites characterized by a relatively high exposure to carbohydrate and diminished salivary effects. Some implications of this concept are discussed.

Key words. Dental Caries, Carbohydrates, Streptococcus, Hydrogen Ion Concentration, Saliva.

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Introduction

Dental caries development is considered to involve a triad of indispensable factors: bacteria (dental plaque), carbohydrates (the diet), and susceptible teeth (the host) (Keyes and Jordan, 1963). The essential process involves demineralization of tooth enamel, and likely also of root surfaces, by high concentrations of organic acids produced by bacteria in dental plaque from dietary carbohydrates. The microbiota of plaque is known to consist of a variety of acidogenic, non-acidogenic, and base-producing organisms and to differ in composition in different dentition sites. A crucial issue is whether caries induction does or does not reflect the activity of only certain specific acidogenic plaque bacteria (concept of specific *vs.* non-specific bacterial etiology). The former concept has been viable since the early 1900's. The first organisms to be implicated as specific cariogenic agents were the lactobacilli. Their demise, as major etiologic agents, was followed by a period dominated by the concept of non-specific bacterial etiology (MacDonald, 1960), whereas during the 1960's the concept of a specific bacterial etiology was revived with the re-discovery of *Streptococcus mutans*. At present, a few specific organisms in plaque are considered by many to play a special role in coronal caries development (for review, see Hamada and Slade, 1980; Loesche, 1986; Tanzer, 1989; van Houte, 1980). Less-detailed information suggests that a broader spectrum of bacteria is involved in root-caries development (for review, see Bowden, 1990; Jordan, 1986). Clarification of the cariogenic role of different plaque organisms has obvious implications for preventive and therapeutic anti-caries measures, the topic of this symposium.

Developments in caries research during the past two decades have conclusively established the dynamic relationship between the earlier-cited indispensable triad factors. This means that the plaque microbiota must be considered as only one component of an integrated system, which includes saliva

in addition to the other triad factors. The nature of this system is exemplified by the profound effect of dietary carbohydrate consumption on the composition and activities of plaque and, hence, caries activity. As will be discussed, carbohydrate-induced shifts in plaque's microbial composition toward certain specific organisms may be postulated to be a prerequisite for caries development. If the view of caries is taken as an ecological problem, this scenario may be described as the breakdown of microbial homeostasis (a stable microbial plaque community) by an environmental stress factor, excessive carbohydrate consumption, which leads to a predominance of specific cariogenic organisms (Marsh, 1989). The profound influence on caries development of the host factor saliva has long been known, and its dynamic relationship with all three triad factors has also become increasingly evident. It should be expected that a prime focus on the interrelatedness of all these factors by workers of various disciplines will greatly accelerate progress in our understanding of the complex caries process. An outstanding example of this is the work by Kleinberg and co-workers over the years (Kleinberg, 1970a,b, 1985, 1987; Kleinberg *et al.*, 1976, 1982a,b). The main purpose of the following discussion is the attempted formulation of an integrated concept of the microbial etiology of caries. Unless noted otherwise, all information discussed pertains to tooth enamel.

Cariogenicity of different plaque bacteria

Evaluation of different plaque organisms as caries-etiologic agents can be discussed conveniently under three headings: (1) physiological cell traits, (2) cariogenic potential in experimental animals, and (3) association with caries in humans. The information indicates that plaque organisms form a hierarchy with respect to a variety of putative cariogenic traits, including acidogenesis and acid tolerance (van Houte, 1980). Acid tolerance, as used here, denotes growth as well as survival of bacterial cells in an acid milieu. As indicated later, its perhaps crucial significance as a cariogenic bacterial trait has become increasingly evident (Bradshaw *et al.*, 1989; Marsh, 1989; van Houte and Russo, 1986).

Lactobacilli

The first organisms to be implicated as caries-etiologic agents were the acidogenic lactobacilli. Lactobacilli rank very high with respect to acid tolerance and their acidogenicity in an acid milieu (van Houte, 1980) and must be considered as potent acidogens at the low pH values in plaque which are conducive to tooth surface demineralization. At present, the organisms belonging to this group still deserve consideration as cariogenic agents in view of the demonstrated cariogenic potential of strains of different *Lactobacillus* species in rodents (Fitzgerald *et al.*, 1981) and the positive correlation of the cell levels of lactobacilli in plaque and saliva in humans with caries activity (Loesche, 1986; van Houte, 1980). The translation of this correlation in terms of caries-etiologic significance has been difficult, however. The available data suggest that, in plaque in fissures, between teeth, or on buccal/lingual surfaces of the crowns of teeth, lactobacilli generally constitute a low or negligible proportion of the plaque microbiota; the same is true for plaque beneath which caries is initiated. By comparison, their prevalence and cell levels in more advanced (cavitated) lesions appear more frequently elevated (Loesche, 1986; van Houte, 1980). The data so far indicate that lactobacilli may play a significant role only during the initiation of a low percentage of coronal caries lesions but may be more important in their progression (Loesche, 1986; van Houte, 1980). Much more limited data indicate a similar situation for root surface caries development (Bowden, 1990; van Houte *et al.*, 1990).

