Turku sugar studies XX

Microbiological findings and plaque index values in relation to 1-year use of xylitol chewing gum

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The aim was to study possible alterations in the microbial flora of plaque and saliva in relation to partial substitution of dietary sucrose with xylitol. The development of plaque index values was observed simultaneously. These observations were carried out during a 1-year clinical trial, the effects of sucrose (S) and xylitol (X) chewing gum on the incidence of dental caries being observed in 100 young adults. Paraffin-stimulated saliva samples were diluted stepwise and cultivated on Rogosa S.L. agar and Sabouraud agar aerobically. Lyophilized dental plaque samples were cultivated on phenol red agar under anaerobic and aerobic conditions. The pH-values were measured after incubating the mixed plaque flora for 1 and 7 days in the presence of various sugars. Both the arithmetic and geometric means of the total CFU values on Rogosa S.L. agar decreased in the S-group at the 6-month phase but returned to the starting level after one year, whereas in the X-group they decreased or remained on the starting level. At the 6-month phase the difference between the groups was significant (U-test, $p = 0.0013$) and almost significant (U-test, $p = 0.0569$) at the end of the study. No significant differences or changes could be seen between or within the groups on Sabouraud agar. The geometric mean values of *S. sanguis* and *S. mutans* as well as the total CFU values on phenol red agar decreased considerably in both the S- and X-groups, but no significant differences could be detected in any of the streptococcal counts between the groups. The pH of the carbohydrate-containing culture media infected with mixed dental plaque significantly decreased, with the exception of the xylitol containing ones in which the pH values were not lowered even after 7 days' incubation. A significant decrease in plaque formation in relation of chewing *per se* was demonstrable. The difference in the plaque index values equalling or exceeding 2 was significant between the S- and X-groups. No bacterial adaptation to utilize xylitol occurred during the trial.

Key-words: Xylitol; sucrose; dental plaque; chewing gum; streptococcus; lactobacillus;

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Previous microbiological findings in these series of studies have indicated that the substitution of dietary sucrose (S) with xylitol (X) or fructose (F) did not affect the proportion of major microbial categories in highly diluted saliva or dental plaque samples (*Larmas et al.*, 1975). On the other hand, the mean values of viable *Streptococcus mutans* in dental plaque (*Gehring et al.*, 1975) and salivary colony forming units on highly selective Rogosa S.L. and Sabouraud antibiotic agar were

significantly lower in the X-group than in the S- or F-groups (*Larmas et al.*, 1975). The consumption of X also reduced the wet weight of plaque in these subjects (*Mäkinen & Scheinin*, 1975). The present study was carried out in relation to the 1-year clinical trial evaluating the caries incidence as affected by partial replacement of dietary S (*Scheinin et al.*, 1975). Microbiological studies in dental plaque and saliva, supplemented with quantification of plaque, were thus carried out in order to observe the possible differences brought about by the intake of a S- or X-containing chewing gum and simultaneous consumption of dietary S *ad libitum*.

MATERIAL AND METHODS

Subjects. A description of the subjects, 50 in the S-group and 50 in the X-group, their dental conditions and dietary habits has been given separately (*Scheinin et al.*, 1975).

Collection and treatment of the samples. Supragingival plaque samples as well as paraffin-stimulated saliva were collected, quantitated and dispersed by sonication as described previously (*Larmas et al.*, 1975). The plaque samples were freeze-dried and sent by air to the Department of Experimental Dentistry, University of Würzburg, for analysis.

Growth media and sample plating. A tenfold serial dilution of saliva in 0.05 % yeast extract was made and dilutions of 1 : 10, 1 : 100 and 1 : 1000 were plated on *Rogosa S.L.* agar (Orion Diagnostica, Helsinki, Finland). Dilutions of 1 : 10 and 1 : 100 were plated on Sabouraud dextrose agar with 12 mg/ml penicillin, 53 mg/ml streptomycin and 0.5 mg/ml actidione (Orion Diagnostica), and the samples were incubated aerobically as described earlier (*Larmas et al.*, 1975).

The lyophilized plaque samples were dispersed with a Potter S homogenizer (B. Braun, Melsungen, Germany) in a solution containing 1.0 ml Bacto-NIH-Thioglycollate broth (Difco Laboratories, Detroit, Michigan, U.S.A.) and 2.0 ml saline solution. Tenfold serial dilutions were made and plated on *phenol red* agar supplemented with 5 % sucrose. These incubations were carried out anaerobically in a 95 % N_2 and 5 % CO_2 atmosphere (BBL GasPak-jars, Baltimore Biological Laboratories, Baltimore, Md., U.S.A.) as well as aerobically as described in detail previously (*Gehring et al.*, 1975).

Identification of the microbes. On Rogosa S.L. agar the following microbial categories were identified: (1) lactobacilli, (2) streptococci and diphteroids and (3) candida as described previously (*Larmas et al.*, 1975). On the Sabouraud agar candida and other yeast species as well as the total colony-forming unit (CFU) values were counted as before (*Larmas et al.*, 1975).

The identification of isolated strains of streptococci (*S. mutans*, *S. salivarius* & *S. sanguis*) as well as the count of total CFU-value on phenol red agar was performed as described previously in detail (*Gehring et al.*, 1975). The simple test scheme of *Shklair & Keene* (1974) was followed in the diagnosis of *S. mutans* biotypes.

pH-measurements. Changes in the pH-values of culture media containing 1 % respectively X, sorbitol, S or F or no carbohydrate addition were measured as described in detail previously (*Gehring et al.*, 1975).

