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DECREASED CARIOGENICITY OF A MUTANT OF *STREPTOCOCCUS MUTANS*

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Summary—A strain of *Streptococcus mutans* was treated with a mutagenic agent. This resulted in isolation of a mutant which, compared to the original strain, had lost the ability to form sticky deposits on hard surfaces in sucrose medium. Apart from colonial morphology, the mutant had not changed in any other characteristic studied. In a 9-week experiment with SPF Syrian hamsters the average caries score induced by the mutant was significantly lower than that induced by the parent strain. In a 5-week experiment using germfree rats, the mutant had virtually lost its cariogenicity in sharp contrast to the original *Strep. mutans*. In all instances the implanted microorganisms could be recovered from the animals during all phases of the experiments.

AN IMPORTANT characteristic of the human oral microorganism *Streptococcus mutans* is the production of extracellular dextran-like glucans from sucrose. Cultures of *Strep. mutans* form sticky, gelatinous deposits on hard surfaces in the presence of sucrose, but not in media containing other sugars (JORDAN and KEYES, 1966; GIBBONS *et al.*, 1966). The formation of these deposits has been suggested to be dependent upon the synthesis of insoluble dextran, other soluble dextran being formed simultaneously (GUGGENHEIM and SCHROEDER, 1967; GIBBONS and NYGAARD, 1968). In the present investigation, an attempt was made to determine if the ability of *Strep. mutans* to form sticky deposits *in vitro* is associated with its cariogenicity in experimental animals. It was decided to use a mutant of *Strep. mutans* which had lost its ability to form sticky polysaccharide in a sucrose medium.

Streptococcus mutans strain C67-1 (closely resembling NCTC strain 10449), isolated from smooth-surface plaque of a caries-active child (DE STOPPELAAR, VAN HOUTE and BACKER-DIRKS, 1969) was used in this study. Mutation was performed by treatment with a mutagenic agent, ethyl methane sulphonate (LOVELESS and HOWARTH, 1959). One millilitre of an 18-hr anaerobic culture was incubated at pH 7·0 and 37°C for 1 hr with 1 ml of 1:25 diluted ethyl methane sulphonate (Koch–Light Laboratories,

Colnbrook, England). The reaction was stopped by washing with 5 per cent sodium thiosulphate and the treated culture was subcultured for 18 hr, after which serial dilutions were streaked on sucrose agar (DE STOPPELAAR, VAN HOUTE and DE MOOR, 1967), incubated at 37°C in an atmosphere of 90 per cent H_2 and 10 per cent CO_2 for 4 days. Possible mutants were isolated by selecting smooth colonies among the normal rough, heaped colonies of *Strep. mutans*. The selected colonies were tested for their ability to form sticky deposits on the wall of a test tube by inoculation in sucrose broth, incubated anaerobically for 3–4 days. In this manner strain C67-25 was isolated, which failed to stick to hard surfaces and was selected for inoculation in the animal experiments. This strain, compared to the parent strain, had not changed in any other characteristic studied, including fermentation spectrum, rate of growth and acid production, the production of extracellular soluble dextran-like polysaccharide in the fluid-phase of sucrose broth and the ability to produce intracellular glycogen. Both the parent and mutant strain reacted with an antiserum prepared against NCTC 10449. During frequent subculturing, the mutant strain did not give rise to colonies with the original morphology and never spontaneously regained the property of sticking to hard surfaces. However, back mutation (reversion) of the mutant with the method described above proved to be possible. This suggests strain C67-25 is a genuine mutant.

