Colonization and Cariogenicity of Streptococcus ferus in Rats

A. L. COYKENDALL* AND M. L. FREEDMAN

Department of Oral Diagnosis, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut 06032

Streptococcus ferus, which is indigenous to wild rats, is a member of the mutans group of streptococci. We tested its ability to colonize and to cause caries in laboratory rats by comparing two strains of S. ferus with the very cariogenic Streptococcus sobrinus strain 6715. Groups of rats were fed either finely ground mouse chow or a 56% sucrose diet, or they were switched from chow to the sucrose diet. All three strains colonized the mouths of rats regardless of diet. However, the infectants reached higher proportions of the total flora more quickly in the rats consuming sucrose. Similarly, the percentage of the oral flora represented by an infecting organism increased numerically when rats originally fed chow were switched to the sucrose diet. S. ferus formed plaques on the teeth of the rats, but these plaques did not proliferate over smooth tooth surfaces as extensively as did those of S. sobrinus. Although S. ferus colonized and accumulated, it was non-cariogenic in rats fed sucrose compared both with rats fed similarly but infected with S. sobrinus 6715 and with uninfected controls. In vitro measurements suggested that S. ferus produced acid less rapidly than S. sobrinus. Thus, the lack of cariogenicity in S. ferus may result from an inability to form copious plaques on smooth tooth surfaces and from low acid production and, therefore, may represent a natural absence of the pathogenic potential usually inherent in the mutans streptococci.

Streptococcus mutans and some phenotypically similar species are the only oral microbes which are consistently capable of causing multisurface dental decay in experimental models (13, 23, 27, 33) and whose occurrence and population sizes in human mouths can be correlated with caries (7, 20, 24, 25, 32). These organisms adhere to teeth and make abundant acid from common dietary sugars, such as glucose and sucrose. Adhesion of bacterial aggregates is promoted by water-insoluble, extracellular glucans, which these species synthesize from sucrose (9, 11, 14); the metabolic acids (8) produced in such cohesive dental plaques (12) are believed to mediate decalcification. The relationship between the pathophysiology of S. mutans and dental caries has been reviewed recently (17).

The discovery of the "mutans-like" Streptococcus named Streptococcus ferus (4) in wild rats, some of which ate considerable amounts of sucrose (6) and some of which did not (5), presented an opportunity to test the colonization requirements and the cariogenic potential of mutans streptococci isolated from feral animals and to test the hypothesis that mutans streptococci are inherently cariogenic and that this cariogenicity is expressed when the diet of a host becomes refined and sucrose rich (3). Although rats which ate sugar cane originally had some evidence of caries, the teeth of such rats wear rapidly in the natural environment, and the extent of decay could not be assessed.

In the experiments described below, we examined (i) the degree to which S. ferus could colonize and decay the teeth of laboratory rats that were fed sucrose, (ii) whether this organism could colonize rats fed a conventional laboratory diet low in sucrose, (iii) the effect of changing the diet from a conventional diet to a sucrose-rich diet on plaque proliferation and disease, and (iv) in vitro acid production by such wild rat strains. Comparisons were made with a strain of known virulence and with uninfected controls.

MATERIALS AND METHODS

Bacterial strains. Two strains of S. ferus were used. Strain HD3 was isolated from a wild rat trapped at a landfill dump, where rats presumably had little, if any, access to sucrose (5). Strain 8S1 was isolated from a wild rat which was trapped in a sugar cane field, where the animals ate the sucrose-rich cane (6). Both strains fermented manitol and sorbitol and formed adherent plaques on nichrome wires suspended in broth cultures containing 5% sucrose. Deoxyribonucleic acid from HD3 hybridized extensively with deoxyribonucleic acid from 8S1 but not with S. mutans deoxyribonucleic acid or with deoxyribonucleic acids.
from other mutants streptococci. The biochemical, genetic, and antigenic features of *S. ferus* have been described previously (4, 5). The very cariogenic *Streptococcus sobrinus* (formerly *S. mutans* serogenetic group IIg [1, 4, 16]) strain 6715-13 was used as a positive control in these experiments. In all cases, the organisms used as infectants were derived from naturally occurring mutants which were resistant to 200 μg of streptomycin per ml.

