

Role of *Streptococcus mutans* in Human Dental Decay

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INTRODUCTION

Dental infections such as tooth decay and periodontal disease are perhaps the most common bacterial infections in humans. Their non-life-threatening nature and their ubiquitousness have minimized their significance in overall human health. Yet the economic burden for the treatment of these dental infections can be staggering. In the United States the annual cost for the symptomatic treatment of dental infections in 1977 was over 11 billion dollars (69), and this had increased to about 24 billion dollars in 1984 (118). More than 90% of these costs are related to the restoration of teeth or tooth substance lost to dental decay (69). Despite the enormity of these dollar figures, they represent the cost for only those estimated 40 to 50% of the public who regularly visit the dentist and receive treatment (347). Put into this perspective, tooth decay and, to a lesser extent, periodontal infections are perhaps the most expensive infections that most individuals have to contend with during a lifetime.

Dental infections are costly because the treatment is symptomatic and does not include a viable preventive approach based upon the control of the bacterial factors

involved in both decay and periodontal disease. The prevailing opinion in clinical dentistry has been that the accumulation of bacterial communities on the tooth surfaces, in aggregates known as dental plaque, causes both decay and periodontal disease. This opinion results from the failure of 19th century dental scientists to identify within the plaques a microbial flora that was uniquely associated with dental pathology (25, 248). This led to the conclusion that dental infections were the result of bacterial overgrowth on the tooth surfaces and that therapy should accordingly be directed toward the prevention of this overgrowth.

This concept of dental infections as being bacteriologically nonspecific offers no rationale for antimicrobial treatment other than the daily debridement of the tooth surfaces by modern versions of ancient implements, such as toothbrushes, floss, and toothsticks. There is no convincing evidence that careful mechanical debridement by the patient has ever reduced the incidence of dental decay, although such procedures can reduce the amount of dental plaque and the level of gingivitis (inflammation of the gingiva or gums) (10, 121, 151, 240). However, if the mechanical debridement is performed by a dental professional at biweekly (9) or

trimonthly (11) intervals, and includes fluoride treatments, both decay and periodontal disease essentially cease to occur. This level of professionally delivered tooth debridement is so labor intensive that its cost would make it economically unavailable to most individuals.

But what would the treatment costs be if decay and periodontal disease were specific bacterial infections related to the overgrowth of one or more bacterial types in the dental plaque, i.e., the specific plaque hypothesis (207)? Then prevention would have as its goal the identification of "infected" individuals followed by treatment procedures which would eliminate or suppress the putative pathogens from the dental plaque (33, 209).

In this review data will be presented that indicate that, among the 200 to 300 species that may be indigenous to the human dental plaque (254, 305), only a finite number may be considered as dental pathogens, i.e., odontopathogens. If this is so, then dental caries and periodontal disease can be considered as specific, treatable infections. In particular, the evidence that implicates the mutans streptococci (MS) (81, 209) and the lactobacilli (LB) as being responsible for the majority of human dental decay will be examined. The evidence implicating specific plaque species in periodontal disease is not as complete as with the MS and will not be explored other than to note that a pattern is emerging in which a completely different group of organisms, such as spirochetes (204, 216, 221), black-pigmented bacteroides (221, 304, 306, 354), *Actinobacillus actinomycetemcomitans* (361), *Eubacterium* (255), and *Wolinella* sp. (328), are associated with the diverse clinical entities that are classified as periodontitis (221, 268).

DENTAL PLAQUE

The tooth surface is unique among body surfaces in that it is a nonshedding hard surface, which selectively adsorbs various acidic glycoproteins (mucins) from the saliva, forming what is known as the acquired enamel pellicle (AEP) (111, 203, 265, 278, 286). The AEP is an amorphous membranous layer which varies in thickness from 0.1 to 3 μ m, and as it contains a high number of sulfate and carboxyl groups, it further increases the net negative charge of the tooth surfaces (278). As bacteria also have a net negative charge, there then exists an initial repulsion between the tooth surface and those bacteria in the saliva which approach this surface. This innate defense mechanism breaks down when plaque formation occurs.

Various mechanisms for plaque formation have been considered, such as the acid precipitation (61), the enzyme precipitation (199), and nonselective (279, 309) or selective (106, 113, 147) microbial adherence theories. Of these, the concept of selective adherence via specific ionic (106), hydrophobic (262), and lectinlike (113, 308) interactions is best able to explain the initial colonization of the AEP by *Streptococcus sanguis* and *S. mitis* (106). Subsequent attachment of other bacteria involves a variety of specific coaggregation reactions of which those involving *Actinomyces viscosus* or *A. naeslundii* with *S. sanguis* are the best described (49). The MS are not particularly good colonizers of the tooth surface (342), so that their emergence as dominant plaque species needs to be explained by mechanisms other than a high affinity for receptors on the AEP. Among these would be the ability to synthesize adherent glucans from sucrose (110, 131, 308) and the ability to survive in the microenvironment created by the topography of that particular tooth surface.

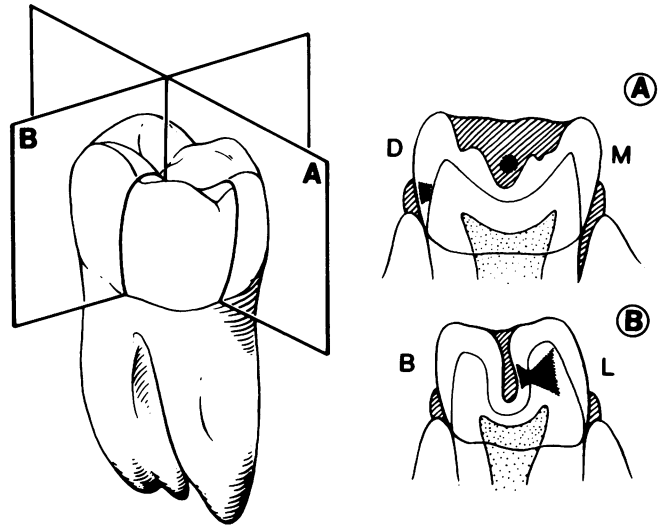


FIG. 1. Saggital (A) and cross-sectional (B) sections through a permanent molar. D, Distal; M, mesial; B, buccal; L, lingual surface. Note fissure decay (shaded area midway down in fissure) and approximal decay (subsurface shaded area on distal surface). Figure is composite of data described in references 100, 101, 165, 293, and 300.

Tooth Anatomy

The morphology of the tooth dictates to a surprising extent the bacterial composition of the various plaque ecosystems. Each tooth consists of a crown or coronal portion that extends into the oral cavity and is bathed by the saliva and a root portion that is attached by the collagen fibers of the periodontal membrane to the jaw (Fig. 1). The crown portion is above the gingival tissue and the plaque which accumulates on the crown is called the supragingival plaque (Fig. 1a and b). In health, there is a shallow (1- to 3-mm) crevice or sulcus about the teeth formed by the approximating gingival tissue. The plaque which forms at the dentogingival margin and extends down into this crevice is called the subgingival plaque. The extension of subgingival plaque toward the apex of the root is concomitant with a periodontal pocket forming between the root and gingival surfaces (see M, Fig. 1a), and it is such plaques that are associated with periodontal infections.

The crown of the tooth has five surfaces that have different propensities for supporting a plaque flora that can become either cariogenic or periodontopathic. The smooth surfaces on the buccal/labial and lingual aspects of the tooth are most disposed to plaque formation (Fig. 1b), yet become decayed only in extreme situations related to xerostomia (low salivary flow) (70) or excessive contact with fermentable substrates, such as occurs in the nursing-bottle syndrome (274). The approximal surfaces on the mesial (anterior) and distal (posterior) surfaces of the tooth are also disposed to plaque formation, and these surfaces are prone to both decay and periodontal disease. The occlusal surfaces, which are the chewing surfaces of molar and premolar teeth, are traversed by developmental grooves or fissures that are colonized by a scant flora relative to the smooth and approximal surfaces (Fig. 1A and B). These fissures, as well as developmental pits on the smooth surfaces, are the most caries prone sites on the teeth (22, 38, 202). The incisal surfaces on the top edge of anterior teeth are not colonized by appreciable numbers of bacteria and are normally caries-free.

TABLE 1. Propensities of tooth type and tooth surfaces to accumulate plaque and experience dental decay or periodontal infections^a

Tooth type	Tooth surface	Plaque		Dental decay	Periodontal infections
		Supragingival	Subgingival		
Molars	Buccal	5	3	1	3
	Lingual	4	3	1	2
	Approximal	5	5	4	5
	Occlusal	2	0	5	0
Premolars	Buccal	4	3	1	2
	Lingual	3	2	1	2
	Approximal	4	4	3	4
	Occlusal	2	0	4	0
Incisors and canines	Labial	5	4	1	3
	Lingual	5	4	1	3
	Approximal	5	4	2	4
	Incisal	1	0	0	0

^a Relative scoring based upon data contained in references 12, 22, 38, 74, 100, 116, 209, 221, 268, 277, 298, and 299. 1 = lowest; 5 = highest; 0 = not applicable. Values in boxes indicate plaque-, decay-, and periodontitis-prone tooth sites.

The supragingival plaque has access to soluble nutrients present in the diet and saliva and, to persist, must withstand the abrasive forces associated with mastication and various oral hygiene procedures. This plaque is dominated by saccharolytic, facultative, and adhesive organisms. The subgingival plaque, especially when a pocket is present, has little access to saliva and the diet, but derives nutrients from host products present in the gingival crevicular fluid (48). Many of these organisms are asaccharolytic, anaerobic, weakly adherent, and often motile (209). Thus, the gingival margin about the tooth surface distinguishes two distinct microbial ecosystems.

Bacteriological Sampling of Plaques: Caries-Prone Sites

Dental decay occurs at discrete sites on certain teeth (Table 1) within months to a few years after tooth eruption (22, 38). Accordingly, bacteriological studies dealing with the etiology of decay are best performed in children and young teenagers. Such plaque samples are usually removed from fissures, approximal contact points, or incipient (white spot) lesions that can be observed on the smooth surfaces. The sampling of the fissure creates a problem as the majority of the flora is inaccessible (Fig. 1). A dental explorer (219) or small-gauge needle (218) can remove about 10⁶ colony-forming units from the fissure orifice but gives no information concerning the identity of bacteria present within the depths of the fissure. If the fissure is diagnosed as decayed, then the contents of the fissure can be collected during the drilling procedure and cultured (239). A direct comparison of the needle-sampling method with the fissure removal method indicated that the needle recovered about 18% of the fissure flora (238). In certain fissures the needle failed to detect the MS that were present deeper in the contents, a finding which could explain those instances where decay was diagnosed but MS were not detected by the needle-sampling procedure (218, 219).

Plaque can be removed from a single approximal site by means of a sterile dental floss which is passed between the contact point of the adjacent teeth (76, 217), by introducing a contoured abrasive strip (26) from the buccal or labial aspect, or by using a dental explorer (158). Because of the close proximity of the gingival margin the approximal sample may contain gram-negative anaerobes characteristic of subgingival plaque (26, 128). Buccal and lingual smooth

surfaces are the easiest to sample, as the associated plaque can be removed either by dental instruments (66) or by a needle (73) without any contamination by subgingival plaque. The isolation of the MS in plaque samples was greatly facilitated by the development of the selective MSB medium (117). MSB medium is composed of mitis-salivarius agar (M), 20% sucrose (S), and 0.5 µg of bacitracin (B) per ml and is selective for *S. mutans*, *S. sobrinus*, and *S. rattus*, but not for *S. cricetus* (117). (See next section.) The MSB medium underestimates the actual levels of MS present in plaque (212) and in saliva (331), and for this reason improved formulations containing either 5% glucose, 5% sucrose, tellurite, and bacitracin (GSTB agar) (331) or tryptone, yeast extract, cysteine, sucrose, and bacitracin (TYCSB agar) (346) have been developed. The GSTB medium yielded higher recoveries of MS in 72% of 300 salivary cultures and lower recoveries in 8% (331). The GSTB and TYCSB media, because of their newness, have not been extensively used; thus, the majority of data to be described will be based on investigations which used the MSB medium.

Taxonomy of the MS

The MS are those streptococci which are found in plaque and which ferment mannitol and sorbitol, produce extracellular glucans from sucrose, and, with the exception of *S. ferus* (93), are cariogenic in animal models (54). In 1924 Clarke isolated such organisms from human carious lesions and called them *S. mutans* because on Gram stain they were more oval than round and thus appeared to be a mutant form of a streptococcus (50). Clarke associated *S. mutans* with human decay, but other investigators were unable to find *S. mutans* and this organism eventually became a nonentity. It was rediscovered in the 1960s (39, 77, 124) as investigators sought to identify the streptococcus shown to cause a transmissible infection in rodent models (85, 87, 176). In retrospect the reasons for the former obscurity of *S. mutans* can be traced to its low levels in nondiseased plaques and salivas. Once investigators sampled plaques from single carious sites or saliva from caries-active individuals, *S. mutans* was routinely associated with human decay (209).

