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Competitive Displacement of Mutans Streptococci and Inhibition of Tooth Decay by *Streptococcus salivarius* TOVE-R

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The ability of *Streptococcus salivarius* TOVE-R to displace virulent representatives of the most prevalent human mutans streptococci from the teeth of rats, and thereby to inhibit caries, was studied. *Streptococcus mutans* 10449S- or *Streptococcus sobrinus* 6715-13WT-infected specific-pathogen-free rats consuming a high-sucrose diet were inoculated by TOVE-R. The infectants were differentially recovered from swabs of the teeth over the time course of infection and from sonically treated material of extracted teeth and excised tongues. Despite initial colonization of the teeth by the mutans streptococci, TOVE-R colonized the teeth, unlike other essentially nonvirulent plaque formers already described. It did not colonize the tongues of the rats. TOVE-R emerged and persisted as a prominent member of the plaque ecology. There was an associated decline in the mutans streptococci on the teeth, and this decline was associated with significant inhibition of the caries component attributable to 10449S infection (56%) and to 6715-13WT infection (52%). TOVE-R did not reliably inhibit the component of fissure caries attributable to the nonmutans indigenous flora of the rats. TOVE-R itself induced no detectable decay. The data suggest the potential therapeutic utility of TOVE-R to inhibit caries by displacement of mutans streptococci from the teeth. These results supplement the already reported ability of TOVE-R to preempt initial colonization of teeth by the mutans streptococci.

The so-called mutans streptococci, whose most prevalent human isolates have officially been redesignated *Streptococcus mutans* and *Streptococcus sobrinus* (formerly *S. mutans* serotype c and *S. mutans* serotype d/g, respectively [6]), are, in all likelihood, the principal cause of decay of the crowns of human teeth (7, 9, 11, 12, 21). Transmission of this infection among humans often occurs early in life, most frequently from the female to her offspring (1, 3, 18, 19, 22), and transmission of infection is an antecedant of the development of human tooth decay in childhood and adolescence (1, 12, 18, 19). Once colonization of the host by the mutans streptococci has occurred, no way has yet been described to cure the infection or to suppress it other than transiently, without continuous treatment with antibiotics, topical antiseptics, or virtually absolute avoidance of sucrose consumption. Such conditions are not readily achievable by the vast majority of the population (8, 19, 34–36).

Rodents infected by the mutans streptococci have been productively used to study the microbial, dietary, and host factors of human dental caries (for a review, see reference 28). Certain mutants of the mutans streptococci can colonize surfaces of teeth of rodent caries models consuming a sucrose-rich diet without inducing significant decay (13, 31, 33). In some cases these variants not only compete with the indigenous nonmutans oral flora of rats, and thus assume ecological stability and persistance (31, 33), but also preempt colonization of tooth loci by virulent mutans streptococci (31). Nonetheless, the virulent mutans streptococci are apparently not displaceable from the teeth by their nonvirulent mutants, at least in the case in which this has been reported; thus, the first mutans streptococcus to colonize teeth appears to win the ecological competition for mutans-available tooth sites (31).

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Like the mutans streptococci (27, 32), TOVE-R, a rough colonial variant of *Streptococcus salivarius*, has remarkable abilities to effect sucrose-dependent colonization of solid surfaces both in vitro and on teeth in vivo (29) and to synthesize water-insoluble glucans from sucrose (14). Unlike the mutans streptococci (27, 32), TOVE-R is unable to decay the teeth of specific-pathogen-free Osborne Mendel rats. If such rats are first inoculated by TOVE-R, it then is difficult for *S. mutans* to colonize their teeth (29).

This paper describes the ability of strain TOVE-R to displace both *S. mutans* and *S. sobrinus* from the teeth of rats and thereby to inhibit tooth decay.

MATERIALS AND METHODS

Microorganisms. Streptomycin-resistant, colonially rough *S. salivarius* TOVE-R (29), *S. mutans* NCTC 10449S (27, 33), and *S. sobrinus* 6715-13WT (27, 32) were studied. Strains were maintained in lyophilized state until tested in laboratory animals, and inocula were cultured in fluid thioglycolate medium (Difco Laboratories, Detroit, Mich.) supplemented with 20% (vol/vol) meat extract (Difco) and excess $CaCO_3$. The high virulence of 10449S and 6715-13WT in rodents has been previously described (27, 29, 32); both were originally isolated from humans and represent the most frequent mutans streptococcal species isolated from humans (4, 23, 27).

Animals, diets, inoculations, and experimental design. Specific-pathogen-free Osborne Mendel breeding rats that were colonized by normal rodent gut flora, as previously detailed (27, 31, 32), were maintained in a laminar flow hood with bacteriological air filters (portable containment system PCS-80; Hazelton Systems, Inc., Aberdeen, Md.) and provided with autoclaved chow 5010 (Ralston-Purina, St. Louis, Mo.) and sterile demineralized water. The colony had been

maintained free of mutans and salivarius streptococcal infection. After weaning at 20 days of age, experimental rats were removed from the laminar flow hood, and several litters were pooled in a large stainless steel cage and thenceforth provided caries test diet 2000 (Zeigler Brothers, Gardeners, Pa.) containing 56% sucrose (17) 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 detailed in the protocols below.

