MICROBIOLOGICAL REVIEWS, June 1980, p. 331-384
0146-0749/80/02-0331/54$02.00/0

Vol. 44, No. 2

Biology, Immunology, and Cariogenicity of *Streptococcus mutans*†

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INTRODUCTION

Whether there is any one bacterium which may *always* be found in decayed dentine, and which might therefore be entitled to the name of *the* bacterium of tooth decay, or whether there are various kinds which occur with considerable constancy, we are not able to say. It is now apparent, however, that various microorganisms are essential in the pathogenesis of dental caries. Orland (451) first demonstrated that selected streptococcal species, namely, enterococci, produced dental caries in germfree rats when fed a high-sucrose diet. Furthermore, indirect evidence that antibiotics suppressed experimental dental caries in rodents (396) strongly suggested the involvement of certain penicillin-susceptible bacteria in dental caries.

Since that time, various investigations have been carried out to elucidate the causative relationship between specific oral bacterial species and dental caries. In 1960, some streptococcal strains, isolated from carious lesions of rats and hamsters, produced dental caries in "caries-resistant" rats and hamsters, respectively (157, 302).

Using a fluorescent-antibody technique, several streptococcal strains which shared immunological specificity with the cariogenic streptococci derived from rats and hamsters were isolated from human carious lesions (275, 628, 629). These strains also produced severe dental caries in germfree animals. Since then, similar streptococcal species have been isolated from human carious lesions by several investigators (183, 202, 321, 322). Carlsson (54, 55) indicated that properties of these cariogenic streptococci were similar to those originally isolated from human carious teeth by J. K. Clarke (81) in 1924 to which he had given the species name *mutans*. Thus, the rediscovery of *Streptococcus mutans* followed the original observation by about 36 years.

S. mutans is now considered to play an important role in the development of dental caries in animals and humans. Extensive research on this microorganism has been done during the last 10 years. Unfortunately, however, *S. mutans* is not given an independent species position in the newest edition of *Bergey's Manual of Determinative Bacteriology* (43). It will be recognized from the evidence described below that *S. mutans* is the best-defined species among the oral streptococci.

The present review is an attempt to define the current state of knowledge concerning *S. mutans*. Numerous reviews and books have recently appeared on microbiological or immunological aspects of dental caries, or oral streptococci (27, 46, 196, 265, 303, 397, 438, 445, 494, 549).

ORAL MICROBIAL FLORA

The oral microflora is a complex ecosystem which contains a wide variety of microbial species (Table 1). The mouth is colonized by various microorganisms before teeth erupt, although newborn infants are essentially free from microorganisms (394). With the eruption of teeth, dental plaque, distinctive patches primarily of microbial origin, develop on exposed enamel surfaces which are covered by a pellicle that is an amorphous, almost invisible film composed primarily of salivary glycoprotein (162, 194). Large microbial masses develop on the teeth surfaces unless proper oral hygiene measures are taken,

TABLE 1. *Distribution of bacteria on various sites in the human mouth*[a]

Bacterial group	Site			
	Plaque	Tongue	Saliva	Gingival crevice
Gram-positive facultative cocci	28.2	44.8	46.2	28.8
Streptococci	27.9	38.3	41.0	27.1
S. mutans	(0-50)	(0-1)	(0-1)	(0-30)
S. sanguis	(40-60)	(10-20)	(10-30)	(10-20)
S. mitior	(20-40)	(10-30)	(30-50)	(10-30)
S. salivarius	(0-1)	(40-60)	(40-60)	(0-1)
S. milleri	(3-25)	(0-1)	(0-1)	(14-56)
Staphylococci	0.3	6.5	4.0	1.7
Gram-positive anaerobic cocci	12.6	4.2	13.0	7.4
Gram-negative anaerobic cocci	6.4	16.0	15.9	10.7
Gram-negative facultative cocci	0.4	3.4	1.2	0.4
Gram-positive facultative rods	23.8	13.0	11.8	15.3
Gram-positive anaerobic rods	18.4	8.2	4.8	20.2
Gram-negative facultative rods	ND[b]	3.2	2.3	1.2
Gram-negative anaerobic rod	10.4	8.2	4.8	16.1
Spirochetes	ND	ND	ND	1.0

[a] Modified from Gibbons and van Houte (194, 196) and Mejare and Edwardson (403). Data are expressed as a percentage of total cultivable count on anaerobically incubated blood agar. Data in parentheses are expressed as a percentage of the total facultative streptococcal counts.

[b] ND, Not detected.

whereas desquamation of epithelial cells does not permit the heavy accumulation on oral mucosal surfaces such as the dorsum of the tongue (195). The number of bacteria in dental plaque can reach 10^8 per mg (wet weight) (192).

As shown in Table 1, predominant microbial species are significantly different in different sites. Irrespective of variation from sample to sample, streptococci, gram-positive rods, and veillonellae comprise the majority of the total viable count. In plaque and gingival crevice, higher proportions of gram-positive and -negative rods are observed. More recently, it has been demonstrated that samples obtained from deep periodontal pockets in patients with advanced periodontitis and periodontosis consist of a significantly higher percentage of gram-negative anaerobic rods (440, 536, 537).

Clinical observations in humans and animals indicate that plaque formation is an essential requirement for both dental caries and periodontal disease. It is of interest to note that a limited number of bacterial species in the oral flora can be detected on the tooth surface. Ritz (472) described the shift in microbial population in developing plaque from a preponderance of coccal forms in very early plaque with an increase of rods and filament forms with age. However, streptococci make up the greater number of the total bacterial population in plaque throughout the period. Most of the streptococci can be identified as one of the following species: *S. mutans*, *S. sanguis*, *S. mitior*, *S. salivarius*, and *S. milleri* (52, 54, 55, 126, 202, 245, 403).

It appears that certain oral streptococcal species have a predilection for colonizing particular sites in the mouth. *S. sanguis* and *S. mutans* preferentially colonize the human tooth surfaces and prosthetic devices (54, 59, 62). *S. salivarius* is present in low numbers in plaque, whereas there is no preferred site for *S. mitior* in the oral cavity. Whereas *S. salivarius* is an early colonizer in the mouth after birth, *S. sanguis* is not usually found until the teeth erupt (59, 60). Similar findings have been obtained with *S. mutans*. The preferred habitat of *S. mutans* appears to be tooth surfaces. The numbers of *S. sanguis* isolated from previously cleaned teeth were much higher than those of *S. salivarius*, indicating the importance of the selective ability of streptococci to attach to oral surfaces. The observed affinity of these species for an oral surface is reported to correlate positively with the proportions that are found in vivo (195).

ISOLATION AND IDENTIFICATION OF *S. MUTANS* AND OTHER ORAL STREPTOCOCCI

In general, there are many difficult technical problems in obtaining representative samples from different oral sites, and in dispersing, cultivating, and enumerating the microorganisms. No single cultivation method of examining the complex and variable dental plaque flora will satisfy all the necessary conditions. Strictly anaerobic procedures will be required in many cases. It is fortunate, however, that most oral streptococcal species can be isolated from various sites in the mouth by using a selective medium, mitis salivarius (MS) agar (Difco Laboratories, Detroit, Mich.). Although MS agar was originally devised by Chapman to isolate fecal streptococci, the use of MS agar has dominated other cultural methods for the isolation of oral streptococci, including *S. mutans*, because of its selective and differential properties. The increasing attention associated with the occurrence of *S. mutans* in the various lesions of the human teeth has resulted in a more refined methodology regarding its isolation, quantitation, and species identification.

On MS agar medium, most oral streptococci show a characteristic colonial morphology which permits their provisional differentiation. Usually, the agar plate is incubated in an atmosphere of 95% nitrogen and 5% carbon dioxide at 37°C for 1 to 2 days, followed by incubation in air for another 1 to 2 days. A candle jar or the GasPak system (BBL Microbiology Systems, Cockeysville, Md.) can also be used for primary isolation of oral streptococci from clinical samples.

In addition to the characteristic colonial morphology, oral streptococci can be differentiated by their ability to ferment certain sugars (especially mannitol and sorbitol) and to adhere to smooth surfaces in the presence of sucrose (245). Techniques for these tests are described in detail (92).

Characteristic Properties of Oral Streptococci

S. mutans. *S. mutans* was isolated from human carious lesions by Clarke (81) in 1924. His description is as follows:

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

The occurrence of *S. mutans* in human carious lesions was confirmed (108, 183, 220, 321, 382). Extensive taxonomic studies revealed that these organisms formed a fairly homogeneous group of nonmotile, catalase-negative, gram-positive streptococci (55, 126, 143, 202). A number of

investigators have also revealed an association between the occurrence of *S. mutans* and the development of caries (vide infra).

As tabulated in Table 2, most streptococcal strains that ferment mannitol and sorbitol in addition to various other sugars, and synthesize adherent water-soluble glucan from sucrose, are considered *S. mutans*. They do not usually deaminate arginine to produce ammonia. *S. mutans* is mostly α- or γ-hemolytic on sheep blood agar, but a few β-hemolytic strains (621) have been reported. A further characterization of these β-hemolytic strains is needed before they can be identified as *S. mutans*. *S. mutans* has been subclassified into several types based on immunological, biological and genetic properties. These properties will be discussed below in detail.

The natural habitat of *S. mutans* is the human mouth. The organism can be isolated frequently from feces in humans (149, 307, 550) and rats (261, 576). Although *S. mutans* appears not to be widely distributed in wild animals, Dent et al. (104) isolated *S. mutans* from the Patas monkey and Indian fruit bat of 18 animal species examined. Coykendall et al. (96) and Lehner et al. (343) also isolated *S. mutans* from wild rats inhabiting sugar cane fields and from rhesus monkeys. It has also been isolated from experimental rats and hamsters (174).

To compare and differentiate other oral streptococcal species from *S. mutans*, the following brief summary of the other principal species is given.

S. sanguis. The species name was given by White and Niven (614) to the α-hemolytic streptococci, isolated from the blood of patients with subacute endocarditis, that split arginine and esculin and produce glucan from sucrose. They produce hydrogen peroxide when grown aerobically. Carlsson (52) demonstrated that the main habitat of *S. sanguis* in humans is the oral cavity, especially in plaque. Low levels of *S. sanguis* were reported in human feces (595).

On MS agar, *S. sanguis* produces small zoogleic colonies with a firm consistency which are embedded in the medium and which deform the surrounding agar. Many *S. sanguis* strains produce spreading zones typical of twitching motility on blood agar plates (252).

Strains with a similar colonial morphology which do not hydrolyze arginine and esculin but synthesize glucan are considered to be another type of *S. sanguis* (55). Although other investigators considered the latter strains as glucan-producing *S. mitior* (91, 97, 98), they are included within the species *sanguis* for convenience and are separated into biotypes A and B as shown in Table 2 (144, 579).

Serological studies on *S. sanguis* strains demonstrate the presence of at least three (488) or four (579) types. The close relationship of *S. sanguis* to group H streptococci has been suspected for many years, but still remains to be defined (88, 144, 146, 245, 252, 490). In spite of the complexity of its antigenic structure, *S. sanguis* is not difficult to identify because of the unique physiological properties and colonial morphology on sucrose agar.

S. mitior. *S. mitior*, frequently called *S. mitis*, is an α-hemolytic, bile-sensitive streptococcus that does not hydrolyze arginine and esculin. It is peroxidogenic, but does not ferment inulin, sorbitol, and mannitol. On MS agar, it elaborates

TABLE 2. *Generalized key characteristics for identifying the predominant streptococcal species*[a]

Organism	Fermentation						Hydrolysis		Polysaccharide from sucrose	Peroxide	Hemolysis on sheep blood agar plate
	Mannitol	Sorbitol	Melibiose	Raffinose	Esculin	Inulin	Arginine	Esculin			
S. mutans[b]											
a	+	+	+	+	+	+	−	+	Glucan ≫ fructan	+	δ
b	+	+	+	+	+	+	+	+	Glucan ≫ fructan	−	γ
c/e/f	+	+	+	+	+	+	−	+	Glucan ≫ fructan	−	γ
d/g	+	±	−	−	−	+	−	+	Glucan ≫ fructan	+	δ
S. sanguis[c]											
A	−	−	−	+	+	+	+	+	Glucan	+	α
B	−	−	−	−	−	−	−	−	Glucan	+	α
S. mitior	−	−	−	±	±	−	−	−	±	+	α
S. salivarius	−	−	−	+	+	±	−	−	Fructan ≫ glucan	−	γ
S. milleri	−	−	−	−	+	−	+	+	−	−	α/γ

[a] Collected data from references 55, 91, 144, 202, 221, 245, 403, 459, and 522.
[b] Serotypes according to Bratthall (31) and Perch et al. (459).
[c] Biotypes according to Torii (579).

soft, round, black-brown colonies. Some "*S. mitior*" strains produce extracellular glucan from sucrose, and develop colonies that are indistinguishable from those of *S. sanguis*. Deoxyribonucleic acid (DNA) base sequence studies showed the presence of two types of moderately homologous strains (97). Perhaps "glucan-producing *S. mitior*" can be included in the biotype B *S. sanguis* (Table 2), or perhaps these strains should be given a new taxonomic designation (144).

S. salivarius. *S. salivarius* primarily produces levan (fructan) from sucrose, and therefore forms unique large, domed colonies on MS agar. It does not ferment sorbitol and mannitol. Colonies of *S. salivarius* are nonhemolytic on blood agar and are not peroxidogenic. The tongue is its main habitat in the oral cavity (55, 91).

S. milleri. The species name *milleri* was originally proposed by Guthof (214) for streptococci which had been isolated from dental abscesses. They deaminate arginine, do not ferment mannitol and sorbitol, do not produce extracellular polysaccharides from sucrose, and do not produce peroxides. They can be isolated from the gingival crevice and dental plaque (Table 1). *S. milleri* shows some resistance to sulfonamides and bacitracin (403), and therefore can be grown on the selective medium devised for isolation of *S. mutans* (see Selective Isolation of *S. mutans*). Although *S. milleri* constitutes a fairly homogeneous group based on its cultural and biochemical characteristics, it appears that immunological specificity is heterogeneous.

Selective Isolation of *S. mutans*

MS agar is most widely used to isolate *S. mutans* as well as other oral streptococcal species. Although MS agar is available commercially, recent investigations (349, 545) have demonstrated significant discrepancies between data from different MS agar preparations manufactured by different companies for the recovery and quantitative enumeration of freshly isolated and stock strains of *S. mutans*.

Linke (353) indicated that trypan blue in MS agar will inhibit growth of most *S. mutans* strains. Furthermore, addition of Chapman tellurite solution to MS agar resulted in a significant reduction in the number of colonies (360).

MS agar has been modified to be more selective for the isolation of *S. mutans* by adding either sulfonamide (MC agar; 53), bacitracin (MSB agar; 198), polymyxin (154), or even supplemental sucrose (MS40S agar; 266). However, it has been suggested that some serotype *d/g* strains are susceptible to sulfonamide (133, 360), and incorporation of bacitracin into MS agar completely inhibited growth of serotype *a* strains (349, 545).

More recently, Linke (353) devised a new selective MSFA medium for *S. mutans* which includes mannitol, sorbitol, basic fuchsin, and sodium azide. On the other hand, BCY (267) and MMIO sucrose agars (369) are nonselective media which allow total bacterial counts and enumeration of polysaccharide-synthesizing streptococci, including *S. mutans*.

Comparative evaluation of various selective media under standardized conditions indicates that the growth of most serotypes of *S. mutans* is depressed by the use of such media. The variable cultural results prevent the unqualified use of a single medium for isolation and enumeration of *S. mutans* (133, 360).

CLASSIFICATION OF *S. MUTANS*

Immunological Typing of *S. mutans*

As described in the preceding section, isolation and identification have relied primarily on phenotypical characteristics rather than immunological specificity. Strains of *S. mutans* are phenotypically homogeneous, as confirmed by numerical taxonomic studies (55, 116). However, recent investigations have revealed a great degree of heterogeneity of *S. mutans* when surveyed serologically, genetically, and biochemically.

Zinner et al. (628) first demonstrated a serological heterogeneity in *S. mutans* strains FA1 and HS1, which were of rat and hamster origin, respectively. In a later study, Bratthall (30, 31) described the presence of five serotypes, *a*, *b*, *c*, *d*, and *e*, within the species. Subsequently, Perch et al. (459) revealed two additional serotypes, *f* and *g*. The serological techniques used in these studies were the capillary precipitin test, originally introduced by Lancefield (334) in classifying beta-hemolytic streptococci; immunodiffusion; comparative immunoelectrophoresis; and immunofluorescence. The specific antigens of each serotype have been purified and characterized chemically (see Serotype-Specific Antigens of *S. mutans*).

Coykendall (94) has reported analysis of DNA base composition and DNA base sequence similarities of *S. mutans* strains, showing the presence of four genetic groups (I through IV), or "genospecies." These four groups correlated with four serotypes, *c*, *b*, *a*, and *d*, respectively. More recently, he (95) proposed to give the subspecies of *S. mutans* species names (Table 3). A considerable overlap in the mole percent guanine plus cytosine content of these species is apparent.