When *S. mutans* strains were collected from different sources it became apparent that considerable serological (31, 269) and genetic heterogeneity existed (53, 75). Eight serotypes could be recognized on the basis of carbohydrate antigens (269) and deoxyribonucleic acid (DNA) hybridiza-

TABLE 2. Differential characteristics of the MS^a

MS	Characteristic										
	Cariogenic		G + C DNA content (mol%)	Serotype(s)	Cell wall carbohydrates	Acid production from:			Resistance to bacitracin	Arginine hydrolysis	Predominant glucan ^b
	Animals	Humans				Raffinose	Starch	Inulin			
<i>S. mutans</i>	+	+	36-38	<i>c, e, f</i>	Glucose, rhamnose	+	-	+	+	-	D > M
<i>S. sobrinus</i>	+	?	44-46	<i>d, g, h</i>	Glucose, galactose, rhamnose	-	-	-	+	-	M > D
<i>S. cricetus</i>	+	-	42-44	<i>a</i>	Glucose, galactose, rhamnose	+	-	+	-	-	M > D
<i>S. rattus</i>	+	-	41-43	<i>b</i>	Galactose, rhamnose	+	-	+	+	+	D > M
<i>S. ferus</i>	-	-	43-45	<i>c</i>	?	-	+	+	-	-	
<i>S. macacae</i>	?	?	35-36	<i>c</i>	Glucose, rhamnose	+	-	-	-	-	

^a Data were taken from information contained in references 17, 31, 53-55, 75, 131, 185, 241, 269, 297 and 298. G + C, guanine-plus-cytosine content.

^b D, Dextran type, water-soluble glucan; M, mutan type, water-insoluble glucan.

tion studies revealed the existence of four genetic groups (54). These genetic groups were elevated to species status and given epithets which reflected the original mammalian source of isolation (55). Thus, *S. mutans* was assigned to those human isolates that resembled Clarke's original description and the representative strain of *S. mutans* that was present in the National Collection of Type Cultures under the number NTCC 10449. *S. mutans* contains strains which possess the *c, e, or f* antigens (Table 2), and as the *c* serotype accounts for about 70 to 100% of the human isolates of MS (Table 3), it is appropriate that *S. mutans* be the specific epithet for the human type "mutans streptococcus." Most remaining human isolates of MS possess *d, g, and h* carbohydrate antigens and are called *S. sobrinus*.

S. rattus (serotype *b*) and *S. cricetus* (serotype *a*) were the epithets assigned to MS isolated from laboratory-bred rats

and hamsters, respectively (55). *S. rattus* and *S. cricetus* have occasionally been isolated from human plaques (32, 131, 177, 297) and *S. mutans* has been isolated from monkey plaques (201). A serotype *c* MS has been isolated from wild rats and because of its genetic unrelatedness to *S. mutans* and the other MS was named as the new species *S. ferus* (56). Recently certain serotype *c* strains from monkeys have been shown on the basis of guanine-plus-cytosine content, sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns, and phenotypic characteristics to be sufficiently different from *S. mutans* to warrant the naming of a new species, *Streptococcus macacae* (17).

S. mutans, S. sobrinus, S. rattus, and S. cricetus are cariogenic in animal models (209). This similarity in pathogenicity has led most investigators to call all MS by the specific epithet *S. mutans*. These MS are sufficiently dif-

TABLE 3. Frequency distribution of MS among various populations

Population (no. of individuals)	% of population or sample in which detected					Reference
	<i>S. mutans</i> (<i>c/e/f</i>) ^a	<i>S. sobrinus</i> (<i>d/g</i>)	<i>S. rattus</i> (<i>b</i>)	<i>S. cricetus</i> (<i>a</i>)	Multiple	
United States						
Infants (53)	74	22	0	0	7	313
6-yr-old children (31)	95	0.4	0.4	0	0	34
>90	>90	0	0	0	>25	337
Hawaiian children (55)	90	12	2	0	8	297
Naval recruits (273)	96	10	0	16	12	297
Naval recruits CF (77)	83	4	0	0	14	350
Naval recruits CA (22)	91	5	0	0	18	350
Canadian children (104)	97	3	0	0		272
Europe						
Dutch military CF (20)	80	5	0	0	15	153
Dutch military CA (20)	100	40	0	0	40	153
Several countries (115)	56	52	8	3		32
Asia						
Japanese infants (22)	82	36	0	0		232
Japanese children (10)	>90	30	0	0		130
Japanese 10-25 yr (40)	25	45	5	3		32
Chinese preschoolers (242)	58	41	0.2	0		— ^b
Africa-Near East						
Egyptian 11-25 yr (20)	10	45	50	50		32
Saudi Arabian adults (217)	76	53	0	0	49	297
Mozambique (462)	81	26	83		19	40
Tanzanian children CF (15)	0	17	20	0		177
Brazilians 10-21 yr (20)	65	35	0	0		32
Columbia 8-14 yr (111)	>65	25	0	0	>33	337
Australians 10-25 yr (40)	30	35	0	0		32

^a Serotype.

^b C. Hsi-Tzch, personal communication.

ferent (Table 2) that to continue to refer to all of them as *S. mutans* is incorrect and can be confusing.

For example, among caries-free Tanzanian children, strains designated as *S. mutans* were found in appreciable numbers (177), and this would seem to be contrary to the general observation that *S. mutans* is associated with decay. However, 83% of the MS isolates were serotype b strains and the remainder were serotype d strains. Accordingly, these children harbored in their plaques *S. rattus* and some *S. sobrinus* strains, but no *S. mutans* strains. *S. rattus* is distinctive among the MS in hydrolyzing arginine (297) and in being the least aciduric (63, 140). As arginine-utilizing organisms may not lower the pH to values associated with demineralization (180), *S. rattus* might be expected to be less cariogenic (63). Thus, by referring to the MS isolated from the Tanzanian children as *S. rattus* there is no confusion or contradiction concerning the *S. mutans*-decay association. In this review, wherever it is possible, the MS will be referred to by their taxonomically valid names (55).

Most clinical studies have not identified which of the MS were associated with decay, but this would be *S. mutans*, based on epidemiological studies which showed that *S. mutans* accounted for 74 to 100% of the MS in diverse populations (Table 3), with the exception being the caries-free Tanzanian children who harbored *S. rattus*. *S. mutans* is among the first MS to colonize infants shortly after their teeth erupt and in one study was the only MS isolated from caries-active infants (5). *S. sobrinus* is the second most common MS isolated (Table 3), but its involvement in decay is less certain, as there is only one study in which in a small number of subjects it was, along with *S. mutans*, associated with approximal decay (153).

The distinction in isolation frequency between *S. mutans* and *S. sobrinus* is important when considering virulence mechanisms and therapeutic strategies based thereupon. In this regard *S. sobrinus* has been extensively studied in rodent models because the relationships between dietary sucrose, plaque formation, and dental decay can be associated with the presence or absence of various glucosyltransferases (GTF) and their resultant glucan products (see section, "Virulence Factors"). Sucrose and GTFs are important in the colonization of the teeth by *S. sobrinus* (109, 280), but may be minimally involved in the colonization of *S. mutans* (109, 343). This is because the attachment of *S. mutans* to the AEP is mediated by an adhesin(s) which interacts directly with salivary components, whereas *S. sobrinus* lacks this adhesin (109). As a result *S. sobrinus* attaches minimally and in a nonspecific manner (309) to the AEP but, once attached, it can, in the presence of sucrose, accumulate by glucan formation (109, 308). Any GTFs present in the AEP can also promote the initial attachment of *S. sobrinus* (109, 280). Despite this unequivocal relationship between sucrose and the colonization and accumulation of *S. sobrinus* on teeth, it is *S. mutans* which is the dominant human-type MS, and it is *S. mutans* that is overwhelmingly associated with human dental decay. This suggests that glucan formation is not the most important microbial determinant of cariogenicity in humans.

BACTERIOLOGY OF DENTAL DECAY

The demonstration of bacterial specificity in dental decay is difficult given the complexity and variability of the plaque flora and the fact that the putative etiologic agents, MS and LB, appear to be present on all dentitions. In this review evidence will be provided that shows that the MS, which in

most instances would be *S. mutans*, and to a lesser extent LB levels or proportions in plaque are statistically related to the presence or the onset of dental decay. The physiologic characteristics of these organisms which might be determinants of their cariogenicity will be examined from the vantage point of identifying strategies for the prevention or control of dental decay.

Clinical Studies

Dental decay is measured clinically as a cavitation on the tooth surface. However, cavitation is a late event in the pathogenesis of decay, having been preceded by a clinically detectable subsurface lesion known as a white spot and prior to that by subsurface demineralization that can only be detected microscopically (300). From a diagnostic and treatment perspective, the lesion should be detected at the white spot stage. This usually cannot be done without rigorous descriptive criteria (not all white spots are due to the decay process) and because the white spot stage in the caries-prone fissures and approximal surfaces cannot be directly visualized during a dental examination. Thus, in some of the bacteriological studies which have been performed, there is the possibility that the flora being associated with the decay is the result, not the cause, of the cavitation.

The prevalence of dental morbidity is documented in terms of the number of teeth (T) or tooth surfaces (S) that have obvious decay (D), contain a dental restoration or filling (F), or are missing (M). These DMF teeth (DMFT) and DMF surface (DMFS) scores do not discriminate as to the relative proportion of the score that is due to decay and how much is due to fillings and extractions. This insensitivity of the DMFT and DMFS scores in quantitating the actual decay independent of morbidity led in early clinical studies to unimpressive associations between the MS and DMFT or DMFS scores (168, 190). However, when the comparison was limited to individuals with decayed teeth (190, 218, 322) or when the plaque samples were taken from a decayed tooth site (66, 205, 222), a significant association between MS and decay was evident.

This indicates that in describing the caries status of an individual it is necessary to state whether he/she is one of the following: caries active (CA), i.e., at least one decayed tooth surface, $D > 1$; caries inactive (CI), i.e., no decayed tooth surfaces, $D = 0$; or caries-free (CF), i.e., no decayed, filled, or missing (because of decay) tooth surfaces, $DMFS = 0$. Additional information may be obtained if the CA or CI individuals are subdivided according to the magnitude of their current or past caries experience. For instance, when a DMFS score of 5 was used to identify individuals with low or high initial caries scores, a relationship between LB and initiation of decay was suggested in the low-CA subjects, whereas a highly significant relationship between *S. mutans* and initiation was found in the high-CA children (219). In the studies to be reviewed, the findings will be analyzed whenever possible as to whether the patient was CA, CI, or CF and whether the sampled tooth surface was decayed or not.

Natural history of dental decay. Epidemiological surveys consisting of a one-time recording of DMFT or DMFS scores on a cross section of a population indicate that dental decay is mainly a disease of youth (74, 231), it occurs in teeth shortly after their eruption (3, 247), it does not occur uniformly on all teeth or tooth surfaces (22, 202), and it tends to be symmetrical (38, 74). The prevalence of decay is highest on the occlusal surfaces of first and second molars and lowest on the lingual surfaces of mandibular anterior

teeth (202) (Table 1). The prevalence of decay on the approximal surfaces is of an intermediate level and reflects the length of time each mesial or distal surface had had an adjacent deciduous or permanent tooth next to it (352). This distribution pattern follows directly from the anatomy of the teeth and access to saliva. Thus, where stagnant or retentive sites exist on the tooth surfaces, such as the fissures and approximal contact sites between teeth (Fig. 1), the saliva is not as able to effectively buffer any pH drops in the plaque resulting from fermentation of dietary carbohydrates, nor is it able to replenish any lost tooth mineral. (See section, "Pathophysiology of Dental Decay.") This indicates both the importance of saliva as a defense mechanism against decay and the fact that a cariogenic flora must be relatively acid tolerant or aciduric.

To better understand caries initiation and progression, repeated measurements on the same individuals are necessary. Ideally such studies should begin prior to tooth eruption and extend for several years. Only a few longitudinal studies of this type have been performed (22, 38), and they indicate that in CA populations up to 100% of the occlusal fissures of first molars become decayed within the first few years after eruption. In contrast, the approximal and smooth surfaces develop decay at a slower rate and a large percentage of these lesions do not progress beyond the white spot stage. In one study of 624 approximal radiolucent enamel lesions that were diagnosed radiographically at age 11, only 57% progressed into the dentin by age 15 (12). In another study of 399 approximal enamel lesions, 53% were still confined to the enamel 3 years later (21). An even greater arrest rate was apparent in smooth-surface lesions (13). In 8-year-old children 72 buccal surface lesions were diagnosed at the white spot stage, but 7 years later only 13% had progressed to cavitation, whereas 51% had remineralized and were declared clinically sound.

This ability of a white spot or early enamel lesion to stabilize or actually reverse complicates the interpretation of bacteriological findings because the investigator does not know if the plaque sample was taken from a progressive, a stabilized, or a healing lesion. Presumably the flora of these various types of lesions would differ, as has been suggested by an increased frequency in the isolation of LB from progressive lesions (29).

These clinical findings describe a differential attack rate of the various tooth surfaces as a function of tooth morphology, access to saliva, and length of time in the mouth. This differential attack pattern also corresponds to the distribution pattern of MS on the tooth surfaces, as the frequency of isolation of MS exhibits the following hierarchy: fissures of molars > approximal surfaces of molars > approximal surfaces of maxillary incisors > approximal surfaces of mandibular incisors (41, 153, 158, 209, 277, 298, 299) (Table 1). These data indicate that efforts to show a specific microbial etiology of dental decay would be most successful when performed on newly erupting teeth, particularly in the fissure surfaces, or in individuals uniquely susceptible to decay due to decreased salivary flow or excessive sugar intake or both.