Mutans streptococci

A much stronger case, and at present the strongest one, can be made for the mutans streptococci (MS), a group of relatively well-defined species. In many Western European countries and the US, among others, the MS in plaque are predominately *S. mutans*, with significant, but lesser, numbers of *Streptococcus sobrinus*. Both species possess an unique array of putative cariogenic traits (Hamada and Slade, 1980). Similar to the lactobacilli, they possess a very high hierarchical status among the plaque microbiota with respect to their acid tolerance and their acidogenicity in an acidic plaque milieu (Harper and Loesche, 1984; van Houte, 1980; Denepitiya and Kleinberg, 1984). MS also possess the ability to synthesize extracellular glucans from dietary sucrose specifically. Considerable evidence implicates glucan synthesis as an important virulence trait of these organisms. As will be discussed later, this process may increase plaque's cariogenicity by enhancing plaque mass, by promoting

Table 1. Mean plaque pH values before and after a sugar rinse for subjects with different levels of caries activity

Subject Group	"Resting" Plaque pH before Sugar Rinse		Minimum Plaque pH after Sugar Rinse	
	UAT[a]	LAT[a]	UAT	LAT
Caries-free	7.1	7.2	5.5	6.1
Caries-inactive	7.0	7.4	5.4	5.9
Slight caries activity	6.8	7.2	4.9	5.4
Marked caries activity	6.2	6.7	4.6	5.0
Extreme caries activity	5.5	5.7	4.3	4.8

[a]Upper (UAT) and lower (LAT) anterior teeth (after Stephan, 1944).

the colonization of MS on the teeth, and by changing the diffusion properties of the plaque matrix. *S. mutans* and *S. sobrinus* are highly cariogenic in experimental animals, with caries affecting all the different types of tooth surfaces if diets with sucrose are used. MS are often found to be very widespread in the human dentition and far exceed the lactobacilli in this respect (Loesche, 1986). In humans, MS exhibit a strong positive correlation with caries development (Loesche, 1986; van Houte, 1980). Thus, tooth surfaces destined to become carious are often associated with plaque in which MS constitute a high proportion of the total cultivable flora prior to and during caries initiation; cavitated lesions also often contain high levels of MS. By contrast, the MS proportions are often much lower in plaque on tooth surfaces that are and remain sound in caries-positive subjects or in plaque on the teeth of caries-free subjects. In certain subjects, *e.g.*, children with "nursing bottle caries", a rampant form of caries, the dominance of MS can be so high that the plaque microbiota associated with the lesions or the carious material itself consists almost completely of these organisms (van Houte *et al.*, 1982). Collectively, the existing data indicate that MS play a major role in the initiation as well as the progression of caries lesions. MS may also play a significant role in root surface caries development. This is indicated by their frequently high proportions in plaque over initial root caries lesions and in cavitated lesions, and their often much lower proportions in plaque on sound root surfaces (Bowden, 1990; van Houte *et al.*, 1990).

Other organisms

A wide variety of organisms other than the lactobacilli and MS is usually found in plaque. Numerically-predominant groups include the non-mutans streptococci, actinomyces, and veillonellae, which together often comprise over one-half of the total cultivable flora (van Houte, 1980). Non-mutans streptococci and actinomyces as well as less-frequently-encountered organisms such as strains of *Neisseria, Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Propionibacterium,* or *Rothia* all have acidogenic potential. The (old) question can therefore be raised why lactobacilli and MS have been singled out by many as caries-etiologic agents. The answer is that, so far, other organisms have often appeared to be less acidogenic or acid-tolerant, have often exhibited a lower or no cariogenic potential in experimental animals, or have not shown a positive correlation with caries activity in humans (Loesche, 1986; Tanzer, 1989; van Houte, 1980). Nevertheless, certain studies indicate that coronal caries can occur in the absence of significant plaque proportions of MS (de Stoppelaar *et al.*, 1969; Boyar *et al.*, 1989; Marsh *et al.*, 1989; Macpherson *et al.*, 1990); other studies suggest an etiologic role of organisms identified as *A. viscosus* in root-surface caries (Jordan, 1986; Bowden *et al.*, 1990; Nyvad and Kilian, 1990).

