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INTRAORAL SPREAD OF *STREPTOCOCCUS MUTANS* IN MAN

MONA L. SVANBERG* and W. J. LOESCHE

Dental Research Institute, University of Michigan, School of Dentistry, Ann Arbor, MI 48109, U.S.A.

Summary—Streptomycin-resistant strains of *Streptococcus mutans* were reliably established in 3 subjects by the use of artifical fissures (AF) containing adherent growth of the *Strep. mutans* strain. The intraoral spread of these implanted *Strep. mutans* strains was monitored in the subjects while the AF was *in situ* and for up to 26 weeks after the AF was removed. During the 26 weeks, all tooth surfaces and saliva were repeatedly sampled. The implanted *Strep. mutans* spread to adjacent and antagonistic teeth only on the side of the dentition where the implantations were made. After establishment of a streptomycin-resistant strain in one subject, a sterile AF was inserted multiple times in the same occlusal surface, on which the implantations had previously been made. The sterile AF was colonized by local spread of *Strep. mutans* strains, as was shown by the relationship between the percentage of streptomycin-resistant *Strep. mutans* to total *Strep. mutans* colony forming units (CFU) counts in the AF and the salivary flora. The streptomycin-resistant *Strep. mutans* represented 10.5–93.5 per cent of the total *Strep. mutans* CFUs in the AF, but only 0–1.5 per cent of the *Strep. mutans* CFUs in the saliva during colonization of the AF. The intraoral establishment and spread of the implanted *Strep. mutans* seemed favoured by conditions present in subjects with high salivary concentrations of endogenous *Strep. mutans* strains and high caries experience, as expressed by the high number of filled tooth surfaces.

INTRODUCTION

The cariogenic potential of *Streptococcus mutans* has been clearly demonstrated in animal models (Keyes, 1962, 1968) and some role for this microorganism in dental caries in man seems evident (Krasse *et al.*, 1968; Loesche *et al.*, 1975).

Several attempts have been made to study the intraoral ecology of *Strep. mutans* by the implantation in the human mouth of *Strep. mutans* strains containing, for identification purposes, a streptomycin-resistant marker (Krasse *et al.*, 1967; Jordan *et al.*, 1972; Edman *et al.*, 1975; Svanberg and Loesche, 1978), but most labelled strains were quickly eliminated from the mouth. Edman *et al.* (1975) established labelled strains in approximal sites on one side of the mouth in two volunteers and noted that the implanted strains were mainly recovered from that side. The artificial fissure model (Löe, Karring and Theilade, 1973) can be used to reliably implant streptomycin-resistant strains in single occlusal sites (Svanberg and Loesche, 1978).

MATERIALS AND METHODS

Subjects

The three subjects (G, T and S) who participated had 38, 52 and 78 filled tooth surfaces, respectively, no carious lesions and no missing teeth. Subjects G and S had a normal occlusion and subject T had a class III malocclusion.

An artificial fissure (AF) containing a *Strep. mutans* strain which had been made resistant to 1 mg/ml of streptomycin was placed in a molar tooth in each subject. The AFs had been cultured *in vitro* and contained at the time of insertion 10^7 colony-forming units (CFU) of *Strep. mutans* as adherent growth on the AF (Svanberg and Loesche, 1978). The AFs were each inserted three times in the occlusal surface of an upper first molar in subject G and in a lower first molar in subjects T and S. The implanted *Strep. mutans* strains G, T and S belonged to serotype c and were re-introduced into the same mouth from which they originally had been isolated (Svanberg and Loesche, 1978).

Bacterial samples

The AFs containing strains G, T and S were left *in vivo* for 7–12 days. At 2, 4, 6, 8 and 26 weeks after removal of the infected AF, plaque samples were collected from all surfaces of all teeth in each subject. Twenty-six approximal tooth surfaces were sampled with dental floss and 56 smooth tooth surfaces with flattened hypodermic needles. Twenty-eight plaque samples from margins of fillings and fissures were collected with 26-gauge hypodermic needles. The samples were taken in the morning after the subjects had refrained from all oral hygiene on the evening and morning before sampling. Two to four salivary samples were collected per week during this 26-week period.