Rampant caries. There are a few individuals who exhibit rapid and extensive cavitation of the teeth. These rampant caries individuals usually present with a history of frequent and excessive sucrose intake and elevated levels of MS and LB in their salivas (15, 20, 209). In one study the MS averaged 40% of the cultivable flora in plaques removed from a carious molar in young children who had at least 12 decayed surfaces (250). In another investigation, MS com-

prised 25% of the fissure flora in CF deciduous teeth present in 4- to 7-year-old children who averaged 22 decayed surfaces in their deciduous dentition (209). (The deciduous dentition consists of those teeth which erupt during infancy and are shed between 6 and 12 years of age). The MS averaged about 22% of the flora in CF permanent teeth present in 7- to 14-year-old children who averaged 16 decayed surfaces at the time of sampling (212). These values were significantly higher than those found in pooled plaques taken from CF teeth in CF individuals (218). These findings indicate that children with rampant caries harbor high levels and proportions of MS on both their decayed and remaining CF teeth.

(i) *Xerostomia.* Rampant caries is also found where salivation is reduced for various reasons. The dry-mouth syndrome is found following radiation treatment of head and neck cancer (70), with habitual use of narcotics (223), in individuals taking certain medication, e.g., antihistamines, and in patients with aplasia of the salivary glands (Sjogren's syndrome) (227). Individuals with dry mouths tend to increase their intake of sucking-type candies and hold sweetened or acidic liquids, such as soda pop, in their mouths for long periods before swallowing. This prolonged exposure of the plaque to both sucrose and acidic solutions selects for aciduric organisms and, given the reduced salivary flow, there is minimal remineralization of the tooth surface so that decay is rapid and extensive.

The development of rampant decay in patients receiving radiation treatment for head and neck cancer is so predictable that these individuals have been studied in a prospective fashion to delineate the bacterial changes which predispose to dental decay. The preradiation salivary flow rate of about 1.3 ml/min drops to only 0.2 ml/min (70). To satisfy nutritional needs and for minimal discomfort, the patient eats soft foods, usually having a high sucrose content, on the average of six times per day. New carious lesions become obvious within 3 months after radiotherapy, and it is not uncommon for the patient to average one or more new decayed surfaces per postradiation month (35).

Llory et al. (206) cultured the saliva and plaque of these individuals before and 6 months after radiation treatment. The MS in saliva went from undetectable to 7.3×10^6 colony-forming units/ml and in plaque it increased from 0.6 to 44% of the total streptococci. The salivary levels of LB went from 0.3×10^6 to 13.4×10^6 colony-forming units/ml, while its plaque proportions increased 1,000-fold. No data were provided on the development of decay in these patients. However, Brown et al. (35) showed that during the development of decay a pronounced shift to a highly acidogenic-aciduric flora occurred at the expense of non-cariogenic organisms such as *S. sanquis* and *Bacteroides*, *Fusobacterium*, and *Neisseria* species. The MS and LB were present in low proportions prior to therapy but increased to 6 and 1%, respectively, following 6 weeks of radiation treatment. Three months later the proportions of MS peaked at 17.5% and five new decayed surfaces were present. Thereafter LB became the dominant aciduric species coincident with the lesions becoming larger and more numerous.

This sequence of events indicated that the MS were involved with the initiation of decay, whereas the LB were associated with the progression of the lesion. This scenario was also suggested by findings obtained from another rampant caries situation known as "nursing-bottle caries."

(ii) *Nursing-bottle caries.* Nursing-bottle caries is extensive decay of the maxillary anterior teeth that is associated

with prolonged and frequent daytime, naptime, and nighttime bottle or breast feeding (211, 274). Its prevalence among young preschool children ranges from 2.5 to 13.7%. The maxillary teeth are affected in the order in which they erupt, suggesting that colonization by MS occurs shortly after the first tooth erupts (19, 232) and then spreads to the adjacent newly erupting teeth. The localization of decay to the maxillary anterior teeth can be explained by the manner in which the infant sucks the nipple (274). During sucking, the nipple rests against the palate, while the tongue lies over the lower teeth, effectively isolating them from events which are occurring on the upper teeth. Liquid from the mother's breast or nursing bottle may bathe all of the teeth except the lower incisors. If the child sleeps with the nipple in its mouth, the liquid will pool against the upper incisors.

The bacteria on these teeth will have prolonged access to any fermentable substrates in the liquid, such as lactose or sucrose. As the salivary flow is reduced during sleep, conditions are apt for the selection of microbes capable of exploiting this stagnant milieu. The MS proportions in plaque taken from both carious and noncarious sites of maxillary teeth were over 50% and represent the highest averages for MS that have been reported on human teeth (20, 341). The proportions of LB were about 5%, and again these values are among the highest reported for these organisms in human plaque.

Longitudinal studies on the development of decay on the central incisors have been reported for Japanese (232), Finnish (5), and Canadian Indian infants (249). Twenty-two Japanese infants ranging in age from 5 to 13 months exhibited no decay at the initiation of the study, but 12 children developed one or more carious lesions during the period of observation. In seven sites, for which there were bacteriological data, MS were not detected in 82% of the plaque samples prior to the diagnosis of decay, whereas MS were always present after decay was diagnosed, usually as a high proportion of the streptococcal flora (232).

In the Canadian infants (249), the five labial surfaces which developed decay usually did not have detectable MS prior to the diagnosis of a white spot lesion, whereas once the lesion developed the frequency and magnitude of the MS infection increased. When the data were analyzed by parametric statistics, no differences could be shown between the proportions of MS, LB, and *Veillonella* spp. on caries-susceptible sites which developed a lesion and those caries-susceptible sites which remained caries-free during the 1-year observation period.

The authors interpreted their findings as indicating that, given a MS flora on a site, additional local factors may be necessary to initiate a lesion. Among these could be differences in access to saliva, variations in fluoride levels in a given tooth (352), and the presence of other microorganisms in the plaque. In the cited study no differences in the isolation frequency of *S. sanguis*, *S. mitis*, *A. viscosus*, *A. naeslundii*, and *A. odontolyticus* could be found between caries-susceptible sites which became decayed and those which did not. The frequency of isolation of LB increased in carious sites after the diagnosis was made.

The age at which *S. mutans* could be detected in the plaque of Finnish children was a reliable predictor of subsequent caries activity (5). Children who harbored *S. mutans* in their plaque by age 2 developed 10.6 DMFS by age 4. In contrast, children in whom *S. mutans* was detected between ages 2 and 4 developed 3.4 DMFS by age 4, and children in whom *S. mutans* could not be detected were essentially caries-free by age 4, i.e., 0.3 DMFS. These data indicate the

diagnostic value of early *S. mutans* detection and suggest that treatment strategies and tactics that delay the colonization of *S. mutans* should cause a reduction in decay (182, 209).

Studies of individual tooth surfaces. Because of the different susceptibilities of the tooth surfaces to decay, it is prudent to examine the bacteriological data for each surface separately.

(i) *Smooth.* Buccal and lingual smooth surfaces normally are not highly colonized by MS; i.e., 0 to 28% of these plaques had detectable MS (153, 215, 277, 298), and only about 5% of these tooth surfaces ever become decayed (22, 202). Yet the smooth surface, because it can be viewed directly, offers an opportunity both to diagnose the clinical status of the surface in a more precise fashion than can be done on fissure or approximal surfaces and to collect plaque only from the site of the lesion. Thus, it was possible for de Stoppelaar et al. (66) to demonstrate significantly higher proportions of MS in sites diagnosed as white spots or decayed compared to sites diagnosed as sound. Subsequently, the development of decay on buccal surfaces was associated with a significant increase in the proportions of MS in the plaque (64). These studies in 1969 were the first unequivocal demonstration in humans of a relationship between MS and development of decay.

The MS infection is localized to the white spot lesion, as plaque samples taken from sound enamel immediately adjacent to the lesion often yielded from 10- to 100-fold fewer MS than were found in the lesion itself (73). This observation becomes important when interpreting results of studies which used approximal and fissure plaques. In these instances the plaque removed from a single approximal and fissure tooth surface would contain contributions from decayed and nondecayed sites, and this could dilute out the MS levels.

(ii) *Fissure.* Fissure surfaces are the most caries-prone sites on the teeth (22, 38, 178, 202). Before water fluoridation and usage of fluoridated products, it would not be unusual for the fissure surfaces of all first molars to become decayed within a few years after their eruption. Every investigation of fissure surfaces has shown a highly significant association between MS and decay, regardless of whether the plaque samples were removed by a dental explorer (158, 205, 222), by a needle (220, 221), or by collection of the fissure contents in an aerosol during dental drilling (239).

In one longitudinal study the MS proportions increased in the plaque at 0 or 6 months before the development of decay, while LB, if detected, became a sizable proportion of the flora only after the appearance of decay (159). In another longitudinal study a significant increase in MS occurred exactly at the time that a dental explorer was able to detect a catch or early cavitation in the fissure surface (219). In this case, the median proportions of percent MS increased 18-fold at the time of diagnosis compared to the values made 6 months earlier. The majority of the CF fissures in CF individuals or in low-CA individuals had no detectable MS. The few fissures which became decayed in the low-CA individuals had elevated proportions of LB, raising the possibility that in a few instances LB could be the primary odontopathogen (219).

In a study involving over 400 children who were initially 6 to 7 years of age, the proportions of MS, *S. sanguis*, *Veillonella* sp., and LB were monitored at 6-month intervals in initially CF fissures of mandibular first molars (37). Decay was diagnosed by the presence of softness or a definite break in the continuity of the enamel surface. Teeth destined to

become decayed exhibited a significant increase in the proportions of MS from 6 to 24 months before the diagnosis of decay. LB were sporadically detected, but when present were almost always associated with decay.

Although the study commenced within a year after tooth eruption, many teeth by this time already had high proportions of MS, i.e., over 20% MS. About 10% of the monitored teeth erupted during the period of observation. In these teeth there was a clear incremental increase in percent MS in the 12-month period and in the percent LB in the 6-month period prior to the diagnosis of decay (213). The median values for MS were ca. 20 to 30 times greater than the LB at all times preceding decay. *S. sanguis* and *Veillonella* sp. could not be associated with decay.

A few occlusal fissures can become decayed in the absence of detectable MS (218, 219). This could reflect that either the MS were not involved in the decay process or the method of sampling the fissure orifice did not reveal the presence of MS deep within the fissure contents. The latter possibility was suggested from studies in which artificial fissures containing streptomycin-resistant (Str^r) strains of *S. mutans* were implanted in vivo into various teeth (320). In samples taken from the orifice of the artificial fissure, the Str^r strains were undetected in 13 of 18 occasions in which these strains were demonstrably present in the contents. Such a finding suggests that the plaque removed from the orifice and the plaque present deeper within the fissure may be so dissimilar in regard to MS, LB, and possibly other organisms that the data described above associating MS with fissure decay could be questioned.

This issue was resolved by Meiers and colleagues, who sampled the entire fissure contents by collecting the aerosol which results when a carious or a noncarious fissure is completely removed by a high-speed dental drill, using a water-air coolant (239). All of the carious fissures had detectable MS, and of the organisms monitored, i.e., MS, *S. sanguis*, *S. faecalis*, *A. viscosus*, and LB, only the MS were found in significantly greater numbers in decayed versus nondecayed fissures.

In another study involving six teeth in which the plaque at the fissure orifice and the plaque in the fissure contents were separately cultured, the MS were found in five of six orifice samples and in all six contents samples (238). In a modification of this sampling method, the fissure plaque was removed from the fissure orifice and from zones approximately 0.5, 0.5 to 1, and >1 mm deep into the fissure (237). Approximately 67% of the cultivable bacteria resided in the uppermost zone. About 70% of the MS were found in this upper zone, whereas about 60% of the LB were found between 0.5 and 1 mm deep.

This indicates that the orifice samples generally reflect the MS proportions deeper within the fissure, but that on occasion they will yield a false-negative result. This observation then could explain those instances in which fissures became decayed without any detectable MS in the samples taken from the fissure orifice (219). There could exist the situation where the contents are negative for *S. mutans*, but the fissure orifices are positive. As the crucial location for MS in terms of caries initiation is within the fissure, then this spatial arrangement of MS would be noncariogenic. This could explain those exceptional cases where MS were persistently elevated in orifice samples, but no decay developed (219).

(iii) *Approximal*. The caries rate on approximal surfaces can range from 5 to 60% depending on the tooth type and the age of the patients (22, 38, 202). The decay on the ap-

proximal surfaces initiates apical to the contact point between adjacent teeth and cannot usually be visualized. This location makes bacteriological sampling of the actual lesion site difficult, and accordingly samples removed by means of dental floss (76, 217), abrasive strip (26), curettes (246), or tooth picks (191) may contain an admixture of plaque from both caries-active and -inactive sites. Also, if MS invade the tooth, into either the white spot (30, 208, 295) or a beginning cavitation, the flora that is removed may not be representative of the flora in the lesion. This was indicated by a study which found that the MS accounted for about 3% of the cultivable flora in plaque removed from over a carious lesion, but for 28% of the flora recovered from the carious dentin of the same lesion (220).