Plaque Index. Plaque was scored according to *Silness* and *Löe* (1964) from the four gingival areas of six teeth: 16, 12, 24, 44, 32 and 36. The subjects were instructed to refrain from all oral hygiene

measures during the preceding evening and the day of the examination, i.e. for 12—24 hours prior to the quantification of plaque.

During the study plaque was scored at the beginning, after 6 months, and at the end of the trial. The calculations of the Pl I values included the arithmetic mean and the median, and additionally, the frequency distribution of the Pl I values equalling or exceeding 2, the latter assessment thus providing information of visible plaque.

Statistical treatment. Variations in plaque and salivary CFU-values during the study within the S- and X-groups were tested with the Friedman test and the differences between the S- and X-groups with the Mann-Whitney U-test. The alterations of the pH-values occurring between days 1 and 7 were examined for significance by means of Wilcoxon's test for paired differences.

The differences between the S- and X-groups with regard to the Pl I values and their distribution was tested for significance with the Mann-Whitney U-test. The correlation between the Pl I value and sugar intake in liquid and solid form between meals (*Scheinin et al.*, 1975) was further evaluated by using the tests of Kendall and Spearman (*Bradley*, 1968; *Sachs*, 1972).

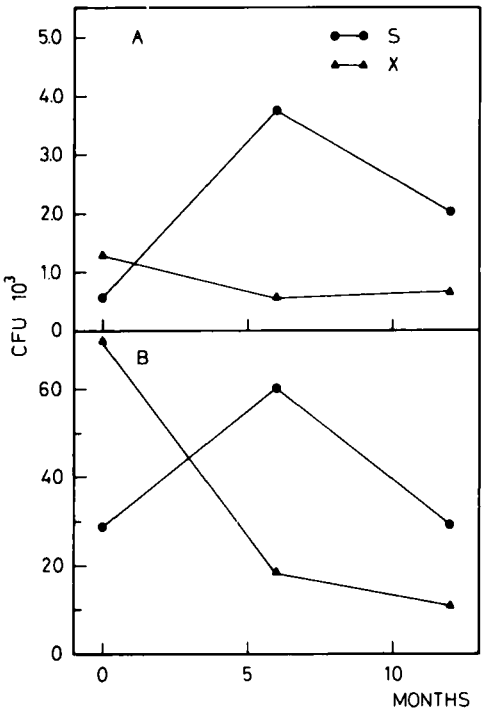

Fig. 1. The geometric means (A) and arithmetic means (B) of the total salivary CFU-values on Rogosa S.L. agar as a function of time and chewing gum group. S = sucrose, X = xylitol.

RESULTS

Salivary microbial counts. The arithmetic mean values of the total CFU on Rogosa S.L. agar varied between 29—60 $\times 10^3$ per ml saliva in the S-group and 11—72 $\times 10^3$ in the X-group (Fig. 1, Table I). Both the arithmetic and geometric mean values had increased in the S-group at the 6-month phase but returned to the starting level after one year. This variation was not significant. In the X-group a trend to almost significant decrease in the total CFU (Friedman, $p = 0.019$) could be seen both in the arithmetic and geometric mean values.

The increase in the S-group of the CFU values of lactobacilli was significant (Friedman, $p = 0.0001$), and almost significant for streptococci and diphtheroids (Friedman, $p = 0.034$). These colony counts decreased or remained on the starting level in the X-group (Fig. 2, Table II and III). Changes in the X-group were not significant in any cases.

The differences in the CFU-values on Rogosa S.L. agar between the S- and X-groups (Mann-Whitney U-test) were not significant at the beginning of the trial. At the 6-month phase a significant

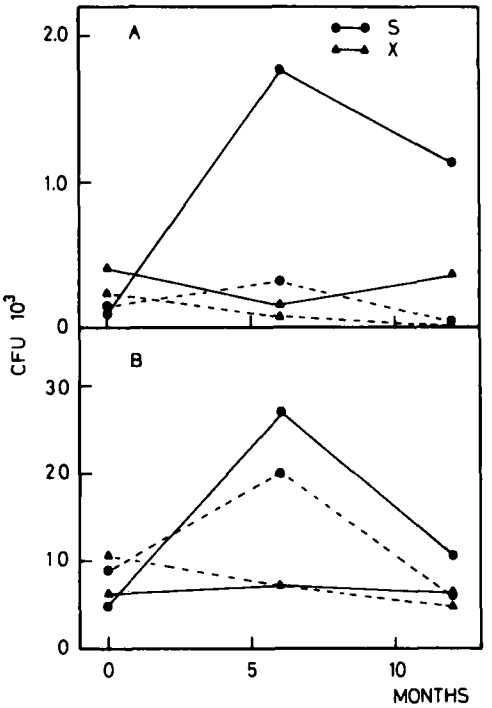

Fig. 2. The geometric means (A) and arithmetic means (B) of the salivary lactobacilli (solid line) and streptococci (dotted line) on Rogosa S.L. agar as a function of time and chewing gum group. S = sucrose, X = xylitol.

