

The Influence of Dietary Sugars and Starch on the Establishment of *Streptococcus mutans* and *Actinomyces viscosus* in Dental Plaque of Specific Pathogen Free Rats

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SUMMARY:

The establishment of *S. mutans* together with *A. viscosus* was investigated in dental plaque of Specific Pathogen Free (SPF) rats fed different carbohydrate diets. Two Tanzanian *S. mutans* strains MM3 and MM24 and one Tanzanian *A. viscosus* strain MM13 were used for this purpose.

The basic diet consisting of 32% skim-milk, 7% yeast extract and 1% soy bean oil was supplemented with either 10% lactose and 50% corn flour, or 10% glucose and 50% corn flour, or 60% amyllum or 60% wheat flour. *S. mutans* and *A. viscosus* were enumerated twenty days after inoculation. *S. mutans* counts were high irrespective of the dietary regime.

A. viscosus counts in the glucose, lactose and amyllum groups were of the same magnitude and significantly higher than those of the wheat flour group. The hypothesis that the establishment of *S. mutans* in sucrose free diets could be facilitated by the extracellular polysaccharides produced by *A. viscosus* was not supported by the present data.

The finding that *S. mutans* can establish

in high number in dental plaque of SPF rats in the absence of sucrose corroborates previous reports indicating high *S. mutans* counts in African populations with a low sucrose intake.

INTRODUCTION:

The oral establishment of mutans streptococci (MS) is known to be stimulated by sucrose through the synthesis of extracellular polysaccharides (Gibbons and Fitzgerald, 1969; van Houte, 1982; Hamada and Slade 1980). Apart from sucrose, there appears to be no evidence that other carbohydrates can act as substrates for the synthesis of extracellular polysaccharides by mutans streptococci (Hassid, 1970).

However, high numbers of mutans streptococci have been reported in Tanzanian infants who consumed breast milk as the main source of nutrition (Matee *et al.*, 1992) and in children on starch rich diets (Matee *et al.*, 1985). These findings suggest that other substrates may play a role in the establishment and multiplication of MS on tooth surfaces.

Studies on the role of lactose on implan-

tation and multiplication of MS has so far produced conflicting results. Guggenheim and co-workers (1966) found little or no implantation of MS in rats fed a lactose diet. On the other hand Schemmel *et al.* (1982) recovered substantial numbers of MS in dental plaque of rats, irrespective of whether lactose or sucrose was fed. When lactose is incorporated in diets at high concentrations rats tend to eat poorly, develop cataracts, and often die prematurely (Frostell *et al.*, 1967). Probably for this reason, caries studies using lactose diets and mutans streptococci have produced conflicting results (Schemmel *et al.*, 1982; Green and Hartles, 1969). To maintain reasonable health among animals, lactose concentrations have to remain low (Pearce and Sissons, 1987). This experiment was aimed at determining whether lactose at low concentrations could support the oral establishment of MS as observed in the breast fed children.

In the mouth, cooked starches are readily hydrolyzed by α -amylase to maltose, a disaccharide of glucose, as the predominant product (Birkhed and Skude, 1978). Glucose can be utilized by *A. viscosus* to synthesize heteropolysaccharides (van der Hoeven 1974), providing a plaque matrix which can entrap a number of other oral microorganisms, including MS. It is therefore tempting to speculate that the presence of MS in children on carbohydrates rich diets devoid of sucrose, is due in part, to the synthesis of heteropolysaccharides by *A. viscosus*.

This study investigated the colonization of *S. mutans* together with *A. viscosus* in dental plaque of rats, harbouring an indigenous microflora, using diets with different carbohydrate composition.

MATERIALS AND METHODS:

Micro-organisms, media and culture conditions:

Two *S. mutans* strains MM3 and MM24 and one *A. viscosus* strain MM13 were isolated from supragingival plaque of Tanzanian children (Matee *et al.*, 1992). *S. mutans* strains were identified biochemically using API 20 Strep, while the *A. viscosus* was identified using API Rapid 132A identification system, according to the instructions of the

manufacturer (Analylab, Montalieu - Vercieu, France). The strains were stored in skim milk (Difco, Detroit, MI, USA) at -80°C . For inoculation of the rats, the micro-organisms were grown in TPY broth containing 20 g/L Trypticase peptone (BBL, Cockeysville, MD), 20 g/L yeast extract (Difco) and 5 g/L galactose (Merck, Darmstadt, Germany). The plaque samples from the animals were diluted in saline (0.85% NaCl) and plated on blood agar plates. Blood agar was composed of 25 g/L BHI (Difco), 10 g/L Bacto peptone (Difco), 1 g/L KNO_3 , and 20 g/L Bacto agar (Difco). After sterilization and cooling to 56°C , a 100 mL quantity of sterile defibrinated sheep blood was added per litre.