The design of the experiment with 10449S is given in Fig. 1a; there were 12 or 13 animals per group. The design of the experiment with 6715-13WT is given in Fig. 1b; there were nine animals per group. Rats were inoculated orally by micropipette with about 6×10^8 cells of either of the mutans streptococci (10449S or 6715-13WT) grown in the thioglycolate medium or were uninoculated.

Either 8 days later (in the case of the 10449S experiment) or 7 days later (in the case of the 6715-13WT experiment) half of the previously uninoculated and half of either the 10449S- or 6715-13WT-infected rats were orally inoculated with about 6×10^8 cells of TOVE-R grown in thioglycolate medium. The latter inoculation was repeated twice.

Recovery of microorganisms. The teeth of all animals were swabbed at various intervals after inoculation. Recovery dates were given the notation: number of days after mutans inoculation/number of days after TOVE-R inoculation, and are so designated in figures; thus, 25/18 denotes 25 days after mutans inoculation and 18 days after TOVE-R inoculation. The cotton swabs were immediately placed in buffered yeast extract, vortex mixed, and plated on appropriate media (see below) within 1 h, 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). Previous data show that percent recoveries of *S. mutans* 10449S monitored by careful swabbing of teeth were not statistically different from percent 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 by means of a small rongeur. The plaque on them and bacteria trapped in their fissures were dislodged, and clumps and chains were disrupted by a model W185 sonic cell disrupter (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) by using its microtip for 5 s at 80 to 90 W. Sonification was carried out in 9 ml of buffered yeast extract contained in test tubes (18 by 150 mm) with the tip of the oscillating probe placed halfway into the depth of the fluid. These conditions had been demonstrated to give maximal recoveries of total recoverable flora.

Typical *S. salivarius* is known to colonize the tongue epithelium of humans in greater proportions than their teeth. Therefore, in one experiment tongues as well as teeth were excised and similarly sonified and cultured for evaluation of absolute numbers as well as relative numbers (percentages) of 10449S and TOVE-R among the total recoverable flora.

Both swabbed and sonified cultures were plated on mitis salivarius agar containing Chapman tellurite (MS) (Difco), MS supplemented with 200 μg of streptomycin per ml, and Trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.). The very large TOVE-R colonies grew rapidly on all the agars, both in the presence and absence of CO_2 atmospheric supplementation. The relatively small colonies of *S. mutans* 10449S and *S. sobrinus* 6715-13WT grew well, but only with CO_2 atmospheric supplementation. They had typical "mutans" morphology on MS and MS supplemented with streptomycin. CFU counts on Trypticase soy agar supplemented with sheep blood were used for estimation of the total recoverable facultative flora; CFU on MS supplemented with streptomycin allowed quantitation of the mutans streptococci and of TOVE-R. Thus, recoveries of the streptomycin-labeled TOVE-R, 10449S, and 6715-13WT infectants in swab samples could be differentially quantitated and expressed as percentages of total flora, but because swab samples varied in size, absolute counts could not be computed. In sonified tooth and tongue samples counts could be expressed in both relative and absolute terms because the entire tongue surface and teeth were sampled. Also, the presence of possible non-streptomycin-resistant streptococcal infectants could be detected on MS. Periodically, the identities of selected reisolates were confirmed by well-established biochemical-physiological and morphological techniques (30).