Heterogeneity has also been observed in var-

TABLE 3. *Chemical composition and immunological determinant of type-specific antigen preparations from S. mutans*

Serotype	Strain	Source	Extraction procedure	Composition (wt %)						Proposed antigenic determinant[a]	Reference
				Galactose	Glucose	Rhamnose	Galactosamine	Protein	Phosphorus		
a	HS6	Cells, walls	Boiling water	54	10	—	5	5.0	0.3	Glc-β(1,6)-Glc	426
b	FA1	Cells, walls	Cold TCA[b]	27	—	47	2	5.4	2.3	α-Gal	425
c	Ingbritt	Cells	Cold TCA	—	29	69	—	0.5	0.5	Glc-α(1,4)-Glc	355
c	GS5	Walls	Hot formamide	—	29	43	—	ND[c]	ND	Glc-α(1,4)-Glc	612
d	B13	Cells, walls	Cold TCA	62	33	—	—	1.6	0.3	Gal-β(1,6)-Glc	357
e	MT703	Cells	Hot saline	—	37	56	—	5.0	0.3	Glc-β(1,6)-Glc	231
e	V-100	Walls	Hot formamide	—	24	52	—	ND	Trace	Glc-β(1,4)-Glc	613
f	OMZ175	Cells, walls	Hot TCA	—	47	49	—	Trace	0.2	Glc-α(1,6)-Glc	216
f	MT557	Cells	Hot saline	—	39	59	—	Trace	0.2	Glc-α(1,6)-Glc	216
g	6715	Cells	Hot buffer	61	10	—	—	9.5	0.4	β-Gal	264

[a] Glc, Glucose; Gal, galactose.
[b] TCA, Trichloroacetic acid.
[c] ND, Not described.

ious enzyme proteins, such as dehydrogenases (39, 40), glucosyltransferase (75), aldolases (375), and invertases (385, 565) within the species *S. mutans*.

In contrast, Shklair and Keene (521) proposed a biochemical scheme for the separation of *S. mutans* into five biotypes, which they reported to correlate with serotypes *a* to *e*. The biotyping was based on the fermentation of mannitol (with or without bacitracin), sorbitol, raffinose and melibiose and the production of ammonia from arginine. They later refined their scheme to include additional serotypes *f* and *g* and designated them biotypes I to V (522) (see Table 3). It is of interest to note that bacitracin inhibited acid production by serotype *a* (biotype III) strains but not serotype *c*, *e*, and *f* strains (biotype I) that were otherwise similar to biotype III (Table 2).

However, a study of 137 clinical isolates composed of serotypes *c* or *e* (221) indicates that serotypes *c* and *e* are essentially similar with respect to fermentation of melibiose, a key character in the differentiation of biotypes I and V. Use of this scheme for differentiation of serotype *c* and serotype *e* *S. mutans* would result in an error in the count of serotype *e* *S. mutans*. Therefore, it appears that biotyping is not correlated with serotyping, and the suitability of biotyping as a taxonomical tool remains in doubt.

It should be added here that all the clinical isolates (221) belonging to serotype *d* or *g* produced a markedly zoogleal colony on MS agar, exhibited alpha-hemolysis on sheep blood agar, and were peroxidogenic, whereas those belonging to serotype *c*, *e*, or *f* developed a small, rough, raised and undulated colony as shown in Fig. 1 and were nonhemolytic and nonperoxidogenic (Table 2). Moreover, 23 isolates of serotypes *d* and *g* were strongly agglutinated shortly after addition of dextran T2000 (500 μg/ml, final concentration), and 40 out of 41 serotype *c*/*e*/*f* strains were not agglutinated even after 18 h of incubation. Similar results have been obtained with reference strains of *S. mutans* (622). These results indicate that serotypes *d* and *g* and serotypes *c*, *e*, and *f* constitute two major subgroups, which correlate with the genospecies proposed by Coykendall (95).

In summary, to minimize taxonomical confusion of *S. mutans*, we prefer serotypes to genospecies or biotypes. Serotyping is a routine procedure and is as valuable as that used with other streptococcal immunological groups and types.

Serotype-Specific Antigens of *S. mutans*

The antigenic components of *S. mutans* can be extracted in a soluble form by various methods from whole cells or cell walls. These methods involve the use of hot physiological saline (50, 216, 220, 230, 231, 234), 5 to 10% trichloroacetic acid (216, 356, 357, 425, 529, 586, 601), formamide (39, 172, 612), dilute hydrochloric acid (31, 334),

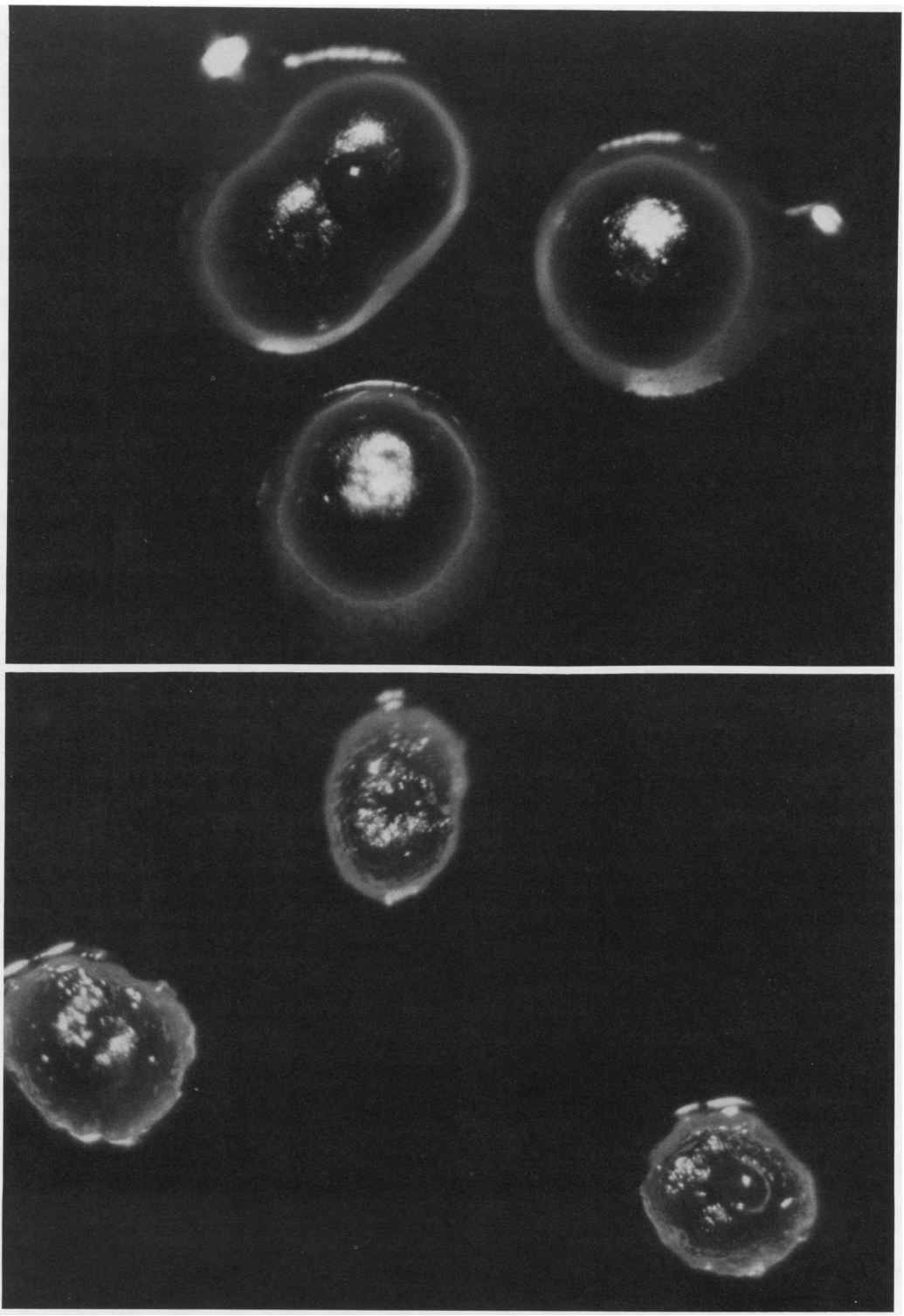

FIG. 1. *Typical colonial morphologies of S. mutans on MS agar. Colonies of serotype d and g strains are surrounded by a puddle (zooglea) with a gelatinous consistency (top), whereas those of serotype c, e, and f strains are small, raised, irregular in margin, and adherent, but do not show the zooglea (bottom). (Reproduced with permission, reference 221.)*

phosphate buffer, pH 7.3 (264), cell wall lytic enzyme "mutanolysin" (230), or even water (426). The crude antigen extracts usually contain contaminants such as nucleic acids, unidentified cellular proteins, and cross-reacting polyglycerophosphate (PGP) antigens as well as the type-specific antigens (234). Therefore, serotype-specific antigens of *S. mutans* have been purified by a variety of column chromatographic procedures, including gel filtration, ion-exchange chromatography, and affinity chromatography.

The purified antigens from serotype *a* to *g* *S. mutans* strains are polysaccharides located in or on the cell wall (Table 3) (354, 531). They are composed primarily of a combination of either glucose, galactose, or rhamnose. They differ from the group-specific polysaccharides of certain β-hemolytic streptococci (326) in the absence of significant quantities of *N*-acetylglucosamine and *N*-acetylgalactosamine. A small quantity of these amino sugars is present in the *a* and *b* antigen. Table 3 summarizes the composition of the seven polysaccharides and the probable antigenic determinant of each. The chemical composition and recovery of these polysaccharides (Table 3) illustrate a variation in their state of purity. This is due in large measure to the source of the polymer and to the various methods of extraction and purification. However, reasonably good agreement has been obtained in the gross composition of the antigen in each of the two strains of types *c*, *e*, and *f*. Confirmation of the immunological determinant must await studies on more highly purified material.

The *a* (strain HS6) (426), *d* (strain B13) (357), and *g* (strain 6715) (219, 263, 264) serotype polysaccharides are composed principally of glucose and galactose. Although the cell walls of these *S. mutans* serotypes have been reported to contain a significant quantity of rhamnose (22, 244), the purified antigens do not contain rhamnose (Table 3). Considerable cross-reactivity has been observed among serotypes *a*, *d*, and *g* (31, 219, 356, 459).

TABLE 4. *Taxonomic relationship between serotypes and biotypes of S. mutans and proposed genospecies*

S. mutans		Proposed species name[c]	DNA base content (mol% G+C)[d]
Serotype[a]	Biotype[b]		
c, e, f	I	*S. mutans*	36–38
b	II	*S. rattus*	41–43
a	III	*S. cricetus*	42–44
d, g	IV	*S. sobrinus*	44–46
c	—[e]	*S. ferus*	43–45
e	V	—	—

[a] From Bratthall (31) and Perch et al. (459).
[b] From Shklair and Keene (522).
[c] From Coykendall (95).
[d] From Coykendall (95). G+C, Guanine plus cytosine.
[e] —, Not described.

A rhamnose-rich polysaccharide has recently been isolated from cell walls of strain B13, serotype *d* (464). It is composed of rhamnose and glucose, whereas the serotype antigen from this strain is a galactose/glucose polymer (357). It is immunologically distinct from the latter. Other distinct antigenic polysaccharides will probably be found in the cell walls of *S. mutans*.

The earlier literature reflects confusion in the serotyping of some *S. mutans* strains (31). For example, strain 6715 was originally reported as serotype *d* (264), but was later reclassified as serotype *g*, using appropriately absorbed typing serum (219). Furthermore, although strain AHT was originally reported to be serotype *a*, it has been found that some AHT substrains, including the one deposited in the National Collection of Type Cultures, London, are serotype *g* and not *a* (219; R. R. B. Russell, personal communication).

Quantitative precipitin inhibition tests indicate that the serotype *a*, *c*, *e*, and *f* antigenic determinants depend mainly upon a glucose-glucose sequence (216, 231, 355, 426, 612, 613). The specificity appears to be related to the presence of either *a* and *b* forms and (1 → 4) and (1 → 6) linkages (Table 3). On the other hand, the serotype *d* and *g* specificities depend on a configuration of galactose and glucose within the antigen molecules (264, 357). However, another group (42) reports that the serotype *a* antigenic determinant may be D-galactose rather than D-glucose and that the *d* specificity depends upon a terminal D-glucose. The result of Brown and Bleiweis (42) confirms the D-galactose specificity and the *a-d* antigenic sites as reported by Mukasa and Slade (426) and Linzer and Slade (357).

Serotype *c* (strains Ingbritt and GS5) (355, 612), *e* (strains MT703 and V-100) (231, 613), and *f* (strains OMZ175 and MT557) (216) antigenic polysaccharides are essentially composed of glucose and rhamnose. Hapten inhibition studies suggest that an α-glucosyl residue is the immunological determinant of the serotype *c* and *f* antigens, whereas a β-glucose residue is the serotype *e* determinant (216, 355, 612, 613).

The type *f* antigen from strain OMZ175 is related to dextran. This is indicated by the presence of α-1,6-glucosidic linkages (90% inhibition of the precipitin reaction by isomaltose and α-methyl-D-glucopyranoside), adsorption to and release from a concanavalin A (ConA)-Sepha-

rose column, and reaction with antidextran serum (216).

This is the only *S. mutans* type antigen which reacts with ConA and dextran antiserum. The cross-reaction between type *a* and *d* strains is due to a common antigenic site (*a-d*) which is the same in both cases. The two antigenic specificities (*a* and *a-d*; *d* and *a-d*) are present on a single polysaccharide molecule in each case (356). This specificity is due mainly to α-D-galactose-1 → 6-glucose (Table 3). The D-galactose specificity of the *a-d* site has recently been confirmed (42).

Serotype *e* antigen cross-reacts with Lancefield group E antiserum (31). However, this antigen has two different immunological specificities. One shares a specificity with group E streptococci (543), and the other is specific for serotype *e S. mutans* (230, 231). The immunological distinction, based upon a single antigen molecule, differs from the original description of the antigen (31). We use antiserum against the whole cells of serotype *e S. mutans* rather than antiserum against Lancefield group E cells, because the former serum possesses far more intensive immunological reactivity with serotype *e S. mutans* (220, 230, 231).

A cross-reaction between serotype *c* antiserum and one of the serotype *e* antigens has been reported (613). The cross-reacting antigen may be a degraded product resulting from drastic formamide extraction, thus exposing internal α-glucosidic linkages which may be reactive with serotype *c* antiserum. Some other aspects of cross-reactive phenomena will be discussed in the next section.

The nature of the serotype *b* antigen has not been adequately explained. Mukasa and Slade (425) obtained two forms of polysaccharide antigen by chromatographic purification from strain FA1. The chemical composition and electrophoretic mobility of the two forms are considerably different; however, they possess an identical immunodeterminant. These antigens contain low amounts of phosphorus and glycerol, but they are negatively charged. They are adsorbed to diethylaminoethyl-Sephadex A-25 resin (234). On the other hand, Vaught and Bleiweis (601) purified two antigenic components from another serotype *b* strain, BHT. One antigen appears to be identical to one of the polysaccharide *b* antigens of Mukasa and Slade (425), but the second antigenic component, which is more negatively charged, is reported to be a glycerol teichoic acid substituted with a galactosyl moiety. Hapten inhibition studies suggest that the immunological determinant of serotype *b* is a β-D-galactoside (425, 601). Furthermore, all serotype *a* to *g* antigens except *b* antigen can be extracted by 0.1 M NaOH at 60°C for 30 min. The *b* antigen as well as the PGP/lipoteichoic acid (LTA) antigen (vide infra) appear to be destroyed by this procedure.

In this context, the electrophoretic mobility of 16 strains of *S. mutans* was compared by a microelectrophoresis technique, and it was found that two serotype *b* strains examined showed the highest surface potential (448). This may be due to the protein content of the two antigenic forms of the type *b* antigen (425). One form contains 30% protein, in addition to polysaccharide. Many of the amino acids in this protein were those not present in the peptidoglycan of *S. mutans* (534). The antigenic specificity was released from cell walls with lysozyme, although the walls were not dissolved. Also, trypsin and pepsin did not release it (426a). Consequently, the protein bound to the immunologically specific carbohydrate is not the peptide of the cell wall peptidoglycan but another peptide/protein located in the wall.

As discussed above, immunological cross-reactions are frequently observed among some combinations of serotypes, i.e., serotypes *c*, *e*, and *f* and serotypes *a*, *d*, and *g*. These close relationships correlate with the DNA base sequence data (195) and the biochemical separation data (221, 459, 522). These cross-reactions are evident by immunodiffusion, whole cell or cell wall agglutination, and immunofluorescence (31, 32, 37, 219, 220, 244, 356, 402). Serotype specificity was obtained after appropriate adsorption procedures.

It has also been shown that many gram-positive bacteria, including all strains of *S. mutans*, possess a common antigenic component, PGP (72, 90, 234, 312, 313, 395, 426, 617). Crude antigen extracts of *S. mutans* serotypes *a* to *g* reacted with anti-PGP serum in agar gel. The cross-reactive PGP antigen was adsorbed with an anion-exchange resin (234). Many batches of antiserum against *S. mutans* whole cells react with heterologous antigen extracts of various serotypes and species of streptococci when examined by the passive hemagglutination technique (223).

Recently, it was found that antiserum specific for serotype *e S. mutans* glycosyltransferase (GTase) almost completely inhibited the GTase activity of types *c*, *e*, and *f S. mutans*, whereas the GTase of types *a*, *d*, and *g* was not affected by the antibody (236). On the other hand, it has been reported that antiserum against serotype *a S. mutans* GTase reacted with GTase of type *a*, *d*, and *g* strains, but not with those of type *b* and *c* strains (171). Several other reports (139, 175, 229, 332, 538) also indicate that serotypes *c*, *e*, and *f* and types *a*, *d*, and *g S. mutans* can be

separated into two major groups on the basis of the immunological relatedness of GTase protein molecules.