Current focus on one or two groups of organisms as caries-etiologic agents could be considered unrealistic insofar as it represents a choice among an array of organisms which exhibit only quantitative differences with respect to important cariogenic traits. A fundamental problem with predictions about the cariogenic role of different acidogenic plaque organisms is that acidogenic organisms are not necessarily cariogenic. For example, in studies with rodents, a weakly acidogenic and acid-tolerant *Actinomyces* strain proved to be non-cariogenic in contrast to a *S. mutans* strain with higher acidogenicity and acid tolerance; *in situ* determination of the pH of the plaque mass formed by the test organisms showed that the *S. mutans* plaque had a much greater pH-lowering potential (Charlton *et al.*, 1971). In contrast to these *in vivo* results, prolonged exposure of enamel *in vitro* to acids formed in an *Actinomyces* plaque from sugar leads to "caries" development (Clarkson *et al.*, 1987). This suggests that, under the conditions prevailing in the human mouth, the frequency and intensity of bacterial acid production in plaque must exceed a critical level before the alternating periods of de- and remineralization lead to net demineralization.

Table 2. Dietary-induced sequence of events leading to an increase in the pH-lowering and cariogenic potential of plaque

- More frequent carbohydrate consumption
- More frequent plaque acidification
- Higher proportions of more acid-tolerant bacteria
- Plaque acidogenesis at acidic pH increased
- Lowering of potential pH minimum in plaque
- Probability of net mineral loss over time increased

The *in vivo* plaque pH response

Human dental plaque *in vivo* has a typical pH response to brief sugar exposure, consisting of a rapid decrease of the pH from the initial "resting" level to a minimum, maintenance of the pH at this level for a variable period of time, and a slower increase of the pH to its initial level (Stephan, 1944). A classic finding by Stephan was that the pH range in which this profile is located becomes increasingly lower with increasing caries activity (Table 1). This observation was made with different groups of subjects exhibiting a range of caries activities and involved plaque on sound tooth surfaces but also carious surfaces in some of the highly caries-active subjects. Generally, these and subsequent pH profile determinations (Fejerskov *et al.*, 1992) indicate that the most dramatic lowering of the range of the pH profile occurs with plaque associated with initial "white spot" or deep advanced caries lesions.

General analysis

In view of the general acceptance of the "acid theory" of caries, clarification of the determinants of this pH profile *in vivo* is of cardinal significance for our understanding of caries etiology (Kleinberg, 1970a; Kleinberg *et al.*, 1976). The discussion for this purpose will be in the context of the earlier-mentioned triad of factors which are considered indispensable for caries development.

As such, it will focus on the parameters dietary carbohydrate and microbial composition of plaque as well as plaque pH profile and caries activity. As already suggested by Stephan (1944), the pH response of plaque *in vivo* also appears to be a function of

saliva, and this factor will be discussed subsequently.

Available evidence indicates that carbohydrate consumption is essential for caries induction and that its level is positively correlated with the level of caries activity (van Houte, 1980; Marthaler, 1990). Sucrose appears to be the most important of all fermentable carbohydrates (simple sugars and starches). This may be due not only to its high (and frequent) consumption but also, perhaps, to its earlier-noted specific function as a substrate for extracellular glucan synthesis by the MS (Newbrun, 1967). A positive correlation also appears to exist between carbohydrate consumption and the proportions of MS or lactobacilli in plaque. This conclusion is amply supported by studies with humans, sub-human primates, or rodents involving drastic changes in dietary carbohydrate intake, including feeding by stomach tube (van Houte, 1980). Further, MS and lactobacilli, except for the "low-pH" non-mutans streptococci (to be discussed later), are the only organisms among the plaque microbiota which have been shown to exhibit a positive correlation with caries activity. There appears, therefore, to be a direct relationship among carbohydrate consumption, the plaque proportions of MS and lactobacilli, and caries activity.

