Inhibition of Ecological Emergence of Mutans Streptococci Naturally Transmitted between Rats and Consequent Caries Inhibition by Streptococcus salivarius TOVE-R Infection

JASON M. TANZER,1,2* ANDREA B. KURASZ,1 AND JONATHAN CLIVE3
Departments of Oral Diagnosis,1 Laboratory Medicine,2 and Behavioral Science and Community Health,3 University of Connecticut Health Center, Farmington, Connecticut 06032

Received 28 January 1985/Accepted 12 April 1985

The ability of Streptococcus salivarius strain TOVE-R to inhibit the ecological emergence of virulent representatives of the most prevalent human mutans streptococci on the teeth of specific pathogen-free Osborne-Mendel rats was studied. Rats which were infected by TOVE-R, or either S. mutans 10449S or S. sobrinus 6715-13WT, or uninfected were transiently co-caged so as to allow natural fecal transfer of organisms due to coprophagy. The infectants were differentially recovered from swabs of the teeth over the time course of the experiments and from sonified teeth at termination. Data were expressed on both relative (percentage) and absolute (CFU) bases. Initial oral colonization of rats by TOVE-R inhibited the ecological emergence of fecally transmitted S. mutans 10449S and S. sobrinus 6715-13WT. There was a generally inverse relationship between the percentages and absolute numbers of TOVE-R and the mutans streptococci on the teeth, which strongly suggested their competition for tooth sites. Absolute numbers of total recoverable flora from the teeth upon sonification were correlated with caries scores, thus suggesting that total recoverable flora counts substantially reflect cavitation status. TOVE-R itself induced no apparent caries activity and its transmission to rats already infected by 10449S or its colonization of rats before 10449S infection inhibited caries induction by this S. mutans strain; similar anticaries effects were not statistically significant for TOVE-R against 6715-13WT in these experiments. These data on the inhibition of the ecological emergence of the mutans streptococci supplement the already reported ability of TOVE-R to preempt initial colonization of teeth and partially displace the colonization of teeth by the mutans streptococci.

Streptococcus salivarius strain TOVE-R, like the mutans group of streptococci, synthesizes alkali-soluble, cell surface-associated glucans from sucrose and sticks to solid surfaces in vitro and teeth in vivo (13, 27, 29, 32, 34). Unlike the mutans streptococci, however, it is not detectably cariogenic in rats (29, 34). TOVE-R-colonized rats are difficult to colonize later by virulent S. mutans strain 10449S (29). Remarkably, when rats were first colonized by 10449S or the virulent S. sobrinus strain 6715-13WT, TOVE-R inoculations partially displace those mutans streptococci and reduce caries (34).

We report here that the initial colonization of rats by TOVE-R inhibits the ecological emergence of natural, fecally transmitted strains 10449S and 6715-13WT from other rats. Initial colonization of rats by TOVE-R thereby inhibits caries associated with mutans infection.

MATERIALS AND METHODS

Microorganisms. Streptomycin-resistant, rough-colony S. salivarius strain TOVE-R (29, 34), S. mutans NCTC 10449S (27, 33), and S. sobrinus strain 6715-13WT (formerly S. mutans serotype dig [4, 6, 23, 27, 32]) were studied. Strains were maintained in a lyophilized state until tested in laboratory animals, and inocula were cultured in fluid thioglycollate medium (Difco Laboratories, Detroit, Mich.) supplemented with 20% (vol/vol) meat extract (Difco) and excess CaCO3. The high virulence of strains 10449S and 6715-13WT in rodents has been described (27, 29, 32–34); both were originally isolated from humans and represent the mutans streptococcal species most frequently isolated from humans (4, 23, 27).