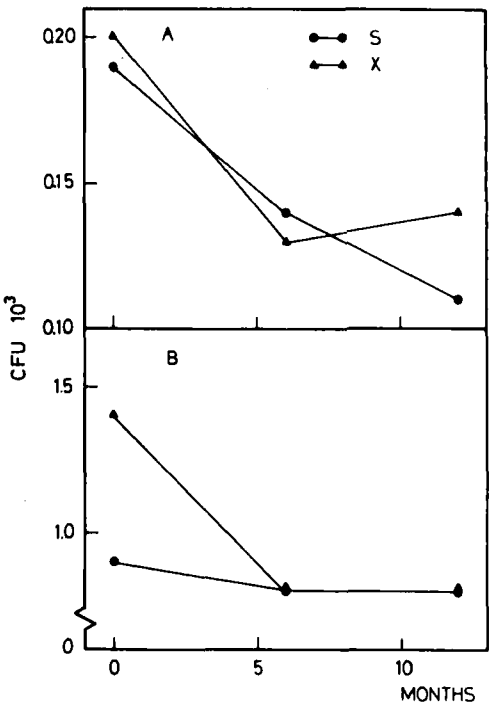

Fig. 3. The geometric means (A) and arithmetic means (B) of the total salivary CFU-values on Sabouraud antibiotic agar as a function of time and chewing gum group. S = sucrose, X = xylitol.

difference was seen in the CFU-value of lactobacilli (U-test, $p = 0.0008$), and in the total CFU-value of Rogosa S.L. agar (U-test, $p = 0.0013$) and almost significant (U-test, $p = 0.0539$) in the counts of streptococci and diphtheroids. These differences were not demonstrable at the end of the study. The difference in the total CFU on Rogosa S.L. agar was almost significant, (U-test, $p = 0.0569$).

The arithmetic mean of the total CFU-values on Sabouraud agar varied between 0.8—1.4 $\times$ 10^3 per ml saliva (Fig. 3, Table IV). No significant differences or changes could be seen between or within the groups during the whole trial.

Streptococci in dental plaque. The geometric mean values of anaerobic *Streptococcus sanguis* and *S. mutans* as well as total CFU-values on phenol red agar considerably decreased in both the S- and X-groups during the trial (Table V). The CFU-values of anaerobic *S. mutans* and especially aerobic *S. salivarius* were smaller and thus their proportion less (Table V). The proportion of biotype »c» of *Streptococcus mutans* was on the order of magnitude of 78 %—88 % of the total CFU-value of *Streptococcus mutans*. The »serotype a» was not found in any of the subjects. No significant differences could be detected in any of the streptococcal counts between the two sugar groups, although there was a trend towards a lower incidence of *S. mutans* in the X-group. On the other hand, in the beginning of the trial there was a significant difference

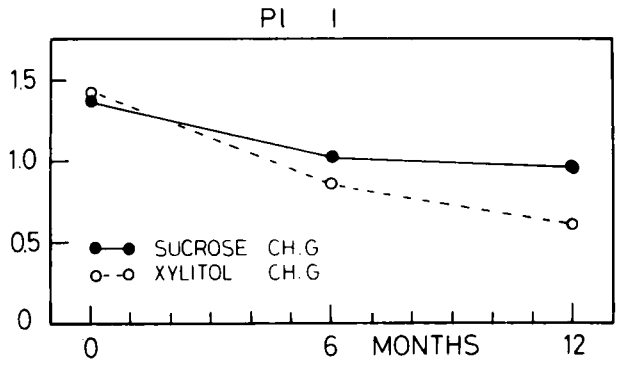

Fig. 4. Development of PI I during the trial in the S- and X-groups.

(Fisher $\alpha = 0.0008$) regarding the qualitative occurrence of *S. salivarius* (in the S-group in 38 % of the cases, and in the X-group in 64 % of the cases.) This difference was not demonstrable thereafter.

pH-values. The pH values of S- or F-containing culture media, which were infected with mixed dental plaque, significantly decreased to a pH value of 4.2—4.3 in both the S- and F-groups after one day's incubation, whereas they remained on the level, of the control value in X or sorbitol containing medium throughout the whole study (Table VI). The pH remained at the value of about 4 in S and F containing media after seven days' incubation and decreased to about pH 5 in sorbitol containing media, whereas it remained on the control level in the X containing ones (Table VI). The pH-values in the X containing media were the only ones which were not significantly lowered at the end of the study, even after 7 days' incubation in both the S- and X-groups.

Plaque Index. The findings at the baseline examination, and at the 6- & 12-month phases are shown separately for the S- and X-groups (Table VII). The difference between the first and last examination was also calculated, all these results being given separately for the minimum and maximum values, medians, means and standard deviations for individual teeth (Table VII). The difference between the S- and X-groups with regard to the development of the PI I values during the study was found to be significant for individual teeth (Table VIII). On an individual level (Table IX) the difference between the S- and X-groups was not significant at the beginning of the study, nor at the 6-month phase. On the other hand, this difference was found to be significant at the end of the study, and also with regard to the change occurring between the baseline examination and that at the termination of the study (Table IX, Fig. 4).

The results were separately examined with regard to visible plaque, i.e. PI I values equalling or exceeding 2. This analysis was carried out with regard to the overall development and the differences between the S- and X-groups. Irrespective of the sugar groups, a noticeable decrease in the frequency of the PI I values equalling or exceeding 2 was observed during the course of the trial (Tables X & XI, Figs. 5 & 6). Additionally, the difference between the S- and X-groups was found to be significant at the end of the study (Table XII, Fig. 7).