In contrast, an antiserum against an *S. mutans* type *a* (HS6) purified GTase, which produced 95% water-insoluble glucan, inhibited the GTases from serotypes *a*, *b*, *c*, and *d* (358). Antisera against similar purified GTase preparations from other type *a* strains need to be tested to clarify these results.

In this connection, Russell (495) has shown on the basis of sodium dodecyl sulfate-gel electrophoresis of whole cell proteins that strains of serotypes *a* and *b* have unique patterns. Serotype *d* and *g* strains show a very close relationship to each other, as do the strains of serotypes *c*, *e*, and *f*. This observation corresponds to the genetic subdivision (95), as discussed above.

Reactivity of *S. mutans* with Lectins

Plant lectins (phytohemagglutinins) have been found to react specifically with sugar residues of polysaccharides and glycoproteins (359). The lectins bind to the surface components of microbial cells, frequently resulting in agglutination (217, 317, 318).

It was shown that ConA, a jack bean lectin reactive with α-D-glucopyranosyl or α-D-mannopyranosyl residues, agglutinated the cells of 13 of 15 strains of *S. mutans* in an 18-h incubation (217). Among these, type *a*, *d*, *f*, and *g* strains agglutinated within 2 h. Cells of seven *S. sanguis* group H streptococcal strains and various other bacterial species were also agglutinated within 2 h by ConA. Binding of ConA to the surface of serotype *f S. mutans* was confirmed using ^{3}H-labeled ConA (217). Furthermore, ConA will also induce the agglutination of *S. sanguis* and *S. faecium* (287, 452).

Ricinus communis agglutinins (RCA I and RCA II), castor bean lectins reactive with galactose residues (441, 442), agglutinated cells of serotype *a*, *d*, and *g S. mutans*, but not those of serotype *b*, *c*, *e*, and *f S. mutans*, after a 2-h incubation (217).

These results with *S. mutans* indicate that the two lectins react with a surface polysaccharide polymer. The binding of ConA, however, did not inhibit the binding of GTase to heat-treated *S. mutans* cells and subsequent adherence due to glucan synthesis (217). If binding of ConA did occur at the type-specific polysaccharide or glucan sites, the lectin did not occupy a position which affected the action of GTase. Many other lectins with different specificities (359) will possibly bind to and agglutinate the cells of various oral streptococci. On the other hand, Staat et al. (546) found that *Persea americana* lectin inhibited the in vitro adherence of *S. mutans* 6715 (serotype *g*) to a glass surface. In this case, the lectin may have bound to the polysaccharide component of GTase.

Cell Wall Structure of *S. mutans* and Other Streptococci

The streptococcal cell wall contains four major antigenic polymers: peptidoglycan, group- and type-specific polysaccharides, protein, and the glycerol form of teichoic and lipoteichoic acids. A considerable volume of evidence indicates, in contrast to a layer type structure (326, 326a), the existence of a mosaic structure in which each of these polymers is accessible for reactions at the cell surface (532). The outer and inner structure of the cell wall of *S. mutans* is seen in Fig. 2. The protoplast membrane is not clearly defined.

Fluorescein-labeled antibodies specific for polysaccharide or a protein component give a uniform surface stain of group A (89) and group F (619) streptococci. Ferritin-labeled antibody to the group A streptococcal M protein (602) and group C polysaccharide (602a), and the LTA of *S. mutans* (588), show a surface location of these antigens. The rapid agglutination of some *S. mutans* strains by ConA indicates a similar location of the polysaccharides (217). The binding of bacteriophage by the peptidoglycan of the group A streptococcus indicates a ready accessibility of the latter to the virus (82).

These data support the concept that these antigenic polymers are available at the cell surface to react with other polymers. Electron micrographs of streptococci show filamentous-type structures on the cell surface (130, 432, 490, 558) which react with ferritin-labeled antibody (558, 588, 602). Selective extraction procedures have distinguished the M protein from the teichoic acid structures at the cell surface of the group A streptococcus (13). These filamentous structures may be termed "fimbriae."

A model of the structure of the streptococcal cell wall is shown in Fig. 3 (532). The lattice-like cross-linking of the peptidoglycan is shown, although all the possible linkages are not illustrated for purposes of clarity. The teichoic acid and LTA are shown in transit across the wall (618) from the site of synthesis in the membrane (524, 533). Protein and polysaccharide may also be synthesized in the membrane. Ferritin-labeled antibody studies show that the fimbriae are composed mainly of protein, polysaccharide, and teichoic acid. Open spaces between the fimbriae to the peptidoglycan may allow the binding of bacteriophage. The close association of these polymers in the wall helps to explain the release of both polysaccharide and protein by proteo-

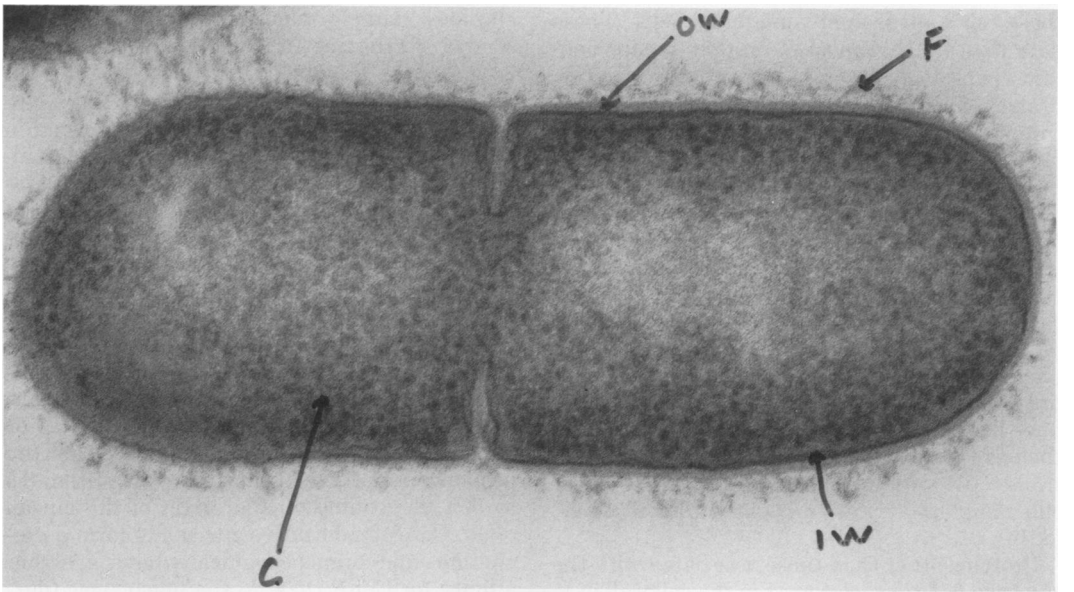

FIG. 2. *Electron micrograph of thin section of S. mutans, serotype d, strain B13. F, fimbriae; OW, outer wall; IW, inner wall; C, cytoplasm. ×125,000.*

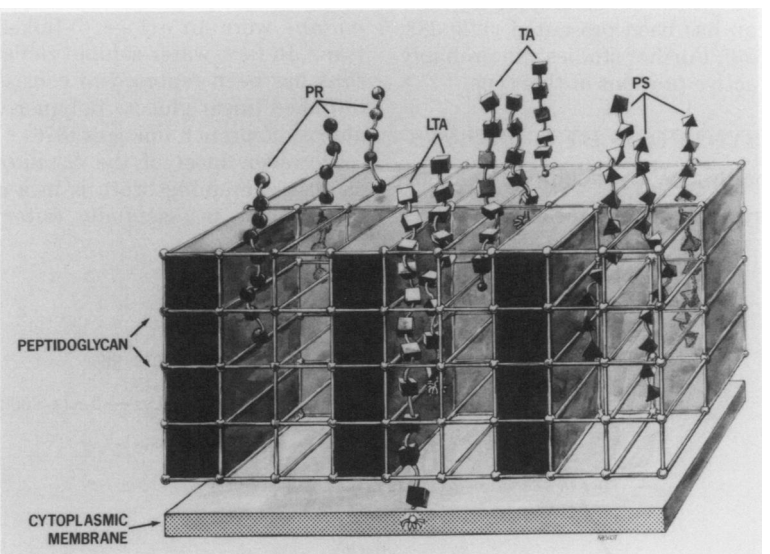

FIG. 3. *Model of the streptococcal cell wall. PR, protein; LTA; lipoteichoic acid; TA, teichoic acid; PS, polysaccharide. (Reproduced with permission, reference 532.)*

lytic and saccharolytic enzymes (179), the "umbrella" effect of antibody (531), and their function as binding sites for enzymes and sites of enzymatic glucan synthesis in in vitro and in vivo adherence (13, 130, 531).

The rigid nature of the bacterial cell wall is due in large measure to a bag-shaped macromolecule (606) which is composed of *N*-acetyl amino sugars plus *N*-acetylmuramic acid and numerous peptides. This basal polymer has been designated "peptidoglycan" (513). The chemical composition of the cell walls of *S. mutans* has been reported (22, 272, 532). *S. mutans* peptidoglycan consistently contains glutamic acid, alanine, lysine, glucosamine, and muramic acid in the approximate molar ratio of 1:2–4:1:1:1. In addition to these major amino acids, the presence of threonine was reported in the cell walls of serotypes *a* (22, 272, 534), *d* (22), and *g* (272). The molar ratio of threonine to glutamic acid in

these cell walls is approximately 0.7–1:1. These data indicate that most *S. mutans* strains contain a peptidoglycan cross-linked by interpeptide bridges consisting of L-alanyl oligopeptide or threonyl-alanyl peptide. Figure 4 shows a possible structure of the peptidoglycan of *S. mutans* BHT (serotype *b*) proposed by Inoue et al. (271). The bonds probably hydrolyzed by the *Flavobacterium* cell wall lytic enzymes are indicated.

Figure 2 also illustrates the filamentous structures on the surface of *S. mutans*. Their chemical nature has not been defined. Those of group A streptococci are individually either protein or teichoic acid (13). These structures on *S. mutans*, seen on practically all gram-positive cocci, have been termed "fuzzy coat" (432). We prefer the term "fimbriae," although they are not identical to the structures on the gram-negative bacilli. The latter are frequently termed "pili" (281a).

Proteins other than those associated with the serotype *b* antigen also exist in the cell wall of *S. mutans*. A protein which functions as a binding site for glucan has been isolated (387, 391). Evidence of proteins which act as binding sites for GTase or glucan has been presented (178, 333, 427, 428, 622, 623). Further studies will probably identify other active proteins of this type.

POLYMER SYNTHESIS BY *S. MUTANS*

Extracellular Polysaccharides

S. mutans synthesizes extracellular polysaccharides, namely, glucans and fructans, from sucrose by the enzymatic action of GTase (EC 2.4.1.5) and fructosyltransferase (FTase; EC 2.4.1.10). These polysaccharides, especially glucans, are considered to be critically important in dental plaque formation and hence in the pathogenesis of dental caries, because they are water insoluble and possess a marked ability to promote adherence when synthesized de novo on various solid surfaces (Fig. 5). Since various aspects of *S. mutans* glucan have been reviewed recently (204, 233, 436, 603), only certain topics will be discussed briefly.

Glucans. In general, all bacterial glucans contain $\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 3)$ glucosidic linkages, with the occasional occurrence of $\alpha(1 \rightarrow 2)$ or $\alpha(1 \rightarrow 4)$ linkages. It has been reported that the proportion of $\alpha(1 \rightarrow 3)$ linkages varies from 0.5 to 60%, depending on the origin of the glucan (603). ConA binds native glucans to form a precipitate, and branched glucans have a higher affinity for ConA than do the linear ones (dextrans) (483).

Earlier investigations (182, 212) indicated that extracellular polysaccharides produced by *S. mutans* were an $\alpha(1 \rightarrow 6)$-linked linear "dextran." In fact, water-soluble glucan from *S. mutans* has been reported to consist of an $\alpha(1 \rightarrow 6)$-linked linear glucose polymer with $\alpha(1 \rightarrow 3)$ glucosidic branch linkages (376).

However, most of the *S. mutans* glucan in sucrose-containing broth is in a cell-associated form, which is essentially water soluble. The

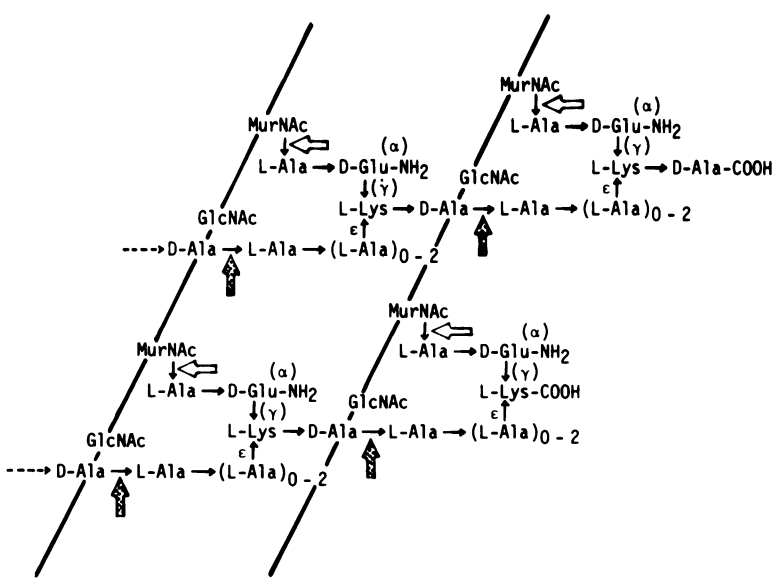

FIG. 4. *Possible structures of peptidoglycan of S. mutans strains BHT cell walls and points of attack of a cell wall lytic enzyme from Flavobacterium sp. strain L-11. GlcNAc, N-acetylglucosamine; MurNAc, N-acetylmuramic acid; Glu, glutamic acid; Ala, alanine; Lys, lysine. Horizontal arrows, N-acetylmuramyl-L-alanine amidase; vertical arrows, D-alanyl-L-alanine endopeptidase. (Reproduced with permission, reference 271.)*

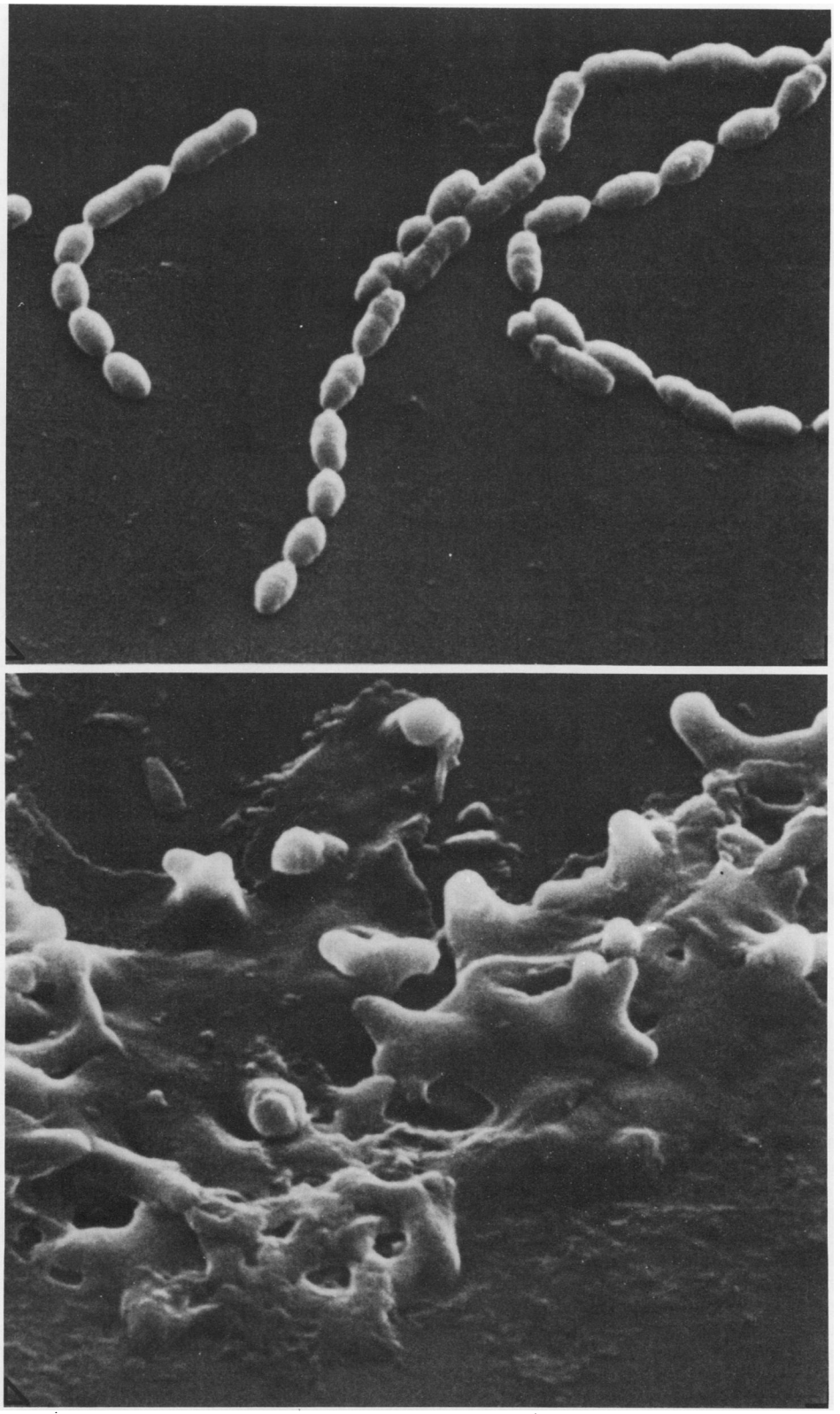

FIG. 5. *Scanning electron micrograph of S. mutans strain OMZ176 (serotype d) grown in glucose broth (top) and sucrose broth (bottom). Cells grown in the presence of sucrose were covered with amorphous capsule-like material of heavy thickness which was adherent to a glass surface. (Reproduced with permission, reference 224.)*

insoluble glucan can be extracted in an alkaline solution, followed by ethanol precipitation. The precipitate can be separated into structurally different water-soluble and insoluble fractions. The latter possesses more $\alpha(1 \rightarrow 3)$ glucosidic linkages than does the former (163, 443, 581) and is more resistant to the enzymatic action of $\alpha(1 \rightarrow 6)$ glucanase, i.e., dextranase (224).