Animals, diets, inoculations, and experimental design. SPFOM (specific pathogen-free Osborne-Mendel) rat breeders colonized by normal rodent gut flora as previously described (27, 31, 32) were maintained in a laminar flow hood with bacteriological air filters (Portable Containment System, PCS-80; Hazellon Systems, Inc., Aberdeen, Md.) and provided with autoclaved chow no. 5010 (Ralston Purina Co., St. Louis, Mo.) and sterile demineralized water. The colony was maintained free of mutans and salivarius streptococcal infection. After weaning at 20 days of age, rats were removed from the laminar flow hood, and several litters were pooled in a large stainless steel cage and provided with caries test diet 2000 (Zeigler Brothers, Inc., Gardeners, Pa.) containing 56% sucrose (16) and sterile demineralized water ad libitum. One day later the rats were randomly distributed, one per cage, and inoculated with test organisms at various times as described in the protocols below.

The design of the experiments is given in Fig. 1. Rats were simultaneously inoculated orally with a micropipette; each animal was either inoculated two times with about 6 × 106 cells (per inoculation) of the mutans streptococci (10449S or 6715-13WT) or TOVE-R grown in the thioglycollate medium or left uninoculated.

After suitable cultures were taken (see below) to confirm that the rat teeth were heavily colonized by the inoculants, mutans-infected rats were transiently co-caged with TOVE-R-infected rats, uninfected rats, or other mutans-infected rats. The ability of Streptococcus salivarius strain TOVE-R to inhibit the ecological emergence of virulent representatives of the most prevalent human mutans streptococci on the teeth of specific pathogen-free Osborne-Mendel rats was studied. Rats which were infected by TOVE-R, or either S. mutans 10449S or S. sobrinus 6715-13WT, or uninfected were transiently co-caged so as to allow natural fecal transfer of organisms due to coprophagy. The infectants were differentially recovered from swabs of the teeth over the time course of the experiments and from sonified teeth at termination. Data were expressed on both relative (percentage) and absolute (CFU) bases. Initial oral colonization of rats by TOVE-R inhibited the ecological emergence of fecally transmitted S. mutans 10449S and S. sobrinus 6715-13WT. There was a generally inverse relationship between the percentages and absolute numbers of TOVE-R and the mutans streptococci on the teeth, which strongly suggested their competition for tooth sites. Absolute numbers of total recoverable flora from the teeth upon sonification were correlated with caries scores, thus suggesting that total recoverable flora counts substantially reflect cavitation status. TOVE-R itself induced no apparent caries activity and its transmission to rats already infected by 10449S or its colonization of rats before 10449S infection inhibited caries induction by this S. mutans strain; similar anticaries effects were not statistically significant for TOVE-R against 6715-13WT in these experiments. These data on the inhibition of the ecological emergence of the mutans streptococci supplement the already reported ability of TOVE-R to preempt initial colonization of teeth and partially displace the colonization of teeth by the mutans streptococci.

Streptococcus salivarius strain TOVE-R, like the mutans group of streptococci, synthesizes alkali-soluble, cell surface-associated glucans from sucrose and sticks to solid surfaces in vitro and teeth in vivo (13, 27, 29, 32, 34). Unlike the mutans streptococci, however, it is not detectably cariogenic in rats (29, 34). TOVE-R-colonized rats are difficult to colonize later by virulent S. mutans strain 10449S (29). Remarkably, when rats were first colonized by 10449S or the virulent S. sobrinus strain 6715-13WT, TOVE-R inoculations partially displace those mutans streptococci and reduce caries (34).

We report here that the initial colonization of rats by TOVE-R inhibits the ecological emergence of natural, fecally transmitted strains 10449S and 6715-13WT from other rats. Initial colonization of rats by TOVE-R thereby inhibits caries associated with mutans infection.

MATERIALS AND METHODS

Microorganisms. Streptomycin-resistant, rough-colony S. salivarius strain TOVE-R (29, 34), S. mutans NCTC 10449S (27, 33), and S. sobrinus strain 6715-13WT (formerly S. mutans serotype dig [4, 6, 23, 27, 32]) were studied. Strains were maintained in a lyophilized state until tested in laboratory animals, and inocula were cultured in fluid thioglycollate medium (Difco Laboratories, Detroit, Mich.) supplemented with 20% (vol/vol) meat extract (Difco) and excess CaCO3. The high virulence of strains 10449S and 6715-13WT in rodents has been described (27, 29, 32–34); both were originally isolated from humans and represent the mutans streptococcal species most frequently isolated from humans (4, 23, 27).