Colonization of sterile AFs

Strain S established on the natural tooth surfaces in subject S for a prolonged period of time. During this interval, sterile AFs were inserted several times in the same tooth as that used earlier for the AF containing adherent growth of *Strep. mutans*. The

* Present address: Göteborgs universitet, Odontologiska kliniken, Fack 400, 33 Göteborg 33, Sweden.

sterile AF was left *in situ* for 1 and 2 days each on 2 occasions, for 5 days on 3 occasions and for 21 days on 1 occasion, whereafter the content of the AF was cultured. The bacterial samples were immediately placed in reduced transport fluid (RTF) (Syed and Loesche, 1972) and dispersed by sonification for 5 s (Branson model W 185 D, N.Y.).

Bacteriological procedures

The saliva and AF samples were serially diluted in RTF, whereas the plaque samples from the tooth surface were placed in 2.5 ml of RTF and not further diluted. 0.05-ml aliquots from appropriate dilutions were spread on mitis-salivarius bacitracin agar (MSB) (Gold, Jordan and van Houte, 1973) and on MM10 sucrose agar (Syed and Loesche, 1973) with and without 0.2 mg/ml streptomycin. The inoculated plates were placed in an anaerobic chamber within 30 min of collection of samples and incubated at 37°C for 48 h in an atmosphere of 85 per cent N_2, 10 per cent H_2 and 5 per cent CO_2.

Identification of colonies of *Strep. mutans* strains on the various media was based on their characteristic colonial morphology, supplemented if necessary with biochemical tests (Shklair and Keene, 1974), and by examination with fluorescent antibody directed against the various *Strep. mutans* serotypes (Grenier, Eveland and Loesche, 1973). The *Strep. mutans* counts were obtained from MSB agar and from MM10 sucrose agar. The counts of the streptomycin-resistant *Strep. mutans* were obtained from the MM10 sucrose agar with streptomycin. The counts of *Strep. mutans* on MM10 sucrose agar minus the counts of *Strep. mutans* on MM10 sucrose agar with streptomycin were considered to represent the counts of endogenous *Strep. mutans*. The total colony-forming unit counts (CFU) was obtained from the MM10 sucrose agar.

RESULTS

Intraoral spread

The AFs containing the streptomycin-labelled strains served to inoculate the other tooth surfaces while they were *in vivo*. After removal of these AFs, the streptomycin-resistant strains of *Strep. mutans* could be detected during the next 26 weeks on a varying number of tooth surfaces (Table 1). In subject G, two tooth surfaces were colonized at the 2 weeks sampling, but thereafter no streptomycin-resistant *Strep. mutans* could be detected. In subject T, 8 surfaces were colonized after 8 weeks and, in subject S, the implanted strain could be detected on 6 tooth surfaces 26 weeks after the removal of the AF. In subject S, as many as 20 tooth surfaces had detectable streptomycin-resistant *Strep. mutans* 4 weeks after removal of the AF. The implanted strain spread to adjacent and antagonistic teeth only on the same side of the dentition as that on which the AF had been inserted (Figs. 1a–c). From 2 (subject G, Fig. 1a) to 26 (subject S, Fig. 1c) separate tooth surfaces were colonized at some time by the implanted *Strep. mutans* strains. In subject T (Fig. 1b), the spread to antagonistic teeth included both the maxillary premolars which, because of a class III malocclusion, were in occlusion with the mandibular molar that contained the AF. All tooth surfaces colonized by the implanted strain also contained detectable levels of endogenous *Strep. mutans*. In all subjects some surfaces which contained endogenous *Strep. mutans* did not become colonized by the implanted strain.