**Animals and diets.** A group of 72 Sprague-Dawley rats bred in our laboratory was divided into the following three diet groups: (i) chol, (ii) diet 2000, and (iii) switch from chol to diet 2000. These groups were further divided into the following four bacterial infection groups: two groups infected with *S. ferus* (strains HD3 and 8S1), one group infected with *S. sobrinus* 6715-13, and one group that remained uninfected.

were examined for the presence of the infecting powdered mouse chol (type 9F; Purina), which was selected because of its low sucrose and fluoride contents. The sucrose content of the chol was determined as the amount of glucose oxidase-positive material in an invertase-treated, water extract of the desiccated diet; appropriate standards and non-invertase-treated controls were included. The diet pellets were ground into a powder to reduce the cleaning action caused when the rodents gnawed. Rats consuming sucrose ate caries test diet 2000 (22), which contained 56% sucrose. Rats in the third (diet switch) group were fed chol for 28 days then switched to diet 2000 for 56 days; other groups were fed a single diet for 56 days. Food and deionized water were available to the rats ad libitum.

**Experimental protocol.** Animals were weaned at 19 to 21 days, weighed, caged in pairs, and immediately fed the appropriate diet. After 2 days, the mouths of the rats were examined for the presence of mutants streptococci by swabbing with sterile cotton-tipped applicators and streaking the swabs onto mitis salivarius agar (BBB Microbiology Systems, Cockeysville, Md.). If no mutants streptococci were detected on these plates after 24 h of incubation in candle jars at 37°C, all animals were infected on day 3. Each rat was infected with the appropriate organism by instilling 0.5 ml of a fresh fluid thiglycollate broth suspension into its mouth. Such suspensions were prepared from overnight cultures which had been harvested and resuspended with their cell densities adjusted so that the infecting doses were roughly equal for all strains (10⁵ cells). Animals that were to remain uninfected were inoculated with 0.5-ml portions of sterile broth.

At 10 days after infection, the mouths of the rats were examined for the presence of the infecting organisms (or for the absence of these organisms in the case of the uninfected control group). Applicators were streaked onto the surfaces of mitis salivarius agar plates which contained 200 μg of streptomycin per ml. At 20 and 49 days after infection, the proportions of infecting organisms relative to the total streptococcal flora were assessed as follows. The mouth of each rat was swabbed with an applicator which had been moistened with sterile phosphate-buffered saline. The swab was broken into a culture tube containing 4.5 ml of phosphate-buffered saline and agitated with a Vortex mixer. The resulting bacterial suspensions were serially diluted, and 0.1-ml samples were spread onto mitis salivarius agar containing 200 μg of streptomycin per ml for estimation of the streptomycin-resistant test organism and onto mitis salivarius agar for enumeration of the total streptococcal population. (Although we tried to have the swab contact the buccal, lingual, and occlusal surfaces of all molar teeth, we recognized the problems inherent in this technique.)

The flora of the animals which were switched from chol to diet 2000 was quantitated at day 10 as well as at day 20, and these animals were also sampled after 35, 47, and 77 days.

**Plaque and caries scores.** The teeth were flooded with 2% safranin to stain plaque in situ. The extent of plaque in each quadrant was assigned a score of from one to four. Thin plaques which were visible in occlusal sulci were assigned a score of one. Thicker plaques which filled the sulci were given a value of two. (These sulcular plaques probably represented food debris as well as bacteria and the products of bacteria.) When plaque filled the sulci and extended onto smooth surfaces, it was scored three, and extensive smooth surface plaques were scored four. The heads were then defleshed by dermestid beetles, and enamel caries were scored by the method of Keyes (21) and analyzed statistically.

**Acid production.** Cultures (100 ml) of the three streptococcal strains were grown to stationary phase in Todd-Hewitt broth (Difco Laboratories). The cells were harvested, and then they were washed with and held overnight in buffer (0.001 M KPO₄, 0.5 M KCl, pH 7; 4°C) at an optical density at 600 nm of 0.6 to deplete endogenous nutrient pools. Acid production at 37°C by stirred cell suspensions exposed to 0.001 M glucose was monitored with a pH meter.

**RESULTS**

**Colonization.** All rats were free of mutants streptococci at the start of the experiment. Uninfected control animals remained free of mutants streptococci, including those strains used to infect the other rats, throughout the experiment (Fig. 1A and 2A). The mouse chol contained 0.17% sucrose. When supplemented with an aqueous extract of mouse chol 9F, Todd-Hewitt broth supported slight *S. sobrinus* plaque formation on nicherme wires in vitro (unpublished data). The fluoride content according to the manufacturer was 6.4 μg/g.