Animals, diets, inoculations, and experimental design. SPFOM (specific pathogen-free Osborne-Mendel) rat breeders colonized by normal rodent gut flora as previously described (27, 31, 32) were maintained in a laminar flow hood with bacteriological air filters (Portable Containment System, PCS-80; Hazellon Systems, Inc., Aberdeen, Md.) and provided with autoclaved chow no. 5010 (Ralston Purina Co., St. Louis, Mo.) and sterile demineralized water. The colony was maintained free of mutans and salivarius streptococcal infection. After weaning at 20 days of age, rats were removed from the laminar flow hood, and several litters were pooled in a large stainless steel cage and provided with caries test diet 2000 (Zeigler Brothers, Inc., Gardeners, Pa.) containing 56% sucrose (16) and sterile demineralized water ad libitum. One day later the rats were randomly distributed, one per cage, and inoculated with test organisms at various times as described in the protocols below.

The design of the experiments is given in Fig. 1. Rats were simultaneously inoculated orally with a micropipette; each animal was either inoculated two times with about 6 × 106 cells (per inoculation) of the mutans streptococci (10449S or 6715-13WT) or TOVE-R grown in the thioglycollate medium or left uninoculated.

After suitable cultures were taken (see below) to confirm that the rat teeth were heavily colonized by the inoculants, mutans-infected rats were transiently co-caged with TOVE-R-infected rats, uninfected rats, or other mutans-infected rats.
rats. Similarly, TOVE-R-infected rats were transiently co-caged with mutans-infected rats or other TOVE-R-infected rats; uninfected rats were co-caged with other uninfected rats or with mutans-infected rats. By this method seven groups of rats (with 9 or 10 animals per group) were formed and given the following designations which describe the initial colonizing organism of the rat and the colonizing organism of the transient cage-mate: mutans-infected/TOVE-R-infected, mutans-infected/uninfected, mutans-infected/mutans-infected, TOVE-R-infected/mutans-infected, TOVE-R-infected/TOVE-R-infected, uninfected/uninfected, and uninfected/mutans-infected. We chose not to study uninfected/TOVE-R-infected and TOVE-R-infected/uninfected groups because the number of rats of exactly the same age was limited and because we knew that uninfected rats challenged by TOVE-R become colonized by this strain but develop no additional (though sometimes slightly fewer) carious lesions (29, 34).

It is well known that rats consume their own feces and that of their cage-mate's, thereby transmitting the streptococcal bacteria between cage-mates and recycling their gut flora by oral reinoculation of fecally cleared bacteria (22, 28). We determined in preliminary experiments that newly co-caged rats 21 to 35 days of age do not reliably share their feces before being co-caged for at least 24 h. This was determined by measuring the oral recovery of streptomycin-tagged, fecally excreted TOVE-R and mutans streptococci colonizing the transient cage-mate. To limit the magnitude of the natural transmission challenge that was incident to co-caging, animals were separated after 48 h and housed singly in freshly autoclaved cages. We know of no way to inhibit the recycling of flora by an animal's ingestion of its own feces without adversely affecting the health of the rats (22).

**Recovery of microorganisms.** The teeth of all animals were swabbed at intervals after inoculation and after separation from cage-mates. The cotton swabs were immediately placed in buffered yeast extract, vortex mixed, and plated on appropriate media (see below) within 1 h by using a spiral plater (Spiral Systems, Cincinnati, Ohio). The problems of sampling plaque from the teeth of small animals and of plate counts of streptococci are well known (27, 28, 32, 34). Previous data had shown that neither TOVE-R nor the mutans streptococci colonize the tongues of rats (34) and that the percentage recoveries of *S. mutans* 10449S monitored by careful swabbing of teeth were not statistically different from the percentage recoveries monitored by directly scraping plaque from the teeth (31). Nonetheless, at the termination of experiments, three molar tooth crowns from one hemimandible of each animal were harvested with a small rongeur as described previously. The plaque on these teeth and bacteria lodged in their fissures were dislodged, and clumps and chains were disrupted by sonification under conditions which gave maximal numbers of total recoverable flora (34).
TABLE 1. Percentage of S. mutans 10449S in competition trial between TOVE-R and 10449S