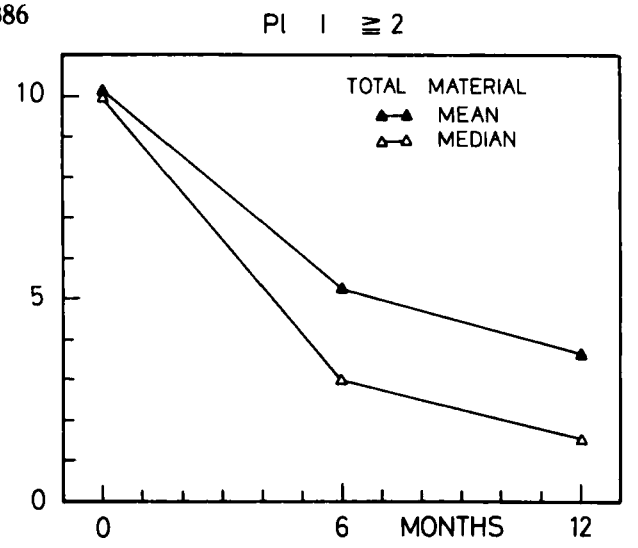

Fig. 5. Mean and median of Pl I values ≧ 2 during the trial in the combined S- and X-groups.

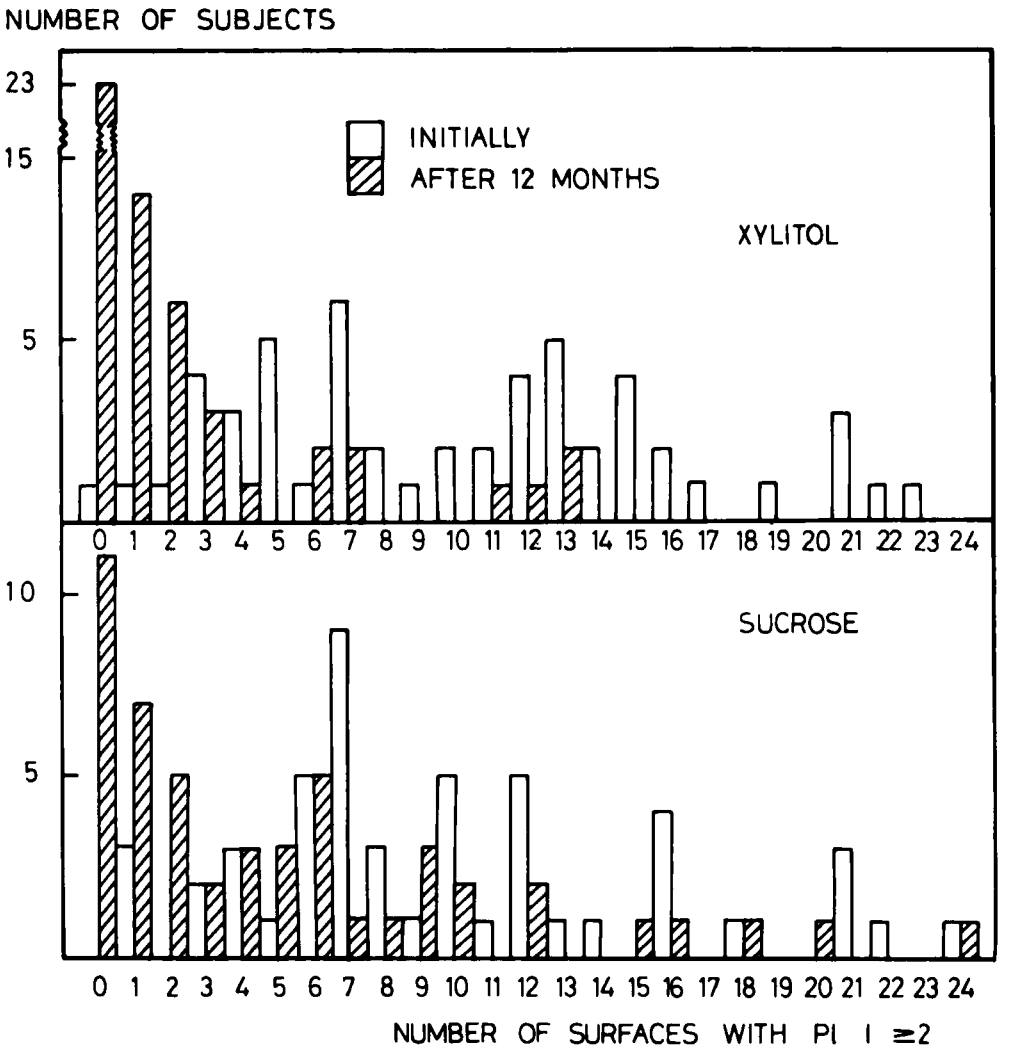

Fig. 6. Distribution of Pl I values ≧ 2 at the beginning and end of the trial in the S- and X-groups.

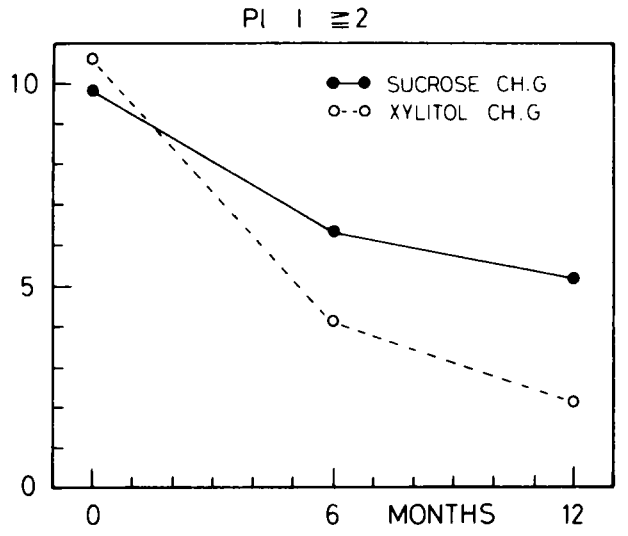

Fig. 7. Mean PI I values ≧ 2 during the trial in the S- and X-groups.