Another problem relates to whether the lesion is progressive, quiescent, or arrested. As noted previously, about 50% of approximal lesions do not seem to progress (12, 21). In cross-sectional studies, therefore, the investigator does not know whether the observed lesion is progressive or not. This diagnostic problem was recognized early, and most bacteriological studies on approximal surfaces have been longitudinal in nature (137, 191, 246, 322).

The most ambitious of these studies was performed by Bowden, Hardie, and their colleagues on plaques removed from the distal approximal surfaces of upper first premolars at 3-month intervals over a 3-year period in 50 children (100 test surfaces) initially 12 to 13 years of age. Interim reports involving 9 (26) and 15 (137) decayed sites as judged by radiographic examination after 1 and 2 years respectively, indicated that the MS can dominate a site which subsequently developed a carious lesion. However, domination by MS was not obligatory, as a combination of organisms which may include moderate levels of MS and LB, especially *L. casei*, could be associated with a lesion.

Black-pigmented bacteroides were common in these plaque samples and, when present, tended to be in higher proportions than the MS (26, 137). This suggested that the abrasive metal strip which was inserted below the contact point removed subgingival plaque as well as plaque over the lesion. This would dilute the levels of MS and introduce an unknown as to whether the plaque over the actual lesion site was completely or only partially removed.

This problem is inherent with the sampling of approximal surfaces and may account for other results which were unable to demonstrate an association between MS and approximal decay (245, 246). Thus when plaque was removed by means of a curette from proximal surfaces of deciduous molars in preschool children, the median values of MS as a percentage of the total streptococci were quite low, being <1% (246). Nevertheless, in those initial lesions, i.e., no cavitation, which developed there was a peaking of MS at 25% of the streptococcal flora at 1 year, but not at 6 months prior to the clinical diagnosis.

Approximal plaque samples taken by means of dental floss, however, showed a statistical relationship between decay and proportions of MS (150, 173, 174, 298, 299). Even when such plaque samples were pooled with buccal surface plaque, it was possible to demonstrate a relationship between proportions of MS and decay (322). In a 2-year study in which pooled plaque was taken from first molars in 575 children (initially 6 to 9 years of age), the relative percentages of MS in the original sample correlated with the caries prevalence of the subjects and predicted the caries incidence over the next 2 years. A level of >1% MS was necessary before statistically discernible differences in mean caries scores occurred. Above this minimum infective level of MS,

the increased caries rate appeared to parallel the increased proportions of MS.

Toothpicks have also been used to sample approximal surfaces. In a 2-year study, 700 surfaces present in 28 children, who were 13 years old at base line, were sampled by inserting a wooden toothpick into the interproximal space between molars and premolars. Both sides of the toothpick were then pressed against the surface of an MSB agar plate and the MS levels were determined (191). More new carious lesions and progressive lesions were found on surfaces positive for MS than on those negative for MS, and the more MS detected, the greater the likelihood of the surface being decayed. Ninety-six percent of the CF surfaces had no detectable MS throughout the study, indicating that the absence of MS could predict the CF state. However, the predictive value of the presence of MS on subsequent caries development was only 24%. This confirms the need to quantify the level of the MS infection to be able to predict caries development (191, 213).

In many of the longitudinal studies only a small fraction of the monitored surfaces become decayed as detected by radiographic examination (137, 191, 246). While these studies showed a statistical association of MS with the development of the radiographic lesion, there may be other factors that determine which of these lesions will progress to the stage of cavitation. To determine whether certain microbial factors were involved, Boyar and Bowden monitored at 6- to 12-week intervals the plaque flora from 32 incipient approximal lesions in deciduous teeth of 4- to 9-year-old children (29). Increases in the proportions and isolation frequency of *S. mutans*, *L. casei*, and *A. odontolyticus* were significantly associated with the progressive lesions.

While *S. mutans* was the numerically dominant member of this trio, it was frequently isolated from nonprogressive lesions and from CF sites. *L. casei*, however, was present in 85% of the progressive lesions before the clinical diagnosis of progression was made and was never isolated from nonprogressive lesions or CF sites. A similar pattern was observed with *A. odontolyticus*. LB had previously been associated with lesion enlargement in teeth of teenagers (159) and in adults with radiation xerostomia (35), but the association of *A. odontolyticus* with caries progression was a novel observation. Its cariogenicity in animal models apparently has not been evaluated, nor has it been actively sought in clinical studies.

A report in which plaque samples were taken from intact approximal surfaces present in 18- to 20-year-old military recruits has associated the frequency of isolation of *S. sobrinus* with the caries experience of the individual (153). *S. mutans* was present in both CF and CA individuals, whereas *S. sobrinus* was found almost exclusively in CA individuals. This represents the only data that *S. sobrinus* is a human odontopathogen. A longitudinal investigation of 14 approximal surfaces which became decayed in these individuals showed an increase in proportions of MS, but no association with *S. sobrinus* was commented on (153).

These studies of approximal plaque implicate the MS as odontopathogens, but because of the difficulty of sampling the exact site where decay is occurring, and the uncertainty associated with the diagnosis of incipient decay, the findings were equivocal compared with those obtained with fissure and smooth surfaces. Evidence was obtained for viewing decay as a two-stage process in which *S. mutans* is associated with the initial lesion and LB, especially *L. casei* and possibly *A. odontolyticus*, are associated with the progression of the lesion.

(iv) *Root*. The root surfaces can become decayed if the gingival tissue about the teeth recedes. As this recession is often secondary to periodontal disease or periodontal treatment, or both, root surface decay is encountered mainly in older individuals (171). In rodent models, root surface decay was shown to be a transmissible infection (169, 170) due to an organism subsequently identified as *A. viscosus* (170). Human isolates of *A. viscosus* will cause similar pathology in rodents (170) and *A. viscosus* has been associated with human root surface decay (145, 323). However, the MS and LB can also be found in high proportions from many (80, 323) of these lesions, and an *Arthrobacter* species has been isolated from the advancing front of these lesions (317). Motile organisms thought to be *Capnocytophaga* have been observed with the scanning electron microscope within the lesion (2).

These observations suggest that a diverse flora is associated with root surface decay. In longitudinal studies, subjects with a high salivary LB count were more likely to develop root surface caries following periodontal treatment than were subjects with a low LB count (273). In this particular study MS levels were not determined. Also, the presence of MS and LB in plaque samples taken from elderly institutionalized subjects at the base-line examination could identify subjects who were to become root surface caries active during the subsequent 32-month observation period (80). These longitudinal data suggest that root surface decay may be similar to coronal surface decay in involving MS and LB.

Virulence Factors

These clinical studies indicated that only *S. mutans*, and to a lesser extent *S. sobrinus* and *L. casei*, of the 200 to 300 species which can be isolated from plaque can be consistently associated with dental decay. What makes these three organisms cariogenic relative to all other bacterial types found in the plaque?

Miller, in the late 19th century, linked microbial acid production from dietary substrates to the etiology of dental decay in what he called the chemoparasitic theory of decay (248). But Miller and his followers (25, 248) were not able to associate any single acidogenic species with decay and concluded that decay was bacteriologically nonspecific and due to the increased amounts of acid formed when bacteria accumulated in plaque on the tooth surfaces, i.e., the non-specific plaque hypothesis (207). Miller noted that decay occurred at retentive sites on the teeth and recommended mechanical and chemical debridement of these sites as the best method of reducing decay.

While Miller's clinical observations were correct, he had no way of determining that the retentive sites are caries prone because they provide the microenvironment which selects for *S. mutans*, *S. sobrinus*, and *L. casei*. In this section we shall examine those attributes of the MS and LB which enable them to be successful on retentive sites and show that they constitute, in effect, the virulence factors which make these organisms specific odontopathogens.

Sucrose in the diet. Considerable evidence from historic (138, 256) epidemiologic observations (157, 263, 295) and animal experiments (85, 176, 262, 330) indicate that, shortly after sucrose is introduced into the diet, there is a notably higher incidence of decay. For example, among the Eskimos (351), the Tahitians (14), the Bushmen (163), institutionalized adults (129), institutionalized children (142), and Englishmen (256), the incidence of decay increased drama-

tically when refined sucrose became part of their diets. The increased availability of a readily fermented carbohydrate should cause population shifts within the supragingival plaque flora, but this shift has never been described during an actual dietary change within a society. However, when human volunteers switched from their usual diets to ones high in sucrose, the plaque proportions of MS, LB, *Veillonella* sp. and yeasts increased, while those of *S. sanguis* decreased (62, 252, 303, 307).

Conversely, when sucrose was restricted in the diet as occurred among European (338) and Japanese (327) populations during World War II, the decay rate declined significantly. The teeth showing the greatest reduction were those that had erupted during the period of dietary restriction. It appeared that a vulnerable period in the life of the tooth had been passed and that these teeth were no longer susceptible to decay even when sucrose was restored to the diet. This was reminiscent of the ability of animal teeth to resist a combined sucrose-*S. mutans* challenge if the newly erupted teeth were permitted to mature in the mouth prior to the challenge (88, 198, 294).

Sucrose restriction lowers the salivary levels of LB (15, 161, 189) and the plaque levels of MS (67). Thus, while there probably was no reduction in the levels of plaque and salivary bacteria in these wartime-undernourished children, there should have been a decline in the levels of MS and LB. Teeth erupting during this period would have their fissures colonized primarily by noncariogenic organisms. If these organisms were not displaced by more aciduric species at a later date, then the progeny of these original colonizers could exclude subsequent colonization of the fissure depth by MS and LB simply by their prior occupation of the available living space.

This exclusion phenomenon was encountered with an artificial fissure model in that fissures inserted into teeth during a period when salivary MS levels were $<10^3$ /ml failed to become colonized by MS (319). Later when the salivary levels of MS increased, these same fissures remained free of MS. Evidence that such a scenario could occur on natural fissures can be surmised from the relationship between *S. mutans* colonization and subsequent decay of deciduous teeth. If *S. mutans* was not detected on the teeth during the first 4 years of life, i.e., the fissure retention sites were colonized by other bacteria, decay was found in only 1 of 34 children. However, in those youngsters in whom *S. mutans* was detected, 21 of 43 experienced decay (182). In another study, infants who harbored *S. mutans* in their plaque before the age of 2 averaged 10.6 DFS at the age of 4, while infants in whom *S. mutans* could not be detected had 0.3 DFS surfaces at the age of 4 (5).

Thus, the main dietary relationship between sucrose and dental decay is probably mediated by the levels of MS available for the colonization of fissure surfaces during tooth eruption. If the MS colonization is delayed until after the fissures are impacted with bacteria other than MS, then the incidence of decay is greatly reduced. The importance of this timing of a cariogenic infection in the fissures can be surmised from recent epidemiologic data from Nigeria (3, 4). Dental decay had not been prevalent among Nigerians until the 1970s when the revenue from oil production brought a sudden increase in sucrose products into the diet. The effect of this dietary change on caries prevalence has been documented within a dentition by the different caries scores in first and second molars. First molars that had erupted prior to this dietary change had a low caries prevalence, whereas second molars, within the same mouth, that had erupted

during and after the period of greater sucrose availability had four times the caries morbidity found in the first molars.

The importance of tooth eruption and the timing of an MS infection on caries scores have been repeatedly shown in studies in both germfree (GF) and conventional animals (88, 198, 294, 344). However, this finding did not attract the same attention as the observation that the MS formed copious amounts of plaque and decay on all tooth surfaces when the animals were fed diets containing 50% sucrose or more. As such, these animal studies were models for rampant caries. They led to the subsequent elucidation of the role of glucans in the adherence of the MS to smooth surfaces, which in turn provided an explanation as to how the MS could colonize the teeth, could extend their niche from the fissures, and could cause smooth-surface decay (131). The success of these glucan studies obscured the primacy of the timing of fissure colonization by the MS, especially *S. mutans*, in caries etiology.

Animal models. In 1960 Fitzgerald and Keyes (87, 175) demonstrated in hamsters that decay was the result of a transmissible infection involving an MS, later identified as *S. cricetus*. Their experiments provided the first definitive evidence for bacterial specificity and led to studies which demonstrated that human isolates of MS, which included *S. mutans*, were cariogenic in the animal models (362). One of the important initial observations was that acid production per se was not an exclusive determinant of decay as the overwhelming majority of acidogenic bacteria introduced into the GF rat did not cause decay (85, 176). This was a surprise because Miller's chemoparasitic theory had stated that microbial acid production was the sole determinant of decay (248). Yet among more than 30 acidogens evaluated in the GF rat, only *S. mutans*, *S. sobrinus*, *S. cricetus*, *S. rattus* (85, 242), *L. casei* (85), *S. faecalis*, *S. sanguis* and *S. salivarius* (71, 72), and *L. acidophilus* (86) caused decay. The MS caused extensive decay on both fissures and smooth surfaces, whereas the other organisms usually caused a minimal amount of decay that was confined to the fissures.

Most GF studies were performed in the presence of diets containing 25 to 60% (wt/wt) sucrose and in the absence of any competing microbes. In conventional animals such as rats (176), hamsters (176, 363), and monkeys (28), only the MS caused decay on all tooth surfaces, whereas the LB caused fissure decay in rats (154). This indicated that the MS and some LB possessed virulence factors that were needed in addition to acid production for the initiation of decay in animals harboring a "normal" flora.