<table>
<thead>
<tr>
<th>Animal/cage-mate</th>
<th>S. mutans 10449S (%) at day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Uninfected/10449S</td>
<td>18 ± 7b</td>
</tr>
<tr>
<td>10449S/uninfected</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>TOVE-R/10449S</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>10449S/TOVE-R</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>10449S/10449S</td>
<td>43 ± 4</td>
</tr>
</tbody>
</table>

* The animal/cage-mate designation indicates the condition of infection of the animal/the condition of infection of the transient cage-mate. The values are for the "animal" of the animal/cage-mate pairs. They are given as the mean ± standard error of the mean for the percentage of the total recoverable flora which was S. mutans 10449S at swab-sampling intervals after initial inoculation.

b P < 0.01 versus the TOVE-R/10449S group.

RESULTS

In each experiment, seven groups of singly caged rats were studied (Fig. I): (i) uninoculated, transiently co-caged with other uninoculated rats or (ii) with mutants- (either 10449S or 6715-13WT) inoculated rats; (iii) TOVE-R-inoculated, transiently co-caged with other TOVE-R-inoculated rats or (iv) with mutants-inoculated rats; and (v) mutants-inoculated, transiently co-caged with mutants-inoculated, (vi) TOVE-R-inoculated, or (vii) uninoculated rats. Never was (i) an uninoculated rat not co-caged with a mutants-infected rat observed to harbor either streptococci-sensitive or streptomycin-resistant mutants streptococci or S. salivarius or (ii) a rat not exposed to design either TOVE-R or a mutants Streptococcus sp. observed to harbor either of them. Thus, there was no evidence of extraneous or unwanted cross-infection of rats or of reversion of the streptomycin-resistant phenotypes to streptomycin-sensitive ones. There were no statistically significant differences among mean body weight gains of animal groups; thus, there was no evidence of effects of inoculations other than those described below.

Recoveries of TOVE-R and mutants streptococci by tooth swabbing. Uninfected animals had essentially no plaque on their teeth at the time of sacrifice, whereas animals which had been infected by either of the mutants streptococci strains or TOVE-R had generally moderate plaque quantities. However, no differences in quantities of plaque could be photographically demonstrated among the infected rat groups.

In both co-cage competition experiments with strains 10449S (Table 1) and 6715-13WT (Table 2), prior colonization of rats by TOVE-R significantly inhibited the emergence of the mutants streptococci compared with the ecological emergence of the mutants streptococci in previously uninoculated rats (uninfected/mutants versus TOVE-R/mutants). These differences are evident by multiple t-test comparisons at different bacterial recovery sampling dates and by ANOVA (see table footnotes for statistical significance data). In addition, the nonparametric probability of observing these consistent directional differences for the 10449S and 6715-13WT trials is P < 0.02. Thus, colonization by TOVE-R inhibited the emergence of the mutants streptococci in the oral ecology.

By contrast, the relative recoveries (percentages) of strain 10449S from uninoculated rats by 10 days after co-caging (20 days after initial inoculation) with 10449S-infected rats (uninfected/10449S) were lower (P < 0.05) than from 10449S/uninfected and 10449S/10449S rats. However, by 25 days after co-caging the relative recoveries were not statistically different from those percentages recovered from rats.
initially colonized by 10449S (i.e., 10449S/uninfected, 10449S/TOVE-R, and 10449S/10449S), and this lack of difference persisted (Table 1). Analogously, the percentages of strain 6715-13WT recovered from uninfected rats by 9 days after co-caging (15 days after initial inoculation) with 6715-13WT-infected rats (uninfected/6715-13WT) were not statistically different from those percentages recovered from rats initially colonized by 6715-13WT (i.e., 6715-13WT/uninfected, 6715-13WT/TOVE-R, and 6715-13WT/6715-13WT), and this lack of difference also persisted throughout the experiment. The lower percentage of 10449S and 6715-13WT in animals first colonized by TOVE-R, therefore, cannot be explained as the result of the shortened duration of exposure to these strains.

