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POTENTIAL CARIOGENICITY OF LYCASIN® 80/55 BEFORE AND AFTER REPEATED TRANSMISSIONS OF THE DENTAL PLAQUE FLORA IN RATS

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Summary—Five successive experiments, with rats fed *ad libitum* on diets containing sucrose or Lycasin® 80/55, were carried out. In experiment I, the rats were inoculated with *Streptococcus mutans* alone or with *Strep. mutans* in combination with *Actinomyces viscosus*. In three successive transmission experiments (II, III, IV), the rats were inoculated with plaque of the rats of the preceding experiment. The rats of experiment V were inoculated with the original strains or with plaque derived from experiment IV. After five successive transmissions of the plaque flora, no alterations were demonstrated in the numbers and percentages of *Strep. mutans* and *A. viscosus* or in the fermentation rate of Lycasin by the plaque flora *in vitro*. Lycasin® 80/55 was virtually non-cariogenic compared with sucrose ($p < 0.001$) irrespective of whether the rats were inoculated with *Strep. mutans* alone or in combination with *A. viscosus*, with the original strains or with plaque from the preceding experiments with rats on a Lycasin® 80/55-containing diet.

INTRODUCTION

Powdered Lycasin® type 55, manufactured by Roquette Frères (Lille, France) is a hydrogenated starch hydrolysate, which contains sorbitol (6–8 per cent), maltitol (50–55 per cent) and higher hydrogenated saccharides ($\simeq$40 per cent). It is commercially available as a clear colourless syrup containing 75 ± 1 per cent dry substances (Lycasin® 80/55). Bacteriological tests (Soyer and Frank, 1979; Havenaar *et al.*, 1979) showed that Lycasin 80/55 is slowly fermented by *Streptococcus mutans* and *Lactobacillus casei*. After mouth rinses with Lycasin 80/55 (Frostell and Birkhed, 1978) and during consumption of Lycasin 80/55-containing sweets (Imfeld, 1977; Mühlemann, 1983), the pH in dental plaque did not fall below the critical value of pH 5.7. Caries experiments in rats fed under programmed feeding conditions and inoculated with *Strep. mutans* showed that Lycasin 80/55 is virtually non-cariogenic (Havenaar *et al.*, 1984). However, fermentation experiments *in vitro* demonstrated that repeated cultivation of *Strep. mutans* and *L. casei* in a medium containing Lycasin 80/55 resulted in adaptation of these strains to metabolize Lycasin (Havenaar *et al.*, 1979). This adaptation found expression in a faster and lower pH drop.

Alterations of the dietary-carbohydrate content may affect the plaque flora, e.g. the numbers of *Strep. mutans* in man (De Stoppelaar, Van Houte and Backer Dirks, 1970) as well as in rodents (reviewed by Van Houte, 1981). Whether selection or adaptation of the oral bacteria take place when Lycasin 80/55 is consumed frequently over long periods is unknown.

Our aim was to investigate whether the plaque flora of rats, harbouring *Strep. mutans* and *Actinomyces viscosus*, adapted to metabolize Lycasin 80/55.

MATERIALS AND METHODS

General experimental design

Five successive experiments were carried out on Osborne–Mendel rats with feeding *ad libitum*. The first experiment tested the potential cariogenicity of Lycasin type 55 in comparison with sucrose in rats inoculated with *Strep. mutans* serotype *c* and combinations of *Strep. mutans* serotype *c* or *d* and *A. viscosus*. In three successive transmission experiments (II–IV), the rats were inoculated with plaque of the rats of the preceding experiment which harboured *Strep. mutans* serotype *c* and *A. viscosus* and were fed on a sucrose or Lycasin-containing diet. The fifth experiment tested the potential cariogenicity of Lycasin type 55 in comparison with sucrose in rats inoculated with the original *Strep. mutans* serotype *c* plus *A. viscosus* strains, or with plaque harbouring these strains that had passed through the rats of the four previous experiments (I–IV) on a sucrose- or Lycasin-containing diet.