Insoluble glucan can be more conveniently obtained by incubating cell-free GTase and sucrose. The glucan is obtained by centrifugation and then washed extensively with water and lyophilized. Using the glucan thus obtained, Guggenheim (203) showed that water-insoluble glucan from *S. mutans* strain OMZ176 contains a markedly high proportion (up to about 90%) of $\alpha(1 \rightarrow 3)$ glucosidic linkages. He proposed the name "mutan" to distinguish this water-insoluble glucan from typical linear-linked "dextran." In contrast to the insoluble glucan from *S. mutans*, gelatinous glucan from *S. sanguis* strain 804 has equal amounts of $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ glucosidic linkages (203).

Ample evidence has accumulated supporting the original finding by Guggenheim (203) regarding the chemical structure of water-insoluble glucans from various strains of *S. mutans* as well as other oral streptococcal species (8, 65, 125, 247, 384, 444). It appears that the large proportion of $\alpha(1 \rightarrow 3)$ glucosidic linkages found in the insoluble glucan explains the insoluble nature of this polymer. The techniques of periodate oxidation, Smith degradation, and methylation analysis have shown that the consecutive $\alpha(1 \rightarrow 3)$ glucosidic linkages form long chains as the backbone of a highly branched insoluble glucan (125, 247). A similar type of $\alpha(1 \rightarrow 3)$ glucan from the cell walls of certain fungi is a structural component and is also water insoluble (6).

There are some significant differences in the quantities and chemical nature of the extracellular glucans synthesized by various serotypes. According to Trautner et al. (582), their "type d" *S. mutans* strains synthesized significantly higher amounts of glucans than the "type c" strains, and the ratio of insoluble to soluble glucans was higher with the "type d" strains. The difference is explained by the presence of the higher proportion of $\alpha(1 \rightarrow 3)$ linkages in the "type d" glucans (580), as is discussed above. Glucans produced by incubating sucrose and cell-free GTase of *S. mutans* strains (serotypes *a* to *g*) can be separated further into one water-insoluble and three soluble fractions. Each fraction possesses a different content of $\alpha(1 \rightarrow 3)$ glucosidic linkage, a different molecular weight, and a different reactivity with ConA or *S. mutans* cells (273). The regulatory mechanisms for the synthesis of water-soluble glucans and their relation to the water-insoluble ones remain to be elucidated.

Fructans. Certain strains of *S. mutans* have been reported to synthesize fructans in addition to glucans from sucrose (56, 124, 224, 427, 474, 507). In the earlier phase of these studies, the fructan was considered to be levan consisting of $\alpha(2 \rightarrow 6)$ fructofuranoside linkages.

Baird et al. (8) suggested that the predominant linkage of the fructan from *S. mutans* was an inulin-type $\beta(2 \rightarrow 1)$ fructofuranoside linkage rather than $\beta(2 \rightarrow 6)$. This has been confirmed by other investigators (19, 124, 491). *S. mutans* fructans occur in both water-soluble and water-insoluble states (124, 507), and the production of fructans appears to differ from strain to strain, depending on cultural conditions.

Polysaccharide-Synthesizing Enzymes

S. mutans can produce extracellular GTase or FTase constitutively (609), which allows the synthesis of water-soluble, adherent glucans in addition to certain amounts of fructans from sucrose (233). Notwithstanding the complex chemical structure of glucans, only GTase(s) appears to be responsible for the synthesis of glucan. It catalyzes the transfer of a glucosyl moiety from sucrose to a terminal site on the growing glucan molecule:

$$n \cdot \text{sucrose} \rightarrow (\text{glucose})_n + n \cdot \text{fructose}$$

The equilibrium of this reaction is almost irreversibly to the right. Practically, sucrose is the sole substrate for GTase. However, Figure and Edwards (148) have shown that α-D-glucosyl fluoride can act as the donor for GTase of *S. mutans* FA1 to synthesize insoluble glucans.

Purification of *S. mutans* GTase has been attempted in various laboratories. Guggenheim and Newbrun (209) obtained three major GTase fractions from the supernatant fluid of a culture of strain OMZ176 (serotype *d*), using hydroxyapatite (HA) chromatography followed by isoelectric focusing. These fractions possessed different isoelectric points (pH 4.24 to 5.65) and different pH optima (pH 5 to 7). The multiple nature of GTase may account for the heterogeneity of the product glucans (75).

On the other hand, Fukui et al. (170) separated GTase and invertase, another sucrose-splitting enzyme, from culture supernatant of *S. mutans* HS6 (serotype *a*). GTase was separated into two fractions by agarose chromatography. The lower-molecular-weight fraction synthesized water-soluble glucan, whereas the higher-molecular-weight fraction synthesized water-insoluble glucans. Mukasa and Slade (429) obtained a GTase fraction synthesizing insoluble adherent glucan from the same strain and another fraction

synthesizing water-soluble glucan. The former preparation contained significant portions of glucose polymer, suggesting that the enzyme resembles a glycoprotein. Similar findings have been obtained with the serotype *c* strain GS5 GTase (330) and the serotype *b* strain FA1 GTase (500). More recently, Mohan et al. (421) have suggested that soluble and insoluble glucan syntheses are catalyzed by interconvertible forms of the same enzyme protein.

A highly active GTase fraction obtained from strain 6715 (serotype *g*) contained two separate bands on polyacrylamide gel electrophoresis (71). The purified GTase fraction had 30 to 40% carbohydrate, which coincides with the finding described above. The GTase activity was also found to be completely dependent upon a primer dextran (176). Also, purified GTase was resolved into two different components which were responsible for the synthesis of water-soluble and water-insoluble glucans, respectively (73, 429).

High concentrations of mono- and divalent cations promote the synthesis of insoluble glucan by an enzyme from strain 6715 (424). Detailed biochemical properties of the GTase enzyme from various *S. mutans* strains have been reviewed elsewhere (233, 436).

Intracellular Polysaccharides

Many plaque bacteria can synthesize intracellular iodine-staining polysaccharides (IPS) from high concentrations of various sugars. Most *S. mutans* strains produce a storage IPS which may contribute to the pathogenicity of *S. mutans* (16, 17, 191, 594). Stored IPS may be the source of acid when exogenous sugar is not sufficient or is absent.

Among various serotypes of *S. mutans*, serotype *d* and *g* strains produce and metabolize less IPS than serotype *c* and *e* strains. IPS-synthesizing strains degrade the IPS to produce acid(s) when external carbohydrates are deprived (164). It is reported that strains 6715 (serotype *g*) and OMZ176 (serotype *d*), like other serotype *d* and *g* strains, produce little or less IPS, whereas they produce marked dental caries in experimental animals (111, 202, 229, 567). Therefore, IPS appears not to be a prerequisite for the cariogenicity of *S. mutans*. Mutants of serotype *c* strains which are weak in their ability to synthesize IPS show diminished cariogenic activity (166, 568).

IPS is a glycogen-like glucan with $\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$ linkages which are susceptible to α-amylase (99, 596). IPS forms a complex with I_2-KI and produces a brownish-yellow color with an adsorption maximum at 520 nm (111).

Two enzymes, adenosine diphosphate (ADP)-glucose pyrophosphorylase (EC 2.7.7b) and ADP-glucose-glycogen glucosyltransferase (EC 2.4.1a), have been shown to be involved in IPS synthesis in *S. mutans* (20, 240). ADP-glucose is synthesized from adenosine triphosphate and glucose 1-phosphate by the former enzyme, and the latter enzyme catalyzes formation of glycogen, using ADP-glucose as the glucosyl donor.

IPS metabolism appears to be influenced mainly by the pH of the external environment (164). *S. mutans* will produce ethanol and acetic acid in addition to lactic acid from IPS under the limitation of exogenous glucose, whereas only lactic acid is formed in the presence of excess glucose (262).

In the deep region of plaque, the cell walls of gram-positive coccal bacteria become thickened and the majority of the cells contain scattered IPS granules in the cytoplasm. On the other hand, cells located in the superficial portion of the plaque possess normal cell wall morphology and fewer IPS granules (597). Rifampin treatment of *S. mutans* cultures results in accumulation of IPS and thickening of the cell walls accompanying inhibition of ribonucleic acid (RNA) synthesis (383), whereas tetracycline treatment causes cell wall thickening accompanying inhibition of protein synthesis but little accumulation of IPS (384). These findings indicate that IPS synthesis may be influenced by various cultural conditions.

Lipoteichoic Acid

LTAs are a glycerol form of teichoic acid covalently linked to a lipid moiety (616, 617). LTA occurs as a cellular surface component and extracellular product of a number of gram-positive bacterial species, including all serotypes of *S. mutans* (90, 223, 285, 381, 519). PGP is the backbone structure of LTA and is responsible for a common antigenic specificity among *S. mutans* (72, 234, 311, 312, 395). The amphipathic nature of LTA strongly influences the immunobiological activities of this unique polymer. LTA possesses most of the biological activities of the lipopolysaccharides of gram-negative bacteria. Among these are immunogenicity, spontaneous sensitization of erythrocytes, bone resorbing activity in organ culture, complement fixation, and stimulation of nonspecific immunity (249, 618). LTAs and certain lipids have also been found to inhibit cellular autolysis of *S. faecalis* (83). The general features and biological characteristics of LTAs have been extensively reviewed (21, 321, 313, 617, 618).

Evidence indicates that LTAs are closely associated with the cytoplasmic membrane (524, 533), and they were frequently called "membrane teichoic acids" or "intracellular teichoic acids." It appears that membrane association depends upon a covalent linkage between PGP

and a glycolipid protein of the membrane. An electron microscope study using the ferritin-labeled antibody technique revealed membrane association more directly in *Lactobacillus fermenti* and *L. casei* (588). It was further demonstrated that the label extended from the outer membrane layer through the matrix of the cell wall beyond the cell surface and into the external environment (617, 618). Chorpenning et al. (72) reported that glycerol teichoic acid was found in a phenol extract from purified cell walls of *S. mutans*. Furthermore, a muramidase (mutanolysin) lysate of *S. mutans* cell walls reacted with antiserum specific for PGP (S. Hamada, unpublished data), indicating the "presence" of glycerol teichoic acid or even LTA in the cell walls.

Extracellular LTAs are found in the cell-free culture supernatant of many gram-positive bacteria, and in particularly high amounts in *S. mutans* strains (285, 381). The greatest recovery of extracellular LTA was obtained by *S. mutans* organisms growing at a low dilution rate at pH 6 to 6.5 in a chemostat (276). It is likely that extracellular LTAs are present as a result of active excretion rather than cellular lysis or as a result of turnover during cell growth. It thus appears that LTAs are in transit from a cellular to an extracellular location in the *S. mutans* cell (285, 301).

Both cellular and extracellular LTAs can usually be separated into two peaks by Sepharose 6B gel filtration. One is the acylated form, and the other is in the deacylated form. Only the former LTA can sensitize erythrocytes, and it contains higher levels of fatty acids (381). Both components are synthesized and excreted by logarithmically growing cells of *S. mutans*.

However, in contrast to the LTA from *S. mutans*, only deacylated LTA is detected in the culture fluid of an *S. faecium* strain. Most probably this extracellular deacylated LTA is derived from cellular LTA by enzymatic deacylation (300).

Most investigators conveniently use a phenol-water extraction method to obtain "native" LTAs from bacterial cells (21, 90, 223, 312, 313, 423, 616, 617). Various extraction methods also yield an antigenic component in the extract which reacts with antibody specific for the PGP backbone of LTA. However, drastic procedures usually split the linkage between PGP and the lipid moiety or cause the hydrolysis of phosphodiester linkages (223, 312). LTA is also released from *S. mutans* cells by treatment with leukocyte hydrolases or with lysozyme or phenol (518).

More recently, Silvestri et al. (526) developed a more refined method to purify LTAs by using gel filtration, hydrophobic interaction chromatography, and adsorption to synthetic membranes (phospholipid vesicles). The latter are very effective in separating various contaminants from cellular and extracellular acylated LTAs of *S. mutans* strains.

There is much speculation concerning the possible functions of LTAs in mammalian tissue and the organism itself (21, 83, 259, 618). We have found that almost all of the hemagglutinating antigen in the culture supernatant of *S. mutans* strains can be recovered by 50% ammonium sulfate precipitation. Immunological tests using an antibody specific for PGP demonstrate that the hemagglutinating activity is due to extracellular LTAs. The extracellular LTA is closely associated with glucosyltransferase activity, and it is difficult to separate the two (223). Both cellular and extracellular LTAs are effectively adsorbed to HA powder (76, 223). Phosphate and fluoride inhibit adsorption of LTA. Phosphorus contamination of extracellular glucans synthesized by oral streptococci (404) may be explained by the strong affinity between glucosyltransferase and extracellular LTA molecules, thus forming complexes of glucan-LTA-glucosyltransferase. It has also been shown that sucrose-grown *S. mutans* binds higher amounts of calcium than do glucose-grown cells. In light of these findings, it is proposed (484–486) that the calcium-binding ability of LTA should be a major selective factor in the adherence of gram-positive bacteria to enamel surfaces. It should be added here that oral streptococcal strains do not necessarily have LTA. It has been reported that a considerable number of *S. mitior* (489) and biotype B *S. sanguis* (S. Hamada and J. Mizuno, unpublished data) strains lack LTA. Furthermore, a new amphipathic antigen has been isolated from *Actinomyces viscosus* (615). LTA-negative *S. sanguis* also has an erythrocyte-sensitizing antigen which is immunologically different from LTA/PGP (Hamada and Mizuno, unpublished data).

Interaction of Glucosyltransferase with Various Agents

The distribution of GTase in broth cultures of *S. mutans* is strongly influenced by various factors. In many cases, almost all of the GTase activities are found extracellularly in sucrose-free media (237, 389, 474), although the occurrence of significant cell-associated GTase activity in addition to cell-free GTase activity is reported by some investigators (187, 298, 277, 278, 329, 422). Ample evidence indicates that the presence of, or the addition to culture media of, sucrose results in the synthesis of cell-associated GTase (232, 237, 389, 474, 544).

McCabe and Smith (389) consider that GTase is reversibly bound to the insoluble glucan during the synthesis of the glucan by sucrose-grown

S. mutans cells. The enzyme then becomes irreversibly bound, and is finally inactivated as insoluble glucan accumulates. Reversibly bound GTase can be eluted in solutions of clinical dextran or guanidine hydrochloride. This property is used to carry out affinity chromatography for purification of GTase (274, 391, 392, 541).

Some commerical media, such as Todd-Hewitt broth and Trypticase soy broth (BBL), contain trace amounts of sucrose; when *S. mutans* was grown in these media, essentially all GTase activity was in a cell-associated form (237). Pretreatment of these culture media with yeast invertase before autoclaving resulted in an *S. mutans* culture which contained increased amounts of extracellular GTase. Related to this, most of the GTase activity produced by *S. mutans* grown in sucrose-free chemically defined medium has been found to be extracellular; this was in contrast with growth on Todd-Hewitt broth or Trypticase soy broth (232, 277, 505). In view of these findings, extracellular and cell-associated GTases are most likely alternate states of the same enzyme protein.

The addition of soluble dextran stimulates the reaction of GTase with sucrose (170, 176, 330, 389, 429). This activation is due to a requirement by GTase for a primer molecule; its nonreducing ends are required for new glucan synthesis. The primer may also act as the site to which newly synthesized units are added (178). Equal weights of dextrans with different molecular weights show a similar priming effect on new glucan synthesis by GTase (176, 238). On the other hand, Robyt and Corrigan (475) have reported that the activation of GTase by dextran cannot be due to a primer reaction with the nonreducing end because of the nonavailability of the nonreducing ends of the dextrans chemically modified by reaction with trypsyl chloride or hydrolysis with an exodextranase (475).

The addition of increasing amounts of soluble dextran will cause a decrease in the synthesis of insoluble glucan and an increase in the synthesis of soluble glucan (422, 475). Certain sugars such as maltose and fructose significantly reduce the yield of insoluble glucan (238).

The enhancement of GTase activity by various humoral fluids such as rabbit antiserum (139), rat oral fluid (45), and monkey antiserum (26) has been reported.

Fukui et al. (169) reported that the secretory component of secretory immunoglobulin A (IgA) caused a severalfold acceleration of GTase activity as compared with the control without additives. However, results with purified secretory IgA component have not confirmed this study (88).