With respect to the Stephan pH profiles (Table 1), two other significant relationships are the one between the plaque pH profile and the plaque proportions of MS and lactobacilli, and the one between the plaque pH and carbohydrate consumption. Support exists for the first relationship. For example, a higher rate of acid production for suspensions of caries-associated plaque with very high MS levels than for sound surface-associated plaque with negligible MS levels has been observed *in vitro* by Minah and Loesche (1977). Recent *in vitro* studies have also shown that suspensions of plaque from tooth surface areas with initial "white spot" lesions exhibit a lower pH minimum and a higher pH drop rate than suspensions of plaque from sound surfaces (Igarashi *et al.*, 1987; van Houte *et al.*, 1991a); moreover, the MS levels in the caries-associated plaque were significantly higher than those in the sound-surface-associated plaque (van Houte *et al.*, 1991a). Similarly, suspensions of plaque from the relatively high-caries-risk posterior dentition area have been found to exhibit a lower pH minimum and higher pH drop rate as well as higher levels of MS than suspensions of plaque from the relatively low-caries-risk lower anterior dentition area of the same subjects (van Houte *et al.*, 1991b). Differences in pH-lowering potential among plaque samples in these *in vitro* studies appear to be related directly to differences in the potential for pH-related acidogenesis of the bacteria present. Support also exists for the second relationship between the plaque pH profile and carbohydrate consumption. Several studies with humans have shown that consumption of a high-sucrose diet leads to a greater acidic plaque pH response than does consumption of a low-sucrose diet (Scheie *et al.*, 1984; Dodds and Edgar, 1986; Sgan-Cohen *et al.*, 1988).

Caries as a dietary carbohydrate—modified bacterial infectious disease

The above discussion suggests a dynamic relationship among carbohydrate consumption, microbial composition of plaque, plaque pH profile, and caries activity. Consequently, insofar as caries is caused by the actions of MS and lactobacilli, it may be formulated as a dietary carbohydrate-modified bacterial infectious disease. This implies that the dietary carbohydrate-induced enrichment of the plaque microbiota with organisms such as the MS and lactobacilli increases plaque's pH-lowering and cariogenic potential; as noted above, evidence to support this concept exists. It should particularly be noted that, according to this concept, the increased cariogenic conditions on the tooth surface reflect the interplay between a carbohydrate-induced enhancement of the cariogenic potential of plaque and an increased exposure to carbohydrate. Stated differently, the cariogenic conditions on the tooth surface reflect, at least in part, matching increases or decreases of the levels of MS and lactobacilli and of the intensity of carbohydrate exposure.

A critical question concerns the mechanism(s) responsible for the changes in the proportions of MS and lactobacilli in plaque. The literature suggests that the bacterial traits of sucrose-mediated glucan synthesis as well as acid tolerance both deserve serious consideration. Available evidence with respect to the MS suggests that glucan synthesis can promote initial cell attachment to the tooth surface, although other adhesive interactions also appear to be involved (Gibbons *et al.*, 1986; Schilling *et al.*, 1989). Thus, glucan synthesis may facilitate an increase in the number of tooth surface sites within the dentition "infected" with MS. Consequently, an increase of such sites during increased caries activity, associated with increased sucrose consumption, should be expected to occur. This is indicated, for example, by the study of Walter and Shklair (1982), involving a very sensitive technique for the detection of very low numbers of MS in plaque. Glucan synthesis can also enhance the accumulation of MS-induced plaque in rodents or the amount of plaque in humans (Carlsson and Sundstrom, 1968; van Houte and Russo, 1986). Such plaque accumulation may be accompanied by an increase of the total numbers of MS *per* given tooth surface area subsequent to initial attachment. However, evidence is lacking in support of a favorable effect of glucan synthesis on the plaque proportions of MS—that is, their numbers relative to those of the other plaque organisms (Carlsson, 1967; van Houte and Russo, 1986). It is significant that data on the association of MS in plaque with caries activity suggest that it is generally a difference between low and high proportions rather than between zero (absence) and high proportions that characterizes sound and carious tooth surfaces (Loesche, 1986). This is consistent with the fact that a very high percentage of sound but caries-prone human tooth surfaces, even in caries-free persons, is "infected" with MS (Lindquist and Emilson, 1990; Walter and Shklair, 1982). Collectively, this information does not support glucan synthesis as a major determinant of the caries-pertinent changes in the plaque proportions of the MS.