In the animals fed chol, *S. ferus* strain 8S1, which was isolated from a sugar cane-eating rat, colonized feebly (Fig. 1B). After 20 days the organism comprised a mean of less than 0.1% of the total streptococcal flora, with wide variations. One rat had no detectable *S. ferus* 8S1. At 40 days the mean level of strain 8S1 increased so that four of the six animals had at least 1% strain 8S1 in their streptococcal flora; one rat remained apparently uncolonized. *S. ferus* strain HD3 colonized well in rats fed chol (Fig. 1C). At 20 days all animals were infected, and at 40 days 1 to 10% of the total streptococcal flora was *S. ferus* HD3. As expected, *S. sobrinus* strain
6715-13 colonized readily in the absence of abundant sucrose and attained high populations after 40 days (Fig. 1D).

All strains colonized and reached high population levels in rats fed the sucrose diet (Fig. 1B through D). After 40 days, in some rats the infecting strains often accounted for one-half of all streptococci, and S. sobrinus 6715-13 (Fig. 1D) often displaced almost all other streptococci, so that this strain was the only Streptococcus strain present in significant numbers. The sucrose diet increased the total number of streptococci recovered.

In animals fed chow and then switched to diet 2000 (Fig. 2B through D, days 10 and 20), the levels of the infectants closely paralleled the
levels observed in rats fed only chow or only diet 2000 (see above). However, the population of streptomycin-resistant infectants increased markedly after the diet of the rats was changed to a diet rich in sucrose (Fig. 2B through D, days 35, 47, and 77).

Plaque scores. Plaque was present, but the scores were low in both infected and uninfected rats fed chow (Table 1). However, the sucrose diet caused plaque (and debris) to accumulate in fissures, even in uninfected animals. Nonetheless, plaque quantity increased with infection and was greater in those rats whose infecting strain colonized better. Thus, strain 8S1-infected animals had less plaque than rats infected with strain 6715-13. This indicated that the bacteriological sampling method did succeed in measuring the differences in accumulation of the mutants streptococci on the teeth. Rats which were infected with the S. ferus strains and whose diet was switched from chow to diet 2000 did not attain quite as high a plaque score as similarly infected animals that always consumed a sucrose diet.

Caries. Although S. sobrinus 6715-13 colonized, it did not decay the teeth of rats fed chow. However, as expected, this Streptococcus strain was very cariogenic for the smooth surfaces of the teeth of rats when the host was fed a sucrose-rich diet. Rats that were infected with strain 6715-13, fed chow, and then switched to diet 2000 also developed smooth surface caries, but not to the extent observed in rats fed sucrose from the start (Fig. 3). Neither strain of S. ferus induced smooth surface or sulcal caries significantly compared with control rats or with S. sobrinus-infected animals fed the same diet (Fig. 3). There was a slight but not significant enhancement of sulcal virulence by S. sobrinus in rats fed sucrose.

![Fig. 3. Cariogenicity of S. ferus 8S1, HD3, and 6715-13.](http://iai.asm.org/)

**Fig. 3.** Cariogenicity of S. ferus 8S1, HD3, and 6715-13. Caries were induced by diet 2000 compared with uninfected controls or diet 2000 switched from chow to diet 2000. The bars indicate standard errors of the means.

**Table 1. Comparison of in situ plaque scores from uninfected controls and from infected rats consuming different diets**

<table>
<thead>
<tr>
<th>Infectant</th>
<th>Mean plaque scoresa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chow</td>
</tr>
<tr>
<td>None (uninfected control)</td>
<td>2.3 (0-4)</td>
</tr>
<tr>
<td>S. ferus 8S1</td>
<td>3.2 (2-4)</td>
</tr>
<tr>
<td>S. ferus HD3</td>
<td>2.7 (0-4)</td>
</tr>
<tr>
<td>S. sobrinus 6715-13</td>
<td>3.3 (2-4)</td>
</tr>
</tbody>
</table>

a Rats were provided ad libitum ground mouse chow 99 (6.4 mg of F^{-1} per ml; 0.17% sucrose; Purina) or caries test diet 2000 (66% sucrose) and deionized water for 56 days or after 28 days were switched from chow to diet 2000 for 56 days.

b Values in parentheses are ranges.