General procedures

The indigenous oral flora of the rats was suppressed by ampicillin (200 parts/10^6 in the drinking water) during the pre-weaning period. At 21 ± 1 days of age, the rats were weaned, weighed and earmarked. The litters were distributed equally among the experimental groups (day 0). They were housed as single-sex pairs in macrolon cages with stainless-steel screen bottoms. The six groups of experiment I consisted of four male and four female rats. Each transmission experiment (experiments II–IV) consis-

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ted of two groups of at least eight animals. Experiment V consisted of six groups (a–f) of seven male and six female rats.

The experimental periods of experiments I and V were 63 days for the female rats and 64 days for the males. The three transmission experiments (II–IV) were of 38, 30 and 28 days, respectively. At the end of the experiments, the animals were killed with Na-pentobarbital (Triotal®) and weighed. After decapitation, the upper molars were extracted and the lower jaws were defleshed. A post-mortem examination was done. Buccal and lingual smooth-surface caries was assessed, after which the mandibular molars were sectioned for scoring approximal and fissure caries.

Environmental conditions, general health control, post-mortem examination, the caries-scoring technique and statistical analysis were described in detail previously (Havenaar *et al.*, 1983, 1984).

Diets and eating habits

The rats of experiment I were fed on a purified diet containing 20 per cent sucrose and 5 per cent glucose (SSP 20/5, Havenaar *et al.*, 1983) during the first four days of the experimental period. From day four, the rats received diet SSP supplemented with 30 per cent uncooked starch and 20 per cent sucrose (control diet) or 20 per cent powdered Lycasin type 55 (test diet). The animals in all other experiments received test and control diet from day zero (d0). To avoid possible metabolic disturbances by Lycasin, the percentages of sucrose and Lycasin were increased stepwise: d0 or d4–d9, 10 per cent; d10–d15, 15 per cent; from d16, 20 per cent. Raw starch and powdered Lycasin type 55 were obtained from Roquette Frères (Lille, France). Diet and tap water (<0.07 parts/10^6 F^-) were available *ad libitum*. In a separate experiment, the eating habits of six 7-week-old female rats on the test and control diets were recorded continuously using six registration units as described by Spengler (1960) and König *et al.* (1964). After an adaptation period of three days, the mean number of meals per day, the meal size and the eating time per meal were recorded during four days for each diet.

Microbiology: inoculation and plaque sampling

The bacterial strains used were: *Strep. mutans* serotype *c*, strain C67-1 (De Stoppelaar *et al.*, 1971), *Strep. mutans* serotype *d*, strain 50B4 (Huis in 't Veld *et al.*, 1978), and *A. viscosus*, strain Ny-1 (Van der Hoeven, 1974). One batch of each strain was distributed into several ampoules and kept in liquid nitrogen (called original strains).

Experiment I

On days one and two, the animals were inoculated using cotton swabs with 125 μl of a washed suspension of the strains indicated in the upper part of Table 1. Each suspension contained between 2×10^8 and 3×10^9 colony-forming units per ml. On day three, four groups were re-inoculated with a suspension of *A. viscosus* containing 4×10^8 c.f.u. per ml. After termination of the experiment, the upper molars were extracted, ground in 10 ml transport medium and treated ultrasonically in order to dislodge and homogenize the plaque. The lower jaws were sonified. These plaque samples were cultured on the elective CBRCA medium (Van Palenstein Helderman and Winkler, 1975) under anaerobic conditions. Total viable numbers and the percentages of *Strep. mutans* serotype *c* and *d* and *A. viscosus* could be determined by their characteristic colony morphology. This plaque sampling and culturing process were described by Havenaar *et al.* (1984). The remaining plaque suspensions of the upper and lower molars were pooled for each group and centrifuged for 20 min at 5000 rev/min. The sediment was resuspended in 2 ml saline. After removal of the coarse