Major evidence supports, instead, the view that dietary carbohydrate-induced changes in the plaque proportions of MS and lactobacilli are directly related to the relatively high acid tolerance of these organisms, noted earlier (van Houte, 1980; van Houte and Russo, 1986; Bradshaw *et al.*, 1989; Marsh, 1989). Thus, the acid tolerance of the MS and lactobacilli may

provide them with a selective growth advantage over other less-acid-tolerant plaque organisms during frequent acidic episodes in plaque. On this basis, a scenario can be proposed in which an increased frequency of plaque acidification, connected with an increased carbohydrate intake, leads to a selective increase of the plaque proportions of MS and lactobacilli (Table 2; van Houte, 1980; Kleinberg *et al.*, 1982a; Kleinberg, 1985). As noted earlier, a cardinal feature of the classic findings of Stephan (1944) is the increasingly lower range of the pH profile with increasing caries activity (Table 1). The above hypothesis may therefore be extended by postulating that a progressive shift toward higher proportions of acid-tolerant organisms such as MS and lactobacilli in plaque would lead to increased acidogenesis at progressively lower pH values (van Houte, 1980; Kleinberg, 1982a). This would lead to a progressive increase in plaque's pH-lowering and cariogenic potential (Table 2). Of course, a decreased consumption of carbohydrate would have the opposite effect.

Microbial composition of plaque and plaque pH profile

With respect to the effect of the microbial composition of plaque on its pH profile *in vivo* following exposure to carbohydrate, two other potentially-significant parameters deserve mentioning. The first is the well-documented bacterial synthesis and degradation of, mainly intracellular, glycogen-like polymers in plaque. The original work by Gibbons (Gibbons and Socransky, 1962; Gibbons and Kapsimalis, 1963) indicated the widespread ability of human plaque organisms to synthesize these polymers from a variety of sugars and supported the potential significance of polymer degradation to acid as a determinant of plaque's pH-lowering potential. In this connection, it was suggested that, when exogenous carbohydrate sources were absent, the metabolism of these polymers could contribute to the low "resting pH" of plaque in caries-active individuals (Table 1), since the proportions of glycogen-synthesizing organisms were positively associated with caries activity. Other aspects of bacterial glycogen synthesis and degradation include its association with enhanced bacterial acidogenicity in the presence of environmental carbohydrate (van Houte *et al.*, 1969) and bacterial survival (Tanzer, 1989). Studies with rodents have also suggested that glycogen metabolism is one of the cariogenic determinants of *S. mutans* (Tanzer, 1989). As such, glycogen metabolism by this and other plaque organisms could enhance plaque's cariogenicity.

The second parameter which may influence the pH profile of plaque is the plaque matrix. In view of the latter's significance as a pathway for diffusion, it was already postulated many years ago that insoluble glucan synthesis from sucrose in plaque by MS could form a barrier to the outward diffusion of bacterial acids formed within it and, hence, increase its cariogenic potential (McNee *et al.*, 1982). In principle, the plaque matrix may consist of an array of different bacterial extracellular polymers in addition to glucans as well as other components such as salivary glycoproteins. Consequently, the microbial composition of plaque, by virtue of its impact on the composition and volume of the plaque matrix, could influence in yet another manner the pH response of plaque *in vivo*. Two recent studies support this contention and suggest that the presence of extracellular glucan in plaque may enhance its pH-lowering ability, particularly in the deeper layers near the tooth surface (Dibdin and Shellis, 1988; van Houte *et al.*, 1989). Both studies involved cell masses of MS with various cell densities and glucan content and used various *in vitro* methods as well as computer modeling (Dibdin and Shellis, 1988) and direct experimentation with the cell masses *in vivo* (van Houte *et al.*, 1989). The *in vivo* experiments indicated that a higher glucan content of the cell masses was associated with an increased pH-lowering ability (see also Zero *et al.*, 1986). Predictions from computer modeling (Dibdin and Shellis, 1988) were consistent with this observation. The increased pH-lowering ability was attributed to the increased porosity of glucan-rich cell masses; the latter appeared to result from the glucan-enhanced spacing of bacterial cells which creates a larger volume of a cell mass matrix with a low organic density relative to that of the bacterial cells and, consequently, an increased volume of the diffusion channels (van Houte *et al.*, 1989). Thus, exposure of a cell mass with such an increased matrix volume to sugar will permit a higher amount of the sugar to pass through. This will lead to an enhanced bacterial acidogenesis, a quicker penetration of the sugar deeper in the cell mass, and an enhanced pH drop in the peripheral as well as deeper cell mass regions. With respect to human plaque, several studies have indicated glucan-mediated increased spacing of cells (van Houte *et al.*, 1989). Also, an enhancement of bacterial acidogenesis is possible because the bacterial acid production rate in human plaque appears to remain often well below its actual potential, owing to the high bacterial cell density, which causes restriction of the carbohydrate supply. Such an effect was evident when cell masses of MS with varying glucan content were exposed to solutions with different sugar concentrations (van Houte *et al.*, 1989). Also, past as well as recent studies have indicated that the pH-lowering ability of human plaque *in vivo*, *e.g.*, with respect to the pH minimum reached, can be increased by increasing the sugar concentration in the rinsing solution (Kleinberg *et al.*, 1982b; Margolis *et al.*, 1993). It should be noted that several studies have failed to support the original concept of a diffusion-limiting effect of bacterial glucan in human plaque on bacterial acids formed within it (Dibdin *et al.*, 1983; McNee *et al.*, 1982; Tatevossian, 1985). In fact, a recent study measuring the diffusion rate of lactic acid through cell masses of *S. mutans* with varying glucan content has suggested the opposite (Hata *et al.*, 1992). The concept of a variation of plaque's pH-lowering ability as a consequence of a variation of its matrix volume, due to the presence of glucan or perhaps other matrix components, has recently received additional support (Zero *et al.*, 1992).