The cariogenicity of the parent strain C67-1 and the mutant C67-25 was compared in SPF Syrian gold hamsters and in germfree Osborne–Mendel rats. Weanling animals (24 hamsters, 6 rats) were housed in stainless-steel cages with mesh bottom without bedding. Regarding the gnotobiotic rats, care was taken to keep fissures free from impactions in order not to favour establishment of the microorganisms artificially (KÖNIG and GUGGENHEIM, 1968). In half of the hamsters, the indigenous Gram-positive oral flora was partly suppressed pre-experimentally by administering drinking water containing 0·2 per cent sodium penicillin G for 2 days. Inoculations of thick suspensions of the test microorganisms grown in glucose-containing brain–heart infusion broth were performed at the start of the experimental period with cotton swabs. High-sucrose low-fat diet 2000 was fed to the hamsters and gamma-irradiated (2·5 Mrad) diet 2000 V (FITZGERALD and LARSON, 1967) to the rats *ad libitum* for 9 weeks and 5 weeks, respectively. Plaque samples from the hamsters and oral swabs from the rats were taken and cultured 3 days after inoculation and at regular intervals throughout the experiments. These showed that the inoculated strains were continuously present in all animals. For scoring caries and plaque in the hamsters, a modified (KEYES, 1944) count of surfaces involved in all molars was used. For scoring of caries in the rats, fissure cavities in the lower molars were counted, and for smooth surfaces the unit method of KEYES (1958) was used.

The results of the hamster experiment (Tables 1 and 2) show that there were no significant differences in plaque formation, which showed higher variation in the non-suppressed groups. However, inoculation of the test strains markedly increased caries activity. The original *Strep. mutans* C67-1 was significantly more cariogenic than the mutant C67-25 without preceding penicillin treatment ($P_t < 0{\cdot}05$) and with penicillin suppression ($P_t < 0{\cdot}01$). While three of the hamsters died in the first days on experiment by an unknown cause, food intake and weight gains were normal and homo-

TABLE 1. AVERAGE PLAQUE SCORE AND STANDARD DEVIATION OF 6 GROUPS OF HAMSTERS ($N = 4$) INOCULATED WITH *Strep. mutans* C 67-1 AND ITS MUTANT C 67-25, WITH OR WITHOUT PRE-DEPRESSION WITH PENICILLIN

Inoculation	No penicillin		Penicillin	
None	25·5	±15·3	34·7*	±3·8
C 67-1	38·0	±22·7	30·0	±9·9
C 67-25	18·2	±11·4	31·0†	±8·5

* $N = 3$. † $N = 2$.

TABLE 2. AVERAGE CARIES SCORE AND STANDARD DEVIATION OF 6 GROUPS OF HAMSTERS ($N = 4$) INOCULATED WITH *Strep. mutans* C 67-1 AND ITS MUTANT C 67-25, WITH OR WITHOUT PRE-DEPRESSION WITH PENICILLIN

Inoculation	No penicillin		Penicillin	
None	6·5	±4·9	14·3*	±5·9
C 67-1	51·8	±32·6	41·5	±7·2
C 67-25	24·0	±4·8	14·5†	±7·8

* $N = 3$. †$N = 2$.

TABLE 3. CARIES INCIDENCE IN THE MANDIBULAR MOLARS OF 6 O–M RATS (I–VI) MONOCONTAMINATED WITH *Strep. mutans* C 67-1 OR C 67-25

Types of carious lesions	Animal No.	Strain C 67-1	$\bar{x}$	Animal No.	Strain C 67-25	$\bar{x}$
No. of fissure cavities	I	14		IV	0	
	II	10	13·3	V	2	0·7
	III	16		VI	0	
Buccal smooth surface units	I	14		IV	0	
	II	10	12·3	V	0	0·0
	III	13		VI	0	
Lingual smooth surface units	I	2		IV	0	
	II	6	4·0	V	0	0·0
	III	4		VI	0	

geneous in the rest of the animals through the 9-week period. Average weight gains and standard deviations were 53·6 ± 12·1, 54·1 ± 6·8 and 48·8 ± 10·1 in the control, C67-1 and C67-25 groups respectively.

The rats did not develop macroscopically visible plaque accumulations. The detailed caries data (Table 3) show that the original strain C67-1 induced high caries activity in both fissures (40 cavities) and smooth surfaces of the mandibular molars. From the

three rats infected with the mutant C67-25, only one animal developed 2 minute fissure lesions.