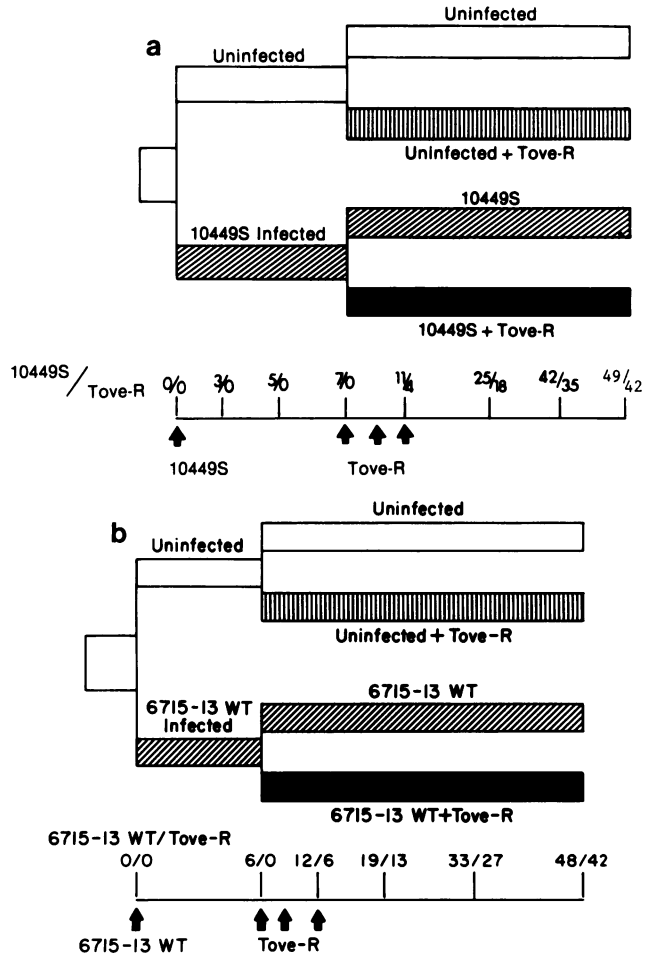

FIG. 1. Experimental designs for study of displacement of *S. mutans* 10449S (a) and *S. sobrinus* 6715-13WT (b) by TOVE-R. The calendar at the bottom of each figure begins with the mutans streptococcus inoculation day. The arrows indicate the dates of inoculations with 10449S, 6715-13WT, or TOVE-R. Oral cultures were taken, as described in the text, with reference to the number of days elapsed since mutans/TOVE-R inoculation. For example, 0/0 signifies culture before both 10449S (or 6715-13WT) inoculation and TOVE-R inoculation; 25/18 signifies culture 25 days after 10449S inoculation and 18 days after TOVE-R inoculation, etc., until termination of the experiments with 10449S and 6715-13WT at 49/42 and 48/42 days, respectively.