All incubations were carried out at 37°C in an atmosphere of 90% N_2 , 6% CO_2 , and 4% H_2 .

Experimental animals

One hundred weaning SPF Wistar rats, 21-22 days old, from different litters were randomly distributed among eight treatment groups on the basis of sex.

The animals were housed in triplets in macrolon cages with a mesh-wire bottom. From weaning, each group was fed one of the four powdered diets, the composition of which is given in Table 1. The skimmilk contained 7% lactose, making the contribution of the

Table 1
Composition of diets (per cent by weight)

Ingredients	Diets			
	Glucose	Lactose	Amylum*	Wheat
glucose	10%	—	—	—
lactose	—	10%	—	—
amylum	—	—	60%	—
cornflour	50%	50%	—	—
wheat flour	—	—	—	60%
skimmilk	32%	32%	32%	32%
inactivated				
yeast	7%	7%	7%	7%
soy oil	1%	1%	1%	1%

* pure soluble starch

disaccharide in the diets to be 2.2%. Diets and drinking water were available *ad libitum*.

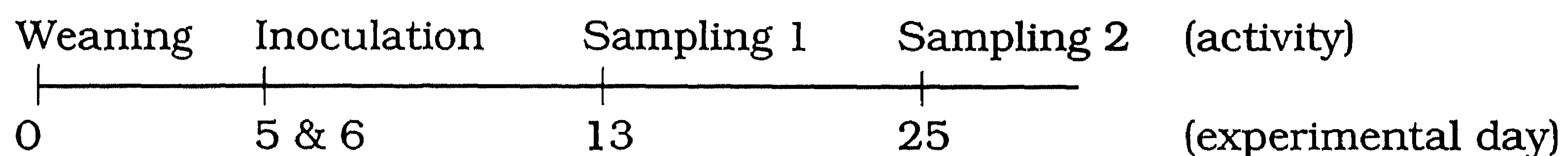
After five days on diet, rats were orally inoculated, by syringe, with 0.1 mL of culture from the late logarithmic phase (18h) containing about 10^8 cells/mL. Each rat was inoculated first with the *A.viscosus* strain

and subsequently with one of the two *S.mutans* strains as outlined in Table 2. The same sequence of inoculations were repeated on the following day. The experimental design is shown in Fig 1.

Table 2
Arrangement of treatment and schedule of inoculation

Treatment No.	1	2	3	4	5	6	7	8
Diet	glucose	glucose	lactose	lactose	amylum	amylum	wheat	wheat
No. of rats	13	12	13	12	13	12	13	12
Strains inoculated on day 5 and 6								
<i>A.viscosus</i> MM13	+	+	+	+	+	+	+	+
<i>S.mutans</i> MM3	+	-	+	-	+	-	+	-
<i>S.mutans</i> MM24	-	+	-	+	-	+	-	+

Fig. 1: Design of the experiment



Bacteriological Studies:

Samples were obtained from twenty six rats 8 days after inoculation and from seventy four rats 20 days after inoculation to investigate implantation and to enumerate bacteria respectively. The animals were killed by CO₂ gas, and the left mandibular molars were extracted aseptically. The molars were ground, using sterile mortars, in 1 mL of 0.85% NaCl solution. The suspension of plaque and adherent food material was dispersed ultrasonically as described by Beckers and van der Hoeven (1982). Suitable dilutions of the samples were plated onto blood agar on which the predominant components of SPF microflora, *A.viscosus* and *S.mutans* could be differentiated and counted. Samples with either undetected *S.mutans* or *A.viscosus* were few and considered to have a species count corresponding to our detection limit (10^4).

Test of Bacteriocin Production:

S.mutans strains MM3 and MM24 were

grown anaerobically in TPY broth containing 0.2% galactose for 18h. The resulting bacterial growth was tenfold diluted in saline and 0.1 mL of suitable dilutions were plated onto TPY agar, composed of 40 g/L Trypticase soy agar (BBL), 5 g/L bacto agar (Difco) and 10 g/L yeast extract (Difco).