A clue as to what one of these factors might be came from hamster studies in which these animals were fed at weaning a diet containing either 56% sucrose or 56% glucose and then were inoculated with an Str^r strain of either *S. cricetus* (186, 187) or *S. mutans* (188). In the sucrose-fed animals, both MS established on the teeth in high numbers and these animals developed extensive decay. In the glucose-fed animals, the MS were recovered in low numbers and the level of decay was similar to that found in uninoculated controls. In another experiment the normal flora of rats was suppressed by erythromycin. The animals were then inoculated with an erythromycin-resistant mutant of *S. sobrinus*, and littermates were fed identical diets differing only in their content of sucrose, glucose, fructose, maltose, or starch (127). Under these well-defined experimental conditions, sucrose caused more fissure decay than did the other tested carbohydrates. Thus, a high sucrose diet was unique in allowing the MS to express maximal virulence for all tooth surfaces.

However, sucrose was not essential for the cariogenicity

of *S. mutans* on the fissure surfaces. This was apparent from studies in which specific-pathogen-free (SPF) rats were fed, after they were infected at weaning with *S. mutans*, diets which differed only in their sucrose or glucose content (329). As expected, *S. mutans* established in higher proportions in the plaque of the sucrose-fed animals and caused extensive smooth-surface and fissure surface decay. But the glucose-fed animals, while they had 86% less smooth-surface decay, had only 26% less fissure decay. This indicated that smooth-surface decay was highly if not exclusively sucrose dependent, but that fissure decay, while maximal with sucrose, seemed to be dependent on the dietary availability of a fermentable carbohydrate. This confirmed an earlier animal study which indicated that extracellular polysaccharide (ECP) formation from sucrose was not required for the initiation of pit and fissure caries (96). This separation of smooth and fissure surface decay was also observed with various ECP-defective mutants (Table 4) and suggested that the MS might have different pathogenic mechanisms on smooth and fissure surfaces. In either case it was necessary to understand how the MS metabolized sucrose.

Sucrose metabolism by MS. Sucrose metabolism by MS is complex, but there is no doubt that the major pathway is concerned with energy metabolism and results, when sucrose is in excess, in lactic acid production (42, 105, 192, 251). However, before the sucrose enters the cells, a certain small percentage, <10% (131, 250, 251), is transformed by a variety of hexosyltransferases into glucans or fructans that either diffuse into the surrounding environment or remain associated with the cell (44, 110). It is these polymers that attracted initial attention to the sucrose-MS-caries association and has led to an extensive literature in this field (for reviews, see references 44, 131, 209). Various studies indicate that in animal models glucan formation is a virulence factor that is important primarily for smooth-surface decay involving an infection with *S. sobrinus* (95, 244, 267, 332). As such its importance in human decay may not be as great as initially suspected (209).

(i) *ECPs*. Several investigators (108, 235) noted that in the presence of sucrose, the MS formed adhesive colonies which stuck to the surfaces of culture vessels or to any wire or object suspended in the culture media. Such colonies were not formed in glucose broth or by the noncariogenic species when they were grown in sucrose broth. This suggested that the ability of the MS to form the adhesive plaque was related to its odontopathic activity (104).

Chemical analysis indicated that the adhesive material was a glucose homopolymer or glucan (107, 125, 126, 356) and that it contained dextran as judged by a positive reaction with antidextran sera (107) and by degradation when incubated with crude dextranase preparations (125). However, other glucans were formed which differed in their water solubility, cross-linkages, and even core linkages (97, 125, 139, 194, 241, 276, 348). In particular, in *S. sobrinus* a hitherto undescrbed glucan with a core α -(1-3) linkage was isolated and given the name mutan (125). A chemical analysis of pooled human plaque revealed that mutan accounted for about 70% of the carbohydrate and presumably was the primary glucan found in plaque (152).

Initially mutan and dextran were thought to be separate linear homopolymers containing the respective α -(1-3) and α -(1-6) core-linked sequences. However, studies with mutants of *S. sobrinus* that were defective in adhesion or agglutination revealed that, while there were two distinct glucan classes, both classes contained mixtures of the two linkage types (59, 93, 131, 139). The α -(1-3)-rich polymer was

water insoluble and cell associated and was involved in smooth-surface decay, whereas the α -(1-6)-rich polymer was water soluble, secreted into the medium, and was not associated with smooth-surface decay (92, 125, 267).

At least two different GTFs are needed to synthesize these glucans (44). One enzyme called GTF-S synthesized a soluble α -(1-6)-branched dextran, whereas the other enzyme, called GTF-I, synthesized an insoluble α -(1-3) D-mutan (44, 259, 349). Both enzymes are difficult to purify, and many preparations with high specific activity often contain other enzyme activities (44). The GTFs isolated from *S. mutans* generally are in the molecular weight range of 150,000 to 180,000 (44). There is evidence for both isozymes (234) and self-degradation (283), which gives rise to a family of lower-molecular-weight proteins all with enzyme activity. The GTFs isolated from *S. sobrinus* also show great heterogeneity in their molecular weights (44) and contain isozymes (234).

S. mutans appears to form primarily GTF-S (43, 46), whereas *S. sobrinus* has both GTF-S and GTF-I activities (43, 45, 195, 259). In *S. sobrinus* the maximum amount of GTF activity occurred at slow growth rates and consisted mainly of GTF-S activity (349). However, in vitro at high growth rates, such as might occur in plaque during exposure to dietary sucrose, the proportions of GTF-I increased, resulting in increased production of mutan (349). If this finding is extrapolated to humans, then frequent sucrose pulses could allow *S. sobrinus* via mutan formation to accumulate on smooth surfaces and this could contribute to the increase in smooth-surface decay associated with frequent eating in humans (16, 129). Mutants of *S. sobrinus* with increased GTF activity formed more water-insoluble glucan and smooth-surface decay than the wild type did (267) (Table 4).

Sucrose plus GTF-S, or their reaction product, soluble dextran, can serve as a primer which stimulates insoluble glucan formation by cell-free preparations of GTF-I isolated from *S. sobrinus* and its close relative *S. cricetus* (97, 103, 131, 139, 349). However, multiple forms of GTF-S exist in *S. sobrinus*, and one form designated as GTF-S4 was primer independent and stimulated glucan synthesis by both a primer-dependent GTF-S1, S2, and GTF-I by at least 10-fold (233). No such primer activity for dextran has been associated with the GTFs from *S. mutans* (44). Yet the GTF-S enzyme(s) of *S. mutans* can make both soluble and insoluble glucans (194, 241). How this can occur is not known other than the observation that under conditions where the enzyme tends to aggregate, various amounts of insoluble glucans can be produced (195). Aggregation may result from complexing with teichoic acids (279) which would be present in the plaque, with phospholipids such as lysophosphatidylcholine which would be present in the saliva (289), or with small amounts of insoluble glucans (195) or by a high ionic environment such as 1.5 M NH_4SO_4 (265) or the supersaturated levels of calcium which would be present in the plaque and saliva (258). These observations suggest that the GTF activities of *S. mutans* and *S. sobrinus* result in insoluble glucan formation by different mechanisms (195, 265).

The complexity of the GTF enzymes isolated from the MS has led investigators to clone the GTF genes into *Escherichia coli*, using plasmid (275) or lambda (193) vectors. The gene product expressed, when the *gtfA* gene from *S. mutans* was cloned into *E. coli* with a plasmid system, was a 55,000-molecular-weight protein which produced a low-molecular-weight, water-soluble glucan both in vitro and in

E. coli (59). There was no primer requirement for synthesis of the glucan and the enzyme could degrade its own product, but not α -(1-6)-glucans (275). When a GTF gene from the same GS-5 strain of *S. mutans* was introduced into *E. coli*, using a lambda vector, the enzyme activity was contained in two protein bands of 163 and 153 kilodaltons (193). The enzyme had a pI of 5.0 which contrasted with that of 7.4 obtained for the homologous enzyme purified directly from strain GS-5 (194). The cloned enzyme was not primer dependent, produced mainly soluble glucan, and was stimulated by the presence of 1.5 M NH_4SO_4 . This GTF gene failed to complement other mutants of *S. mutans* defective in insoluble glucose synthesis or in both soluble and insoluble glucan synthesis. These data suggest that there are two or more distinct GTF genes in *S. mutans* involved in soluble glucan synthesis (193).

A similar multiplicity of GTF genes apparently exists in *S. sobrinus*. Two distinct genes for GTF have been cloned and expressed in *E. coli*, using the lambda vector L47.1 (114). A gene designated *gtfs* expressed a GTF-S which synthesized a water-soluble, dextranase-sensitive glucan which was not dependent upon dextran T-10 as a primer. Another gene designated *gtfi* synthesized a water-insoluble, dextranase-resistant glucan which was primer dependent. The GTF-S and GTF-I enzymes were between 150 and 160 kilodaltons.

These genetic studies confirm the multiplicity of the GTF enzyme systems in *S. mutans* and *S. sobrinus* and introduce another layer of complexity in regard to the differences between GTFs expressed in *E. coli* and in MS. Some of these differences may be clarified by mutating the *gtf* genes in *E. coli* and then returning them to the host *S. mutans* or *S. sobrinus* strain to determine which effect the mutation has upon the expression of GTF activity in the natural environment of the gene (58). These studies have become possible by using the transformation tactics described by Perry et al. in *S. mutans* (270).

(ii) *Mutants with altered cariogenicity.* The cariogenicity of the MS indicates that, while considerable genetic variability can exist among the various species, those characteristics associated with dental virulence are conserved. Attempts to identify these virulence factors by genetic methods have been hampered by the fact that classical methods of gene transfer for mapping and complementation studies, i.e., sex, transducing phage, and transformation, have not been identified (57, 95) until recently when both transformation methods (57, 270, 353) and recombinant DNA procedures have been described (57, 58, 114, 193, 225, 275). Most prior genetic research with the MS had depended upon the recognition of atypical colonies on sucrose agar that arose spontaneously or after exposure to physical or chemical mutagens (93). With this approach there is no certainty that only single mutational events were being examined, so that these putative single mutant strains needed to be carefully compared to the wild type in as many characteristics as possible (93). This was not routinely done, so that conclusions drawn from some genetic studies should be considered tentative.

The use of mutants in caries research followed quickly after the recognition of the importance of glucan polymers in caries formation. de Stoppelaar et al. (65) treated *S. mutans* with methane sulfuric acid ethylester and observed that certain isolates, which exhibited a smooth colony when grown on sucrose agar, were unable to form adherent plaque on wires suspended in sucrose broth and caused decreased fissure and smooth-surface decay in hamsters and GF rats

(64, 65). However, the tested strain, C67-25, may have had multiple defects, including decreased aciduricity (68), which by itself could have accounted for the lowered virulence observed on both fissure and smooth surfaces.

This observation of a smooth colony provided a simple means for identifying strains of MS which had lost their ability to adhere to surfaces via a defect in glucan synthesis. Subsequently a second class of glucan-defective mutants were identified by their inability to be aggregated or agglutinated by exogenously added glucan (92). By using these selection criteria, a large number of glucan-defective mutants have been isolated (94) which apparently have not lost their ability to form acid at low pH values (99), with the exception of the first such mutant described (68).

(a) *S. sobrinus.* The most extensively studied MS strain has been *S. sobrinus*, strain 6715, a serotype g, Str^r strain that was isolated from CA hamsters in 1959, after they had been inoculated with plaque taken from a human with rampant decay (P. Keyes, personal communication). Two classes of mutants have been isolated following exposure to *N*-methyl-*N'*-nitro-*N*-nitroguanidine, the first being adhesion defective but aggregation competent (92, 167, 260), and the second, adhesion competent but aggregation defective (92, 260). In addition, a mutant with enhanced adhesion compared to the wild type has been found (267).

The adhesion or plaque-defective mutants have decreased ability to synthesize the insoluble α -(1-3) linkage-rich glucans and overproduce the α -(1-6) linkage-rich, water-soluble glucans (95, 261) (Table 4). In some cases this overproduction of water-soluble glucans can inhibit in vitro plaque formation by the parent strain and by certain plaque-producing mutants of *S. sobrinus* (261). In the case of mutant strain UAB108, the inhibitory glucan, designated as glucan 108, was a branched α -(1-6)-glucan with a molecular weight of about 2×10^6 (325). This glucan when added to a GTF preparation from *S. sobrinus* strain 6715 inhibited the formation of an adhesive water-insoluble glucan, while promoting the synthesis of a nonadhesive water-insoluble glucan. Thus not all water-insoluble glucans are adhesive, a finding previously suggested by Fukushima et al. (98).

The virulence of the wild type and mutant strains have been assayed in GF and SPF rats (59, 93). The mutant strain 4 caused about 90% less smooth-surface decay but exhibited undiminished virulence for the fissures in SPF rats relative to the wild type (Table 4). Additional evidence for the importance of insoluble glucan formation in smooth-surface decay came from studies which showed that a revertant of mutant strain C4, i.e., strain C4R1 in Table 4, produced as much decay as the wild type (157). Also, if GTF produced by the wild type was added to the diet of GF animals colonized by strain C4, there was a significant increase in caries activity on the smooth surfaces (149). No such increase was observed when glucan was added to the diet (Table 4). This finding shows the importance of GTF as an adhesin for the colonization of *S. sobrinus* on smooth surfaces (109, 280). In other experiments, mutants were selected for which formed more insoluble glucans per unit time than the wild type, with the expectation that these organisms would be more virulent. This was the case, as mutant strain C211, which exhibited increased levels of GTF activity, caused 40% more smooth-surface decay (Table 4) and almost 100% more proximal decay than the wild type (267).