Table 1. Incidence of fissure caries in the mandibular molars (16 fissures at risk), viable numbers and percentages of *Strep. mutans* and *A. viscosus* in plaque of the maxillary and mandibular molars of experiment I (means ± SD)

Diet SSP; carbohydrates:						
Starch	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent
Sucrose	20 per cent	—	20 per cent	—	20 per cent	—
Lycasin	—	20 per cent	—	20 per cent	—	20 per cent
Inoculation:	*Strep. mutans* serotype *c*	*Strep. mutans* serotype *c*	*Strep. mutans* serotype *c* *A. viscosus*	*Strep. mutans* serotype *c* *A. viscosus*	*Strep. mutans* serotype *d* *A. viscosus*	*Strep. mutans* serotype *d* *A. viscosus*
Caries lesions:						
Total (A + T + B + C)	13.1 ± 1.7	2.5 ± 2.1	12.3 ± 2.6	2.0 ± 1.4	11.3 ± 2.1	1.5 ± 1.2
Initial (T + B + C)	9.3 ± 2.3	0	10.0 ± 4.3	0	8.0 ± 2.3	0
Advanced (B + C)	5.0 ± 3.1	0	8.3 ± 4.7	0	5.1 ± 1.7	0
Very advanced (C)	2.9 ± 2.4	0	5.5 ± 4.8	0	4.4 ± 1.4	0
Number of *Strep. mutans* × 10^6:						
Maxillary molars	88 ± 34	30 ± 11	147 ± 50	28 ± 12	29 ± 10	9 ± 5
Mandibular molars	26 ± 13	6 ± 5	29 ± 18	9 ± 4	13 ± 26	5 ± 3
Number of *A. viscosus* × 10^6:						
Maxillary molars	0	0	318 ± 205	15 ± 8	362 ± 179	18 ± 10
Mandibular molars	0	0	45 ± 31	1 ± 0.7	30 ± 24	2 ± 1.5
Percentage of *Strep. mutans*:						
Maxillary molars	92 ± 3	66 ± 8	33 ± 9	44 ± 10	10 ± 8	17 ± 11
Mandibular molars	81 ± 10	43 ± 16	34 ± 10	37 ± 20	19 ± 18	24 ± 9
Percentage of *A. viscosus*:						
Maxillary molars	0	0	62 ± 11	26 ± 20	80 ± 19	30 ± 13
Mandibular molars	0	0	48 ± 19	15 ± 22	50 ± 20	15 ± 18

remainder of the upper molars, a suspension of the plaque of each group was used to inoculate the rats in a subsequent experiment.

Experiments II–IV

In experiment II, on day one, the sucrose and Lycasin groups were inoculated with the plaque suspensions derived from the female rats of the sucrose or Lycasin groups respectively of experiment I, harbouring *Strep. mutans* serotype *c* and *A. viscosus*. On day two, the rats were re-inoculated with plaque suspensions derived from the male rats in the same way. This procedure was repeated in experiments III and IV with the plaque suspensions derived from experiments II and III, respectively (upper part of Table 2). At the end of each experiment, the numbers and percentages of *Strep. mutans* and *A. viscosus* were determined as mentioned under experiment I. However, in these experiments both the maxillary and mandibular molars were extracted and ground.

Experiment V

The groups a and b of experiment V were inoculated on day one and two with the original *Strep. mutans* serotype *c* and *A. viscosus* strains as described under experiment I. The next two groups (*c* and *d*) and the last two groups (*e* and *f*) were inoculated with plaque suspensions of the sucrose and Lycasin groups respectively of experiment IV (the upper part of Table 3). At the end of the experimental period, plaque samples were taken, cultured as in experiment I and the numbers and percentages of *Strep. mutans* and *A. viscosus* determined. The plaque sample from the maxillary molars of each rat was cultured in a fermentation medium containing 5 per cent glucose or 5 per cent Lycasin type 55 and incubated under anaerobic conditions for 18, 42 and 96 h (male rats) or 24, 72, 120 168 h (female rats). At each point, the pH was measured.