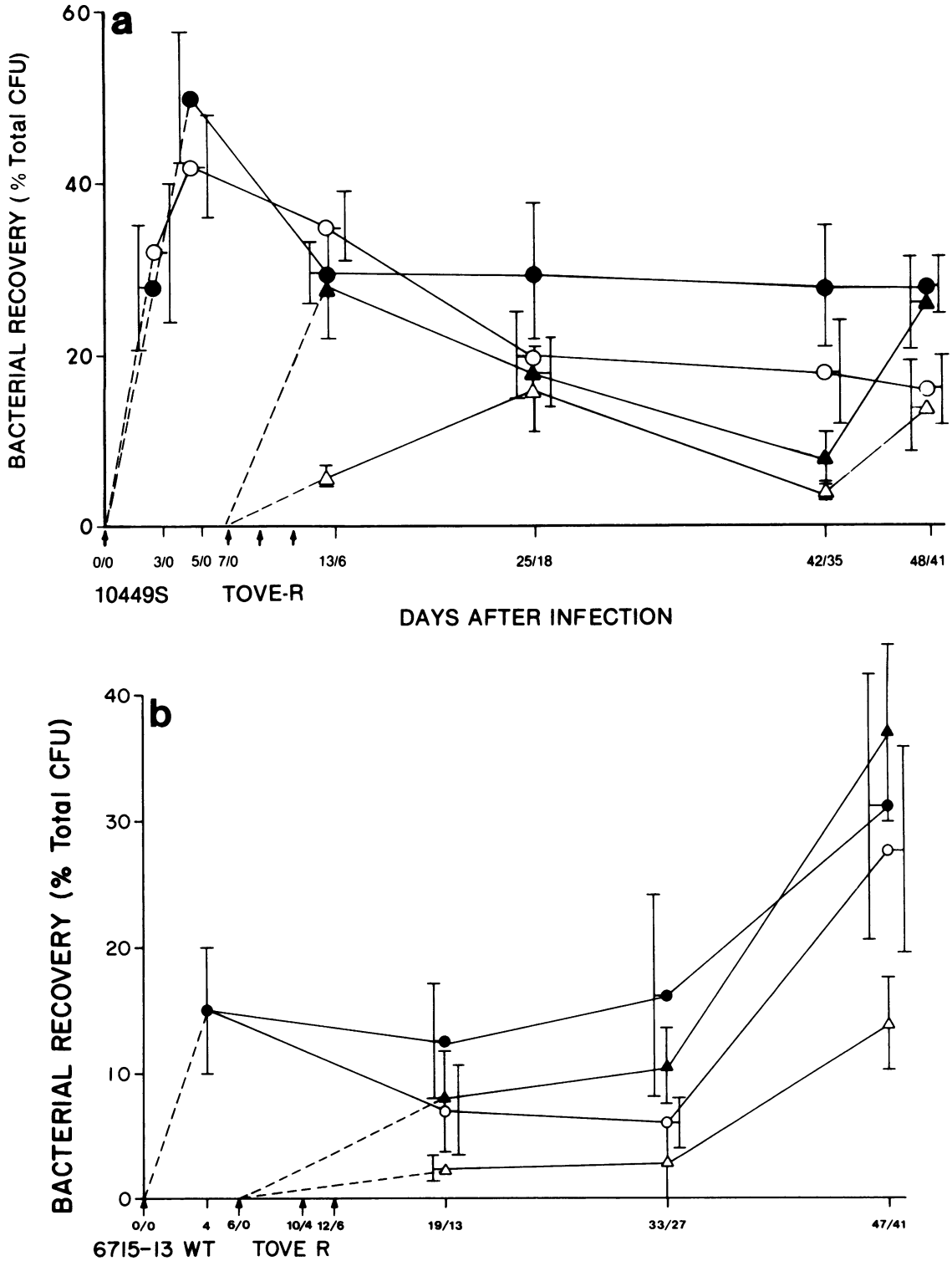

FIG. 2. Mean recoveries by tooth swabs of mutans streptococci and of TOVE-R as percentages of the total recoverable flora (± standard error of the mean). Those rats not inoculated by either mutans streptococci or TOVE-R had negative recoveries of these streptomycin-resistant infectants and negative recoveries of non-streptomycin-resistant extraneous infectants; thus, the data for these animals are not plotted. The inoculation and sampling dates are recorded on the *x* axis and are expressed in number of days after mutans inoculation/number of days after TOVE-R inoculation, as described in the text. Symbols (a): ●, 10449S recoveries from rats inoculated by 10449S only; ○, 10449S recoveries from rats first inoculated by 10449S, then by TOVE-R; ▲, TOVE-R recoveries from rats inoculated by TOVE-R only; △, TOVE-R recoveries from rats first inoculated by 10449S, then by TOVE-R. Symbols (b): ●, 6715-13WT recoveries from rats inoculated by 6715-13WT only; ○, 6715-13WT recoveries from rats first inoculated by 6715-13WT, then by TOVE-R; ▲, TOVE-R recoveries from rats inoculated by TOVE-R only; △, TOVE-R recoveries from rats first inoculated by 6715-13WT, then by TOVE-R.

Statistical analyses of recoveries were carried out with percentage data arcsin transformed to meet the requirements of the parametric analysis of variance and *t* test (26). Data were also analyzed by the nonparametric Dixon-Mood sign test (25). Recoveries of more than 0 but less than 1% were treated as though they were 1%. The numbers of absolute bacterial CFU were analyzed statistically without transformation.

Plaque and caries evaluation. Excised, coded hemimandibles with teeth in situ were viewed microscopically for plaque presence after staining with 0.25% aqueous safranin for 20 s. They and the similarly coded maxillas were defleshed by dermestid beetles and scored for carious enamel areas by the method of Keyes (16), as modified by Larson (20). Because carious lesions are quite symmetrically represented bilaterally (15), the caries score for the molars of a single hemimandible was doubled before adding it, for each animal, to the score for its maxillary molar teeth. Statistical evaluations of mean caries scores were carried out by analysis of variance (26).

RESULTS

In each experiment, four groups of singly caged rats were compared (Fig. 1): those uninoculated; those inoculated by TOVE-R; those inoculated by a mutans streptococcus, either 10449S or 6715-13WT; and those inoculated by either of these mutans streptococci plus TOVE-R. Never was (i) an uninoculated rat observed to harbor either streptomycin-susceptible or streptomycin-resistant mutans streptococci or *S. salivarius* or (ii) a rat not inoculated by either TOVE-R or a mutans streptococcus observed to harbor them. Thus, there was no evidence of extraneous or cross-infection of rats or reversion of the streptomycin resistance phenotypes to streptomycin-sensitive ones. There were no statistically significant differences among mean body weight gains of animal groups, and thus, there was no evidence of effects of inoculations other than those described below.

Recoveries of TOVE-R and mutans streptococci by tooth swabbing. Uninoculated animals had essentially no plaque on their teeth at the time of sacrifice, whereas those which had been inoculated had generally moderate plaque quantities. However, no differences in quantities of plaque could be photographically demonstrated among the TOVE-R only, mutans plus TOVE-R, and mutans only groups.

The mean percentage of TOVE-R in the total recoverable flora increased more slowly in animals previously inoculated with mutans streptococci than in previously uninoculated animals (Fig. 2a and b) and was at every sampling date lower in those animals first inoculated by the mutans streptococci. The nonparametric probability of observing this consistent difference was $P < 0.02$. By the parametric analysis of variance, this difference was also highly significant ($F = 19.03$; $P < 0.005$). This suggested that TOVE-R colonization of the teeth occurred in competition with mutans streptococci already colonizing them and that TOVE-R may, in fact, displace at least some of the mutans streptococci already established in the ecology.

In correlary fashion, for the doubly infected animals the percentage of 10449S or 6715-13WT among the total recoverable flora on tooth swabs became lower in those animals that had been superinfected by TOVE-R (Fig. 2a and b). Thus, by the second recovery date after TOVE-R inoculation in the illustrated 10449S trial (25/18), 10449S was lower in doubly infected than in singly infected rats and remained so; by the first recovery date after TOVE-R inoculation in the 6715-13WT trial (19/13), 6715-13WT was lower in doubly

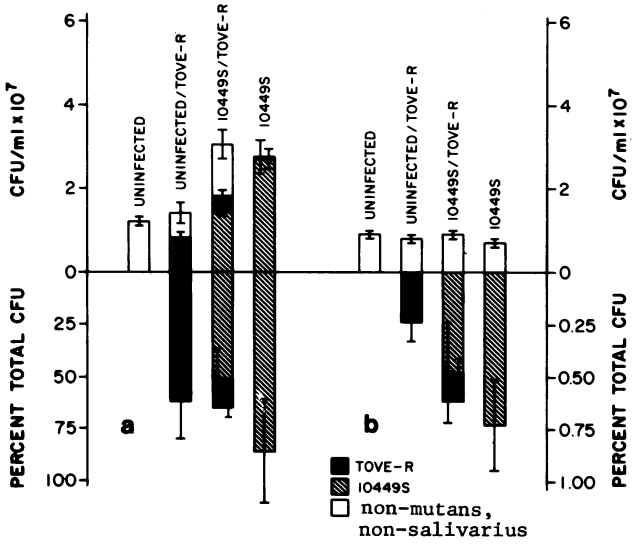

FIG. 3. Histogram of absolute CFU count recoveries for total recoverable flora, 10449S, and TOVE-R as well as relative (percent) recoveries, plotted as mean ± standard error of the mean at day 49/42 for the 10449S/TOVE-R experiment. The absolute CFU counts are above the horizontal and the relative numbers are below the horizontal. (a) Data for the three sonified mandibular molars of the rats of the infected experimental groups; (b) data for the sonified tongues of the same experimental groups. Because uninoculated rats gave no recoveries of these or other mutans or salivarius streptococci, no data for such rats are presented.