Further analysis of the plaque index values in relation to sucrose consumption showed a negative correlation between frequency of sucrose intake in solid and liquid forms between meals, and the difference in the PI I value between the initial and final examination. This correlation was found to be almost significant only in the S-group (Kendall's τ = —0.19, α = 0.0529; Spearman's Σ = —0.28, α = 0.0487).

DISCUSSION

The increase in the total CFU-value on Rogosa S.L. agar during the first 6-month period in the S-group is thought to be mainly due to the consumption of both S-containing chewing gum and S-intake between meals (*Scheinin et al.*, 1975). A decline in the consumption during the course of the study was regularly noticed when comparing the intake of S-containing products (*Scheinin et al.*, 1975). This is probably reflected in the decrease in the total CFU-values during the second 6-month period (Fig. 1). These observations strongly support the view that a consumption of caries-inducing carbohydrates invariably results in high salivary lactobacillus counts (*Jay*, 1947; *Becks*, 1950; *Krasse*, 1954). This microbiological finding is in full agreement with recent clinical observations that S in chewing gum in definitely cariogenic (*Scheinin et al.*, 1975; *Glass*, 1975).

The reduction of the acidogenic and aciduric salivary flora caused by partial replacement of S by X is thought to be due to the irrelevancy of five carbon polyols for microbial metabolism (*Mäkinen et al.*, 1975; *Larmas et al.*, 1975). This view is further supported by the findings of the changes in the pH values of the growth media: the presence of X or the total absence of sugar in the growth media caused the pH to remain above 6.0 after 1 and 7 days' incubation with the mixed plaque flora from all subjects. This indicates that the plaque flora after one year's use of X-containing chewing gum does not form acid from X. These findings are in agreement with the previous findings of the total replacement of dietary sucrose with X (*Gehring et al.*, 1975).

The mean count values on the growth media are of a similar order of magnitude as those reported earlier (*Gehring et al.*, 1975; *Larmas et al.*, 1975). Such comparisons are possible due to the use of exactly identical methodology. Thus, although anaerobic incubation would have been possible, the advantages of this procedure being recognized (*Frostell & Nord*, 1972), the maintenance of the same methodological standard as in previous studies has a logical motivation.

The drastic decrease in the CFU-values of *S. mutans* in both the S- and X-groups remains obscure. The chewing process in itself stimulates salivary secretion and influences its ion concentration and buffering capacity, etc., which may affect the ecology of the indigenous oral flora (see: *Geddes & Jenkins*, 1974). Some explanation may also be found from the present finding that a noticeable decrease in the number of subjects whose plaque index value was equal to or exceeded 2 was observed during the course of the trial, irrespective of the sugar groups.

Nearly complete replacement of dietary S by X resulted in a significant reduction in the *S. mutans* counts of dental plaque (*Gehring et al.*, 1975). In contrast, the present findings did not reveal significant differences between the S- and X-groups, whereas the *S. mutans* biotype distribution was similar. Observations of *van Houte & Duchin* (1975) on the presence of *S. mutans* in the mouths of children with congenital sucrase deficiency suggest that restriction of S intake is of little value in either preventing initial infection by *S. mutans* or in eliminating it. However, it is clear from many studies (e.g. *Newman & Poole*, 1974) that *S. mutans* variants are involved in caries formation because of their ability to produce acids and extracellular polysaccharides, to survive in acidic environments, and because of their relative tolerance to large amounts of dietary S (see: *Newman & Poole*, 1974). Perhaps it can be concluded that the role of both *S. mutans* as well as other streptococci on one hand and that of lactobacilli on the other, is qualitatively similar in the pathogenesis of dental caries, whereas their diagnostic or quantitative significance in the estimation of caries activity does not completely overlap.

REFERENCES

Becks, H. 1950. Carbohydrate restriction in the prevention of dental caries, using the lactobacillus count as one index. J. Calif. Dent. Assoc. 26, 53—58

Bradley, J. V. 1968. *Distribution-free statistical tests.* Prentice-Hall Inc. Englewood Cliffs, New Jersey, N.Y., U.S.A.

Frostell, G. & Nord, C. E. 1972. A comparison between different methods of culturing lactobacilli from human saliva. Swed. Dent. J. 65, 553—557

Geddes, D. A. M. & Jenkins, G. N. 1974. Instrinsic and extrinsic factors influencing the flora of the mouth, in *The normal microbial flora of man.* Ed. by *Skinner, F. A. & Carr, J. G.*, Academic Press, London and New York, pp. 85—100

Gehring, F., Mäkinen, K. K., Larmas, M. & Scheinin, A. 1975. Turku sugar studies. X. Occurrence of polysaccharide-forming streptococci and ability of the mixed plaque microbiota to ferment various carbohydrates. Acta Odont. Scand. 33, Suppl. 70, 223—237

Glass, R. L. 1975. Personal communication

Van Houte, J. & Duchin, S. 1975. *Streptococcus mutans* in the mouth of children with congenital sucrase deficiency. Archs Oral Biol. 20, 771—773

Jay, P. 1947. Reduction of oral lactobacillus acidophilus counts by the periodic restriction of carbohydrate. Amer. J. Orthodont. 33, 162—184

Krasse, B. 1954. Relationship between caries activity and the number of lactobacilli in the oral cavity. Acta Odont. Scand. 12, 157—172

Larmas, M., Mäkinen, K. K. & Scheinin, A. 1975. Turku sugar studies. VIII. Principal microbiological findings. Acta Odont. Scand. 33, Suppl. 70, 173—216