RESULTS

Experiment I

Between the sucrose groups, there were no statistically-significant differences in the incidence of fissure lesions (Table 1). Approximal carious lesions were found only in the three sucrose groups. The mean incidence of the advanced approximal lesions (not shown in the Table) were 0.8 ± 1.1, 1.1 ± 1.5 and 1.0 ± 1.2, respectively. The mean incidence of the fissure carious lesions in the Lycasin groups was significantly lower than in the sucrose groups ($p < 0.001$), irrespective of whether the rats were inoculated with *Strep. mutans* serotype *c* only or with *Strep. mutans* serotype *c* or *d* in combination with *A. viscosus*. The three Lycasin groups had similar numbers of the slightest fissure lesions but no severe lesions.

In all separate plaque samples of the maxillary and mandibular molars, the inoculated bacteria could be recovered in high numbers. The mean numbers of *Strep. mutans* and *A. viscosus* in the maxillary and mandibular molar plaque were significantly lower in the Lycasin groups compared with the corresponding sucrose groups ($p < 0.02$). However, as percentages

Table 2. Viable numbers and percentages of *Strep. mutans* and *A. viscosus* in plaque of the maxillary and mandibular molars of experiments II, III and IV (means ± SD)

	Experiment II		Experiment III			Experiment IV	
Diet SSP; carbohydrates:	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent
Starch	20 per cent	—	20 per cent	—	20 per cent	—	—
Sucrose	—	20 per cent	—	20 per cent	—	—	20 per cent
Lycasin							
Inoculation with the pooled plaque suspension of:	Sucrose group of experiment I	Lycasin group of experiment I	Sucrose group of experiment II	Lycasin group of experiment II	Sucrose group of experiment III	Sucrose group of experiment III	Lycasin group of experiment III
Number of *Strep. mutans* × 10^6:							
Maxillary molars	96 ± 21	36 ± 12	149 ± 25	51 ± 15	209 ± 68	65 ± 28	
Mandibular molars	110 ± 24	42 ± 10	118 ± 60	48 ± 19	117 ± 37	59 ± 23	
Number of *A. viscosus* × 10^6:							
Maxillary molars	83 ± 30	9 ± 11	58 ± 29	6 ± 3	125 ± 10	22 ± 16	
Mandibular molars	28 ± 19	6 ± 6	18 ± 8	5 ± 3	21 ± 13	9 ± 6	
Percentage of *Strep. mutans*:							
Maxillary molars	50 ± 7	61 ± 7	72 ± 10	63 ± 9	63 ± 14	53 ± 13	
Mandibular molars	76 ± 10	65 ± 8	82 ± 8	71 ± 7	86 ± 12	65 ± 10	
Percentage of *A. viscosus*:							
Maxillary molars	43 ± 10	9 ± 8	22 ± 9	8 ± 5	28 ± 12	19 ± 9	
Mandibular molars	18 ± 8	10 ± 8	16 ± 10	7 ± 4	8 ± 5	13 ± 9	

there were significant differences only for *A. viscosus* ($p < 0.02$). The mean percentage of *Strep. mutans* in the Lycasin group which was inoculated with *Strep. mutans* only was lower than in the corresponding sucrose group ($p < 0.02$).

Experiments II–IV

All rats in the three successive transmission experiments harboured *Strep. mutans* serotype *c* and *A. viscosus* (Table 2). As in experiment I, the numbers of both strains and the percentages of *A. viscosus* in the maxillary and mandibular molar plaque were significantly lower in the Lycasin groups in comparison with the corresponding sucrose groups ($p < 0.02$). The percentages of *Strep. mutans* were not statistically different between the sucrose and Lycasin groups. Comparing experiments II, III and IV with each other, the ratio between the numbers of both strains in the Lycasin and in the sucrose group were similar.