infected than in singly infected rats, but this difference was not impressive at the last swabbing date (47/41). Nonetheless, the consistency of these mutans streptococcal recovery percentages for the doubly infected compared with singly infected animals was impressive. The nonparametric probability of observing this consistent difference is $P < 0.02$; by the parametric analysis of variance, this difference was also highly significant ($F = 6.98$; $P < 0.01$). Thus, there was strong evidence that TOVE-R, in fact, partially displaced the two most prevalent mutans streptococcal types from the teeth.

This phenomenon was independent of whether 10449S or 6715-13WT was used as the mutans infectant ($F = 0.007$) and was perhaps even more impressive because the levels of colonization (i.e., percentage) achieved by 10449S and 6715-13WT were different from each other ($F = 6.427$; $P < 0.01$). Nonetheless, the data suggested that 6715-13WT by the last sampling date (47/41) was overcoming the displacement effect of TOVE-R ($t = 0.3063$; $P > 0.05$).

Recoveries of TOVE-R, mutans streptococci, and total recoverable flora by sonification of teeth and tongues. For the 10449S experiment, sonification of crowns of three mandibular molar teeth at day 49/42 gave recoveries of microorganisms (Fig. 3a) which paralleled those from the swab samples of the teeth at day 48/41 (Fig. 2a.). Thus, 10449S recoveries from 10449S-infected rats were very high and significantly higher that those from 10449S/TOVE-R-inoculated animals ($P < 0.005$), both on absolute count and percentage bases. TOVE-R recoveries from sonified teeth similarly paralleled those from swabs of teeth 1 day earlier and were significantly higher ($P < 0.01$) from rats previously not inoculated by 10449S.

Recoveries of both 10449S and TOVE-R from sonified tongues averaged less than 1% of the total recoverable flora for all groups (Fig. 3b). Clearly, TOVE-R, like the mutans

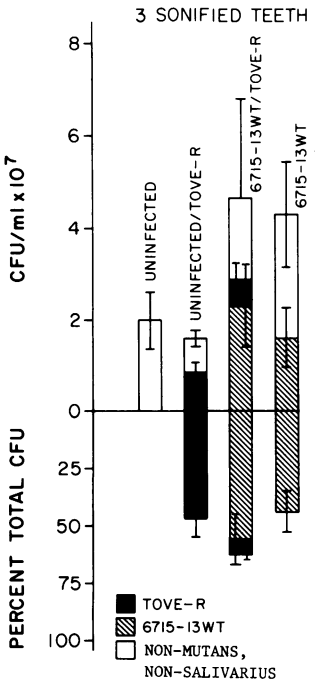

FIG. 4. Histogram of absolute CFU count recoveries for total recoverable flora, 6715-13WT, and TOVE-R as well as relative (percent) recoveries, plotted as mean ± standard error of the mean at day 48/42 for the 6715-13WT/TOVE-R experiment. The absolute CFU counts are above the horizontal, and the relative numbers are below the horizontal. Because uninoculated rats gave no recoveries of these or other mutans or salivarius streptococci, no data for such rats are presented.

streptococci, heavily colonized the teeth but not the tongue of rats.

It was of interest that the absolute number of total CFU recovered from the teeth, but not the tongues, of the two 10449S-infected groups, in which caries scores were highest (see below), was greater than that number from either of the groups not infected by 10449S ($P < 0.01$). However, there was no difference in total CFU between either the two 10449S-infected groups or the two groups not infected by the *S. mutans* strain.

For the 6715-13WT experiment, the recoveries from sonicates of the three molars of one hemimandible at day 48/42 (Fig. 4) confirmed that the displacement of 6715-13WT by TOVE-R, as revealed by tooth swabbing at day 47/41, was being overcome at the end of the trial. Thus, there was no statistically significant difference between the absolute or relative numbers of 6715-13WT between the 6715-13WT-infected and the 6715-13WT/TOVE-R-infected groups. Although the absolute CFU counts of TOVE-R were not different from sonicates of the molars of uninfected/TOVE-R-infected rats compared with 6715-13WT/TOVE-R-infected rats, on a percentage basis TOVE-R constituted a higher proportion ($P < 0.01$) of the total recoverable flora in the tooth sonicates from animals not previously infected by 6715-13WT.