"Low-pH" non-mutans streptococci

A recent study, involving measurement of the pH drop in suspensions of human plaque, indicated that plaque samples may exhibit a very high pH drop rate and a very low pH

minimum in spite of very low levels of MS and lactobacilli (van Houte *et al.*, 1991a). This suggested that other plaque organisms are capable of contributing to a very low plaque pH. Consequently, subsequent studies (van Houte *et al.*, 1991b; Sansone *et al.*, 1993) examined the ability of non-mutans streptococci for acidogenesis at a low pH by evaluation of their "final pH" in sugar broth. Attention was focused on these organisms because they are very numerous in human plaque, possess a widely-varying "final pH" in sugar broth (Carlsson, 1967; Kilian *et al.*, 1989), and include many strains with considerable cariogenic potential in experimental rodents (Willcox *et al.*, 1990). The "final pH" in Phenol Red Dextrose Broth of non-mutans streptococci present in different types of human plaque samples was found to range from 4.05 to over 5.0; *S. mutans* strains yielded a "final pH" between 3.95 and 4.1. Comparison of plaque from caries-free and caries-positive subjects after categorization of the non-mutans streptococci according to their "final pH" indicated a significant shift toward strains with a "final pH" below 4.4 in plaque from caries-positive subjects (Sansone *et al.*, 1993). Furthermore, even in plaque from caries-positive subjects, the MS were generally far outnumbered by the "low-pH" non-mutans streptococci. A similar shift among non-mutans streptococci toward strains with a low "final pH" was also observed in plaque obtained from the relatively high-caries-risk posterior dentition area as compared with plaque from the relatively low-caries-risk lower anterior dentition area of the same subjects (van Houte *et al.*, 1991b). These findings suggest the existence of another group of plaque streptococci capable of acidogenesis at low pH during increased caries-conducive conditions. They also further support the proposed scenario for the regulation of plaque's pH-lowering and cariogenic potential. The identification of the "low-pH" non-mutans streptococci is so far unsatisfactory. "Low-pH" strains isolated from human plaque by us have been identified as *S. salivarius*, *S. mitis*, *S. milleri II*, *S. sanguis I/1* and *S. sanguis* II with the API Rapid STREP system. A number of "laboratory" strains, identified with the system of Kilian *et al.* (1989), have also been tested for their "final pH". Largely in agreement with the data on "final pH" of Kilian *et al.* (1989), strains labeled as *S. anginosus*, *S. gordonii*, *S. mitis*, and *S. oralis* were all categorized as "low-pH" non-mutans streptococci, whereas strains of *S. sanguis* were not.

Effects of saliva

Besides the microbial composition of plaque, saliva must be considered as by far the most important (and controversial) determinant of the pH response of plaque *in vivo* (Abelson and Mandel, 1981; Kleinberg, 1970b, 1987). Separation of the relative effects of the microbial composition of plaque and of saliva during the pH response of plaque to sugar exposure is very difficult. Salivary effects on plaque acidity may involve its buffering activity, substrates for bacterial base production, and the removal of plaque acids (Kleinberg *et al.*, 1982b; Mandel, 1987), as well as the clearance of dietary carbohydrate (Hase and Birkhed, 1988; Swenander Lanke, 1957). A distinction may also be made between the "short-term" effect of saliva on the pH response of plaque directly after being rinsed with sugar solution and possible effects of saliva over time. A number of studies have clearly demonstrated that a restriction of salivary flow leads to lower pH values after sugar exposure than when access of saliva to the teeth is normal (Abelson and Mandel, 1981; van Houte, 1980). Stimulation of salivary flow during a pH decrease in plaque following sugar exposure has the opposite effect and causes an immediate upturn of the pH (Kleinberg and Jenkins, 1964; Jensen, 1986).