Experiment V

Between the sucrose groups there were no statistically-significant differences in the incidence of fissure lesions (Table 3). Approximal carious lesions were found only in the three sucrose groups. The mean incidence of the advanced approximal lesions (not shown in the Table) were 0.9 ± 1.3, 1.8 ± 1.7 and 0.8 ± 1.5, respectively. The incidence of fissure caries in the Lycasin groups was significantly lower compared with the sucrose groups ($p < 0.001$), irrespective of whether the rats were inoculated with the original *Strep. mutans* and *A. viscosus* strains or with *Strep. mutans* and *A. viscosus* strains which passed several rat experiments on either a sucrose or a Lycasin-containing diet (Table 3). The three Lycasin groups had equal numbers of the slightest fissure lesions but no severe lesions. *Strep. mutans* and *A. viscosus* were found in all separate maxillary and mandibular plaque samples. Within the three sucrose or Lycasin groups, there were no differences (Table 3). The mean numbers of both strains and the percentages of *A. viscosus* were significantly lower in the Lycasin groups in comparison with the sucrose groups ($p < 0.02$).

Eating habits and general health

The average meal frequency per day, the meal size and the eating time per meal were the same for both diets (Table 4). None of the animals died prematurely and all were in good general health. The consistency of the faeces was normal in all groups. The mean body-weight gains of the female rats fed on the Lycasin diet were the same as those on the sucrose diet. The body weight of the male rats of experiments I and V were slightly (5–6 per cent) lower in the Lycasin groups compared with the sucrose groups. Post-mortem examination showed no differences between the Lycasin and sucrose groups in the absolute and relative weight (2.4 times) compared with the sucrose groups.

In-vitro *fermentation*

The pH values indicating fermentation of glucose or Lycasin 80/55 under *in-vitro* conditions by the plaque flora of the maxillary molars of the groups a–f of experiment V are shown in Fig. 1. The pH falls caused by the fermentation of glucose were the same in all six groups: considerably faster and to a lower final pH than in the case of Lycasin. However, during the fermentation of Lycasin, some differences were present between the groups fed on sucrose or Lycasin. The pH values of the three Lycasin-diet-groups (b, d and f) were significantly lower than the pH values of the three sucrose-diet-groups (a, c and e) after 42 and 96 h (Fig. 1A; $p < 0.02$) and after 72 and 120 h (Fig. 1B; $p < 0.05$). The plaque flora of group f which passed through rats on the Lycasin diet during five successive experiments showed slightly lower pH values than the Lycasin groups b and d, although not statistically significant.

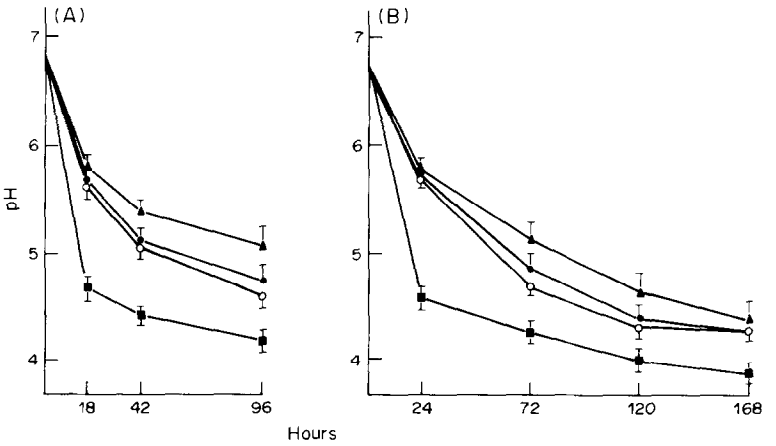

Fig. 1. Mean pH ± SD in fermentation medium containing 5 per cent glucose (■) or 5 per cent Lycasin 80/55 (▲, ●, ○) and inoculated with maxillary molar plaque of the groups a–f of experiment V. (A) male rats, $n = 7$; (B) female rats, $n = 6$. (■—■) Plaque of the sucrose and Lycasin groups (a–f). (▲—▲) Plaque of the sucrose groups (a, c and e). (●--●) Plaque of the Lycasin groups b and d. (○—○) Plaque of Lycasin group f.