Mäkinen, K. K. & Scheinin, A. 1975. Turku sugar studies. VII. Principal biochemical findings on whole saliva and plaque. Acta Odont. Scand. 33, Suppl. 70, 129—171

Mäkinen, K. K., Ojanotko, A. & Vidgren, H. 1975. Effect of xylitol on the growth of three oral strains of *Candida albicans.* J. Dent. Res. 54, 1239

Newman, H. N. & Poole, D. F. G. 1974. Structural and ecological aspects of dental plaque, in *The normal microbial flora of man.* Ed. by *Skinner, F. A. & Carr, J. G.*, Academic Press, London and New York, pp. 111—134

Sachs, L. 1972. *Statistische Auswertungsmethoden.* Dritte neubearbeite und erweiterte Auflage mit neuer Bibliographie. Springer-Verlag, Berlin, Heidelberg, New York

Scheinin, A., Mäkinen, K. K., Tammisalo, E. & Rekola, M. 1975. Turku sugar studies. XVIII. Incidence of dental caries in relation to 1-year consumption of xylitol chewing gum. Acta Odont. Scand. 33, 269—278

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

Silness, J. & Löe, H. 1964. Correlation between oral hygiene and periodontal condition. Acta Odont. Scand. 22, 121—135

TABLES

Table I. *Frequency of occurrence and total colony-forming unit values on Rogosa S.L. agar per ml saliva ($n \times 10^3$) as a function of time and chewing gum group*

Time	N	N—	N+	Logarithmic scale $\bar{x}_{log}$	S.D.$_{log}$	Min.	Max.	Med.	$\bar{x}_{geom.}$	Arithmetic scale 68% interval	$\bar{x}_{arith.}$	S.D.$_{arith.}$
Sucrose												
— 0 month	50	12	38	6.41	4.59	—0.98	13.1	7.70	0.608	0.006— 59.67	29.9	86.3
6 months	50	5	45	8.26	3.66	—0.98	13.6	9.00	3.865	0.100—149.70	60.8	158.0
12 »	50	7	43	7.64	3.97	—0.98	12.3	8.60	2.082	0.033— 91.88	29.6	53.6
Xylitol												
0 month	50	9	41	7.20	4.49	—0.98	13.7	7.9	1.343	0.015—119.84	72.6	183.8
6 months	50	8	42	6.39	3.75	—0.98	13.0	7.2	0.593	0.014— 25.14	19.1	70.2
12 »	50	9	41	6.50	3.96	—0.98	11.6	7.6	0.662	0.013— 34.59	11.3	20.5

Table II. *Frequence of occurrence and total colony-forming unit values of lactobacilli per ml saliva ($n \times 10^3$) on Rogosa S.L. agar as a function of time and chewing gum group*

Time	N	N—	N+	Logarithmic scale $\bar{x}_{log}$	S.D.$_{log}$	Min.	Max.	Med.	$\bar{x}_{geom.}$	Arithmetic scale 68% interval	$\bar{x}_{arith.}$	S.D.$_{arith.}$
Sucrose												
— 0 month	50	18	32	4.62	4.46	—0.98	10.6	6.1	0.102	0.001— 8.83	5.0	9.0
6 months	50	6	44	7.49	3.67	—0.98	12.9	8.6	1.789	0.045—70.17	28.0	70.0
12 »	50	8	42	7.04	3.86	—0.98	11.3	8.3	1.145	0.024— 5.40	11.0	17.0
Xylitol												
0 month	50	11	39	6.01	4.04	—0.98	10.6	7.3	0.408	0.007—23.30	7.0	10.0
6 months	50	12	38	5.22	3.94	—0.98	11.9	6.0	0.185	0.004— 9.52	8.0	24.0
12 »	50	11	39	5.92	4.01	—0.98	10.8	7.4	0.371	0.007—20.39	7.0	12.0

Table III. *Frequency of occurrence and total colony-forming unit values of streptococci and diphtheroids per ml saliva ($n \times 10^3$) on Rogosa S.L. agar as a function of time and chewing gum group*

Time	N	N—	N+	Logarithmic scale $\bar{x}_{log}$	S.D.$_{log}$	Min.	Max.	Med.	$\bar{x}_{geom.}$	Arithmetic scale 68% interval	$\bar{x}_{arith.}$	S.D.$_{arith.}$
Sucrose												
— 0 month	50	19	31	4.99	4.87	—0.98	11.3	7.6	0.146	0.001—19.01	9.1	17.4
6 months	50	16	34	5.84	4.90	—0.98	13.0	7.8	0.344	0.003—46.04	20.2	65.8
12 »	50	24	26	4.06	5.02	—0.98	11.3	5.3	0.580	0.004— 8.76	7.3	14.2
Xylitol												
— 0 month	50	16	34	5.49	4.80	—0.98	10.8	6.9	0.243	0.002—29.46	11.2	16.5
6 months	50	19	31	4.46	4.56	—0.98	11.9	5.7	0.860	0.009— 8.23	7.5	22.8
12 »	50	27	23	3.37	4.86	—0.98	10.8	0	0.290	0.002— 3.74	4.6	9.3

Table IV. *Frequency of occurrence and total colony-forming unit values on Sabouraud antibiotic agar per ml saliva ($n \times 10^3$) as a function of time and chewing gum group*