Strain UAB108, which produced high levels of glucan 108, caused about 50% of the caries activity of its parent 6715 strain (325). As glucan 108 inhibited in vitro plaque formation by strain 6715, it was of interest to determine whether

TABLE 4. Effect of various mutations on cariogenicity of MS in SPF or GF rats

Species	Mutations in ECP formation				Animal	Caries score (%)		Reference(s)
	ECP		Adhesion to surface	Aggregation		Fissure	Smooth	
	Mutan	Dextran						
<i>S. sobrinus</i>								
WT 6715-13	++	++	+	+	GF	100	100	90, 92-95, 332
					SPF	100	100	
Mutant 4	+	+++	-	+	GF	33 ^a	0 ^a	
					SPF	98	13	
Mutant 18	++	+	+	-	GF	110	140	
					SPF	115	110	
WT 6715	++	++	+	+	GF	100	100	58-60, 149, 150, 157, 243, 244, 267
Mutant C4	+	+++	-	+	GF	44	50	
Revertant C4R1	++	++	+	+	GF	108	89	
Mutant C4	+	+++	GTF added to diet		GF	79	69	
Mutant C4	+	+++	Glucan added to diet		GF	54	34	
Mutant C211	++++	++	++	+	GF		140	
<i>S. mutans</i>								
WT C67-1	ND ^b	ND	+	ND	GF	100	100	65, 66
C67-25	ND	ND	-	ND	GF	5	0	
WT 10449	++	+++	+	+	GF	100	100	229
Mutant PN4	0	++	-	+	GF	91	47	
WT GS5	++	++	+	+	rat	100	100	167
Mutant			-	+	rat		47-57	
WT UAB62	++	ND	+	ND	GF	100	100	58-60, 197
Mutant 230 ^c	+	ND	-	ND	GF	83	51	
Mutant 95 ^d	++++	ND	-	ND	GF	81	65	
230 + 95	++++	ND	+	ND	GF	87	97	
Decreased acid production								
<i>S. mutans</i>								
WT 10449	Terminal pH 4.2				GF	100	100	229
Mutant SN13	Terminal pH 4.7				GF	22	12	
Decreased aciduricity								
WT C-67-7	% survival 5% glucose broth, 95				GF	100	100	65, 66, 68
C-67-25	% survival 5% glucose broth, <1				GF	5	0	
Decreased ICP formation								
WT 104495	1.3 meq of IPS/g of cells				SPF	100	100	94, 333
Mutant 711	0.34 meq of IPS/g of cells				SPF ^e	52	28	
					SPF ^f	21	0 ^g	
LDH defective								
<i>S. rattus</i>								
WT BHT	34 mmol of lactic acid/mg of protein				GF	100	100	146, 166
Mutant BHT-2	<1 to 18 mmol of lactic acid/mg of protein					24	4	

^a Caries score as a percentage of the control.

^b ND, Not determined.

^c Mutant which produces "defective glucan" but can bind normal glucan.

^d Mutant which produces normal glucan but cannot bind glucan.

^e Animals fed ad libitum.

^f Animals fed every 7 h.

^g Animals infected with wild type had only two smooth-surface lesions.

this strain or glucan 108 could also inhibit decay in GF rats infected with strain 6715. Contamination of GF rats with strains 6715 and UAB108 caused about 40 to 50% of the caries levels seen in animals infected with 6715 alone. The addition of glucan 108 to the drinking water also lowered the caries levels due to 6715 by about 50% (325). As glucan 108 redirects the synthesis of water-insoluble glucans from the adhesive to the nonadhesive form, these studies both demonstrate the importance of adhesive water-insoluble glucan in caries formation and provide via strain UAB108 and glucan 108 potential therapeutic modalities of treatment.

Aggregation-defective or agglutination-defective mutants, i.e., strain 18 in Table 4, exhibited no reduced virulence on fissure surfaces of SPF rats, whereas in GF rats there was an increase in caries scores on both surfaces. Thus, strains which produced the α -(1-3) glucan, but showed a loss of

soluble α -(1-6)-rich glucan retained the virulence of the wild type (95).

These experiments with *S. sobrinus* demonstrate that the insoluble α -(1-3)-rich glucans (mutan) are of pathological significance on the smooth surfaces of animal teeth when a high sucrose diet is consumed. These glucans apparently provide a mechanism by which *S. sobrinus* extends its niche from the retentive fissure site to the nonretentive smooth surfaces. It appears that GTF and glucans may play a minor role in fissure decay due to *S. sobrinus*, and perhaps no role at all where *S. mutans* is concerned. This may explain why vaccines directed against the GTF of *S. sobrinus* are mainly protective on smooth surfaces in the rat model (236, 335) and those directed against the GTF of *S. mutans* are without effect in the primate model (200, 284).

(b) *S. mutans*. Genetic studies of *S. mutans* have been

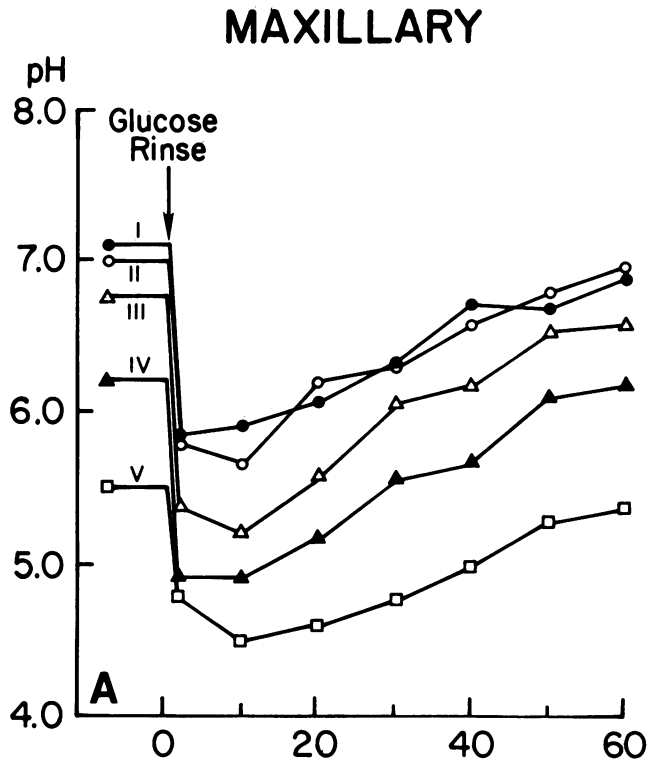


FIG. 2. Plaque pH drop following a glucose rinse. The pH is measured in situ on maxillary teeth with an antimony electrode. The kinetics of the response reflects the caries activity status of the patient. Curve I, CF subjects; curve II, CI subjects; curve III, CA subjects, slight; curve IV, CA, marked; curve V, CA, extreme. Data taken from reference 310.

performed with several mutant strains. The first such mutant, strain C67-25, was unique in that it exhibited reduced decay on both fissure and smooth surfaces (65). Other mutants of *S. mutans*, as was the case with *S. sobrinus*, exhibited decreased virulence on smooth surfaces with little (167) or no (229) decrease on fissure surfaces (Table 4). The selection of mutants by their inability to adhere to a surface would include a broad class of mutations that could be involved in the synthesis of one or more GTF, in the formation of altered glucans, or in the synthesis of glucan- or GTF-binding sites (57, 93, 95). Indeed, studies with different adherence-defective mutants of *S. sobrinus* showed complementation, as was evidenced by increased virulence for smooth surfaces when combinations of these mutants were evaluated in GF rats (Table 4). None of these GTF-glucan mutations affected fissure decay, indicating that strain C67-25 possessed additional genetic changes that were associated with virulence on fissure surfaces.

This strain has not been extensively studied, but there is evidence that it possesses reduced aciduricity (68). When C67-25 was grown in 5% glucose broth, >99.99% of the cells that were viable after 8 h of incubation were nonviable after 30 h. In contrast, only 5% of the wild-type cells were nonviable under the same assay conditions. Survival of the mutant was considerably better in sucrose broth, suggesting that the ECPs that were synthesized were protecting the cells from the killing effects of a low-pH environment.

This loss of aciduricity by strain C67-25 could explain the reduced or absent virulence on both fissure and smooth surfaces. The pH in plaque can drop below pH 5.0 for 30 to

120 min following exposure to a fermentable carbohydrate (257, 290, 310). Most plaque bacteria are not metabolically active at these pH values (63, 141) but *S. mutans* and, to a lesser extent, *S. sobrinus* have a pH optima at about 5.0 to 5.5 (140) and may be selected for in the plaque at these pH values (318). (See subsection, "Selection of an Odontopathogen.") A nonaciduric strain, such as C67-25, could not occupy the low-pH niche present in cariogenic plaques and, accordingly, it would form a noncariogenic plaque in GF animals (65) and perhaps never achieve dominance in plaques present in conventional animals (J. D. de Stoppelaar and R. H. Larson, ORCA, abstr. no. 47, 1974).

There have been few experiments performed with nonaciduric mutants. However, several mutant strains which produced less acid were relatively avirulent on both fissure and smooth surfaces (Table 4). One such mutant, strain SN13, was selected by its inability to lower the terminal pH in glucose broth to the same extent as the wild type (229). This less acidogenic mutant exhibited 80 and 90% reductions of fissure and smooth-surface decay, respectively, relative to the wild type in GF rats (Table 4).

Other mutants that were altered in their acidogenic capabilities were selected for by their inability to form an intracellular polysaccharide (ICP) (94, 333). This amylopectin-like, iodine-staining ICP is formed by most plaque bacteria, including the MS, during a period of nutrient excess such as would occur during eating, and then is degraded during the postprandial period (23, 104). While ICP production is not unique to the MS, this trait could enable these organisms to exploit their aciduricity in the plaque ecosystem by keeping the plaque pH below 5.5 for considerable time periods after each meal (Fig. 2). There is reason to suspect that ICP production is a virulence factor in human decay, as the percentage of iodine-staining bacteria in the plaque can be correlated with caries activity (111) and carbohydrate consumption (214, 340). Also, electron microscopic pictures of plaque taken over a carious lesion, as compared to plaque over a noncarious site, revealed cytoplasmic ICP-containing granules in most bacteria (89, 286).

All the MS, with the exception of *S. sobrinus*, formed copious amounts of ICP when exposed to media with high carbohydrate/nitrogen ratios (91). As *S. sobrinus* was virulent in animal models, this suggested that ICP was not an essential determinant for cariogenicity. However, ICP-negative mutants of *S. mutans* exhibited diminished virulence in SPF rats on both fissure and smooth surfaces (Table 4) (333). These mutants exhibited in vitro a marked diminution of endogenous acid production (94), but in vivo were successfully able to colonize the teeth and to emerge as prominent members of the plaque flora. Thus, the loss of virulence was attributed solely to the diminished ability to form an endogenous nutrient reserve which could be converted to acid in the absence of exogenous carbohydrates.

To determine if this was so, two vastly different feeding schedules were used. The animals either were fed ad libitum, so as to maximize the plaque exposure to dietary nutrients and thereby minimize the importance of ICP, or were given food on a restricted basis, i.e., only 6 to 7 h of feeding per day (333). This latter regimen would exaggerate the difference in ICP production between the wild type and mutant strains and, accordingly, should lead to a greater difference in caries scores. In the ad libitum-fed animals the mutants established and caused significantly less decay on both fissure and smooth surfaces. In the restricted-feeding animals only fissure caries occurred and the animals infected with mutant strain 711 had only 20% of the decay found in

the wild type (Table 4). These results indicated that in *S. mutans* endogenous acid production from ICP must be considered a virulence factor that is operable on both fissure and smooth surfaces.

(c) *S. rattus* and *S. ferus*. Mutants of *S. rattus* have been isolated which exhibited reduced levels of lactic dehydrogenase (LDH) and accordingly formed less lactic acid than the wild type (146). These LDH-deficient mutants adhered to hydroxyapatite and formed more plaque on glass slides submerged in sucrose broth than did the wild type, indicative that their ability to form ECP was intact. The mutant's ability to produce acid per unit of glucose fermented was reduced 16% compared with the wild type at pH 7.0, whereas it was reduced 40% at pH 5.0 (146). This suggested that the defect in lactic acid formation was exaggerated at a low pH, possibly due to other defects involving aciduricity. The GF animals infected with the mutants had 95% less smooth-surface and 75% less fissure decay than that found in the wild type (Table 4). Thus, mutations involved in the synthesis of LDH rendered *S. rattus* essentially noncariogenic in GF rats.

S. ferus is not cariogenic in SPF rats (93). This species in vitro behaved like a naturally occurring analog of the LDH variants of *S. rattus* in that it was less acidogenic and its metabolism was adversely affected at pH 5.0 relative to control studies performed with *S. sobrinus* (93). Thus, the only noncariogenic member of the MS could colonize and establish as a major member of the plaque flora of SPF animals, but could not cause decay because of its diminished acidogenicity and aciduricity.