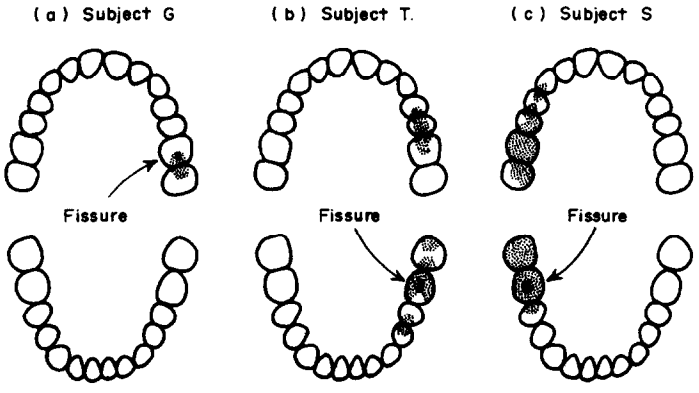
Table 1. Persistence of streptomycin-resistant strains of *Strep. mutans* on tooth surfaces in volunteers

	Weeks after removal of infected AF*				
	2	4	6	8	26
Subject G	2†	0	0	0	0
Subject T	10	6	7	8	0
Subject S	12	20	14	4	6

* Artificial fissure.

† Number of tooth surfaces with streptomycin-resistant strains of *Strep. mutans*.

The implanted *Strep. mutans* strain G was detected in the salivary samples of subject G for a longer period of time than in plaque samples, i.e. salivary samples were positive 8 weeks after removal of the AF, although the bacteria were found on the tooth surfaces only at the 2 week sampling (Table 2). Twenty-four consecutive salivary samples collected from subject G from week 9 to week 14 were negative



Figs. 1(a–c). Intraoral spread of implanted *Strep. mutans* in subjects G, T and S. Dotted fields, tooth surfaces colonized by implanted *Strep. mutans*.

Table 2. Presence of streptomycin-resistant *Strep. mutans* in saliva samples in relation to persistence of the microorganisms on tooth surfaces

Subject		Weeks after removal of the artificial fissure 1–4	4–8	8–12	12–16	16–20	20–26
G	No. of tooth surfaces colonized	2	0	0	ND‡	ND‡	0
	Per cent saliva samples positive	27	14	4	29*	0	ND
T	No. of tooth surfaces colonized	10	7	8	ND	ND	0
	Per cent saliva samples positive	19	29	40	14†	2	0
S	No. of tooth surfaces colonized	20	14	4	ND	ND	6
	Per cent saliva samples positive	45	41†	24	39	72	32

* 1 lump of sugar every waking hour for 11 days.
† Daily fluoride rinses.
‡ Not determined.

for strain G. Subject G was then placed on a high sugar regimen in which he ate a lump of sugar every waking hour for 11 days. Twenty nine per cent of his salivary samples became positive for the streptomycin-resistant strain. When the frequent sucrose ingestion was stopped, no streptomycin-resistant *Strep. mutans* strains were detected in his salivary samples. At the end of the experiment, at week 26, no tooth surfaces were positive for strain G. In subjects T and S, daily fluoride rinses, i.e. 5 ml of 0.4 per cent stannous fluoride and 5 ml of 0.02 per cent acidulated phosphate fluoride (Iradicv®, Janar Co., Grand Rapids, Mich.), were administered for a 4-week period in order to eliminate or reduce the implanted strain. In subject T, the implanted *Strep. mutans* strain was present in 40 per cent of the salivary samples prior to rinsing, but decreased to 14 per cent during rinsing and was not detectable in either the saliva or on the tooth surfaces at week 26 (Table 2). In subject S the fluoride rinsing did not eliminate the implanted strain, as both saliva and some tooth samples remained positive during the whole experimental period. The positive salivary samples in all subjects were due to low levels of the implanted strain, i.e. $\leq 10^3$/ml.