Thus, mutans streptococcal infection after TOVE-R colonization inhibited the relative emergence of both S. mutans 10449S and S. sobrinus 6715-13WT in the oral flora.

The mean percentage of TOVE-R among the total recoverable flora was lower in animals first colonized by strain 10449S (Table 3) or 6715-13WT (Table 4). The nonparametric probability of observing such directional differences is $P < 0.02$ for these experiments. Thus, although TOVE-R, like the mutans streptococci, was transmitted by co-caging, it achieved lower relative levels if animals had first been colonized by the mutants streptococci, suggesting that TOVE-R colonization of the teeth occurred in competition with mutants streptococci already colonizing them.

**Table 3.** Percentage of *S. salivarius* TOVE-R in competition trial between TOVE-R and 10449S*

<table>
<thead>
<tr>
<th>Animal/cage-mate</th>
<th><em>S. salivarius</em> TOVE-R (%) at day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>TOVE-R/TOVE-R</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>TOVE-R/10449S</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>10449S/TOVE-R</td>
<td>4 ± 3</td>
</tr>
</tbody>
</table>

*For an explanation of data, see Table 1, footnote a.

*b* $P < 0.01$ versus the 10449S/TOVE-R group.

*c* $P < 0.05$ versus the 10449S/TOVE-R group.

Recoveries of TOVE-R, mutants streptococci, and total recoverable flora by sonification of teeth. Although many comparisons could be described statistically for the two illustrated experiments, because of their complexity only certain key contrasts are presented here.

For both the 10449S and 6715-13WT trials, terminal recoveries (on days 41 and 50, respectively) of the mutants streptococci and TOVE-R upon sonification of the crowns of three mandibular molars (Fig. 2 and 3) paralleled the percentage of recoveries obtained by swabbing the teeth on that same date (Tables 1 through 4). Recoveries of the two mutants streptococci from rats initially infected by them were very high, significantly higher on both absolute and relative bases than recoveries from rats first infected by TOVE-R ($P < 0.05$; determined by ANOVA). The nonparametric probability of this consistent directional difference was $P < 0.02$ for both experiments.

Some additional specific contrasts should be noted. For the 10449S study, the absolute and relative numbers of 10449S recovered from uninfected/10449S, 10449S/uninfected, 10449S/TOVE-R, and 10449S/10449S rats were not different from one another, although they were higher than for TOVE-R/10449S rats ($P < 0.05$). The absolute and relative numbers of TOVE-R recovered from sonified teeth were higher from rats not previously inoculated with 10449S than from rats so inoculated ($P < 0.05$).

It is interesting that the total CFUs recovered from the teeth of rats infected by 10449S, where caries scores were highest (see below), were greater ($P < 0.05$) than the total CFUs recovered from the uninfected/uninfected, TOVE-R/TOVE-R, and TOVE-R/10449S groups. In addition, there was no difference in total CFUs among the groups first

**Table 4.** Percentage of *S. salivarius* TOVE-R in competition trial between TOVE-R and 6715-13WT*

<table>
<thead>
<tr>
<th>Animal/cage-mate</th>
<th><em>S. salivarius</em> TOVE-R (%) at day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>TOVE-R/TOVE-R</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>TOVE-R/6715-13WT</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>6715-13WT/TOVE-R</td>
<td>6 ± 5</td>
</tr>
</tbody>
</table>

*For an explanation of data, see Table 1, footnote a.

*b* $P < 0.05$ versus the 6715-13WT/TOVE-R group.

*c* $P < 0.01$ versus the 6715-13WT/TOVE-R group.
Caries scores for all animal groups initially colonized by strain 6715-13WT were not different from one another (Fig. 5, Table 6). Also, caries scores for the group initially uninfected but later co-caged with 6715-13WT-infected rats (uninfected/6715-13WT) were lower than those for 6715-13WT/uninfected and 6715-13WT/6715-13WT rats. However, caries scores of the rat group initially colonized by TOVE-R and later co-caged with 6715-13WT-infected animals (TOVE-R/6715-13WT) were lower than those of the three groups of rats initially colonized by 6715-13WT but not significantly lower than those of the uninfected/6715-13WT rats. Scores for uninfected/uninfected and TOVE-R/TOVE-R rats were not different from each other. Therefore, initial and longer-duration infection by 6715-13WT induced higher caries scores than if infection by 6715-13WT had occurred later and for a shorter duration. Prior colonization by TOVE-R was not detectably protective against caries associated with later colonization by 6715-13WT.