These studies with *S. rattus* and *S. ferus* demonstrate the prime importance of lactic acid as a virulence factor. Comparable studies with *S. mutans* and *S. sobrinus* are lacking because of the inability of investigators, until recently, to isolate LDH⁻ mutants from these species. Mutation to the LDH⁻ form requires that the strain have an alternate mechanism for the elimination of pyruvate and excess electrons presumably via the pyruvate formate-lyase pathway (326, 358, 359). The pyruvate formate-lyase system is suppressed in *S. mutans* by the levels of the glycolytic intermediates glyceraldehyde-3-phosphate (358, 359) and dihydroxyacetone phosphate (326) when glucose is present in excess. Abhyankar et al. reasoned that this metabolic control could prevent the pyruvate formate-lyase system from functioning at an adequate rate in LDH⁻ mutants grown in conditions of glucose excess, and accordingly such mutants would not survive (1). When glucose was limiting, these investigators had no difficulty in isolating LDH⁻ strains of *S. mutans*. This same group has also isolated stable LDH⁻ mutants of *S. sobrinus* (285). It now remains to be demonstrated whether such strains have reduced virulence in animal models.

(d) Fluoride-resistant mutants. Factors relating to lactic acid production and aciduricity have not been as extensively studied as has glucan formation, perhaps because there are no simple selection methods for them. However, it is possible that acidogenic or aciduric defective mutants could be selected for by their resistance to fluoride.

Laboratory strains of the MS can be made resistant to levels of fluoride as high as 600 ppm (36, 132, 355). Fluoride resistance (F⁺) presumably can be acquired in vivo, as F⁺ strains of *S. mutans*, and less frequently of *S. sobrinus*, were isolated from patients with radiation xerostomia who were treated daily for 2 years with 1% sodium fluoride gels (316). These F⁺ strains were isolated from patients in whom the MS averaged about 10% of the plaque flora over an 18-month

period (35). These persistently high proportions were not associated with decay, although similar proportions of presumably fluoride-sensitive MS had been associated with rampant decay in patients who received a placebo treatment in the same study. This suggested that the F⁺ strains of *S. mutans* and *S. sobrinus* might not be cariogenic, a supposition subsequently supported by the observation that F⁺ strains of *S. mutans* and *S. rattus*, but not *S. sobrinus*, were less cariogenic in GF animals than were their fluoride-sensitive parent strains (282).

This reduced cariogenicity can be explained by examining the mechanism by which the MS acquire their resistance to fluoride. It has been known for some time that an acid pH potentiates the antimicrobial effect of fluoride (24, 132, 357). For example, when *S. mutans* was grown at a constant growth rate at pH 7.0, fluoride had no effect on the glycolytic rate (135). If the pH was dropped to 6.5 and 8.8 mM fluoride (160 ppm) was added, complete inhibition of glycolysis occurred. If the pH was then returned to 7.0, the inhibition was reversed (135).

A model proposed by Whitford and Schuster (355) has provided insight as to how the pH in the external environment can determine the degree of fluoride toxicity. Two assumptions are made: (1) that the uncharged hydrogen fluoride (HF) molecule is the form of fluoride that enters the cell by diffusion and (ii) that the intracellular pH of the microbes is about 7.0. At pH 7.0 most of the fluoride exists as the fluoride ion, and accordingly very little fluoride enters the cell. At an extracellular pH of 6.5 enough HF is formed that it can enter the cell, whereupon it dissociates to the fluoride ion, which inhibits glucose uptake, thereby reducing glycolysis (132). If the extracellular environment is even more acidic, such as the pH 5.0 which occurs in plaque after eating, then the intracellular uptake of fluoride is so efficient that a small amount of fluoride, i.e., 5 to 10 ppm (5 to 10 µg/ml), can be inhibitory (141a; 357).

The decreased sugar transport in fluoride-poisoned cells has generally been attributed to the inhibition of enolase, which in turn impaired the phosphoenolpyruvate-energized phosphotransferase transport systems (47, 132). However, the phosphotransferase systems in the MS are not normally active at pH 5.0 (230) so that at this pH another transport system must be inhibited by fluoride. As aciduric streptococci, such as the MS, are more sensitive to fluoride than are nonaciduric streptococci such as *S. sanguis* (18, 141a, 172) this other transport system appeared to be linked to the aciduricity trait.

Aciduricity is related to the ability of the cell membrane to maintain a relatively alkaline cytoplasm in an acid environment (18). The resulting pH gradient generates a proton motive force that would be adequate to transport sugars into the cell (253). A mutant of *S. mutans* defective in the glucose phosphoenolpyruvate-phosphotransferase system was able to transport glucose into the cell by a low-affinity transport system (136). This system had the characteristics of a proton motive force created by the extrusion of protons by an N'-N'-dicyclohexylcarbodiimide-sensitive adenosine triphosphatase in which glucose was transported down a proton gradient in symport with protons. The diffusion of HF into the bacterial cell at a low pH would after dissociation release enough protons to both lower the pH below the optima of most cytoplasmic enzymes and dissipate the pH gradient that energizes the proton motive force, thereby curtailing cellular metabolism (78, 79, 133, 172).

Only aciduric strains would be affected by both modes of fluoride inhibition. To combat this inhibition, such strains

could develop mechanisms for hindering the entry of HF into the cells. They could pump out the HF by the membrane-bound adenosine triphosphatase, but this seems unlikely because at acidic pH values the cellular adenosine triphosphate levels in *S. mutans* are nearly depleted (123). Recent isolates of *S. mutans* from children living in a fluoridated community were unable to initiate growth at pH 6.0 in the presence of 20 µg of fluoride per ml (27, 134). When a strain of *S. mutans* was adapted to fluoride in vivo in GF rats and given sucrose to ferment, the amount of lactic acid formed per microgram of plaque DNA was reduced compared to the amount formed by the same strain in GF plaques which had never been adapted to fluoride (339).

This suggests that low-dose fluoride exposure such as occurs with water fluoridation and use of fluoride dentifrices could be selecting for MS strains that have reduced capacity in those aciduric and acidogenic traits that are associated with virulence. When an F⁺ variant of *S. sobrinus*, strain 6715, was incubated with 750 ppm of fluoride (42 nM) and the initial pH was 7.8, significant growth occurred until the pH of the medium reached pH 6.0 (294). However, if the initial pH was 6.1, the F⁺ strain failed to grow. From this, it would appear that this F⁺ strain was neither aciduric nor acidogenic.

These findings suggest that some F⁺ strains manifest their resistance by not lowering the pH, as an acid environment would lead to the translocation of inhibitory levels of HF into the cell. If this is so, then F⁺ strains would not be cariogenic, as they would not lower the pH to the critical pH needed for enamel demineralization. Three of four F⁺ mutants were less cariogenic than their parent types in GF animals (282). As these F⁺ mutants appeared to possess the same adherence characteristics, at least in the case of *S. sobrinus*, as the fluoride-sensitive wild-type strains (316), they provide a class of mutants in which genetic analysis of acidogenicity and acidity could be analyzed independently of adherence to determine which of these characteristics is the dominant virulence factor(s). These studies should provide an explanation as to why some F⁺ mutations are stable, whereas others are lost when the cells are transferred in fluoride-free medium (316, 339).

Pathophysiology of Dental Decay

In this section we examine the pathogenesis of decay and focus on the dynamic pH phenomena that occur at the interface between the plaque and the enamel surfaces.

Microbial acid production in plaque. The nonspecific plaque hypothesis assigned to the plaque microbes the role of acid production when exposed to dietary carbohydrates. The kinetics of acid production were measured by Stephan et al. (311, 312) in patients who were CF or CI or who exhibited various degrees of caries activity. The pH readings were obtained prior to rinsing for 2 min with a 10% glucose solution and at intervals thereafter until the pH returned to its original value. In all instances, there was a rapid pH drop, indicating that the glucose was instantaneously converted to enough acid products, mainly lactic acid (52, 102), to overwhelm the available salivary buffering capacity. The pH levels persisted at or below pH 5 in the plaques of the CA subjects for periods of 20 to 50 min, whereas in the CF or CI subjects the plaque pH dropped to about 6.0 and returned to resting values within 40 min (Fig. 2). Thus comparable glucose exposures resulted in different pH intensities at the plaque-enamel interface that could be related to the current

caries status of the individual. This indicated that the salivary buffers were inadequate in the CA subjects relative to the CF or that the plaques in the CA subjects were producing more acid and at a faster rate than the plaque in the CF subjects or both. This latter supposition proved to be the case, as plaques from CA sites produced about twice the acid per milligram (wet weight) as plaques from CF sites (250). However, this was not recognized at the time and research instead focussed on the role of saliva in this phenomenon.

(i) *Protective role of saliva.* Saliva is remarkably protective against decay, as is evident by the rampant decay that ensues when salivary flow is very low, i.e., in xerostomia patients (70, 227), and by the fact that decay is a relatively minor health problem in the absence of frequent sucrose consumption (129, 264, 296). When food is masticated, salivary flow increases, which not only serves to prepare the bolus for swallowing, but also provides both a large liquid volume for plaque acids to diffuse into and an increased concentration of bicarbonate buffer to neutralize these acids (226, 321). The saliva also contains pH rise factors (181), such as urea (179) and a tetrapeptide called sialin which contains lysine and arginine (180). The hydrolysis of these basic compounds by certain members of the plaque flora liberates ammonia, causing the pH to rise (164).

The saliva and the fluid phase of plaque are supersaturated with respect to calcium and phosphate ions (144). This means that the environment of the tooth surface actually favors mineral deposition, and if saliva were an ordinary fluid, the tooth would be encrusted with ectopic calcium phosphate deposits. The saliva contains certain proline-rich proteins (143) and an unusual tyrosine-rich peptide, called statherin, which in vitro delays both the onset and rate of precipitation of calcium phosphate salts from supersaturated solutions (143). The maintenance of the supersaturated state of these ions provides a constant and powerful remineralizing mechanism on those surfaces that are bathed by saliva.

Salivary proteins or glycoproteins such as lysozyme (271), lactoperoxidase (8), lactoferrin (8, 51), and high-molecular-weight agglutinins (83, 281, 324) possess antibacterial activity. These proteins are chemically distinct from immunoglobulins, are present at relatively constant levels, exhibit broad-spectrum activity, and lack any aspects of immunological memory. Those organisms which colonize the oral surfaces apparently are resistant to these proteins, and this characteristic could have contributed to their selection as members of the indigenous oral flora. For example, *S. mutans* and *S. sobrinus* are minimally affected by lysozyme (156, 271) and these MS are routinely found in human plaque (Table 3). *S. cricetus* and *S. rattus* bind lysozyme at significantly faster rates than the other MS (271) and are inhibited by 1% of the dosage that is required to inhibit *S. mutans* and *S. sobrinus* (156). These findings could explain why it was not possible to implant *S. rattus* in the artificial fissure model in humans (320) and in monkeys (360) and why these animal species cannot be routinely found in human plaque.

These antibacterial proteins are found in similar levels in CF and caries-susceptible individuals (8, 227). In fact, analysis of salivary electrolytes, flow, and buffering capacity in CF or caries-susceptible individuals has failed to show any differences in the measured parameters (228). This suggests that caries susceptibility is not related to any apparent abnormality of the saliva and that the low pH following glucose ingestion in the CA subjects (Fig. 2) reflected greater microbial acid production in their plaques.

(ii) *Critical pH.* The persistent pH drop after exposure to

fermentable dietary carbohydrates (257, 290) can be due to the metabolic activity of increased numbers of bacteria on the tooth surfaces (314) or the increased presence of a particularly efficient carbohydrate fermenter such as *S. mutans* or *S. sobrinus* in the plaque (209, 250). In either case, the tooth begins to lose some of its mineral content. The pH at which this demineralization begins is known as the critical pH and is in the vicinity of pH 5.0 to 5.5 (82, 162).

The critical pH defines an important event, namely, that pH at which the hydroxyapatite of the tooth acts as a buffer. It is not usual to consider the demineralization of the tooth as a buffering phenomenon, but closer examination of the curves in Fig. 2 shows that following the initial rapid fall in pH there is a plateau section in which the pH remains relatively stable for 10 to 40 min. It is during this acid plateau that the tooth mineral dissolves to buffer further microbial acid production. If this tooth buffering were not available, the plaque pH could drop to 3.0 or 4.0, as can be measured in plaques which form on gold or plastic surfaces placed in vivo on the teeth (160, 290). But why would the tooth act as a buffer to maintain the pH in the vicinity of 5.0? If the pH drops to 3 or 4, the surface layer of mineral would be irreversibly lost, as occurs when a tooth is exposed to acid regurgitation from the stomach in vivo or when a tooth is exposed to acid in vitro (82). However, at pH 5.0, the mineral is lost from the subsurface layers in such a way that repair, in the form of remineralization, can proceed once the pH returns to values above the critical pH.

(iii) *Demineralization-remineralization*. The early enamel lesion is characterized by an intact surface with subsurface demineralization (165, 300). The predecessor of this lesion is a histologically undetectable chemical lesion caused by the diffusion into the enamel of undissociated lactic (122) and possibly acetic (84) acids produced by plaque microbes during a nutrient pulse. At some distance below the enamel surface these acids dissociate and react with the hydroxyapatite of the enamel crystals to form soluble calcium and phosphate products. As these ions diffuse outward, some of them may reprecipitate as calcium phosphate salts in the surface layer, so as to create a histologically sound outer layer overlying a porous subsurface structure (301).