Time	N	N—	N+	Logarithmic scale $\bar{x}_{log}$	S.D.$_{log}$	Min.	Max.	Med.	Arithmetic scale $\bar{x}_{geom.}$	68% interval	$\bar{x}_{arith.}$	S.D.$_{arith.}$
Sucrose												
— 0 month	50	24	26	2.94	3.90	—0.98	9.90	4.61	0.019	0.004—0.093	0.9	2.9
6 months	50	27	23	2.65	4.06	—0.98	8.99	—0.98	0.014	0.002—0.081	0.8	1.6
12 »	50	29	21	2.35	4.03	—0.98	9.11	—0.98	0.011	0.002—0.059	0.8	1.8
Xylitol												
— 0 month	50	25	25	3.01	4.17	—0.98	9.62	1.81	0.020	0.003—0.0130	1.4	2.9
6 months	50	26	24	2.54	3.80	—0.98	9.90	—0.98	0.013	0.003—0.056	0.8	2.8
12 »	49	25	24	2.63	3.92	—0.98	9.11	—0.98	0.014	0.003—0.069	0.8	1.8

Table V. *Geometric mean values and approximate 95 % ranges mg plaque ($n \times 10^5$) for the streptococci and total colony forming unit values on phenol red agar*

Strain		Sucrose N	Sucrose $\bar{x}_{geom.}$	Sucrose 95 % range	Xylitol N	Xylitol $\bar{x}_{geom.}$	Xylitol 95 % range
S. sanguis	0 month	50	4.91	0.15 —161.8	50	7.16	0.32 —158.4
(anaerobic)	6 months	48	0.11	0.0001 —142.9	49	0.13	0.0001 —166.5
	12 »	39	0.17	0.0003 — 89.7	40	0.54	0.008 — 35.9
S. mutans	0 month	50	0.002	0.00000—191.4	50	0.001	0.00000—545.3
(anaerobic)	6 months	48	0.010	0.00000—374.8	49	0.0003	0.00000— 16.0
	12 »	41	0.0002	0.00000— 4.0	41	0.0005	0.00005— 41.5
S. salivarius	0 month	50	0.00003	0.00000— 0.03	50	0.00015	0.0000 — 0.06
(aerobic)	6 months	48	0.0002	0.00000— 0.83	49	0.00008	0.00000— 0.03
	12 »	41	0.0002	0.00000— 0.04	41	0.0002	0.00000— 2.28
Total colony	0 month	50	20.99	1.18 —373.4	50	22.31	1.48 —337.0
count	6 months	48	1.52	0.06 — 40.7	49	1.16	0.02 — 85.6
(anaerobic)	12 »	39	2.21	0.17 — 29.3	40	3.16	0.12 — 84.7

Table VI. *Mean pH-values* ($\bar{x}$) *and standard deviations* (S.D.) *of the growth media infected with mixed plaque flora from the subjects and incubated for 1 day and 7 days at +37°C*

Examination		0 month				6 months				12 months			
Decomposition of the carbohydrate of the growth medium		Sucrose group n = 50		Xylitol group n = 50		Sucrose group n = 50		Xylitol group n = 50		Sucrose group n = 42		Xylitol group n = 42	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Xylitol	day 1	6.81	0.19	6.83	0.23	6.85	0.25	6.76	0.20	6.42	0.23	6.39	0.21
(1 %)	day 7	6.46	0.51	6.39	0.50	6.33	0.48	6.34	0.53	6.35	0.34	6.25	0.29
Sorbitol	day 1	6.16	0.76	6.16	0.70	6.51	0.48	6.59	0.33	6.26	0.23	6.18	0.33
(1 %)	day 7	5.13	0.83	4.83	0.45	4.65	0.64	4.89	0.86	5.67	0.79	5.40	0.90
Sucrose	day 1	4.20	0.19	4.19	0.23	4.33	0.68	4.52	0.76	4.32	0.35	4.26	0.17
(1 %)	day 7	4.18	0.13	4.16	0.13	4.12	0.53	4.24	0.50	4.21	0.13	4.21	0.17
Fructose	day 1	4.31	0.45	4.21	0.13	4.29	0.57	4.45	0.52	4.36	0.35	4.31	0.20
(1 %)	day 7	4.24	0.16	4.19	0.13	4.14	0.58	4.25	0.36	4.27	0.36	4.26	0.21
Control	day 1	6.64	0.30	6.66	0.13	6.61	0.25	6.64	0.21	6.17	0.25	6.12	0.16
(no carbohydrate)	day 7	6.48	0.55	6.43	0.47	6.37	0.46	6.43	0.40	6.26	0.38	6.19	0.37

Table VII. *Plaque index values in relation to use of sucrose- or xylitol-containing chewing gum. The findings are shown on a tooth level, at the beginning, the 6-month phase, the end of the trial, and as the difference between initial and final Pl I values*