**Acid production.** Figure 4 shows the differences in the rates of pH drop caused by S. ferus HD3 and 8S1 and by S. sobrinus 6715-13. The pH was monitored until it inhibited endogenous metabolism and, consequently, ceased to decline. Spreading suitable dilutions of all cell suspensions onto mitis salivarius agar plates gave no differences in cell viability at the conclusion of the pH experiments (unpublished data).
DISCUSSION

We hypothesized that S. ferus would initiate dental caries in laboratory rats consuming a nonabrasive, sucrose-rich diet which has potentiated the cariogenicity of other mutants streptococci in experimental caries studies. However, although the S. ferus strains colonized the teeth of rats, they did not decay these teeth. Therefore, some factor(s) present in cariogenic strains, such as S. sobrinus 6715-13, was not present or was not expressed in the S. ferus strains. Among the virulence factors associated with cariogenic species are adherence (plaque production), which is dependent on sucrose, the ability to make acid rapidly and to produce a low extracellular pH, and perhaps the ability to produce and store intracellular polysaccharides for later glycolysis. It has been shown that S. sobrinus does not produce or degrade abundant intracellular polysaccharides; thus, this factor may be dispensable for cariogenicity in some strains (10).

S. ferus does produce plaques on smooth surfaces, such as nichrome wires or glass, when it is grown in broth containing sucrose (6). The sucrose-derived extracellular and cell surface-bound polymers of S. ferus have been analyzed and compared with those of S. sobrinus, and these polymers are glucans (unpublished data). Furthermore, the S. ferus strains colonized the teeth of rats in the same pattern as S. sobrinus; that is, they colonized more completely and attained higher populations in animals consuming sucrose. Although S. ferus strains formed less plaque than S. sobrinus, the accumulation of these strains on teeth should have resulted in at least an augmentation of decay if they are truly cariogenic.

Although plaque formation has been shown to be essential for cariogenicity among mutants streptococci (28), many other bacteria adhere to teeth in large numbers but are either not cariogenic or only slightly cariogenic (7, 13). The very cariogenic mutants streptococci, such as S. mutans and S. sobrinus, are distinguished by the rapid, homofermentative production of copious amounts of lactic acid (29). Thus, cariogenicity seems to require both plaque-forming ability and some minimum degree of acidogenicity, as demonstrated also with specific mutants (19; J. D. Hillman, U. S. patent 4, 133, 875, January, 1979). S. ferus did not produce as low a terminal pH as S. sobrinus, and this may account for its lack of cariogenicity. In broth containing 1% glucose, S. ferus produces a pH of 4.6 to 4.8, whereas S. sobrinus can attain a pH of 4.0. Experiments with washed, resting cells of equivalent viabilities suspended in a weak buffer indicated that S. ferus cells produce acid at a slower rate than S. sobrinus. In mouths, slower acid production could be countered more easily by salivary buffers or diffusion or both.

Previously, we regarded the S. ferus strains as "latent" cariogenic streptococci, having virulence potentials genetically present and awaiting an abundance of sucrose to manifest odontolytic properties. Instead, now it appears that, although these strains do have properties in common with cariogenic mutants streptococci (4; Coykendall and Freedman, manuscript in preparation), at least one trait, acidogenicity, has not developed to an extent sufficient to decay teeth. The lack of cariogenicity in S. ferus implies that non-cariogenic, mutants-like streptococci can persist in nature.

Although the increase in the extent of caries both in a particular mouth and in a population as a whole correlates historically with increases in the availability of refined sucrose (2, 18, 26), it does not seem likely that the present virulent species recently evolved to cariogenicity simply under the selective pressure of sucrose (3). This is supported by evidence that (i) mutans streptococci have been found in isolated primitive people (15), (ii) sucrose, which is ubiquitous in plants, was never completely absent from the diet of humans, and (iii) abundant sucrose is not essential for colonization by S. mutans (30, 31). The lack of virulence in S. ferus 881 isolated from rodents indigenous to sugar cane fields is consistent with this.

Our findings show that (i) mutants-like strains, such as S. ferus, are stable and cannot be aroused to virulence by sucrose alone, and (ii) cariogenic streptococci, such as S. sobrinus, remain quiescent in the absence of sucrose, their pathogenic potentials gaining expression from an abundance of that sugar. The question arises as to whether S. ferus strains represent analogs of the progenitor(s) of the cariogenic streptococci.

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LITERATURE CITED