As with the 10449S experiment, the numbers of total CFU recovered from the teeth of the two 6715-13WT-infected groups, in which caries scores were highest (see below), were greater than those numbers from either of the groups not infected by 6715-13WT ($P < 0.01$). There was, however, no statistically significant difference between the total CFU of the two 6715-13WT-infected groups or between the two groups not infected by this strain.

Caries scores. The caries scores for the two experiments are plotted (Fig. 5a and b) such as to discriminate between sulcal (fissure) and smooth (buccal, lingual, approximal, and morsal) tooth surfaces (24) because of the well-known tendency of smooth-surface caries to be essentially mutans dependent and of sulcal caries not to be mutans dependent, but to be augmented by the mutans streptococci (27).

As expected, infection by either 10449S or 6715-13WT alone induced dramatically higher caries scores than in the uninfected control rats harboring their indigenous mutans- and *S. salivarius*-free floras.

Inoculation of mutans-infected rats by TOVE-R was associated with approximately 30 and 23% reductions of absolute mean caries scores for the 10449S and 6715-13WT experiments, respectively ($F = 53.38$, $P < 0.005$). TOVE-R infection without mutans streptococcal infection was associated with caries scores no higher than those of the uninfected animals harboring their mutans-free, *S. salivarius*-free indigenous oral flora. In fact, in the 10449S trial, TOVE-R infection significantly ($P < 0.05$) reduced fissure caries scores by comparison with those uninfected animals harboring their indigenous nonmutans flora; this same comparison was not statistically significant for the 6715-13WT trial, although the data suggested the same effect.

If one adjusts mean caries scores of singly (mutans only) and doubly (mutans plus TOVE-R) infected rat groups for the mean level of caries of the uninfected control rats, one can compute the reduction of mutans-induced caries caused by TOVE-R infection by the following equation: % reduc-

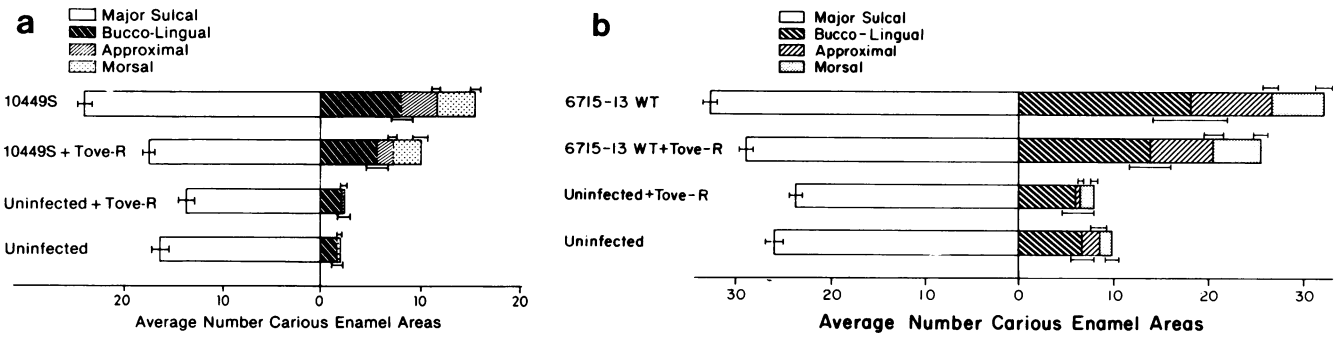

FIG. 5. Average number of carious enamel areas of specific-pathogen-free Osborne Mendel rats infected by mutans streptococci alone, mutans streptococci plus TOVE-R, TOVE-R alone, or uninfected rats for experiments with *S. mutans* 10449S (a) or *S. sobrinus* 6715-13WT (b). The mean and standard error of the mean are plotted.

tion of mutans-induced caries = 1 − (singly infected − control) − (doubly infected − control)/(singly infected − control) × 100.

By this calculation, TOVE-R reduced caries associated with 10449S infection by 56% ($P < 0.001$) and caries associated with 6715-13WT infection by 52% ($P < 0.01$).

No evidence of alveolar bone loss or of root surface caries was noted for any animal.

DISCUSSION

These data demonstrate that *S. salivarius* TOVE-R competes with *S. mutans* NCTC 10449S and *S. sobrinus* 6715-13WT for tooth surface sites. The emergence of TOVE-R in the oral ecology of rats is accompanied by concomitant partial displacement of both of the virulent mutans streptococci.

The emergence of TOVE-R in the oral cavity of rats is inhibited but is not blocked if the teeth of the rats are first colonized by either 10449S or 6715-13WT. This is distinct from the complete inhibition of establishment of *S. mutans* mutant 805 in rats previously colonized by its parent strain, 10449S (31).

There is a significant reduction of caries experience of rats when infection by the mutans streptococci is followed by inoculation with TOVE-R. This reduction is the more impressive because the inception of most carious lesions for rats, like for humans, occurs in the early period after the teeth erupt and the bacteriological and dietary challenge to develop decay is imposed (5, 10, 28). In these experiments, rats were infected by 10449S and 6715-13WT for 7 and 6 days, respectively, soon after weaning and consumed the high-sucrose experimental diet before initial TOVE-R inoculation. These conditions are known to give maximal ecological emergence of mutans streptococci and lead to induction of severe caries (27–33), as confirmed herein.