More recently, lysophosphatidylcholine, a phosphoglyceride, has been found to cause a 2.6-fold increase in water-soluble glucan synthesis by *S. mutans* GTase. The increased rates of glucan synthesis by lysophosphatidylcholine and primer dextran are additive (248). In a subsequent study, phospholipids normally detected in human oral fluids, e.g., saliva from various glands, gingival crevicular fluids, and serum, enhanced the activity of GTase (503). The GTase level is reported to be increased about fivefold in the presence of 1.0% Tween 80; alteration of the fatty acid composition of the *S. mutans* cells also occurs (583). Many other nonionic surfactants promote the activity of GTase, whereas anionic and cationic surfactants inhibit this activity. Lower concentrations of ampholytic surfactants activate GTase activity; this is followed by almost complete inhibition of GTase at high concentrations (0.1% or more) of the surfactants (M. Torii and S. Hamada, unpublished data). Enhanced levels of cell-free GTase are also obtained when cells of *S. mutans* are grown in penicillin (100 to 250 μg/ml)-containing medium (278). This may be due to release of certain lipid components from the cells by an unknown mechanism (258) which in turn results in an enhancement of GTase levels.

Invertase

Invertase (β-fructofuranosidase; EC 3.2.1.26) is a sucrase that catalyzes the hydrolysis of the glucosidic linkage of sucrose, which results in the release of an equimolar ratio of glucose and fructose.

Gibbons (180) first suggested the presence of an intracellular, inducible "sucrase" activity other than GTase and FTase in *S. mutans* GS5 (serotype *c*). Toluene treatment of intact cells that destroys the selective permeability system of the bacterial cell membrane (338) revealed enhanced invertase levels in *S. mutans* GS5 and K1R (serotype *g*) (180, 393). The molecular weight of intracellular invertase has been calculated to be 47,000 to 48,000, and the invertase has a relatively high K_m value for sucrose (35 to 140 mM) (327, 564). The intracellular location of the invertase implies the presence of a sucrose permease system, but little is known about the sucrose transport mechanism of *S. mutans*. Controversial results have been obtained on the inducibility of invertase from various strains of *S. mutans* (327, 393, 564). The biochemical properties of the invertases found in the 37,000 × *g* soluble cell fraction are different in the individual serotypes of *S. mutans* but similar within the same serotypes (565). Invertases from serotypes *e*, *f*, and *g* are reported to be structurally similar to that from serotype *c* (385).

Extracellular invertase also exists (70, 170) and has a molecular weight of 5×10^5 (385).

The physiological role of *S. mutans* invertase

is not fully understood. However, a large portion of available sucrose is hydrolyzed by this enzyme (69). The remainder of the sucrose is converted to the synthesis of glucan or fructan via GTase or FTase.

Recently, strains of *S. mutans* belonging to various serotypes have been found to have highly efficient phosphoenolpyruvate (PEP)-dependent sucrose phosphotransferase activity that can initiate the catabolism of sucrose and produce sucrose phosphate. The K_m value of the enzyme for sucrose is reported to be about 70 μM, indicating that the enzyme, unlike invertase, can function even at low substrate conditions (499, 535).

α(1 → 6) Glucanase

α(1 → 6) Glucanase [α(1 → 6)-glucosidase; EC 3.2.1.11] is synthesized constitutively by some strains of *S. mutans* as well as certain other bacterial species in dental plaque (110, 509, 548).

An endoglucanase specific for the α(1 → 6) linkage has been purified from the culture supernatant of *S. mutans* OMZ176 (serotype *d*) (205). The biochemical property of the enzyme is similar to that of mold dextranases in general. A similar endo-α(1 → 6) glucanase was obtained from the culture supernatant of serotype *g* strains 6715 (131, 177) and K1R (465, 466).

These results indicate that most glucans produced by growing *S. mutans* cells or by "crude" GTase preparations obtained from culture fluids could be synthesized under the influence of intrinsic or contaminating α(1 → 6) glucanase. Therefore, structural heterogeneity of the *S. mutans* glucans may be a result of the combined enzymatic action of GTase and endoglucanase activities. Relative quantities of these enzymes may significantly affect the chemical and physical properties of glucans synthesized by *S. mutans*.

In addition to the polymers described above, *S. mutans* produces proteases active against casein and glycoprotein (93) and against collagen (492), phospholipase A (44), and arylaminopeptidase (456). *S. mutans* also produces intracellular hydroxyapatite crystals which may be responsible for calculus formation (552).

SUGAR METABOLISM BY *S. MUTANS*

S. mutans has been reported to be a homofermentative lactic acid bacterium (115, 282, 569). However, the metabolic pathway of glucose by *S. mutans* varies, depending on environmental factors. The major fermentation product of *S. mutans* is lactate, especially when the organism is grown in the presence of excess glucose, whereas *S. mutans* produces significant amounts of formate, acetate, and ethanol in addition to lactate when glucose is limiting (61). An in vivo study supports the latter finding (584).

Sucrose has also been shown to serve as the energy source during growth of *S. mutans* in addition to its role as the substrate for extracellular glucan synthesis. Most of the glucosyls of sucrose are converted into lactic acid. Only a small portion of sucrose is diverted to extracellular polysaccharide synthesis (473, 562, 566). Furthermore, *S. mutans* is known to utilize sucrose at a significantly faster rate than other oral bacteria such as *S. sanguis*, *S. mitis*, and *Actinomyces viscosus* (418, 450). *S. mutans* produces significant amounts of intracellular polysaccharide from sucrose, which can be converted to lactic acid after prolonged incubation (419). The organism also produces mannitol when high levels of sucrose or glucose are present (370). Comparison of metabolic activities of "cariogenic" and "noncariogenic" plaques indicates that *S. mutans* is metabolically dominant in plaques closely associated with the carious lesion (418). *S. mutans* is more aciduric than other oral streptococcal species (114).

In the presence of sucrose, *S. mutans* grows at the same exponential rate as it does on glucose (100). A previous finding that growth of *S. mutans* is linear in sucrose culture (572) is attributed to an optical artifact based on the formation of visible cell aggregates (100).

S. mutans transports glucose into cells via a membrane-associated PEP-dependent phosphotransferase system (132, 241, 501, 508). Sucrose and lactose are similarly transported in *S. mutans* by this system (49, 499, 535). The nonfermentable glucose analog D-2-deoxyglucose is an effective inhibitor of glucose transport by the *S. mutans* PEP-dependent glucose phosphotransferase system (506).

ADHERENCE OF *S. MUTANS*

The adherence of *S. mutans* and other oral bacteria to tooth surfaces and the formation of dental plaque are of major significance in the development of dental caries. These processes are complex and involve a variety of bacterial and host components. Various aspects of bacterial adherence in the oral cavity have been extensively reviewed (194, 195, 233, 530, 531, 589).

Initial Attachment of *S. mutans* to Smooth Surfaces

Bacterial attachment to the tooth surface is usually preceded by the formation of an acquired pellicle of salivary origin. The initial stages of plaque development on cleaned tooth surfaces require cell attachment to the pellicle suffi-

ciently firm to resist local cleansing forces of salivary flow and muscular movements. The attachment may involve specific interaction of pellicle components with selected bacterial species.

Ørstavik et al. (454) found a significant increase in attachment of *S. mutans*, *S. sanguis*, and *S. salivarius* to pellicle-coated enamel slabs when compared with an uncoated slab. The in vitro adherence of *S. sanguis* was significantly greater than that of *S. salivarius*, and both species adhered in greater numbers than did *S. mutans*.

S. mutans has been found to attach in greater numbers to dextran-coated HA than to pellicle-coated or uncoated HA, whereas the attachment of *S. sanguis* and *S. mitior* was not enhanced by dextran-coated HA (350, 351). The in vitro affinity of each oral streptococcal species for pellicle-coated solid surfaces appears to correlate with the proportions of that species found in vivo (195). It is likely that *S. mutans* does not play a key role in the initial stages of tooth colonization, although the latter experiment was performed in the absence of sucrose.

Recently, a model has been proposed by Rölla (486) suggesting that cells of *S. mutans* and *S. sanguis* behave like negatively charged particles in their electrostatic interaction with HA surfaces in vitro. He demonstrated that calcium and protamine phosphate significantly increased uptake of bacteria, whereas fluorides, phosphate, or even saliva decreased the uptake of the cells. The acidic proteins in saliva are selectively bound by HA. It is considered that pellicle formation by acidic protein results in a reduction of the cationic nature of the surface and reduces the binding of bacterial cells.

It appears that a large number of hydroxyl groups on the surface of sucrose-grown *S. mutans* and *S. sanguis* cells preferentially form hydrogen bonds with the pellicle proteins (487). LTAs are closely associated with extracellular GTase (223) and its product glucan (76, 404). LTAs possess a strong affinity for HA (486).

Divalent cations such as Ca^{2+} were found to enhance the interaction between a negatively charged pellicle surface and a similarly charged bacterial cell surface (293, 484). Related to this, ethylenediaminetetraacetic acid, EDTA, is known to have a strong plaque-disintegrating ability (293, 369); this supports the concept that calcium bridges are essential for the initial binding of the bacterial cell to the pellicle surface. The major discrepancy between the reports of Rölla (486) and those of Liljemark and Schauer (350, 352) and Ørstavik et al. (454) may be ascribed to the use of buffers of high ionic strength in the case of the latter investigations.

Interaction of Salivary Components with Streptococcal Cells

Direct interaction of salivary components with bacterial cells seems to be significant in regulating the attachment and accumulation of different bacterial species involved in plaque formation. Whole saliva is known to possess the ability to agglutinate many plaque bacteria (193). The salivary agglutinating factor was reported to be a high-molecular-weight glycoprotein which is heat-stable and Ca^{2+} dependent and which occurs optimally between pH 5 to 7.5 (193, 250). It is unlikely that IgA is involved in these interactions (136, 288, 620). Different agglutinating factors have been found for *S. mutans*, *S. mitior*, and *S. sanguis* (136, 194, 286). When saliva is pretreated with wheat germ agglutinin, the saliva does not induce the agglutination of *S. mutans* strains. *N*-Acetylglucosamine, for which the lectin shows a specificity, does not block the inhibitory effect of the lectin (420).

These salivary agglutinating factors may resemble lectins in that specific determinants may bind selected bacterial species. The salivary agglutinating factor that is responsible for binding *S. sanguis* was destroyed by neuraminidase or protease treatment, indicating the importance of sialic acids (386). The mucin-like glycoprotein agglutinated both *S. mutans* (serotypes *b* and *d*) and *S. sanguis* strains ATCC 10556 and 10558 (347). Interestingly, elimination of sialic acid from the glycoproteins resulted in a loss of agglutination of *S. sanguis* but not of *S. mutans*. It is suggested that salivary lysosyme may participate in the agglutination of some *S. mutans* strains (463).

Preincubation of various bacteria and saliva reduced the attachment of bacteria to HA surfaces (78, 80, 379). Serotype *c* *S. mutans* cells appeared to bind salivary components (78, 379). Therefore, it is suggested that the agglutinating factor free in saliva competitively inhibits the interaction between salivary coated HA and those surface components of the bacterial cells which contain bound salivary glycoproteins.

Recently, Gibbons and Qureshi (189, 190) found that strains of *S. mutans* and other oral bacteria bind the blood group-reactive (BGR) mucins of saliva after exposure to whole saliva or partially purified mucin preparations. Different serotypes of *S. mutans* bind different components of BGR mucins. BGR salivary mucins are present in the acquired pellicle on the tooth surface (542), which may serve as receptor molecules involved in the attachment of bacteria to teeth, suggesting that a lectin-receptor-type mechanism is involved (181).

Implantation of *S. mutans*

Active transmission of *S. mutans* implies implantation in a receptive host. However, *S. mutans* is not easily implanted in adult humans. Variability in the ability of *S. mutans* to implant in different subjects has been noted, and a gradual decrease in the number of implanted organisms has been observed over an extended period of time (284, 323).

When *S. mutans* was implanted onto human tooth surfaces, the bacterium was recovered in much higher numbers from originally pellicle-free than pellicle/plaque-coated tooth surfaces (493).

An in vivo study with human subjects has revealed that *S. mutans* can be recovered from cleaned tooth surfaces after a few hours of oral exposure when the salivary concentration of this bacterium exceeds a certain level. In adult human subjects with a salivary *S. mutans* count of about 10^4 colony-forming units (CFU)/ml or less, the organisms could not be isolated from the tooth surface (593). On the other hand, at a salivary concentration of 10^3 CFU/ml, artificial fissures inserted in three adult subjects were colonized (555). Fluoride administration is unrelated to the colonization of *S. mutans* on human teeth (590). In a later study (118), the frequency of detection and concentration of *S. mutans* in saliva were higher in older children. However, some infants can acquire *S. mutans* shortly after tooth eruption (15, 382).

When organisms of serotypes *a* and *c* *S. mutans* were implanted in humans, serotype *a* (strain E49) failed to colonize, although a serotype *c* strain of human origin appeared to colonize (556). The intraoral spread of the implanted *S. mutans* was confirmed on the adjacent and antagonistic tooth surfaces (557). Furthermore, surfaces which harbored significant numbers of *S. mutans* tended to remain positive, whereas surfaces which did not possess detectable numbers of *S. mutans* remained at that level, indicating that *S. mutans* does not evenly colonize the surfaces of teeth (184).

Sucrose-Dependent In Vivo Adherence of *S. mutans*

Sucrose has been reported to markedly facilitate the colonization of *S. mutans* on teeth. In early studies using hamsters and rats, it was found that *S. mutans* could be established far more easily when the animals were given sucrose-containing diets (128, 207, 319, 320). It was also found that *S. mutans* could implant in the human oral cavity after inoculation with a pure culture, and the frequent chewing of sucrose gum enhanced the implantation (128).

More recently (600), the colonization of *S. mutans* 6715 occurred in rats fed diets with a sucrose content from 56% to as low as 1%, in which the lowest effective inoculum was 10^5 CFU by a single oral administration (600). More frequent inoculations with about 5×10^8 CFU were needed to establish the organisms on a high-glucose diet. When inoculated with less than 10^7 CFU, however, the cells were gradually eliminated from the teeth.

It should be noted here that streptomycin-resistant mutants of *S. mutans* frequently colonize less effectively than parent strains (12, 158). For example, the minimum dose required for implantation of a streptomycin-resistant mutant of *S. mutans* LM7 was more than 2×10^7 CFU, whereas the minimum dose of the parent strain was about 10^4 CFU (79, 591). The basis of the phenomenon has not been well explained.

Preformed dextran/glucan, whether associated with *S. mutans* cells or with the tooth surface, does not permit the degree of cell attachment that occurs in the presence of sucrose (598). Apparently, de novo glucan synthesis (see next section) leads to a far stronger adherence to the tooth surface than that which occurs in the presence of dextran/glucan precoated on either tooth surface or bacterial cell surfaces, although sucrose may not be indispensable to the initial attachment of *S. mutans* in the oral cavity. In this connection, high numbers of *S. mutans* may be detected in the mouths of children with sucrase-isomaltase deficiency who, therefore, consume a diet with an extremely low sucrose content (592).

In rats, colonization of *S. mutans* occurred with increasingly greater difficulty as the rats became older (599). The actual mechanism responsible for the changes during aging remains to be elucidated. However, the age effect was not observed when rats were fed a sucrose diet, whereas the results from rats fed a glucose diet indicate that changes may have occurred early after weaning.

In *Macaca irus* monkeys fed by stomach tube and provided with oral supplements, the colonization of *S. mutans* was dependent upon sucrose from the drinking water. Withdrawal of the sucrose resulted in complete absence of detectable *S. mutans* on the teeth, although the salivary counts of *S. mutans* remained unchanged (306).

Sucrose-Dependent In Vitro Adherence of *S. mutans*

Active glucan synthesis from sucrose has been found to foster the adherence of *S. mutans* to various solid surfaces (195, 215, 233, 388, 427,

428, 530, 531, 578). Synthesis of the glucan is mediated by the enzymatic action of cell-free (extracellular) or cell-bound GTases. This concept is supported by the finding that mutants of *S. mutans* which lack the ability to synthesize water-insoluble, adherent glucans do not adhere to solid surfaces (107, 165, 314).

Heat-treated cells of *S. mutans* which do not have cell-bound GTase adhered to a glass surface when incubated simultaneously with sucrose and exogenous GTase. No adherence occurred in the absence of de novo glucan synthsis (215, 427, 428). Later studies have clearly demonstrated that the adherence of *S. mutans* in the presence of sucrose depends primarily upon the specific binding of extracellular GTase synthesized by *S. mutans* (218, 235, 237, 428, 530). The nonadherent property of other bacterial species is due to their inability to bind GTase to the cell surface. However, a nonspecific adherence of cells of a variety of bacterial species can be obtained when the cells, GTase, and sucrose are incubated together (218, 530). This process, due to cell-free synthesis of glucan, is a nonspecific trapping mechanism for adherence that may contribute to the development of dental plaque.

Glucan on the surface of *S. mutans* appears to function as a binding site for GTase (232, 237, 333, 531). Antiserum against a glucan synthesized by a type *c* strain blocked binding of GTase and subsequent adherence (237, 427, 428). The strong affinity of GTase to glucan has been reported; however, adherence was not measured in these experiments (314, 389, 391, 392, 472). Antiserum against the type *a* polysaccharide antigen also inhibited adherence. These results indicate that glucan may not be a specific binding site and that other complex polysaccharides may mediate the process. Protein is also involved in the binding of GTase (428). The glycoprotein-like characteristics of GTase (531) may be related to its ability to bind to both polysaccharide and protein molecules. Figure 6 illustrates a possible mechanism of this binding (531, 531a). Also illustrated is the participation of *S. mutans* surface protein in the binding of dextran (see next section).

Cell-free water-soluble glucan "particles" treated by sonic oscillation bind GTase and cause marked adherence to glass accompanied by de novo glucan synthesis (Fig. 7; 237). This finding strongly supports the hypothesis that GTases bound to the surface glucan participate in the adherence of *S. mutans* cells to smooth surfaces when sucrose is present in the oral environment. Other water-insoluble glucans such as amylopectin and cellulose do not bind GTase significantly (238).