After the establishment of strain S on adjacent and antagonistic tooth surfaces in subject S, a sterile AF was inserted on multiple occasions into the molar site. These AFs were removed at various time intervals and the ratio of streptomycin-resistant CFUs of *Strep. mutans* to total *Strep. mutans* was determined for the AF and for the saliva at the time of the removal of the AF. After 1 day, approximately 90 per cent of the *Strep. mutans* in the AF were streptomycin resistant, whereas only 0.1 per cent of the *Strep. mutans* in the saliva were resistant to streptomycin (Table 3). At 2, 5 and 21 days, the proportions of streptomycin-resistant strains decreased in the AF, but these proportions were always at least 100-fold higher than the corresponding ratio of these organisms in the saliva.

DISCUSSION

In all subjects, the intraoral spread of the implanted *Strep. mutans* occurred only on the same side of the dentition as that on which the implantations were made. The extent of the intraoral spread varied widely in the three test persons, which may be due to a variety of host, diet and microflora factors. The dissimilarity of the hosts is illustrated by their different caries

Table 3. Colonization of sterile artificial fissures (AF) by *Strep. mutans*

Days after insertion of AF	% Streptomycin-resistant *Strep. mutans* of total *Strep. mutans* CFU count in AF and in saliva: AF per cent		Saliva per cent	
1	$n = 2$	87.0, 90.0	$n = 6$	ave = 0.1
2	$n = 2$	93.5, 10.7	$n = 10$	0.1
5	$n = 3$	33.0, 10.7, 10.5	$n = 33$	0.1
21	$n = 1$	11.5	$n = 16$	0.004
	Range 10.5–93.5 per cent		0.0–1.5 per cent	

experience, number of retention sites such as margins of fillings and salivary *Strep. mutans* concentrations. Subject S had a high number of filled tooth surfaces and a high salivary concentration of endogenous *Strep. mutans* strains, i.e. 10^6 CFU per ml (Svanberg and Loesche, 1978). Subject G had a lower caries experience and a low salivary concentration of endogenous *Strep. mutans* strains, i.e. about 10^3 CFU per ml, and subject T was intermediate in regards to the various parameters under discussion (Svanberg and Loesche, 1978).

The importance of dietary sucrose in raising the salivary concentrations of the implanted *Strep. mutans* was demonstrated in subject G, when this subject went on a dietary regimen of frequent sucrose ingestion. The implanted strain G, which had been undetected in 24 consecutive salivary samples, re-emerged in the salivary flora, showing that a person can be a carrier of *Strep. mutans* at undetectable levels in plaque samples collected from tooth surfaces using dental floss and sharp needles, as well as in saliva samples. This finding confirms earlier observations (Svanberg and Loesche, 1978) that a *Strep. mutans*-infected retention site, such as an AF, often may remain undetected by the sampling techniques at present available.

The implanted *Strep. mutans* strains spread to adjacent and antagonistic tooth surfaces. The spread was detected only on the side of the dentition where the implantations were made, confirming the observations of Edman *et al.* (1975). This suggests that the number of streptomycin-resistant CFU of *Strep. mutans* shed from the AF is so low that they become diluted in the saliva so that their chance to make contact with teeth on the other side of the dentition is slight. We have shown (Svanberg and Loesche, 1978) that AFs containing more than 10^5 CFU of the labelled *Strep. mutans* shed 10^2–10^3 CFU of these organisms per ml of saliva. The saliva contains about 10^8 CFU of various bacteria per ml, which means that in homogenized whole saliva about one in every 100,000 to 1,000,000 organisms would be of the labelled strain. Homogenized whole saliva, however, is an artifact of our culturing procedure. *In vivo*, the saliva flowing over the side containing the AF would be enriched for the streptomycin-resistant strains, whereas the saliva flowing over the opposite side would most likely have no streptomycin-resistant strains. The higher salivary concentrations of *Strep. mutans* on the implanted side would favour unilateral spread of the labelled organisms. This was so when sterile AFs were inserted in subject S after the establishment of the implanted *Strep. mutans* on adjacent and antagonistic teeth. The local spread of *Strep. mutans* strains was illustrated by the relationship between the percentage streptomycin-resistant *Strep. mutans* of total *Strep. mutans* CFU counts in the AF, i.e. 10.5–93.5 per cent and in the salivary flora, i.e. 0.0–1.5 per cent. The salivary concentrations of the implanted *Strep. mutans* were on all occasions lower than those found previously to be associated with *Strep. mutans* colonization of a sterile AF (Svanberg and Loesche, 1978) and of a smooth tooth surface (van Houte and Greene, 1974). The shedding of *Strep. mutans* strains from infected areas probably increases the local salivary concentrations to such an extent compared to whole saliva that colonization of adjacent and antagonistic teeth is favoured. It is, however, also possible that contact between teeth greatly enhances the spread of *Strep. mutans* strains.