Although the two mutans streptococci are potently cariogenic, TOVE-R is not detectably cariogenic in this animal model, as previously reported (27, 29, 32, 33). It is notable that TOVE-R inoculation of uninfected rats decreased fissure caries activity in the 10449S trial, although this decrease was not statistically significant in the 6715-13WT trial. These data suggest that TOVE-R may also competitively inhibit caries induced by the indigenous nonmutans flora of the rats.

Tooth swab and sonification data assessing bacterial recoveries were parallel in these studies. Because recoveries of TOVE-R from the tongues were very low, like those of 10449S, one cannot ascribe their percentages in tooth swab data to tongue surface contamination of swabs; rather, it is more likely that the TOVE-R and mutans recoveries from tongues reflect organisms shed from the teeth. TOVE-R does not appear to colonize the tongues of rats.

The absolute numbers of total recoverable flora from sonified teeth of mutans-infected rats were higher than from uninfected rats. Both experimental situations evidenced substantial plaque on the teeth if rats had been infected by either TOVE-R or a mutans streptococcus. These observations suggest that absolute counts substantially reflect the degree of cavitation of the teeth and the mechanical retention of bacteria in those cavities, as though they were additional tooth fissures. In fact, a plot of caries score as a function of absolute CFU count of total recoverable flora gives a high level of correlation ($r = 0.603$; $P < 0.001$).

It is, of course, not surprising that the percentages of recoveries of the tooth-adherent mutans streptococci are considerably higher in sonicates than in swab samples. This is due to several factors including (i) the unavoidability of sampling tongue, cheek, and palate when swabbing the teeth of conscious rats; (ii) the sampling of only teeth by the extraction-sonification procedure; (iii) the sought-after breaking of streptococcal chains and dispersal of bacterial clumps by sonification, thereby more nearly achieving the ideal of one live cell giving rise to 1 CFU on agar (an ideal difficult to approach for streptococci without sonification); (iv) the relative sonic fragility of gram-negative cells (although previous data have shown the gram-negative flora of these rats to constitute a very small portion of the total recoverable flora); and (v) the dislodgement of bacteria from deep fissures and carious lesions by sonification, a dislodgement not possible with swabbing. Of course, sonification conditions were used, as detailed above, which had been shown to maximize absolute CFU counts of total recoverable flora.

The presently reported competitive partial displacement of two virulent representatives of the most prevalent human mutans streptococci, *S. mutans* 10449S and *S. sobrinus* 6715-13WT, and the previously reported successful preemption of 10449S infection by TOVE-R colonization (29) suggest that TOVE-R may have clinical therapeutic utility for inhibiting initial infection and suppressing existing infection of humans by the mutans streptococci. Indeed, there is renewed interest in control of diverse bacterial infections by similar means (2).

The mechanistic bases for the lack of cariogenicity of TOVE-R and its ability to compete with the mutans streptococci are under study.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Alaluusua, S., and O.-V. Renkonen.** 1983. *Streptococcus mutans* establishment and dental caries experience in children from 2 to 4 years old. Scand. J. Dent. Res. **91**:453–457.
2. **Aly, R., and H. R. Shinefield (ed.).** 1982. Bacterial interference, p. 183. CRC Press, Inc., Boca Raton, Fla.
3. **Berkowitz, R. J., and H. V. Jordan.** 1975. Similarity of bacteriocins of *Streptococcus mutans* from mother and infant. Arch. Oral Biol. **20**:725–730.
4. **Bratthall, D.** 1970. Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans*. Odontol. Revy **21**:143–152.
5. **Carlos, J. P., and A. M. Gittelsohn.** 1965. Longitudinal studies of the natural history of caries. Arch. Oral Biol. **10**:739–751.
6. **Coykendall, A. L.** 1983. *Streptococcus sobrinus* nom. rev. and *Streptococcus ferus* nom. rev.: habitat of these and other mutans streptococci. Int. J. Syst. Bacteriol. **33**:883–885.
7. **de Stoppelaar, J. D., J. van Houte, and O. Backer-Dirks.** 1969. The relationship between extracellular polysaccharide-producing streptococci and smooth surface caries in 13-year-old children. Caries Res. **3**:190–200.
8. **de Stoppelaar, J. D., J. van Houte, and O. Backer-Dirks.** 1970. The effect of carbohydrate restriction on the presence of *S. mutans*, *S. sanguis* and iodophillic polysaccharide-producing bacteria in human dental plaque. Caries Res. **4**:114–123.
9. **Duchin, S., and J. van Houte.** 1978. Relationship of *Streptococcus mutans* and lactobacilli to incipient smooth surface dental caries in man. Arch. Oral Biol. **23**:779–786.
10. **Fitzgerald, R. J., and R. H. Larson.** 1967. Age and caries susceptibility in gnotobiotic rats. Helv. Odontol. Acta **11**:49–52.
11. **Ikeda, T., and H. J. Sandham.** 1971. Prevalence of *Streptococcus mutans* on various tooth surfaces in Negro children. Arch.