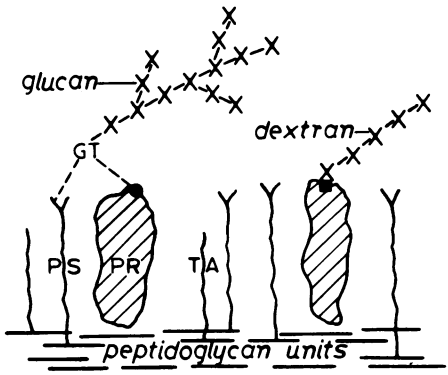

FIG. 6. *Polysaccharide (PS) and protein (PR) as binding sites for GTase and the binding of dextran to protein on the surface of S. mutans. TA, lipoteichoic acid. Symbols: ●, dextran-like site which involves binding of GTase; ■, site which binds the dextran responsible for cell agglutination. (Reproduced with permission, reference 532.)*

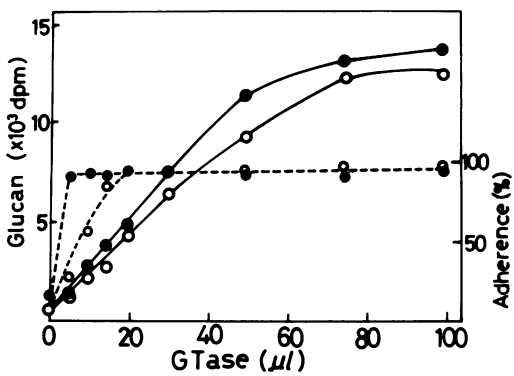

FIG. 7. *Glucan synthesis from [^{14}C]glucose-labeled sucrose (59,000 dpm) by extracellular GTase bound to the surface of heat-treated S. mutans B13 (serotype d) cells and cell-free, water-insoluble glucans and subsequent adherence to a glass surface. To heat-treated cells or insoluble glucans (1.0 mg, dry weight) was added to 0 to 100 μl of extracellular GTase (specific activity, 56.6 mU/μl), and the mixture was incubated for 10 min at 20°C. The suspension was centrifuged and washed twice with phosphate buffer (0.05 M, pH 6.8). New glucan synthesis due to cell- and glucan-associated GTase was measured by incorporation of radioactivity from [^{14}C]sucrose. Adherence to a glass surface was measured as percentage of adherence of cell or glucan. Symbols: ●——●, cell-bound ^{14}C-labeled glucan synthesis; ○——○, glucan-bound ^{14}C-labeled glucan synthesis; ●-----●, adherence of cells due to new glucan synthesis; ○-----○, adherence of glucans due to new glucan synthesis.*

When *S. mutans* is grown in sucrose-free complex medium or the chemically defined medium FMC (575), the organisms do not have enough cell-bound GTase to produce significant adher-

ence to solid surfaces when sucrose is added. On the other hand, cells grown in complex media containing sucrose (e.g., Todd Hewitt broth and Trypticase soy broth) or sucrose-containing FMC have strong cell-associated GTase activity. These cells produce marked adherence to solid surfaces in the presence of exogenous sucrose (232, 237). Furthermore, the presence of sucrose determines the ratio of cell-free and cell-bound GTase (232, 237).

Kuramitsu (328) reported that a preformed glucan layer on a glass surface produced a partial adherence of heat-treated cells of two of four serotypes of *S. mutans*, claiming that glucan synthesis need not be restricted to the cell surface of *S. mutans* for cellular adherence to develop. However, the presence of residual GTase and sucrose in the precoated glucan has been demonstrated, and the function of active GTase in this case cannot be ruled out (215, 230, 314). An in vivo study in which conventional rats were used also supports the latter concept (598; see previous section).

Maltose has been demonstrated to inhibit the in vitro adherence of *S. mutans* to glass surfaces (11, 238, 439). Furthermore, the increased glucan synthesis in the presence of primer water-soluble dextrans inhibits adherence to a glass surface (238).

Cell-to-Cell Adherence: Bacterial Aggregation

Cells of *S. mutans* grown in a complex medium have been shown to agglutinate upon addition of high-molecular-weight dextran T2000 (molecular weight, 2×10^6) (185). This means that whole cell agglutination is due to cells which are bound together by dextran molecules. The binding of dextran has been demonstrated by using radioactive dextrans/glucans (554, 623). Agglutination of *S. mutans* strain 6715 (serotype *g*) is detected at pH 8.5 upon addition of 6 ng of dextran T2000. This corresponds to about three molecules of dextran per cell in the reaction mixture.

Pretreatment of *S. mutans* cells with 4.0 M urea, 0.01 M EDTA, or 0.1% sodium dodecyl sulfate prevents agglutination, and divalent cations reverse the effect of EDTA (293). McCabe and Smith (390) have reported that agglutination is independent of cell-bound GTase activity. GTase activity is almost completely abolished by chemical treatments without adversely affecting the agglutination reaction. However, rabbit antisera specific for GTase are shown to inhibit the agglutination reaction (449). Furthermore, cells grown in sucrose-free complex medium (544) or chemically defined synthetic medium (623) show a markedly decreased ability to agglutinate. Therefore, GTase associated with the surface glucan of the cell may augment the function of a "dextran receptor."

In this context, glucan/dextran-binding proteins have been demonstrated in *S. mutans*. The multiplicity of proteins showing this capacity or GTase activity indicates the complexity of the cell-to-cell and cell-to-surface adherence mechanisms of *S. mutans* (178, 387, 497, 531).

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

Many strains of *S. mutans* agglutinate (homologous cell-cell adherence) upon addition of high-molecular-weight dextran (185). Certain strains of *S. mutans* are also reported to form aggregates with other bacterial cells such as *Nocardia* and *Neisseria* (heterologous cell-cell adherence) (188). Strains of *A. naeslundii* and *A. viscosus* have been shown to form aggregates more often with strains of *S. sanguis* and *S. mitior* than with strains of *S. mutans* (77, 129, 188, 401). Coaggregation between *A. viscosus* and *S. sanguis* is inhibited completely by β-linked galactosides (i.e., lactose) (401). However, when *S. mutans* cells are coated with high-molecular-weight dextran or grown in the presence of sucrose, they form visible aggregates with *A. viscosus* (23).

Another example of heterologous cell-cell aggregation is shown between *S. mutans* and *Candida albicans*. Artificial plaque formation by an *S. mutans* strain is augmented when a *C. albicans* strain is inoculated with the *S. mutans* (416a).

Conversely, certain oral bacteria in plaque and saliva are demonstrated to produce dextranase that may inhibit the adherence of *S. mutans* to smooth tooth surfaces (431, 504, 548, 603).

The increased synthesis of polysaccharides by plaque bacteria during a sucrose-rich diet is accompanied by increased levels of dextranase and levanase of plaque bacteria (173).

It now appears from these various data that the adherence of *S. mutans* and other oral species to pellicle-covered teeth occurs in several steps. The initial attachment of single cells, chains of cells, or aggregated cells may involve divalent ions (such as Ca^{2+}) and the negative charges on the bacterial cell and the tooth pellicle (486). This proposal, however, suffers from many limitations (181). It seems more likely that a complex may form between a glycoprotein in the pellicle and a polysaccharide on the bacterial

surface. The reverse may also occur (181, 190, 233, 235). This may be a lectin-like effect (181). The second phase of the process would depend in large measure on the multiplication of *S. mutans* and glucan synthesis. The maturation of the plaque, containing various gram-positive and occasionally gram-negative species, would be mediated by the synthesis of glucan by *S. mutans*. Glucan would ensure the stability of the plaque. Bacterial species which enter the developing plaque after the phase of initial attachment may do so by random contact with the adhesive glucan of the plaque (233, 235).

GENETIC ASPECTS OF *S. MUTANS*

Lysogenicity and Plasmids

The occurrence of genetic elements such as plasmid and prophage in bacteria may relate to various phenotypes such as ability to ferment sugars, production of toxins, bacteriocins, and antigens, and antibiotic resistance.

In 1971, Greer et al. (200) reported that there was a consistent positive correlation between the lysogenicity and cariogenicity of *S. mutans*. "All" cariogenic streptococci including eight strains of *S. mutans* and one strain of *S. salivarius* underwent lysis after induction with ultraviolet light and mitomycin C. In contrast to cariogenic strains, "no" noncariogenic streptococcal strains displayed the induced lysis. Another group (309) also reported that a phage with similar morphology was induced from 15 cariogenic strains including *A. viscosus* and *S. sanguis* in addition to 9 strains of *S. mutans*.

However, an *S. mutans* that had been cured of its prophage exhibited cariogenicity essentially similar to that of its lysogenic parent strain when examined in animal models (R. J. Fitzgerald, personal communication).

Higuchi et al. (254) found that plasmid-curing agents induced mutants at high frequency; these mutants produced diminished insoluble polysaccharide. A satellite band of plasmid DNA in cell lysates of parent strains of PK1 and JC2 was subsequently found, whereas mutants of these strains, that had lost the ability to synthesize adherent, insoluble polysaccharides had no detectable satellite band of DNA (253).

Other investigators have been unable to find a plasmid in many strains of *S. mutans*, including strains PK1 and JC2 from which Higuchi et al. (253) isolated plasmids. Furthermore, no phenotypic function has yet been ascribed to any of the reported plasmids of *S. mutans*.

Later studies reported (225) that only the parent strain of PK1 (254) carried prophage and that the mutant strains of PK1 were transformed to the "cariogenic" strain with adherence ability by infecting them with the phages or with free phage DNA. In addition, all transformants were reported to acquire a new character, the deamination of arginine. Furthermore, *S. sanguis* strain ATCC 10556 was transfected with free phage DNA of parent strain PK1, and two transfectants which had developed the "cariogenic nature" were obtained (256). However, the cariogenicity of these strains was not tested. No explanation was given of the relationship between prophage and plasmid DNA of parent strain PK1.

A small plasmid has been isolated from *S. mutans* strain LM7 (serotype *e*). The plasmid has a molecular weight of approximately 3×10^6 and is calculated to have 16 copies per chromosomal genome equivalent (120). Since then, more than 100 strains of *S. mutans*, including laboratory-maintained strains and clinical isolates, have been examined for plasmid DNA by examination of cell lysates on cesium chloride-ethidium bromide gradients (84, 377, 378, 471). The frequency of occurrence of plasmid DNA in *S. mutans* of human origin has been reported to be approximately 5%. The value is lower than that of plasmids in other bacterial species, including gram-positive and -negative species (145).

Macrina et al. (377, 378) have reported that the four plasmids isolated by them are identical in molecular weight (3.6×10^6) and are present to the extent of approximately 30 copies per chromosomal equivalent. These results are essentially similar to those obtained with LM7 plasmids obtained by Dunny et al. (120). In spite of their physiological comparison of the plasmid-containing and plasmidless *S. mutans* strains, they failed to find any clues regarding possible function of the plasmids, although production of bacteriocin-like activity was different in the two plasmid-containing strains.

The few occurrences of plasmids in *S. mutans* argue against earlier claims of plasmid-mediated polysaccharide synthesis by this bacterium. Related to this, Donkersloot et al. (112) isolated mutants of *S. mutans* LM7 that had essentially no GTase activity, but still retained plasmid DNA. Therefore, these mutants are different from the plasmidless, GTase-deficient mutants of strains PK1 and JC2 (253). Furthermore, no difference between the mitomycin C-induced lysis of parent and mutant LM7 cultures was observed, which is contrary to previous findings (254, 309). Similar results have been obtained with a "cariogenic" *S. faecalis* strain ND539. Isogenic pairs with or without plasmid (pAM539) are found to exhibit only a marginal degree of caries activity (84), indicating that

these plasmids do not control cariogenic potential of these *S. mutans* strains. In certain bacterial species, the loss of virulence factor has been demonstrated to be not necessarily accompanied by the loss of a plasmid (541a).

It should be added here that Katayama et al. (289) isolated a plasmid from seven "mucoid" strains of *S. mutans*, using an enzyme, mutanolysin, with lytic activity against the cell wall. They feel that use of mutanolysin results in a more consistent detection of the plasmid.

The transfer of genetic elements by conjugation is well known in gram-negative bacteria. LeBlanc et al. (337) have recently reported that a β plasmid from a group F streptococcus, which codes for resistance to erythromycin and lincomycin, is transferred to *S. mutans*, *S. sanguis*, and *S. salivarius* by cell-to-cell conjugal transfer when donor and recipient cells are incubated on a membrane filter but not in broth cultures. The presence of the β plasmid in *S. mutans* confers on this bacterium the ability to serve as a β-plasmid donor to other *S. mutans* strains with more than 50-fold-higher frequency than is obtained in the original transfer from group F streptococcus to *S. mutans*.

Transformation

Transformation has been shown to occur among most groups of streptococci and *S. sanguis* (group H, strain Challis) (460). Davidson et al. (101) have shown that strain Challis is capable of incorporating DNA prepared from streptomycin-resistant strains of *S. mutans*, *S. sanguis*, and *S. salivarius*. However, transfer of other genetic markers such as fermentation of sorbitol and mannitol and the synthesis of water-soluble polysaccharide was not demonstrated. Reciprocal transformation is observed only between SM resistant strain Challis and *S. sanguis* strains ATCC 10556 and ATCC 10557, but not between strain Challis and *S. mutans* (101).

Subsequently, Westergren and Emilson (611) examined the prevalence of competent strains among oral streptococcal isolates and surveyed the ability for transformation of some competent strains when exposed to heterologous DNA. They confirm that DNAs from streptomycin-resistant strains of five oral streptococcal species including *S. mutans* transform *S. sanguis* strain Challis. None of the *S. mutans* strains is transformed to streptomycin resistance after exposure to *S. mutans* DNA. In contrast to *S. mutans*, 13 out of 50 *S. sanguis* strains are found to be competent in response to DNA from streptomycin-resistant *S. sanguis* 804 (611), whereas most spreading and twitching strains of *S. sanguis* from the human throat are reported to be competent for DNA from strain Challis (251).

A later study reported that DNA prepared from a spontaneous rough mutant, G26-R, of *S. sanguis* transforms wild-type smooth strains G26-S, G30-S, and Challis-S of *S. sanguis* into variants with rough colonial morphology. The rough *S. sanguis* G26-R strain shows an increased ability to adhere to solid surfaces in vitro. However, the transformation with DNA from *S. mutans* strain Ingbritt that produces a rough colonial morphology was not successful (610).

BACTERIOCINS OF *S. MUTANS*: MUTACINS

Orginally, the term "bacteriocin" was applied to proteins of the colicin type which cause death of the bacterial host. These proteins had activity against strains of the same or closely related bacterial species, and were adsorbed to specific receptors (470, 560). However, the activity spectra of bacteriocins produced by gram-positive bacteria, including *S. mutans*, are broader than those in gram-negative bacteria, and only a few bacteriocins of gram-positive bacteria are known to have the properties of the colicins (560).

Bacteriocinogeny Among *S. mutans*

Kelstrup and Gibbons (296) first reported that in stab culture using Typticase agar plates several *S. mutans* strains out of 13 oral streptococci tested produced inhibition zones against other streptococcal strains, including those of *S. pyogenes* and enterococci, but not against unrelated bacteria such as lactobacilli, staphylococci, and *Escherichia coli*. Because the inhibition zones were not infective, the possibility of the involvement of bacteriophages was excluded. All bacteriocins were protease sensitive and had a relatively low molecular weight. However, no activity was demonstrated in broth cultures. These bacteriocins required a stabilizing agent such as agar, agarose, starch, dextran, or glycerol.

In subsequent studies, some additional strains of *S. mutans* were found to produce bacteriocins active against several species (298, 476, 624).

Hamada and Ooshima (226), using the stab culture method, found that some of the reference and freshly isolated *S. mutans* strains inhibited the growth of a wide variety of gram-positive bacteria, including mycobacteria, streptomyces, and actinomyces. They proposed that the bacteriocin of *S. mutans* be designated "mutacin" to differentiate it from those produced by other streptococcal species. The name has been accepted (46, 560).

Among 113 clinical isolates of *S. mutans* from

Japanese children (220), 84 strains (74%) inhibited at least one of the ten indicator strains, which included *S. mutans*, *S. salivarius*, *S. sanguis*, and *S. pyogenes* (225). This result indicated the high level of bacteriocinogenic ability of *S. mutans* as compared with other gram-positive bacteria (560, 605). Serotype *c* strains account for 85 of the 113 clinical isolates, and these strains produce mutacins more frequently than those of other serotypes (225). Essentially similar results have been obtained by others (480).

Very few strains are found to produce mutacins extracellularly (225). Stabilizing agents did not support mutacin production (298). Mutacins are generally heat stable, and some are protease sensitive. Mutacins contain at least two kinds of active components which have different molecular weights (225, 479). Production of mutacins is influenced by the culture media used. Furthermore, when the indicator *S. mutans* strains are cultured in broth containing 5% sucrose, sensitivity to the mutacin decreases remarkably (225). A coating of extracellular glucan most likely renders normally susceptible organisms resistant to mutacin action (477).

Strains of *S. mutans* have been characterized and differentiated by the production of, and sensitivity to, mutacins. Mutacin typing may be a useful tool in epidemiological studies (14, 298, 478, 480). The possibility of maternal and/or intrafamilial transfer of *S. mutans* has been suggested based on the similarity of mutacin patterns (14, 478, 382). Rogers (478) reported that one mutacin type of *S. mutans* predominates in the individual human mouth. However, plural serotypes of *S. mutans* were isolated from a single human subject (220, 382). These strains should have different mutacin patterns.