**DISCUSSION**

These data demonstrate that initial oral colonization of rats by *S. salivarius* TOVE-R inhibits the ecological emergence of fecally transmitted *S. mutans* 10449S and *S. sobrinus* 6715-13WT; conversely, prior colonization by these mutants streptococci probably inhibits the ecological emergence of fecally transmitted TOVE-R. There is a generally inverse relationship between the percentages of TOVE-R and the mutants streptococci on the teeth. The data support previously published evidence that these bacteria compete for the same tooth-surface ecological sphere (29, 34). Other studies have shown that TOVE-R, like the mutants streptococci, colonizes the teeth, but not the tongues, of rats (34).

Previous studies of preemptive colonization of teeth by TOVE-R and of displacement of mutants streptococci from teeth by TOVE-R have used oral inoculations of known numbers of cells (29, 34). The present study, employing natural, fecal inoculation resulting from co-caging, provides an intense, although difficult to quantitate, infectious challenge with fecal impaction into the fissures of the teeth probably occurring several times over the course of 2 days. This technique thus tests the effect of TOVE-R on the ecological emergence of mutants streptococci in a model in which transmission of microorganisms is virtually assured.

Oral inoculation with a pipette of both TOVE-R (29) and weakly virulent *S. mutans* mutant 805 (31) and their coloni-
INHIBITION OF MUTANS STREPTOCOCCI BY TOVE-R

zation of the teeth inhibits subsequent implantation of carefully quantitated numbers of inoculated 10449S. However, from this preemptive-inoculation model one cannot ascertain possible anticaries effects; the periods of cariogenic challenge by strain TOVE-R or 805, by strain 10449S, and by the indigenous non-mutans, non-salivarius flora of the rats are all different, making groups incomparable as to consequent caries induction. The present co-caging studies substantially overcome this problem because rats of the same age with the same indigenous flora are simultaneously inoculated with either TOVE-R or mutans streptococci shortly after the cariogenic diet is presented. In addition, co-caging and the consequent coprophagous sharing of flora produces additional animal groups challenged for a shorter time with either the mutans streptococci or TOVE-R and allows tests of whether TOVE-R inhibits the ecological emergence of the mutans streptococci. It must be recognized that the inception of most carious lesions in rats, as in humans, occurs in the early period after the teeth erupt and the bacteriological-dietary challenge to develop decay is imposed (5, 10). Thus,
TABLE 5. Multiple statistical comparisons of total caries scores for each of seven rat groups in the co-cage competition trial of TOVE-R and 10449S depicted in Fig. 4a

<table>
<thead>
<tr>
<th></th>
<th>T/T</th>
<th>U/U</th>
<th>T/10449</th>
<th>U/10449</th>
<th>10449/T</th>
<th>10449/10449</th>
<th>10449/U</th>
<th>10449/10449</th>
<th>U/10449</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U/U</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/10449</td>
<td>U/10449</td>
<td>10449/T</td>
<td>10449/10449</td>
<td>10449/U</td>
<td>10449/10449</td>
<td>U/10449</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Statistical significance is indicated by * at the P < 0.05 level, using the least-significant-difference method to isolate differences revealed by ANOVA. Although there were significant differences at P > 0.01, they are not reported in this table. Abbreviations: T, TOVE-R-infected; U, uninfected; 10449, 10449S-infected. Column and row designations represent the condition of infection of the rats/the condition of infection of the transient cage-mates. The groups are arrayed, from top to bottom and from left to right, in the order of increasing total caries scores.

sucrose-eating rats infected by the mutants streptococci soon after weaning develop the highest caries scores, and later infection or sucrose provision induces fewer carious lesions, as seen here. However, rats infected by TOVE-R develop no higher caries scores than animals harboring their normal mutans- and salivarius-free flora. Thus, TOVE-R again (29, 34) was observed not to be detectably cariogenic.