The absence of assessible gross accumulation of plaque on the tooth surfaces of the rats does not mean that there were no minor bacterial aggregations in the depths of the fissures and in the gingival crevices which may cause carious destruction as indicated by the observations of RANKE *et al.* (1971) on rats infected with streptococci.

The results obtained in the present investigation suggest that the ability of *Strep. mutans* cells to stick together and to the tooth surface is an important property. Mutant strain C67-25 may be lacking one of the glucosyltransferases described by GUGGENHEIM and NEWBRUN (1969) resulting in the inability of this strain to synthesize an insoluble glucan which is adherent to the cells. This is substantiated by our finding that no polysaccharide can be removed by sonic oscillation or alkali extraction of sucrose-grown centrifuged cells of the mutant strain in contrast to the parent strain. The mutant strain, however, is still capable of producing liberal amounts of extracellular polysaccharide which does not adhere to the cells, but is present in the culture fluid. It is possible that the polysaccharide does not remain bound to the mutant cells, due to a change in the cell surface. This assumption was strengthened when dextran-induced agglutination was performed as described by GIBBONS and FITZGERALD (1969). Cell-suspensions of parent strain C67-1 agglutinated readily at pH 7·2 with commercial dextran (MW 150,000), with dextrans produced by strains C67-1, C67-25 and a strain of *Strep. sanguis*, as well as in the presence of sucrose. Mutant strain C67-25 did not agglutinate within 18 hr with any of these substances.

From the above considerations, it may be concluded that it is not the capacity to form extracellular polysaccharide *per se* that determines the cariogenicity of a microbial strain, but rather the capacity to form and bind insoluble polysaccharide to the cell. This appears to be not only important for the development of caries on smooth surfaces, but also for fissure caries in the animal experiments described.

The mutant strain produced only smooth colonies upon re-isolation from the animals. This property and the decreased cariogenicity indicate that this strain is different from the occasionally occurring smooth and mucoid variants of *Strep. mutans* which were shown by EDWARDSSON (1970) to give rise to rough colonies after hamster passage while their cariogenic potential did not differ from the original rough strain.

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Résumé—Une souche de *Streptococcus mutans* a été traitée avec un agent mutagène. Ceci a résulté dans l'isolation d'une mutante qui, comparée à la souche originale, avait perdu la capacité de former des dépôts visqueux sur des surfaces dures, dans un milieu de sucrose. À part la morphologie coloniale, la mutante n'a pas changé aucune caractéristique

etudiée. Dans une expérience de 9 semaines avec des hamsters Syriens SPF, le mombre moyen de caries produites par la mutante a été significativement plus bas que celui produit par la souche apparentée. Dans une expérience de 5 semaines, utilisant des rats exempts de micro-organismes, la mutante avait virtuellement perdu sa cariogénicité, en contraste avec le *Strep. mutans* original. Dans tous les cas, les micro-organismes implantés ont pu être recouvrés des animaux, durant toutes les phases de l'expérience.

Zusammenfassung—Ein Stamm von *Streptococcus mutans* wurde mit einem mutagenischen Mittel behandelt. Daraus ergab sich die Absonderung einer durch Mutation entstandenen Variante, die im Vergleich mit dem ursprünglichen Stamm die Fähigkeit verloren hatte, auf harten Oberflächen im Sucrosemedium klebrige Ablagerungen zu bilden. Ausser Koloniegestaltung hatt sich die durch Mutation entstandene Variante in keiner der anderen untersuchten charakteristischen Merkmale verändert. Im Laufe eines 9-Wochen Experimentes mit SPF syrischen Hamstern erwies sich die mittels der Mutationvariante verursachte, durchschnittliche Knochenfäule als bedeutend geringer als die durch den Mutterstamm verursachte. In einem 5-Wochen Experiment mit keimfreien Ratten hatte die Mutationsvariante im scharfen Gegensatz zu dem ursprünglichen *Strep. mutans* virtuell ihr kariogenes Vermögen verloren. In allen Fällen konnten die eingepflanzten Mikroorganismen von den Tieren während aller Phasen des Experimentes entfernt werden.

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