Examination	Tooth	Sucrose N	Min.	Max.	Md	$\bar{x}$	S.D.	Xylitol N	Min.	Max.	Md	$\bar{x}$	S.D.
Base line	16	50	0.50	2.75	1.50	1.54	0.47	50	0.50	2.50	1.50	1.46	0.44
	12	50	0.00	2.50	1.25	1.34	0.48	50	0.25	2.50	1.50	1.39	0.45
	24	50	0.00	2.50	1.25	1.36	0.46	50	0.25	2.25	1.50	1.40	0.36
	44	50	0.50	2.50	1.25	1.36	0.44	50	0.00	2.50	1.50	1.37	0.48
	32	50	0.00	2.25	1.00	1.12	0.48	50	0.00	2.25	1.25	1.25	0.42
	36	50	0.50	2.50	1.75	1.59	0.45	50	0.35	2.50	1.75	1.62	0.45
6 months	16	48	0.25	2.75	1.25	1.12	0.59	50	0.00	2.00	1.00	1.00	0.55
	12	48	0.00	1.75	1.00	0.94	0.57	50	0.00	2.00	0.75	0.79	0.63
	24	48	0.00	1.75	1.25	1.04	0.56	50	0.00	1.75	1.00	0.91	0.48
	44	48	0.00	2.50	1.13	1.03	0.65	50	0.00	2.00	0.88	0.76	0.61
	32	48	0.00	2.50	0.88	0.80	0.60	50	0.00	1.75	0.63	0.65	0.53
	36	48	0.00	2.00	1.25	1.27	0.60	50	0.25	2.00	1.00	1.02	0.46
12 months	16	50	0.00	2.50	1.00	1.10	0.58	50	0.00	1.75	0.50	0.68	0.47
	12	50	0.00	2.25	1.00	0.96	0.61	50	0.00	1.75	0.50	0.59	0.56
	24	50	0.00	2.00	0.88	0.86	0.45	50	0.00	1.50	0.50	0.62	0.45
	44	50	0.00	2.50	1.00	0.95	0.55	50	0.00	1.75	0.38	0.43	0.50
	32	50	0.00	2.75	0.50	0.63	0.54	50	0.00	1.75	0.25	0.42	0.52
	36	50	0.00	2.25	1.00	1.21	0.64	50	0.25	2.25	0.75	0.82	0.47
Diff. Base line — 1 year	16	50	—1.00	2.00	0.50	0.44	0.63	50	—0.25	1.75	0.75	0.78	0.53
	12	50	—1.00	1.50	0.50	0.38	0.55	50	—0.75	2.00	0.88	0.80	0.62
	24	50	—0.50	1.25	0.50	0.50	0.46	50	—0.75	1.75	0.88	0.78	0.52
	44	50	—0.50	1.50	0.50	0.41	0.55	50	—0.25	1.75	1.00	0.88	0.57
	32	50	—0.75	1.50	0.50	0.42	0.57	50	—0.50	2.25	1.00	0.83	0.58
	36	50	—1.00	1.75	0.38	0.38	0.62	50	—0.75	1.75	0.75	0.80	0.55

Table VIII. *Significance level of differences between sugar groups with regard to change between initial and final Pl I values (Mann-Whitney U-test)*

Tooth	α
16	0.0044
12	0.0007
24	0.0059
44	0.0001
32	0.0017
36	0.0008

Table IX. *Development of plaque index values in the sucrose- and xylitol-groups. The findings include the base line examination, the 6- and 12-month phases, the differences between the initial and final values, and the corresponding significance levels (Mann-Whitney U-test) between sugar groups*

	Sucrose (n = 50)					Xylitol (n = 50)					
Months	Min.	Max.	Md	$\bar{x}$	S.D.	Min.	Max.	Md	$\bar{x}$	S.D.	U-test
0	0.58	2.38	1.33	1.38	0.36	0.42	2.17	1.46	1.41	0.35	0.395
6	0.17	2.21	1.15	1.03	0.52	0.08	1.75	0.88	0.85	0.46	0.070
12	0.17	2.29	0.92	0.96	0.46	0.08	1.58	0.54	0.60	0.38	0.0001
0—12	—0.54	1.17	0.42	0.42	0.40	—0.08	1.67	0.83	0.81	0.41	< 0.0001

Table X. *Distribution of Pl I values equalling or exceeding 2 during the trial in the S- and X-groups*

Number of surfaces with Pl I ≥ 2	Number of subjects (frequency)					
	0 month		6 months		12 months	
	S	X	S	X	S	X
0	0	1	9	11	11	23
1	3	1	5	7	7	9
2	0	1	4	6	5	6
3	2	4	4	7	2	3
4	3	3	2	3	3	1
5	1	5	1	3	3	0
6	5	1	6	2	5	2
7	9	6	1	1	1	2
8	3	2	0	4	1	0
9	1	1	3	1	3	0
10	5	2	2	0	2	0
11	1	2	2	0	0	1
12	5	4	3	0	2	1
13	1	5	0	0	0	2
14	1	2	2	1	0	0
15	0	4	2	1	1	0
16	4	2	1	1	1	0
17	0	1	1	1	0	0
18	1	0	1	1	1	0
19	0	1	0	0	0	0
20	0	0	0	0	1	0
21	3	3	1	0	0	0
22	1	1	0	0	0	0
23	0	1	0	0	0	0
24	1	0	0	0	1	0

Table XI. *Occurrence of Pl I values ≥ 2 during the trial in the combined S- and X-groups*

Months	Min.	Max.	Md	$\bar{x}$	S.D.
0	0.0	24.0	10.0	10.20	5.82
6	0.0	21.0	3.0	5.23	5.39
12	0.0	24.0	1.5	3.68	4.99

Table XII. *Occurrence of Pl I values ≧ 2 and significance level of observed differences between the S- and X-groups during the trial (Mann-Whitney U-test)*

	Sucrose (n = 50)					Xylitol (n = 50)					
Months	Min.	Max.	Md	$\bar{x}$	S.D.	Min.	Max.	Md	$\bar{x}$	S.D.	U-test
0	1.0	24.0	8.0	9.82	5.72	0.0	23.0	11.0	10.58	5.95	0.439
6	0.0	21.0	5.5	6.32	5.80	0.0	18.0	3.0	4.14	4.76	0.069
12	0.0	24.0	3.5	5.18	5.77	0.0	13.0	1.0	2.18	3.53	0.001