There is considerable evidence that saliva may also exert, on the pH profile of plaque, long-term effects which are of a different nature than those imposed during a "short-term" test of the pH response of plaque to sugar exposure. For example, plaque in the lower anterior dentition area, where salivary exposure is high, differs significantly from plaque in other dentition areas, where salivary exposure is reduced. The former exhibits a reduced plaque pH-lowering response *in vivo* as well as *in vitro* (Kleinberg and Jenkins, 1964; van Houte *et al.*, 1991b). This reduction is associated with reduced levels of MS and "low-pH" non-mutans streptococci (Lindquist and Emilson, 1990; van Houte *et al.*, 1991b) and with a relatively low level of caries activity (Backer-Dirks, 1961). A diminished saliva-related exposure to sugar of teeth in the lower anterior dentition area as compared with teeth in other dentition areas has also been recently demonstrated (Dawes and Macpherson, 1993). By contrast, long-term reduced salivary exposure of the teeth, *e.g.*, in humans suffering from xerostomia, leads to rampant caries associated with shifts in the microbial composition of plaque involving the emergence of MS and lactobacilli; the latter can be prevented by dietary exclusion of high-sugar foods (Brown *et al.*, 1976). A much less efficient clearance of carbohydrate from the mouths of such subjects should also be expected (Hase and Birkhed, 1988).

The short-term effects discussed earlier indicate that saliva can alter the pH response of plaque dramatically while its microbial composition remains unchanged. The long-term effects, as exemplified by the lower anterior dentition area and the condition of xerostomia, indicate that changes in salivary flow may lead to fundamental changes in the microbial composition of plaque which are also associated with changes in plaque's pH-lowering and cariogenic potential (Kleinberg *et al.*, 1976; Kleinberg, 1987). Most interestingly, the dynamic saliva-plaque interaction appears to mimic closely the dynamic dietary carbohydrate-plaque interaction hypothesized earlier (Table 1) and to involve likewise a change in plaque acidity as the underlying mechanism.

Further discussion of the effect of saliva on the *in vivo* pH response or on caries may benefit from a focus on the localized nature of dental caries. The preferential development of caries in certain "stagnant" tooth surface sites, such as pits and fissures and areas between the contacting tooth surfaces, has long been known. These are areas prone to the build-up of plaque, food retention, and reduced salivary flow (Kleinberg, 1970a, 1985). Interestingly, even on buccal smooth tooth sur-

faces, more exposed to the oral environment, the caries lesion is often highly localized in a particular tooth surface area (Duchin and van Houte, 1978). Recent studies of oral salivary clearance appear to be highly pertinent to the issue of caries localization. The studies suggest that: (1) the intensity of salivary effects varies markedly within the mouth as well as in different local sites within the dentition—a variation which may reflect the anatomy of the mouth or tooth, proximity to salivary glands, positioning of teeth (*e.g.*, crowding), and many other factors (Dawes and Weatherell, 1990; DiSabato-Mordarski *et al.*, 1991; Kellaway, 1960; Lecomte and Dawes, 1987; Weatherell *et al.*, 1989); (2) salivary effects may be exerted by saliva as a thin film which moves with different velocities over different dentition surfaces (Dawes, 1989; Dawes *et al.*, 1989; Dawes and Macpherson, 1993); (3) the velocity of the salivary film may affect the outward diffusion of substances from plaque as well as the availability of substrates to the plaque (Macpherson and Dawes, 1991a,b); (4) as distinguished from salivary film velocity, saliva may also exert a diluting effect on substrates such as sugar, *e.g.*, a lower sugar concentration in the salivary film on the lingual surfaces of the lower anterior teeth during the chewing of sugar-containing gum as a result of their direct exposure to salivary flow from the submandibular ducts (Dawes and Macpherson, 1993); and (5) the caries experience of smooth facial and lingual dentition surfaces is correlated with the factors salivary film velocity and saliva-mediated substrate dilution effect (Dawes and Macpherson, 1993).