In all subjects, all tooth surfaces that harboured the implanted *Strep. mutans* strains were also colonized by streptomycin-sensitive strains of *Strep. mutans*. The streptomycin-sensitive *Strep. mutans* were presumably of endogenous origin, as the streptomycin-resistant marker is stable *in vivo* (Svanberg and Loesche, 1978). This indicates that strains G, T and S coexisted for a time with the endogenous strains of *Strep. mutans*.

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REFERENCES

Edman D. C., Keene H. J., Shklair I. L. and Hoerman K. C. 1975. Dental floss for implantation and sampling of *Streptococcus mutans* from approximal surfaces of human teeth. *Archs oral Biol.* **20**, 145–148.

Gold O. G., Jordan H. V. and van Houte J. 1973. A selective medium for *Streptococcus mutans*. *Archs oral Biol.* **18**, 1357–1364.

Grenier E. M., Eveland W. C. and Loesche W. J. 1973. Identification of *Streptococcus mutans* serotypes in dental plaque by fluorescent antibody techniques. *Archs oral Biol.* **18**, 707–715.

Jordan H. V., Englander H. R., Engler W. A. and Kulcyzk S. 1972. Observations on the implantation and transmission of *Streptococcus mutans* in humans. *J. dent. Res.* **51**, 515–518.

Keyes P. H. 1962. Recent advances in dental caries research. *Bact. Int. dent. J.* **12**, 443–448.

Keyes P. H. and Fitzgerald R. J. 1962. Dental caries in the Syrian hamster IX. *Archs oral Biol.* **7**, 267–278.

Keyes P. H. 1968. Research in dental caries. *J. Am. dent. Ass.* **76**, 1357–1373.

Krasse B., Edwardsson S., Svensson I. and Trell L. 1967. Implantation of caries inducing streptococci in the human oral cavity. *Archs oral Biol.* **12**, 231–236.

Krasse B., Jordan H. V., Edwardsson S., Svensson I. and Trell L. 1968. The occurrence of certain "caries-inducing" streptococci in human dental plaque material. *Archs oral Biol.* **13**, 911–918.

Löe H., Karring T. and Theilade E. 1973. An *in vivo* method for the study of the microbiology of occlusal fissures. *Caries Res.* **7**, 120–129.

Loesche W. J., Rowan J., Straffon L. H. and Loos P. J. 1975. Association of *Streptococcus mutans* with human dental decay. *Infect Immun.* **11**, 1252–1260.

Shklair I. L. and Keene H. J. 1974. A biochemical scheme for the separation of the five varieties of *Streptococcus mutans*. *Archs oral Biol.* **19**, 1079–1081.

Svanberg M. and Loesche W. J. 1977. Salivary concentrations of *Streptococcus mutans* and *Streptococcus sanguis* and the colonization of artificial fissures in man by these organisms. *Archs oral Biol.* **22**, 441–447.

Svanberg M. and Loesche W. J. 1978. Implantation of *Streptococcus mutans* on tooth surfaces in man. *Archs oral Biol.* **23**, 551–556.

Syed S. A. and Loesche W. J. 1972. Survival of human dental plaque flora in various transport media. *Appl. Microbiol.* **24**, 638–644.

Syed S. A. and Loesche W. J. 1973. Efficiency of various growth media in recovering oral bacterial flora from human dental plaque. *Appl. Microbiol.* **26,** 459–465.

van Houte J. and Green D. B. 1974. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. *Infect. Immun.* **9,** 624–630.