After 24h of anaerobic incubation, the plates were overlaid by a mixture of 4.5 mL of warm TPY agar and 1.5 mL of TPY broth containing an 18h culture of *A.viscosus* MM13 and the overlaid plates were incubated anaerobically for 24h and bacteriocin activity was determined by the presence of inhibition zone around *S.mutans* colonies.

Statistical Analyses:

Analysis of variance (ANOVA) and the student's t-test were used to assess the statistical significance of the observed results.

RESULTS:

Plaque samples collected eight days after

inoculation showed that both *S. mutans* and *A. viscosus* established well in all the dietary groups. Samples taken twenty days after inoculation showed that *S. mutans* established well in the four dietary groups (Table 3). There were no significant differences in *S. mutans* counts between the strains or the diets. Both strains of *S. mutans* produced bacteriocin activity against *A. viscosus* *in vitro*.

A. viscosus counts for the glucose, amylum and lactose diets were in the same range and significantly higher than those found in the flour diet. The *A. viscosus* counts were not affected by the type of *S. mutans* strain. The presented bacteriological data are derived from seventy three rats. The samples of one rat belonging to the amylum group could not be enumerated due to a heavy contamination of the culture plates.

Table 3
***S. mutans* and *A. viscosus* counts in dental plaque of SPF rats according to diet, 20 days after inoculation**

Diet	<i>S. mutans</i> strain	No. of rats	<i>S. mutans</i> counts* Log ₁₀ (X ± SD)*	<i>A. viscosus</i> counts** Log ₁₀ (X ± SD)*	Total Count*** Log ₁₀ (X ± SD)*
Lactose	MM 3	9	7.5 ± 0.3	7.6 ± 0.3	8.1 ± 0.2
	MM 24	9	6.5 ± 1.1	7.1 ± 0.4	7.6 ± 0.2
Glucose	MM 3	10	6.9 ± 1.1	7.7 ± 0.2	7.9 ± 0.1
	MM 24	9	6.8 ± 0.4	7.9 ± 0.3	8.1 ± 0.2
Amylum	MM 3	9	6.9 ± 0.4	7.2 ± 0.5	7.6 ± 0.4
	MM 24	9	6.3 ± 1.1	7.5 ± 0.3	7.8 ± 0.2
Wheat	MM 3	9	7.1 ± 0.3	5.7 ± 0.9	7.4 ± 0.3
	MM 24	9	7.1 ± 0.3	5.1 ± 0.3	7.5 ± 0.2

* mean and standard deviations of log₁₀ counts **Differences in *A. viscosus* counts: F = 3.48 df (3,69) P < 0.001. *Differences in *S. mutans* counts: F = 2.63 df (3,69) n.s. ***Differences in total bacterial count F = 2.37 df (3,69) n.s.

DISCUSSION

S. mutans established well in all dietary groups without significant quantitative differences. The addition of readily fermentable sugars like lactose and glucose did not give an advantage over the amylum and flour diets for the establishment of *S. mutans*. In contrast the establishment of *A. viscosus* was significantly reduced in the wheat flour group.

The proposed mechanism of establishment of *S. mutans* in sucrose free diets involving the entrapment of *S. mutans* cells in extracellular polysaccharides produced by *A. viscosus* is not reflected in our data. According to that proposal, the counts of the two organisms were expected to be highest in the glucose diet, because glucose would stimulate the synthesis of heteropolysaccharide plaque matrix by *A. viscosus* (Van der Hoeven, 1974).

It can be argued that the inclusion of skim milk in diet might have influenced the establishment of *S. mutans* in the non-lactose groups. However, lactose from skim milk accounted for only 2% of the total diet, an amount which might be too low to contribute significantly towards the observed *S. mutans* counts. The non-sucrose dependent establishment of *S. mutans* observed in this study is compatible with observation in breast fed infants in Tanzania (Matee *et al.*, 1992b) and in populations in Sudan (El Tayeb *et al.*, 1985), Kenya (Beighton *et al.*, 1989), Mozambique (Carlsson *et al.*, 1985: 1987) and Tanzania (Matee *et al.*, 1985) where sucrose consumption is low, but starch consumption is high. In these countries low sucrose consumption may be responsible for the low level of dental caries but this does not affect the prevalence and level of mutans streptococci.