Eleven of 17 human strains and 7 of 16 rat strains of *S. mutans*, all of which are nonlysogenic, produce mutacins. Most of the nonmutacinogenic rat strains are tetracycline resistant (290).

Extracellular Mutacins

Although many strains of *S. mutans* produce mutacins on solid agar plates, only a few strains have been reported to produce mutacins extracellularly in broth culture of the same composition (225, 294, 458, 479). Addition of yeast extract (2 to 4%, final concentration) to Trypticase soy broth promoted the synthesis of extracellular mutacins (225, 294).

Paul and Slade (458) isolated and partially purified the extracellular mutacin from *S. mutans* GS5 (serotype *c*). It was necessary to add horse serum (5%, vol/vol) to the broth media to obtain consistent activity. The mutacin GS5 is a protein with a molecular weight of about 20,000, and it is sensitive to trypsin and pronase. It is lethal for various streptococcal strains belonging to Lancefield groups A, C, D, G, L, and O, but inactive against *S. mutans* strains of serotypes *a*, *b*, *c*, and *d*. The mutacin binds to the sensitive cells as well as to the cells resistant to its lethal action. Recently, Perry and Slade (461) isolated the specific receptor molecule for the mutacin from an *S. pyogenes* strain which inhibited the activity of mutacin GS5. The receptor has a molecular weight of about 93,000 and may be a heat-sensitive glycoprotein.

Delisle (102) obtained a bactericidal substance from *S. mutans* strain BHT (serotype *b*) by sonication or agitation of the culture with glass beads. The chemical properties of this mutacin have not been described. The extracellular glucans produced from sucrose by this strain did not prevent mutacin production. Furthermore, sensitivity of the indicator strains producing polysaccharides from sucrose remained unaltered even when sucrose was present (103), contrary to the results of Hamada & Ooshima (225) and Rogers (479).

The only information available on the mechanism of action of bacteriocins of streptococci indicates an inhibition of DNA, RNA, and protein synthesis (512). No involvement of a plasmid in mutacinogeny has been reported, although extensive surveys have been made (102, 225, 226, 290, 294).

More recently, S. Hamada and H. Imanishi (unpublished data) found that a clinical isolate of *S. mutans* (serotype *g*) produced mutacin extracellularly in tryptose phosphate broth. Neither yeast extract nor serum was required for the production of the mutacin. It was heat stable, protease insensitive, and active against some other *S. mutans* strains as well as other streptococcal species. Similar mutacinogenic serotype *g* strains were isolated from the sister and mother of the patient, indicating an intrafamilial transmission, as has been suggested by others (316). Characterization of this mutacin is now in progress.

A possible in vivo role for mutacins has been suggested recently. The streptococcal species killed by a mutacin in vitro were also sensitive to the mutacin in vivo (604). Rogers et al. (481) reported that a mutacinogenic *S. mutans* strain prevented the oral establishment of *A. viscosus* Ny1 when introduced into gnotobiotic rats. However, a nonmutacinogenic *S. mutans* strain did not show such an inhibitory effect. Similar findings have been obtained with a mutacinogenic parent strain and a nonmutacinogenic mutant strain of *S. mutans* (482). Mutacin production by *S. mutans* strains has been shown to occur in vivo, and this ability appears to be

ecologically advantageous to the invading *S. mutans* strain in a microenvironment (585). Furthermore, a cell-free preparation of mutacin from a serotype *c* strain was shown to inhibit caries induction by a mutacin-sensitive *S. mutans* strain (T. Ikeda et al., Int. Assoc. Dent. Res. Abstr. no. 1147, 1979). These results indicate that some mutacins are concerned in the ecology of the oral flora.

IMMUNOLOGICAL ASPECTS OF *S. MUTANS*

Distribution of *S. mutans* Serotypes in Humans

It is essential to know the distribution of *S. mutans* serotypes in human populations before considering the immunological aspects of *S. mutans* and dental caries. After the existence of five serotypes in *S. mutans* was established (32), the geographical distribution of *S. mutans* serotypes in plaque samples obtained worldwide was surveyed (32). Serotypes *c* and *d* were found in every area. Serotypes *a* and *b* were also detected in samples from 6 and 9 areas, respectively, of the 14 studied. All of the plaque sampled from American boys (14 to 16 years of age) contained serotype *a* and *d* strains. Serotypes *b* and *c* were present in 74 and 8% of the samples, respectively, and no serotype *e* was detected (201).

However, later studies with isolates cultured from human plaques have revealed that serotype *c* is the most frequently detected serotype of *S. mutans*, irrespective of age, country, sampling site, or isolation and serotyping procedures. Serotype *c* usually comprises about 80% of the total isolates (38, 220, 368, 382, 459, 467, 577). Other serotypes such as *d*, *e*, *f*, and *g* have been occasionally isolated. However, it is surprising that almost none of serotype *a* and *b* strains were found in most of the recent studies. This finding is in sharp contrast to the earlier reports demonstrating the prevalence of serotypes *a* and *b* in plaque samples (32, 201, 275).

Other investigators also demonstrated, using a biotyping method, that strains similar to serotype *c* predominated (174, 292, 521, 522), although the biotyping method cannot differentiate serotypes *c* and *e* (221, 577).

In Vitro Effects of Antisera Against *S. mutans*

Antibodies raised against various cellular and extracellular components of *S. mutans* have been shown to exert a variety of effects on the biological activities of *S. mutans*. Antisera against whole cells of *S. mutans* markedly inhibit the adherence of homologous or immunologically related cells of *S. mutans* to smooth surfaces (230, 427, 428, 447). The adherence inhibition was found to depend on IgG antibody (447). The antibody specific for the *a-d* site of the serotype *a S. mutans* was reported to inhibit the binding of GTase and subsequent adherence of *S. mutans* cells (427). Antibody specific for serotype *e*, but not cross-reacting group E antigen, inhibited adherence of serotype *e S. mutans* cells. However, these antibodies did not prevent the binding of GTase to serotype *e S. mutans* (230). Similar findings have been obtained with serotype *d S. mutans* cells (237).

Some antisera against whole cells of *S. mutans* significantly inhibit the enzymatic activity of extracellular GTase and hence the subsequent adherence to smooth surfaces (139, 222, 230, 427, 428, 538). Anti-GTase activity is not related to whole cell agglutination titers (222), and the inhibitory activity cannot be diminished by adsorption with the homologous whole cells (139, 222). The inhibition by antiserum is serotype dependent. Serotype *c*, *e*, and *f* and type *a*, *d*, and *g S. mutans* are separated into two major groups. Similar relations have been reported with the antisera directed against extracellular GTase of *S. mutans* (236, 331, 332, 538).

Information on the penetration of antibody into the plaque is limited. In vitro *S. mutans* plaque was found to contain the specific antibody at the plaque surface as shown by immunofluorescence (279). Additional studies are needed.

Opsonization of *S. mutans*, followed by phagocytosis and killing by polymorphonuclear leukocytes, has been demonstrated by using antisera from rhesus monkeys immunized with whole cells of serotype *c S. mutans* (516, 517). The monkey antiserum to serotype *c S. mutans* induced maximum phagocytosis and killing of serotype *c* and *e* strains, which are immunologically related. Serotype *a* and *d* cells were also opsonized but to a lesser degree.

Immunological Responses of Host to *S. mutans*

Various antibodies reacting with *S. mutans* have been detected in serum, saliva, and colostrum by several investigators using different methods. In many infectious diseases, serum antibodies play a protective role. Patients with immunoglobulin dysfunctions have been found to have a greater susceptibility to dental caries (87). In addition to humoral antibody responses, local immunity may be enhanced to contribute to protection against diseases of mucosal surfaces including the oral cavity (397).

Only a slight difference in serum antibody titers was observed between caries-free and

rampant-caries groups (299). Cell-wall agglutination tests were used to detect antibody against *S. mutans* in sera. However, the cell walls were prepared from serotype *a*, *b*, and *d S. mutans*, which were later found to be rare serotypes in the human population as discussed above. More recently, it has been suggested that the immunological response against dental caries is associated with the proportion of IgG to IgA and IgM classes of antibodies to serotype *c S. mutans* (346).

Many oral bacterial species have been found to react with salivary antibody (29). Significant levels of agglutinins specific for the five serotypes of *S. mutans* were detected in normal human colostrum and saliva, whereas relatively low levels were found in serum. The agglutinin activity was identified as secretory IgA (5). It is suggested that antigenic stimulation occurs at a site remote from the oral cavity, because secretory IgA to the bacteria of the indigenous oral flora was found in the colostrum as well as the saliva (398, 414).

Arnold et al. (4) further demonstrated that 8 of 25 patients with selective IgA deficiency had significant levels of IgM in their saliva. In the normal control group, IgA was responsible for antibody activity. These results suggest a biological activity for secretory IgM which compensates for the absence of secretory IgA.

A positive correlation between increased caries incidence and decreased levels of salivary IgA in humans has been reported by different groups of investigators (41, 66, 140, 141, 341, 453). Recently a significant negative correlation has been reported between salivary IgA specific for serotype *b S. mutans* and the dental caries of 20 children (3 to 7 years old), implying that IgA provides protection against dental caries (142).

IgA antibodies reacting with serotype *c S. mutans* in secretions from minor salivary glands of humans have been found (234). Furthermore, parotid saliva from all subjects tested had IgA antibodies to various serotype-specific polysaccharides, LTAs, and the peptidoglycan of *S. mutans* (33–35). It should be noted that a significant variation of antibody titers was observed during the experimental periods (33, 35, 234).

Possible Vaccination with *S. mutans* Antigens

Immunization with *S. mutans* is an attractive concept for the control of dental caries (339). In this respect, two different hypotheses have been proposed for the mechanisms of immunological control against dental caries. One hypothesis, put forward primarily by British groups, is that serum IgG antibodies are mainly responsible for the protective effect (47, 343, 344), whereas American workers suggest that secretory IgA in saliva inhibits adherence of *S. mutans* to tooth surfaces (138, 400, 409, 573). However, it should be noted that these two mechanisms are not necessarily mutually exclusive.

A preliminary study has shown that three irus monkeys vaccinated with whole, live cells of a serotype *c S. mutans* developed significantly fewer carious lesions than control, nontreated monkeys (24). Subsequently, it was demonstrated that whole cell or broken cell vaccines conferred significant protection in monkeys, especially when the immunogen was administered by intraoral submucosal injection (26). It was suggested that the induction of local immunity is not a prerequisite, because good protection was obtained by immunization via both submucosal and subcutaneous routes (85). Protection was not obtained with glucosyltransferase preparations in the monkey test system (26, 85).

Lehner et al. (342, 343) have reported the immunization of rhesus monkeys with serotype *c S. mutans* cells mixed with incomplete Freund adjuvant. They found protection with use of the complete immunogen and a delayed onset of caries with the adjuvant alone. Immunized animals contained demonstrable serum antibody to GTase that inhibited GTase activity (498). Immunization enhanced serum IgG and IgM titers, whereas there was little increase in the salivary IgA titers in the immunized compared with nontreated control monkeys (340). The reduction in caries was associated with a reduction in the number of CFU of *S. mutans* in the fissures (340). A correlation was found between the CFU of *S. mutans* and the number of carious lesions in rhesus monkeys (47). More recently, protection was demonstrated against dental caries in rhesus monkeys infused passively by the intravenous route with antibodies of the IgG class. Intact molecules of IgG, IgA, and IgM have been shown to pass from plasma to the oral cavity via crevicular fluid, and therefore can contribute to local defense mechanisms (67, 343).

In contrast to the findings described above, ample evidence has been reported indicating the primary importance of local immunity due to secretory IgA antibodies. Taubman and Smith (573) demonstrated that local immunization with formalinized whole cells of *S. mutans* resulted in an enhanced salivary IgA response and reduced caries development in both conventional and gnotobiotic rats. It was also found that similar immunization using cell-free GTase preparations with Freund complete adjuvant resulted in the presence of antibodies in saliva of rodents (540, 574) and monkeys (7). Oral immunization of hamsters with the enzyme pro-

duced similar results (540). Reductions in carious lesions were greater on smooth surfaces of teeth than on occlusal surfaces, probably due to interference with adherence of *S. mutans* (325, 574). In these cases, salivary antibody is the most likely protective principle in the rodents.

Local immunization with whole cells of *S. mutans* stimulates a specific salivary IgA response which is protective against caries induction by *S. mutans* infection (400). Similar findings have been demonstrated in irus monkeys by injection of *S. mutans* into the vicinity of the major salivary glands or parotid ducts (134, 138).

The ingestion of Formalin-treated cells of a serotype *g S. mutans* strain has been shown to stimulate specific secretory antibodies in saliva and milk but not in serum of rats. These antibodies were found to be of the IgA class. Orally immunized rats developed significantly fewer carious lesions than nontreated control rats (409). The level of specific salivary IgA antibodies in rats correlated with a reduction in the level of plaque and caries scores and the viable counts of *S. mutans* in plaque (408).

Intravenous or intramucosal administration of vaccines to female rats produced elevated IgG in colostrum, milk, and serum and elevated IgA in colostrum and milk, respectively. Passive transfer of either IgG or IgA has been found to render protection against caries development in rat offspring (399, 407). These results support earlier findings (345).

A recent report (406) has indicated that a secretory IgA immune response is elicited in humans by ingestion of capsules that contain Formalin-treated cells of *S. mutans* strain OMZ176 (serotype *d*). No increase in serum antibody levels was demonstrated. It is of interest to note the simultaneous appearance of antibodies in remote secretory glands such as the salivary and lacrymal glands without a serum antibody response.

Protein-malnourished rats exhibited increased caries susceptibility (405). However, a nutritionally compromised rat can elicit a specific immune response that protects against *S. mutans*-induced caries (411).

It should be noted here that immunological cross-reactions have been observed occasionally between human heart tissue and certain components of *S. mutans* strains (85, 498, 587). These antigens have not been described, but their presence in vaccine antigens is of great concern. It seems possible, however, that certain antigens, GTase for example, may be useful (540). Low concentrations of IgG antibody to this enzyme exist in the serum of young normal adults (22a). Heart-reactive antibody was not adsorbed from pooled acute rheumatic fever sera by *S. mutans* 6715 (587). The quantity of antibody necessary to reduce the caries rate in humans after oral immunization with GTase may not be detrimental to the host (540).

Other antigens of *S. mutans* deserve consideration as a vaccine to reduce the incidence of dental caries. Antibodies to the type-specific polysaccharide and the glucan of serotype *a* (strain HS6) will inhibit the in vitro adherence of this species (427, 428). Type-specific polysaccharide antibodies to the type *e* (strain MT703) have the same property (231). The isolation of a rhamnose-rich polysaccharide from the cell wall of serotype *d S. mutans* (462) indicates that polymers other than the serotype polysaccharides remain to be characterized. Polysaccharides would be expected to produce fewer reactions when used as an antigen in humans.

CARIOGENICITY OF *S. MUTANS* IN EXPERIMENTAL ANIMALS

Caries Induction in Animals

After the epoch-making experiments by Orland and his co-workers (451) in which germfree rats were used, Fitzgerald and Keyes (157) demonstrated in 1960 that certain streptococcal strains isolated from carious lesions of rats and hamsters could produce caries in gnotobiotic rats and "caries-inactive" hamsters (156, 157, 302). These strains are not termed "*S. mutans.*" The "caries-inactive" hamsters have been found to be free from indigenous microflora which could induce dental caries when a caries-inducing high-sucrose diet is fed. Once *S. mutans* is established in the mouth of the animal, caries activity is transmitted from parent to offspring (157, 302).

In the earlier stage of caries research, it was thought that there might be a specificity between the caries-inducing streptococci and the host animal species. However, Zinner et al. (628) demonstrated that human strains of *S. mutans*, which reacted with the antiserum against the hamster strains of *S. mutans*, could produce extensive caries in hamsters. Since then, many streptococcal strains isolated from the human mouth have been shown to be cariogenic in various animal model systems (126, 127, 151, 183, 202, 207, 229, 321, 322, 433). Most of the cariogenic strains belong to the species *S. mutans*. However, organisms other than *S. mutans* can occasionally induce variable levels of caries in animals (for review, see reference 196).

Dental caries have been induced in various kinds of animals, including monkeys (24), gerbils (150), mice (229, 551), rats, and hamsters. The

transmission of *S. mutans* from hamsters to mice and caries induction in mice have also been demonstrated (551).

Strains of *S. mutans*, regardless of their serotypes, almost always induce smooth-surface and pit-and-fissure caries in animals (229, 322, 410). Strains of serotypes *a*, *d*, and *g* *S. mutans* tend to produce smooth-surface caries preferentially in rats (229, 410). However, variations are frequently observed in the pattern and severity of the induced carious lesions in experimental animals (366). In general, young animals are more susceptible to a caries attack (335, 412, 434, 515).

Dietary factors critically influence the composition and pathogenic potential of inoculated *S. mutans* by affecting the implantation, colonization, and metabolic activities of the bacterium. Sucrose has been demonstrated to be most cariogenic and supports the most rapidly progressive pathogenesis, although other sugars, such as maltose, lactose, and fructose, also support the induction of dental caries in animals to some extent (51, 168, 563).

Noncariogenic and Supercariogenic Mutants of *S. mutans*

To identify a virulence factor in the pathogenesis of an infectious disease, mutants which lack one or more characteristic properties possibly responsible for pathogenic processes are a useful tool to analyze the mechanism of the pathogenesis.

In the work cited below, it should be noted that the presence of a single mutation has not been established. It is reasonable to assume, based on the techniques used, that more than one mutation is present. Also, a mutation, if present, may be only indirectly related to the character(s) being considered.