The above information suggests that the local effects of saliva may differ substantially between different dentition areas and, perhaps, different areas on the same tooth surface; they also appear to correlate with caries experience. Further, saliva can affect plaque acidity directly but can also do so indirectly by affecting carbohydrate exposure. Consequently, it may be proposed that caries occurs preferentially in dentition sites characterized by a relatively high exposure to carbohydrate and diminished salivary effects (see also Dawes and Macpherson, 1993). Integration of this concept with the earlier-formulated concept of caries as a dietary carbohydrate-modified bacterial infectious disease leads to a broader concept which includes a major role of saliva in the regulation of the exposure of tooth surfaces to carbohydrate and of plaque acidity and, hence, the microbial composition and pH-lowering and cariogenic potential of plaque (Table 2). The role of saliva in this regard might be more significant in the case of poorly-retained liquid, carbohydrate-containing foods or beverages than in the case of solid, well-retained carbohydrate foods (Kleinberg, 1985).

Additional considerations

As noted earlier, one criterion for evaluation of the caries-etiologic significance of different human plaque organisms has been their numerical association with caries. Thus, the existence of higher levels of MS in caries-associated than in sound surface-associated plaque in individuals (or in caries-positive as compared with caries-free subjects) has been interpreted often as support for the cariogenicity of MS. However, the opposite conclusion has been drawn for organisms such as "*S. mitis*" which in many past studies were found at similar levels in plaque on both types of surfaces (Loesche, 1986; van Houte, 1980). Actually, these conclusions are based on the assumption that the local environment of carious and sound tooth surface areas, including the impact of the major cariogenic determinants, dietary carbohydrate and saliva, is the same. As such, they represent a dangerous application of Koch's postulates to bacterial agents (plaque bacteria) which require a non-bacterial factor (dietary carbohydrate) for expression of their virulence. A different conclusion may be reached if, as proposed, the local environment of carious and sound tooth surface areas is dissimilar and, in the case of the former, is characterized by a higher carbohydrate exposure and diminished salivary effects. First, the similar levels of "*S. mitis*" in plaque on both types of surfaces would indicate that "*S. mitis*", unlike the MS and lactobacilli, is not readily affected by the local variation of carbohydrate exposure and salivary effects. Second, the local conditions for the expression of the cariogenic potential of "*S. mitis*" for caries-associated plaque would be more favorable than those for sound surface-associated plaque. These considerations, therefore, do not necessarily exclude organisms such as "*S. mitis*" as cariogenic agents. They also suggest a potential limitation of the concept of caries as a dietary carbohydrate-modified bacterial infectious disease.

Finally, according to the concepts proposed, the pH response of plaque in discrete dentition sites to sugar exposure will reflect essentially the variable local level of exposure to carbohydrate and saliva, on the one hand, and the pH-lowering potential of the plaque, on the other. The latter variable is dictated by the microbial composition of the plaque which, at least in part, is a reflection of its history with respect to its exposure to carbohydrate and saliva. This concept includes a critical role of an as-yet-incompletely-defined spectrum of specific plaque organisms in caries causation.

Target organisms for caries therapy or prevention

Considerable effort has been focused on the selective elimination of single types of organisms from human plaque. At present, primary focus on the MS in this regard appears justified. If successful, such a direct approach would constitute, potentially, caries therapy as well as clarify the etiologic role of different plaque organisms. Different imaginative approaches tested include interference with the transmission of MS from mother to infant (Kohler *et al.*, 1984), "replacement therapy" involving MS (Hillman and Socransky, 1987), and elimination of MS with anti-bacterial agents such as chlorhexidine (Sandham *et al.*, 1992). The efforts so far have been only partially successful, and no unequivocal data on the impact of MS on caries experience have been obtained. The possibility exists that a major reduction of caries by anti-bacterial therapy will require inclusion of other plaque organisms as targets. As stressed recently by Bowden (1990), this will

require more in-depth characterization of the plaque microbiota. This is exemplified by the findings with the "low-pH" non-mutans streptococci. These organisms, being a segment of the non-mutans streptococci, represent part of what was referred to formerly as *S. sanguis* and *S. mitis*. While this "low-pH" segment in plaque correlates positively with caries experience (Sansone *et al.*, 1993), many studies in the past failed to establish such a correlation for *S. sanguis* and *S. mitis* (Loesche, 1986; van Houte, 1980). Further work will be necessary to identify these "low pH" organisms, to develop rapid methods (*e.g.*, genetic probes) for their enumeration in plaque, to determine their correlation with caries, and to obtain other information which forms the basis for the selection of suitable target organisms for anti-bacterial therapy.

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