Rats infected first by *S. mutans* 10449S and then by naturally transmitted TOVE-R developed fewer carious lesions than if they had not been exposed to TOVE-R. This caries-protective effect was not evident in the 6715-13WT experiment reported here, a failure perhaps due to two factors. In the 6715-13WT trial, animals ate diet 2000 for 51 days and were heavily infected by the highly virulent *S. sobrinus* strain, in addition to harboring their indigenous fermentative flora (27), for 50 days before sacrifice. Also the Keyes’ caries-scoring technique requires that damaged tooth surfaces be scored as carious. Thus, a smooth tooth surface which evidences a cusp fracture or carious involvement due to extension of sulcal caries is nonetheless scored as carious. One cannot be sure whether the caries process originated in the sulcus or on the smooth surface; one can only observe that such a surface is missing or carious (and thus score it as carious). Because of this scoring artifact and the duration of this particular 6715-13WT trial, base-line fissure and smooth-surface caries scores were unusually high, even for uninfected and TOVE-R-infected animals. This perhaps obscured the caries-protective effects of TOVE-R against 6715-13WT that were observed in the 10449S experiment. In fact, TOVE-R protection against caries by displacement of 6715-13WT and 10449S was previously reported (34).

There has been uncertainty in the literature about whether to report percentage recoveries of bacteria from teeth or absolute numbers. The latter, of course, requires extraction of the teeth or jaws and thus the termination of experiments. However, total bacterial recoveries reported in absolute CFUs were suggested to be a reflection of the state of cavitation of the teeth, with the cavities mechanically accommodating more bacteria as though they were additional tooth fissures (34). For our 10449S experiment, the correlation between caries score and total recoverable flora at the time of sonification of teeth was $r = 0.489, P < 0.005$; for the 6715-13WT experiment it was $r = 0.340; P < 0.005$. Thus, total recoverable flora counts are correlated with the caries score, independent of mutans streptococcal infection. It is also clear, however, that the groups of animals with the highest caries scores are those first infected by the mutants streptococci (10449S or 6715-13WT).

It is not surprising that absolute counts of strain 10449S or 6715-13WT recovered from teeth at termination of the experiments do not correlate with caries scores for those same mutans-infected animals because most lesions begin to develop soon after the bacteriological-dietary cariogenic challenge (5, 10). Also, because caries scores for mutans-infected animals and bacterial recoveries from them are rather similar within an experiment, they do not allow spread of the data, thus diminishing the likelihood of detecting correlations of statistical significance. Therefore, counts at the time of sacrifice may not so much reflect causation of disease as they do the eventual dimension of the lesions.

The principal cause of human coronal caries appears to be infection by the mutants streptococci (7, 9, 11, 12, 20), the transmission of which frequently occurs from female to child after the first teeth erupt into the oral cavity (1, 3, 17, 18, 21). No practical ways have yet been demonstrated to suppress or cure such infections (8, 18, 35–37).

The presently reported inhibition by TOVE-R of the ecological emergence in rats of the two mutants streptococcal species most frequently isolated from humans, the previously reported successful preemption of *S. mutans* infection by TOVE-R colonization (29), and the previously reported displacement of virulent *S. mutans* and *S. sobrinus* strains from rats and consequent caries inhibition (34) suggest that TOVE-R or similar microorganisms may have important human clinical therapeutic utility. Initial human studies along these lines are under way. Studies of the mechanistic bases for the lack of cariogenicity of TOVE-R and its ability to successfully compete with the mutants streptococci have also begun. There has been a long and newly revived interest in the inhibition of various bacterial infections by analogous bacterial competitive means (2).

**ACKNOWLEDGMENT**

This study was supported by Public Health Service grant DE-03758 from the National Institute of Dental Research.

The authors thank John Richi and Lynn Laskowski for excellent technical support.

**LITERATURE CITED**

INHIBITION OF MUTANS STREPTOCOCCI BY TOVE-R

83