De Stoppelaar et al. (107) isolated a mutant which failed to synthesize cell-bound glucan in 5% sucrose broth from a serotype *c* strain of *S. mutans*. The inability to synthesize insoluble glucans of an adherent nature was accompanied by a significant reduction of cariogenic potential in experimental animals. The mutant also showed a dramatic loss of viability due to acid production from either glucose or sucrose (113).

Freedman and Tanzer (165) isolated mutants of *S. mutans* 6715 (serotype *g*) that differed from each other in colonial morphology on MS agar. They found that the mutants lost the ability to adhere to a wire surface but retained the ability to agglutinate and form macroscopically visible clumps in the presence of sucrose or exogenous glucans. The mutants were found to produce increased amounts of water-soluble extracellular glucans (163, 165). The latter finding coincides with the fact that cells of these mutant strains bound significantly lower quantities of extracellular GTase from *S. mutans* (235).

Furthermore, the mutants differed from the parent strain in that each failed to form plaque on the smooth surface of the teeth and to produce smooth caries in specific-pathogen-free and gnotobiotic rats (567). However, these mutants produced sulcal caries, although with diminished intensity. These results indicate that surface-associated glucan synthesis by *S. mutans* apparently contributes to the local environment and promotes the pathogenic potential of *S. mutans* on smooth tooth surfaces. This is probably due to a barrier effect of the glucan layer to the diffusion of metabolically excreted lactic acid, which has been considered to be critical in the demineralization of the teeth (567). The above results also indicate that cell-to-surface adherence via insoluble glucan synthesis from sucrose is a more important factor than cell-to-cell agglutination induced by glucan in the pathogenesis of dental caries.

A surface fuzzy coat is suspected to contain a glucan receptor which may be responsible for glucan-induced agglutination in both parent and mutant strains of *S. mutans* 6715. On the other hand, only the parent strain produces extracellular glucans with predominantly fibrillar morphology (432).

Other investigators also isolated similar types of mutants from serotype *g* *S. mutans* strain AHT (308, 314) and serotype *c* strains GS5 (28, 280, 281) and PS14 (415). A mutant of *S. mutans* LM7 (serotype *e*) forming little cell-bound glucan has been reported to attach to the teeth of rats comparably to its parent. However, cariogenic activity of these strains was not compared (79).

Mutants which produce elevated levels of GTase have been isolated independently by two different groups (415, 502). These mutants demonstrate increased ability to adhere to glass surfaces (415, 502) and produce more carious lesions than the parent strain (415). Thus, a clear correlation has been demonstrated between cariogenicity, in vitro adherence, and insoluble glucan synthesis in *S. mutans*.

Other types of mutants that synthesize or degrade less IPS have been isolated from two serotype *c* strains which are strong producers of IPS (166). These mutants had diminished virulence both on smooth tooth surfaces and in fissures (568). The loss of cariogenicity of these mutants is attributed to diminished ability to produce acid from endogenous IPS storage in the absence of exogenous carbohydrates. However, strains of serotype *d/g* which have low

IPS-forming ability (164) do not appear to depend on this property for caries induction as do strains of serotype *c*.

S. MUTANS AND DENTAL CARIES IN HUMANS

Effect of Sucrose on the Proportion of *S. mutans*

The famous Vipeholm study provided strong support for a close relationship between sucrose intake and human caries prevalence (213). Recent studies with gnotobiotic rats (413) have revealed that as little as 0.1% sucrose in the diet can significantly promote the development of dental caries by *S. mutans* 6715, indicating that the consumption of artificially high levels of sucrose is not necessary for the induction of dental caries.

It is well known that dietary carbohydrates and infection with *S. mutans* are essential factors in the development of dental caries (303, 511). Among dietary carbohydrates, sucrose is considered to be directly related to dental caries (51, 168, 380).

Several studies on the effect of dietary sucrose on streptococcal composition in plaque flora have been carried out with human subjects. Carlsson and Egelberg (58) reported that plaque formation was heavier during high-sucrose diet periods than in glucose diet periods. When six subjects were instructed to abstain from any dietary carbohydrates for 17 days, the *S. mutans* count decreased to an undetectable level while the percentage of *S. sanguis* increased (109). Such an inverse relationship between the *S. mutans* and *S. sanguis* population was observed in other investigations (167, 547). Other nutritional interactions between *S. mutans* and *S. sanguis* may be important for the ecology of these organisms in the oral flora (57).

Contrary to an earlier study (58), it was reported that high-sucrose diets had no significant effects on total plaque accumulation, although total viable microbial density and populations of *S. mutans* and lactobacilli increased (547). A low-sucrose diet did not completely eliminate *S. mutans* from the oral flora (547) as was shown in a study with monkeys (306).

Epidemiological Relationship Between *S. mutans* and Caries Development

Many strains of *S. mutans* isolated from humans have been demonstrated to be cariogenic in experimental animals as described above. However, these results do not necessarily apply to human dental caries. To clarify the etiological role of *S. mutans* in caries development in humans, we must depend on epidemiological studies which relate the microbes of the carious lesion or dental plaque to the initiation of caries at the tooth site. The rationale for the hypothesis that *S. mutans* is strongly associated with human caries has been supported by the following epidemiological studies.

S. mutans was isolated from all carious lesions, whereas only 23% of the samples from sound tooth surfaces of children (13 to 14 years old) contained the bacterium (362). Similar tendencies were also found in younger (135, 553) and older (17 to 22 years old) (257, 523) subjects.

In an extensive study, it was concluded that there is a strong association between percentage levels of *S. mutans* in single occlusal fissures and dental caries. Seventy-one percent of the carious fissures retained *S. mutans*, accounting for more than 10% of the viable count, whereas 70% of the fissures free from caries had no detectable levels of *S. mutans* (372). Furthermore, it has been shown that aciduric bacteria such as *Lactobacillus* are detected in significant quantities in the dentinal carious lesion as the decay progresses (373, 525, 561).

More recently, it was demonstrated that the proportion of *S. mutans* in samples from early carious lesions (white spots) of smooth tooth surfaces was significantly higher than that from the adjacent sound surface. No significant numbers of lactobacilli were found in the early lesions (118).

However, the etiological involvement of a bacterium in the oral flora cannot be fully attributed by cross-sectional studies in the case of a chronic disease such as dental caries. To overcome the problem, several longitudinal studies that demonstrate cause-and-effect relationships have been reported. The distribution of *S. mutans* on the tooth surfaces was followed over a period of 18 months. The development of caries was more frequently preceded by colonization with elevated levels of *S. mutans* (268). Subsequently, other investigations (291, 310, 559) have led to similar findings.

On the other hand, no significant relationship between *S. mutans* and the initiation of dental caries in Danish preschool and British school children was found (246, 416). The variable results may be attributed to complex factors such as sampling sites, methods of cultivation, fluoride content, eating habits of the subjects, sucrose intake, and possible immunity in the oral cavity.

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

glands, and an increase in *S. mutans* (363).

In a survey of 22 infants over a period of 30 months, no clear-cut association between the development of caries and previous detection of *S. mutans* was reported (382). However, *S. mutans* was isolated from all 12 of the infants who developed caries. During the test period, changes in the distribution of serotypes were occasionally noted. Serotype *d/g* strains have a tendency to give rise to smooth-surface caries; serotype *c* strains were always present (382).

In this context, as the number of erupted teeth increases, there is a gradual increase in the prevalence of *S. mutans*. Edentoulous newborns or aged men do not harbor significant quantities of *S. mutans* (59, 62, 63, 382). It appears that *S. mutans* is most likely transmitted intrafamilially (14, 15, 316, 382).

PREVENTION OF CARIES CAUSED BY *S. MUTANS*

In theory, dental caries can be prevented by eliminating cariogenic bacteria, especially *S. mutans*, from the mouth, as well as by increasing the resistance of teeth and modifying the diet (303, 305, 366, 437, 511). In the following sections, emphasis has been paid mainly to methods in the first category.

Suppression of *S. mutans* by Antimicrobial Agents

The usefulness of penicillin in preventing experimentally induced caries has been noted since the pioneering work of McClure and Hewitt (396); they suspected *Lactobacillus acidophilus* as a causative agent. Since then, ample evidence has accumulated which shows that most antibiotics with antimicrobial activity against gram-positive bacteria depress the development of dental caries induced in experimental animals (18, 152, 153). Furthermore, young human patients (6 to 19 years old) who had received long-term administration of penicillin and/or tetracycline for treatment of chronic infectious diseases developed about two-thirds fewer caries than did control subjects (242). This observation could be explained by the recent finding that the presence of the very low concentrations of both penicillin G and sulfadiazine markedly inhibits in vitro plaque formation by *S. mutans* (607).

However, Weld and Sandham (608) reported that long-term therapy with penicillin and sulfadiazine did not cause a significant reduction in the proportions of *S. mutans* or lactobacilli, although the organisms isolated from the patients demonstrated high susceptibility to penicillin. No penicillin-resistant strains of *S. mutans* have been described.

S. mutans has been reported to be highly susceptible in in vitro tests with penicillin, ampicillin, erythromycin, cephalothin, methicillin, and many other antibiotics (10, 147, 361). It is of interest that serotype *a* and *b* strains of *S. mutans* are very susceptible to bacitracin and polymixin B, respectively; other serotypes are not susceptible (361).

In spite of the in vitro effectiveness of antibiotics, it is not practical to use them for caries control. Recent investigations, however, suggest that certain antimicrobial agents may be used on a short-term basis to suppress *S. mutans*. Such agents as vancomycin (105, 283), kanamycin (367), and iodine (64, 184, 570) can be used topically for this purpose.

Other agents that have been reported to suppress *S. mutans* and other cariogenic bacteria include fluoride (371, 374, 627), bisbiguanidines (137, 197, 570), and surfactants (9, 571). Many of these antimicrobial and antiplaque agents have been found to inhibit GTase activity of *S. mutans* (74; Torii and Hamada, Abstr. Jpn. Assoc. Oral Biol., 1979).

Another unique method is the use of a bacteriolytic enzyme termed "mutanolysin," which has been purified from a soil bacterium (625, 626). Most *S. mutans* strains, including laboratory stock cultures and fresh clinical strains, are markedly lysed by this enzyme (239). Mutanolysin inhibits the accumulation of dental plaque and the development of caries induced by *S. mutans* strain AHT or KIR in specific-pathogen-free hamsters (455).

Inhibition of Adherence of *S. mutans* by Glucan-Hydrolyzing Enzymes

The synthesis of insoluble adherent glucan from sucrose by *S. mutans* is a prerequisite for the induction of dental caries in experimental animals. Plaque deposits on wire can be removed by a dextranase preparation obtained from *Penicillium funiculosum* (159). In vitro studies indicate that dextranases, $\alpha(1\rightarrow6)$ glucanases, have limited ability to degrade the extracellular glucans produced by *S. mutans* (203, 224, 315, 435, 514). In subsequent studies, $\alpha(1\rightarrow6)$ glucanases of different origins effectively prevented plaque formation and caries induction by *S. mutans* strains in hamsters (155, 159, 161). Similar positive results have been obtained with other animal model systems (25, 227, 228, 463). Human clinical trials of $\alpha(1\rightarrow6)$ glucanases have resulted in conflicting antiplaque effects (48, 117, 199, 304, 364, 430).

Other investigators claimed that $\alpha(1\rightarrow6)$ glucanase exerted no inhibition of plaque formation or of caries induction in a rat test system (208).

They later reported that $\alpha(1\rightarrow3)$ glucanase, termed "mutanase," from a strain of *Trichoderma harzianum* (206) did inhibit caries induction (210, 211). Preparations of $\alpha(1\rightarrow3)$ glucanase have been reported to impair the colonization of *S. mutans* in plaque (295) and to inhibit the formation of dental plaque and gingivitis in humans (297). Several insoluble glucan-hydrolyzing enzymes have been obtained from different origins (123, 270). In vivo effects of these $\alpha(1\rightarrow3)$ glucanases have not been examined. More recently (527), a dextranase from a strain of *Fusarium moniliforme* (528) had a much greater affinity for HA than did the *Penicillium* dextranase. The investigators suggest the occurrence of a more effective interference with the initial attachment of *S. mutans* and subsequent accumulation of dental plaque.

In this connection, yeast invertase that splits sucrose into glucose and fructose is not regarded as having a caries-inhibiting potential (160). Furthermore, Gibbons and Keyes (186) reported that addition of low-molecular-weight dextran into a caries-inducing diet inhibited plaque formation and caries induction as well as the in vitro prevention of insoluble glucan synthesis by *S. mutans* GTase. This finding, however, has not been supported by other investigators (520). A "coupling sugar" preparation significantly reduced caries activity in rats when it was substituted for sucrose in a rat diet (269). The coupling sugar is produced by incubating starch, sucrose, and cyclodextrin GTase from *Bacillus megaterium* (3, 446).

ENDOCARDITIS CAUSED BY *S. MUTANS*

Subacute endocarditis caused by streptococci is frequently due to the alpha-hemolytic and nonhemolytic types. Abercrombie and Scott (1) first reported a case of endocarditis caused by a streptococcus that was considered identical to *S. mutans* proposed by Clarke (81). In a recent study in England, Parker and Ball (457) identified the species of the 317 streptococcal strains which had been isolated from patients with subacute endocarditis. The most numerous are *S. sanguis* (16.4%), *S. bovis* (15.1%), and *S. mutans* (14.2%, 45 strains). It is of interest to note that all of them as well as certain *S. mitior* strains (7.3%) synthesize glucan from sucrose.

Fifty-four strains of *S. mutans* from cases of endocarditis in Denmark have been identified and serotyped. The most prevalent was serotype *c* (459). Seventy-seven stock strains isolated from human blood designated as *S. bovis* were rechecked, and 35 were identified as *S. mutans* (106). There are no appreciable differences between the isolates from human blood (54 strains) and dental plaque (50 strains) in terms of their physiological characteristics (143).

Recently, several investigators have confirmed that subacute bacterial endocarditis can be caused by *S. mutans* (243, 260, 365). The patients had the typical picture of fever, heart murmur, weakness, and repeated positive blood cultures. Most patients had prior known valvular heart disease. Teeth were suspected to be the locus of infection in some cases. All the strains were susceptible to various antibiotics including penicillin G (243, 260). All the patients were treated with penicillin G and streptomycin (243), but a fatal case did occur (365).

It is important that clinical laboratories differentiate *S. mutans* from the enterococcal species. *S. mutans* is susceptible to low concentrations of penicillin G, in contrast to enterococci, which are usually resistant to this antibiotic (10, 243).

Experimental endocarditis due to various bacteria can be readily induced in rabbits by placing a catheter in the left side of the heart (121). With this model, Durack et al. (122) investigated the effect of prior immunization with *S. mutans* and *S. sanguis* on the susceptibility of rabbits to experimentally induced streptococcal endocarditis. Rabbits with a high complement-fixing antibody titer to the infecting organisms developed the disease with a significantly lower frequency than those with lower titers. The results do not support the concept that immunization with *S. mutans* for the prevention of dental caries may increase the susceptibility of the immunized subjects to an endocarditis caused by *S. mutans* (122).

The initial event of the pathogenesis of bacterial endocarditis is the attachment of bacteria to heart valves, particularly those with damaged aortic valves possessing a platelet-fibrin thrombus (2). Cell-bound glucan appears to promote the establishment of *S. mutans* and other glucan-producing streptococci on the heart valves (468, 510). Adherence to damaged valves is approximately five times higher than adherence to normal valves. These results may explain the high prevalence of glucan-synthesizing streptococci, including *S. mutans*, as the causative agent of subacute endocarditis. Thus, the adherence-promoting ability of glucan synthesized by *S. mutans* appears to be the initial step in the pathogenesis of endocarditis as well as dental caries.

SUMMARY

It is likely that *S. mutans* is the primary cariogenic bacterium in both humans and animals. Other bacteria

found in actively progressive carious lesions are considered to be secondary invaders, probably commensal with *S. mutans* with regard to their physiological activities. Only a limited number of species of bacteria other than *S. mutans* are occasionally found to be cariogenic in experimental animals.

Virulence factors of *S. mutans* responsible for its cariogenicity include the ability to adhere to smooth surfaces and acidogenic-aciduric properties. Adherence to smooth tooth surfaces is responsible for cariogenic plaque formation by *S. mutans* and is mediated by the de novo synthesis of a glucose polymer from dietary sucrose. The synthesis is due to the action of cell-free or cell-associated forms of GTases. This explains the marked caries-inducing property of sucrose in diets.

In terms of bacterial taxonomy, the species *S. mutans* includes a number of heterogeneous strains. Various immunological, biological, and biochemical properties and the epidemiological distribution of *S. mutans* have been discussed in this review with special reference to the seven serotypes of the microorganism. The occurrence of *S. mutans* in subacute endocarditis and the possibilities of a vaccine against dental caries have also been discussed.

ACKNOWLEDGMENTS

We thank F. C. McIntire for his generous cooperation in providing facilities in the Department of Oral Biology during the writing of a large part of this review. We are also grateful to Wanda Valentine and Chiaki Kimura for excellent secretarial help, and to Albert E. Vatter for the electron microscopic work.

The funds to support S. H. during his stay in Denver were kindly provided to H.D.S. by the Pioneer Fund. The work reported here in the laboratory of H.D.S. at Northwestern University Medical-Dental Schools was supported by the National Institute for Dental Research, the National Institute for General Medical Sciences, and the National Heart and Lung Institute of the National Institutes of Health, Public Health Service, and by the Pioneer Fund.

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