This remarkably conservative dissolution pattern has great significance in regard to the development of dental decay. Whenever a fermentable dietary substrate diffuses into the plaque and is converted to acid end products, some degree of subsurface demineralization occurs. Then, between meals or snacks, the pH in the plaque returns to neutrality and calcium and phosphate ions in the plaque, driven by the supersaturated concentration gradient, diffuse into the lesion, promoting remineralization (7, 183, 184, 302). These demineralization-remineralization cycles can be documented in the incipient lesion as characteristic zones that reflect large hydroxyapatite crystals due to remineralization (dark zone) and small hydroxyapatite crystals due to demineralization (translucent zone) (302).

Demineralization which progresses to cavitation occurs if the frequency and magnitude of acid production overwhelm the repair process. This situation would occur with frequent eating (129) or if the repair process is compromised by a reduction in salivary flow (70, 227). Remineralization dominates if the plaque acid production is restricted, as occurs with the ingestion of low sucrose diets (15) or the use of sugar substitutes for between-meal snacks (292). Fluoride also promotes remineralization (336), and this may be the main mechanism by which fluoride protects against decay (6). Thus, under most conditions, the tooth and its flora exist

in a balanced relationship in which the tooth surface remains CF. In fact, a once demineralized surface seems to be even more resistant to subsequent acid dissolution than an undemineralized surface (302) due to the formation of both larger crystals and, where fluoride is available, less acid-soluble fluoride compounds, including fluorapatite (336).

Selection of an odontopathogen. The curves shown in Fig. 2 exhibit a rapid pH drop, a plateau, and a recovery phase. It is evident that the magnitude and extent of the various phases differ fundamentally between the CF subjects and the rampant-caries subjects. In the CF sites in the CF subjects, the pH never drops below the critical pH, and the sugar rinse constitutes no cariogenic challenge to the enamel surface. In the CA sites in the rampant-caries subjects, the pH drop is more precipitous and, more importantly, the pH is below the critical pH for a period of 50 min or more. The sugar rinse in these subjects constitutes a substantial challenge to the integrity of the enamel, as subsurface hydroxyapatite is solubilized to buffer the acid which the plaque microbes are producing from the sugar and the recently synthesized ICP and ECP (104, 209).

Why do the plaques from CA subjects differ from CF subjects in their responses to a sugar rinse? Plaques from CA sites have significantly higher proportions of MS than do plaques from CF sites, especially when the CF sites are in CF individuals (213, 219). Other studies have shown that cariogenic plaques per unit of plaque protein are significantly more acidogenic than plaques from noncariogenic sites (250) and that *S. mutans* is more active than other plaque species in its ability to convert sucrose to acids and reserve polysaccharides (251).

The MS have highly efficient and diverse transport systems, which include high-affinity phosphotransferase for hexoses (288, 291) and sucrose (42, 314) and possibly glucose and sucrose permeases (42, 230), as well as a low-affinity proton motive force system (133, 136) whose activity is modulated by the $[K^+]$ of the plaque environment (230). High $[K^+]$ favors acid production, and as $[K^+]$ is present in the plaque fluid at about twice the $[Na^+]$ (334), this proton motive force system would be particularly effective under acidic conditions where the $[K^+]$ could be exchanged for intracellular $[H^+]$ (133).

pH levels of 5.0 or lower may persist in CA plaques for up to several hours (290). This acid plateau reflects that the microbial acid production from ICP and ECP is balanced by the tooth and salivary buffer systems. That the plaque continues to produce acid at these low pH levels is somewhat surprising, as many plaque species in vitro are metabolically sluggish at pH 5. In a survey of the ability of certain plaque species to convert sucrose to lactic acid at pH 5.0, *A. viscosus*, *S. sanguis*, *S. salivarius*, and *S. mitis* were virtually unresponsive, whereas all of the MS and to a lesser extent *L. casei* were quite active (141). In fact, *S. mutans* and *S. sobrinus* were more active at pH 5.0 than at pH 7.0 (140). This suggests that these MS are the principle acid producers in vivo when the plaque pH is buffered on the acid plateau. If this is so, then the frequent ingestion of sucrose could lead to the selection of a microbe that, because of its aciduricity, is most competitive at pH levels that both solubilize the tooth mineral and discriminate against other plaque species.

Aciduricity is also a trait possessed by the LB, a group of organisms that otherwise are not noted for their rate of acid production, especially from sucrose (141, 230), or for ECP production. In fact, the delayed appearance (20, 159, 249) of the LB in the carious lesion is compatible with the pH

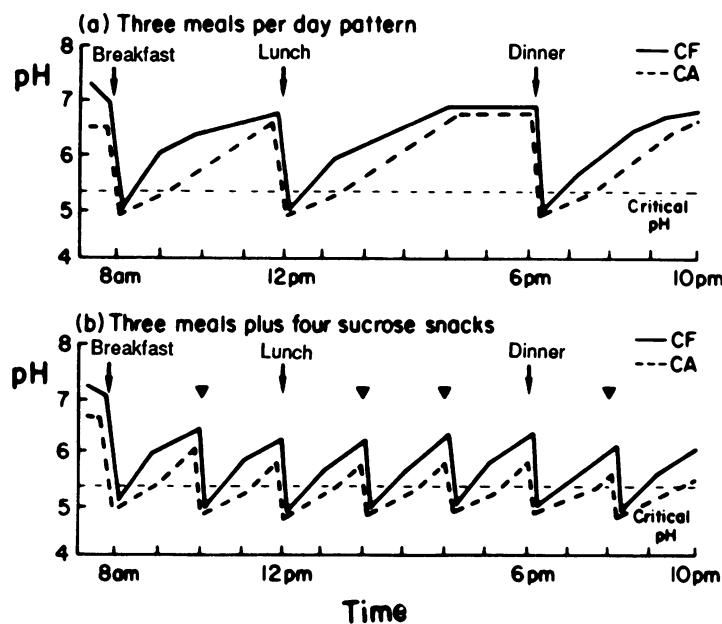


FIG. 3. CA and CF pH profiles in plaque in response to different eating habits. Note the increased amount of time in which the plaque pH is below the critical pH in the CA individuals.

reaching such acidic values that these, the most aciduric of the plaque species, can thrive.

An evolutionary derived protective mechanism. If aciduricity is the critical determinant of virulence for both the MS and LB, why then has dental caries only emerged as a significant health problem since the widespread usage of sucrose in the diet? While sucrose consumption has been linked to decay, there has always been a paradox of sorts in this relationship. Thus, in the Vipeholm study (129), an average of 330 g of sucrose could be consumed per day at meal times over a 2-year period by mentally retarded institutionalized adults with poor oral hygiene without any appreciable increase in caries prevalence, whereas in another clinical investigation only 2 to 3 g of sucrose per day consumed between meals was cariogenic (115). Clearly, humans have a mechanism(s) for coping with large amounts of sucrose when it is presented at meal times, but this mechanism is inadequate when even small amounts of sucrose are presented between meals.

This mechanism consists of the salivary and tooth buffering systems working in harmony with the salivary remineralizing system. This protective mechanism against plaque acid production evolved in the context of food being consumed at a restricted frequency. Also, natural foods contain mostly macromolecular nutrients that are not rapidly degraded or solubilized during transit in the oral cavity. Even when copious amounts of sucrose are present within these foods, as was the case in the Vipeholm study (129), the sucrose is rapidly cleared from the oral cavity via the normal masticatory process (196, 224).

Man in his evolutionary past rarely had the luxury of savoring food. This changed when sucrose became available as a consumer nutrient. The hedonistic appeal of sucrose, its low cost, patterns of cultural imitation, manufacturing practices in the food industry, and marketing tactics have led to the rapid assimilation of refined sucrose into all cultures which have access to it. In the process, a unique modern type of food has evolved, namely, a slow-release device for

sucrose known as a candy that has been recommended for use between meals as an energy food.

When sucrose is ingested between meals at frequent intervals, the protective mechanisms that the tooth has for maintaining its integrity are overwhelmed. This is because the use of candies between meals can cause the plaque pH to drop as much as it would with a full meal. This was demonstrated by intraoral pH telemetry, using volunteers who wore an indwelling pH electrode on denture teeth contained in a partial denture (119). Typical pH responses for various meal patterns are shown in Fig. 3.

Figure 3a shows the plaque pH response to three meals eaten at 8 a.m., 12 noon, and 6 p.m. During each meal there is a sharp drop in pH to below the critical pH and then a slow return to the resting or starting pH. The pH may remain below the critical pH for about 40 to 60 min per meal, meaning that for a total of 120 to 180 min per day the teeth are subject to demineralizing conditions at the plaque-enamel interface. In the intervals between meals there is ample time for saliva to replenish any lost tooth mineral. As the estimated time below the critical pH is about 8 to 10% of the day, it is not likely that the MS would be selected for. This is the typical dietary pattern that predominated throughout human history prior to the ready availability of sucrose and is not overtly cariogenic even when large amounts of sucrose are consumed at meal times (129).

The length of time at which the pH is below 7 can be multiplied by a factor of 10, 100, and 1,000 corresponding to the observed pH of 6.0, 5.0, and 4.0, respectively, so as to give a value called proton hours, which takes into account the magnitude of the acid exposure on the tooth surface (120). This calculation was applied to plaque pH readings which were continuously recorded on a limited number of subjects who were consuming, under strictly controlled conditions, meals containing moderate to high amounts of sucrose (50 to 80 g/day) or meals containing low amounts of sucrose (2 to 6 g/day). The proton hours in the plaque were 3- to 17-fold higher in the same subjects at the higher sucrose

intake even though the actual sucrose intake was 8- to 40-fold higher (120). Apparently at the higher dietary sucrose levels some of the sucrose is physically not bioavailable to the plaque flora or the capacity of the flora to degrade sucrose has been saturated.

Thus, while it is apparent that more sucrose taken at meal times increases the amount of acid present in the plaque, this amount, as measured by proton hours, is less than what would have been predicted based upon actual sucrose content of the diet. The opposite is true for between-meal sucrose ingestion where the proton hours are more than what would have been predicted based upon actual sucrose content.

Figure 3b depicts the pH profile in the plaque when the subject consumes four snacks containing sucrose at about 2-h intervals between his main meals. Each snack sends the pH plummeting to below the critical pH and aborts the return to the resting pH. If we assume that the plaque pH remains below the critical pH for 40 to 60 min per ingestion, then the teeth in this subject are exposed to 280 to 420 min of demineralizing activity per day. In fissures and approximal sites, there may not be ample time for full remineralization of the enamel to occur, and subsurface demineralization could progress to cavitation.

Even this calculation assumes that the composition of the plaque flora remains unchanged, whereas there is reason to believe that under acidic conditions the MS would be selected for (209). For example, when adult volunteers with moderate levels of MS in their plaques rinsed their teeth with various buffers, a pH 3.9 buffer, but not a pH 7 buffer, led to increased proportions of the MS in plaque (318). This selection combined with the metabolic prowess of these organisms at acidic pH levels would prolong the duration of each acid period, thereby compounding the assault upon the tooth. From this scenario it is easy to explain how during the Vipeholm study 330 g of sucrose taken at meal times was not cariogenic, whereas smaller amounts of sucrose (120 g) consumed between meals caused a five- to sevenfold increase in the annual caries rate (129).

Thus, dental decay occurs when the normal demineralization-remineralization cycle that occurs in the subsurface of the enamel following food ingestion is perturbed by events which either increase the acid challenge or decrease the salivary repair functions. A change in dietary pattern to one of frequent ingestion of sucrose leads to the selection of the MS which, because of their aciduricity at the critical pH for enamel demineralization, exploit this sucrose bioavailability to expand their niche in the plaque. If sucrose constituted most of the nutrient in the diet, as seen in the nursing-bottle caries syndrome (274) or in animal models, then the MS niche was large and rampant decay resulted. Under less severe sucrose exposure the metabolic activity of the MS can potentiate the postprandial pH drops at the plaque-enamel interface, thereby interfering with the normal salivary remineralizing system and leading eventually to clinical decay.

SUMMARY

These data provide convincing, albeit circumstantial, evidence that *S. mutans*, possibly *S. sobrinus*, and lactobacilli are human odontopathogens. As such, dental caries is a diagnosable and treatable infection (209). Aciduricity appears to be the most consistent attribute of *S. mutans* that can be associated with both its selection in stagnant areas and its cariogenicity. Other aciduric species such as *S.*

sobrinus appear to be important primarily in smooth-surface decay and, as such, may be a cariogenic determinant when rampant decay occurs.

Colonization by *S. mutans* occurs after tooth eruption, and if the fissures become colonized in their depths, then decay may be inevitable. However, if this colonization is delayed until the fissure depths are occupied by other bacteria, there is the possibility that decay will not occur or its occurrence will be greatly reduced. This understanding of the ecology of *S. mutans* suggests that treatment strategies which interfere with the colonization of *S. mutans* may have a profound effect on the incidence of dental decay in human populations (182).

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant DE02731 from the National Institute of Dental Research.

N. Grossman provided the typing and editing of the manuscript. Roy Curtiss, Roy Russell, M. Russell, I. Hamilton, M. Hughes, R. J. Gibbons, P. Caufield, and S. Michalek provided preprints of manuscripts.

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