Table 3. Incidence of fissure caries in the mandibular molars (16 fissures at risk), viable numbers and percentages of *Strep. mutans* and *A. viscosus* in plaque of the maxillary and mandibular molars of experiment V (means ± SD)

Group:	a	b	c	d	e	f
Diet SSP; carbohydrates:						
Starch	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent	30 per cent
Sucrose	20 per cent	—	20 per cent	—	20 per cent	—
Lycasin	—	20 per cent	—	20 per cent	—	20 per cent
Inoculation:	Original *Strep. mutans* *A. viscosus* strains	Original *Strep. mutans* *A. viscosus* strains	Plaque suspension sucrose group of experiment IV	Plaque suspension sucrose group of experiment IV	Plaque suspension Lycasin group of experiment IV	Plaque suspension Lycasin group of experiment IV
Caries lesions:						
Total (A + T + B + C)	12.9 ± 1.4	1.7 ± 1.3	14.4 ± 1.2	2.2 ± 2.0	13.8 ± 2.1	2.4 ± 2.1
Initial (T + B + C)	11.1 ± 1.5	0.1	13.2 ± 1.5	0	11.2 ± 3.2	0
Advanced (B + C)	7.8 ± 3.6	0	10.8 ± 2.7	0	7.7 ± 4.7	0
Very advanced (C)	5.2 ± 3.1	0	6.9 ± 3.3	0	4.8 ± 4.1	0
Number of *Strep. mutans* × 10^6:						
Maxillary molars	96 ± 36	32 ± 9	144 ± 72	27 ± 7	116 ± 44	27 ± 9
Mandibular molars	20 ± 9	6 ± 4	30 ± 20	8 ± 8	24 ± 13	5 ± 5
Number of *A. viscosus* × 10^6:						
Maxillary molars	122 ± 41	10 ± 7	143 ± 56	7 ± 9	99 ± 38	16 ± 10
Mandibular molars	16 ± 16	0.7 ± 0.5	12 ± 8	0.9 ± 19	9 ± 10	1.4 ± 3
Percentage of *Strep. mutans*:						
Maxillary molars	41 ± 15	59 ± 14	48 ± 14	52 ± 8	51 ± 10	47 ± 15
Mandibular molars	49 ± 14	36 ± 13	61 ± 8	42 ± 10	64 ± 15	29 ± 15
Percentage of *A. viscosus*						
Maxillary molars	52 ± 12	16 ± 10	50 ± 14	10 ± 10	44 ± 10	26 ± 11
Mandibular molars	32 ± 19	4 ± 3	18 ± 9	6 ± 10	19 ± 9	10 ± 5

Table 4. Number of meals per day, meal size in grammes and the eating time per meal in minutes of six rats on the control (sucrose) and test (Lycasin) diet (means ± SD)

	Rats						
	1	2	3	4	5	6	1–6
Number of meals per day:							
Control diet	16.8 ± 1.5	21.0 ± 2.2	17.8 ± 1.7	20.0 ± 4.2	18.0 ± 4.6	16.3 ± 1.9	18.3 ± 3.1
Test diet	14.0 ± 3.6	17.5 ± 2.4	18.5 ± 5.7	24.3 ± 2.6	19.5 ± 4.2	18.3 ± 2.5	18.7 ± 4.6
Meal size:							
Control diet	1.2 ± 0.6	1.0 ± 0.7	0.9 ± 0.6	1.1 ± 0.6	1.4 ± 1.0	1.7 ± 1.2	1.2 ± 0.9
Test diet	1.4 ± 0.7	1.3 ± 0.9	1.3 ± 1.0	0.8 ± 0.4	1.2 ± 0.7	1.0 ± 0.6	1.1 ± 0.7
Eating time per meal:							
Control diet	12 ± 8	11 ± 10	10 ± 8	10 ± 9	9 ± 7	13 ± 12	11 ± 9
Test diet	11 ± 6	10 ± 9	14 ± 12	7 ± 6	11 ± 9	12 ± 10	11 ± 9