Oral Biol. **16**:1237–1240.
12. **Ikeda, T., H. J. Sandham, and E. L. Bradley, Jr.** 1973. Changes in *Streptococcus mutans* and lactobacilli in plaque in relation to the initiation of dental caries in Negro children. Arch. Oral Biol. **18**:555–566.
13. **Johnson, K. P., and J. D. Hillman.** 1982. Competitive properties of lactate dehydrogenase mutants of the oral bacterium *Streptococcus mutans* in the rat. Arch. Oral Biol. **27**:513–516.
14. **Kelstrup, J.** 1981. Extracellular polysaccharides of smooth and rough variants of *Streptococcus salivarius*. Scand. J. Dent. Res. **89**:374–383.
15. **Keyes, P. H.** 1958. Dental caries in the molar teeth of rats. I. Distribution of lesions induced by high carbohydrate low-fat diets. J. Dent. Res. **37**:1077–1087.
16. **Keyes, P. H.** 1958. Dental caries in the molar teeth of rats. II. A method for diagnosing and scoring several types of lesions simultaneously. J. Dent. Res. **37**:1088–1099.
17. **Keyes, P. H., and H. V. Jordan.** 1964. Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. Arch. Oral Biol. **9**:377–400.
18. **Köhler, B., and D. Bratthall.** 1978. Intra-familial levels of *Streptococcus mutans* and some aspects of the bacterial transmission. Scand. J. Dent. Res. **86**:35–42.
19. **Köhler, B., D. Bratthall, and B. Krasse.** 1983. Preventive measures in mothers influence in the establishment of *Streptococcus mutans* in their infants. Arch. Oral Biol. **28**:225–232.
20. **Larson, R. H.** 1981. Merits and modifications of scoring rat dental caries by Keyes' method, p. 195–203. *In* J. M. Tanzer (ed.), Animal models in cariology. Information Retrieval, Inc., Washington, D.C.
21. **Littleton, N. W., S. Kakehashi, and R. J. Fitzgerald.** 1970. Recovery of specific "caries-inducing" streptococci from carious lesions in the teeth of children. Arch. Oral Biol. **15**:461–463.
22. **Masuda, N., N. Tsutsumi, S. Subue, and S. Hamada.** 1979. Longitudinal survey of the distribution of various serotypes of *Streptococcus mutans* in infants. J. Clin. Microbiol. **10**:497–502.
23. **Perch, B., E. Kjems, and T. Ravn.** 1974. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. Acta Pathol. Microbiol. Scand. Sect. B **82**:357–370.
24. **Rinehimer, L. A., and J. M. Tanzer.** 1980. Statistical relationship of morsal to bucco-lingual, approximal and sulcal caries in rats. J. Dent. Res. **59**:745–748.
25. **Sachs, L.** 1982. Applied statistics: a handbook of techniques, p. 316. Springer Verlag, New York.
26. **Snedecor, G., and W. Cochran.** 1967. Statistical methods, 6th ed., p. 593. Iowa State University Press, Ames.
27. **Tanzer, J. M.** 1979. Essential dependence of smooth surface caries on, and augmentation of fissure caries by, sucrose and *Streptococcus mutans* infection. Infect. Immun. **25**:526–531.
28. **Tanzer, J. M. (ed.).** 1981. Animal models in cariology, p. 458. Information Retrieval, Inc., Washington, D.C.
29. **Tanzer, J. M., and J. Fisher.** 1982. Competition of a rough non-cariogenic *Streptococcus salivarius*-like strain with *Streptococcus mutans* in dental plaque, p. 124–125. *In* S. E. Holm and P. Christiansen (ed.), Basic concepts of streptococci and streptococcal diseases. Reedbooks, Ltd., Chertsey, England.
30. **Tanzer, J. M., and M. L. Freedman.** 1978. Genetic alterations of *Streptococcus mutans* virulence. Adv. Exp. Med. Biol. **107**:661–672.
31. **Tanzer, J. M., M. L. Freedman, and J. Fisher.** 1982. Preemption of *Streptococcus mutans* 10449S colonization by its mutant 805. Infect. Immun. **35**:138–142.
32. **Tanzer, J. M., M. L. Freedman, R. J. Fitzgerald, and R. H. Larson.** 1974. Diminished virulence of glucan synthesis-defective mutants of *Streptococcus mutans*. Infect. Immun. **10**:197–203.
33. **Tanzer, J. M., M. L. Freedman, F. N. Woodiel, R. L. Eifert, and L. A. Rinehimer.** 1976. Association of *Streptococcus mutans* virulence with synthesis of intracellular polysaccharide, p. 597–616. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Microbial aspects of dental caries. Information Retrieval, Ltd., New York.
34. **van Houte, J., and S. Duchin.** 1975. *Streptococcus mutans* in the mouths of children with congenital sucrase deficiency. Arch. Oral Biol. **20**:771–773.
35. **Zickert, I., C.-G. Emilson, and B. Krasse.** 1982. *Streptococcus mutans*, lactobacilli and dental health in 13–14-year-old Swedish children. Comm. Dent. Oral Epidemiol. **10**:77–81.
36. **Zickert, I., C.-G. Emilson, and B. Krasse.** 1982. Effect of caries preventive measures in children highly infected with the bacterium *Streptococcus mutans*. Arch. Oral Biol. **27**:861–868.