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REFERENCES:

1. BECKERS, H. J. A. and VAN der HOEVEN, J. S. (1982). Growth rates of *Actinomyces viscosus* and *Streptococcus mutans* during early colonization of tooth surfaces in gnotobiotic rats. *Infect. Immun.* 35, 583-587.
2. BEIGHTON, S., MANJI, F., BAELUM, V., FEJERSKOV, O., JOHNSON, N.W. and WILTON, J. M. A. (1989). Associations between salivary levels of *Streptococcus mutans*, *Streptococcus sobrinus*, lactobacilli and caries experience in Kenyan adolescents. *J. Dent. Res.* 68, 1242-1246.
3. BIRKHED, D. and SKUDE, G. (1978). Relation of amylase to starch and lycasin metabolism in human dental plaque *in vitro*. *Scand. J. Dent. Res.* 86, 248-258.
4. CARLSSON, P., GANDOUR, I. A., OLSSON, B., RICKARDSSON, B. and ABBAS, K. (1987). High prevalence of mutans streptococci in a population with low caries experience. *Oral Microbiol. Immunol.* 2, 121-124.
5. CARLSSON, P., OLSSON, B. and BRATTHALL, D. (1985). The relationship between bacterium *Streptococcus mutans* and dental caries in children of Mozambique. *Arch Oral Biol.* 30, 265-268.
6. EL TAYEB, I. Y., BRATTHALL, D. and CARLSSON, P. (1985). Dental caries and *Streptococcus mutans* in Sudanese children. *Odontostomatol. Trop.* 8, 77-80.
7. FROSTELL, G., KEYES, P. H. and LARSON, R. H. (1967). Effect of various sugars and sugar substitutes on dental caries in hamsters and rats. *J. Nutr.* 93, 65-76.
8. GIBBONS, R. J. and FITZGERALD, R. J. (1969). Dextran-induced agglutination of *Streptococcus mutans* and its potential role in the formation of microbial dental plaques. *J. Bacteriol.* 98, 341-346.
9. GREEN, R. M. and HARTLES, R. L. (1969). The effects of diets containing different mono- and disaccharides on the incidence of dental caries in the albino rat. *Arch Oral Biol.* 14, 235-241.
10. GUGGENHEIM, B., KONIG, K.G., HERZOG, E. and MUHLEMANN, H. R. (1966). The cariogenicity of different dietary carbohydrates tested on rats in relative gnotobiosis with a streptococcus producing extracellular polysaccharide. *Helv. Odontol. Acta* 10, 101-113.
11. HAMADA, S. and SLADE, H. D. (1980). Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* 44, 331-384.
12. HASSID, W. Z. (1970). Biosynthesis of sugars and polysaccharides. In: *The carbohydrates. Chemistry and Biochemistry*: Pigman, W. and Horton, D. (eds.). New York, Academic Press, Vol. IIA, pp 301-373.
13. MATEE, M. I., MIKX, F. H. M., FRENKEN, J. E. F. N., TRUIN, G. J. and RUIKEN, H. M. H. M. (1985). Selection of a micromethod and its use in the estimation of salivary *Streptococcus mutans* and lactobacillus counts in relation to dental caries in Tanzanian children. *Caries Res.* 19, 497-506.
14. MATEE, M. I. N., MIKX, F. H. M., MASELLE, S. Y. M. and VAN PALENSTEIN HELDERMAN, W. H. (1992). Mutans Streptococci and lactobacilli in breast-fed children with rampant caries. *Caries Res.* 26, 183-187.
15. PEARCE, E. I. F. and SISSONS, C. H. (1987). On the cariogenicity of lactose. *New Zealand Dental J.* 83, 32-36.
16. SCHEMMEL, R. A., KROHN-LUTZ, K., LYNCH, P. and KABARA, J. J. (1982). Influence of dietary disaccharides on mouth micro-organisms and experimental dental caries in rats. *Arch. Oral Biol.* 27, 435-441.
17. VAN der HOEVEN, J. S. (1974). A slime producing micro-organism in dental plaque of rats, selected by glucose feeding. *Caries Res.* 8, 193-210.
18. VAN HOUTE, J. (1982). Bacterial adherence and dental plaque formation. *Infection* 10, 252-260.

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