DISCUSSION

Our findings show that Lycasin 80/55 fed *ad libitum* is virtually non-cariogenic in rats inoculated with *Strep. mutans*. Programmed feeding experiments (Havenaar *et al.*, 1984) showed similar results, although the differences in caries scores between the sucrose and Lycasin groups were larger in the *ad-libitum* experiments. As the eating patterns were the same (Table 4), the difference in caries scores between the Lycasin- and the sucrose-containing diets cannot be explained by differences in eating habits of the rats in the test and control groups. The extremely low-potential cariogenicity of Lycasin 80/55 was found in the rats colonized with *Strep. mutans* as well as in the rats harbouring *Strep. mutans* and *A. viscosus* (Table 1). There was no change in the potential cariogenicity of Lycasin 80/55 after five successive transmissions of the total plaque flora of the rats fed on sucrose or Lycasin (Table 3).

The inoculation of *A. viscosus* in combination with *Strep. mutans* serotype *c* seemed to enhance the colonization of *Strep. mutans* in the plaque (Table 1). This combination of microorganisms also showed a higher number of advanced carious lesions, although not significantly. Possible synergistic effects of *Strep. mutans* and *A. viscosis* on plaque formation and caries were also found by Regolati, Guggenheim and Mühlemann (1972).

More *A. viscosus* Ny-1 colonized in the presence of *Strep. mutans* C67-1. This is in contrast to the results of a study in gnotobiotic rats (Rogers, Van der Hoeven and Mikx, 1978), in which the establishment of *A. viscosus* Ny-1 was prevented by the bacteriocin-producing *Strep. mutans* strain C67-1. Because representative numbers of *Strep. mutans* colonies, isolated from the plaque samples of experiment V, had the same bacteriocin activity against *A. viscosus* Ny-1 as a reference strain of *Strep. mutans* C67-1 subcultured in our laboratory, this conflicting finding cannot be caused by alterations in the bacteriocin-production of *Strep. mutans* during the experiments. Differences in experimental conditions (gnotobiotic versus conventional animals) could explain this discrepant finding.

In the three transmission experiments and experiment V, the numbers and percentages of *Strep. mutans* and *A. viscosus* in the maxillary molar plaque were similar within the sucrose groups and within the Lycasin groups (Tables 2 and 3). The ratios between the sucrose and the corresponding Lycasin groups were also similar. This indicates that long-term exposure of the plaque flora to Lycasin 80/55 did not alter the composition of the microflora under our experimental conditions. However, the fermentation of Lycasin 80/55 *in vitro* by the plaque flora of the rats fed on Lycasin was slightly faster than that of the rats fed on sucrose. Some extra enzymic activity may have been induced during long-term exposure to Lycasin. These findings agree with a study in man (Birkhed *et al.*, 1979), in which no changes were found in the proportions of total anaerobic streptococci and *Strep. mutans*, and also in plaque pH, before and after three months frequent consumption of the Swedish Lycasin. Long-term consumption of maltitol-containing lozenges (Lycasin type 55 contains 50–55 per cent maltitol), however, caused a significant increase in the percentage of *Strep. mutans* and a lower pH in plaque after the test period. Birkhed *et al.* (1979) could not explain this because the *Strep. mutans* strains tested by Edwardsson, Birkhed and Mejáre (1977) did not ferment maltitol. In our previous *in-vitro* study (Havenaar *et al.*, 1979), maltitol as well as Lycasin 80/55 were fermented slowly by cell suspensions of *Strep. mutans* C67-1, especially after an adaptation period.

Our study shows clearly that there was no adaptation or selection of the plaque flora towards Lycasin 80/55 and no alteration in the extremely low cariogenicity of Lycasin 80/55.

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