Turku sugar studies

IV. An intermediate report on the differentiation of polysaccharide-forming streptococci (*S. mutans*)

F. GEHRING, K. K. MÄKINEN, M. LARMAS & A. SCHEININ

Department of Experimental Dentistry, University of Würzburg, Germany, and Institute of Dentistry, University of Turku, Finland

Gehring, F., Mäkinen, K., Larmas, M. & Scheinin, A. Turku sugar studies. IV. An intermediate report on the differentiation of polysaccharide-forming streptococci (*S. mutans*). *Acta Odont. Scand.* 32, 435—444, 1974.

Colony count determinations were carried out in order to detect quantitative changes of *Streptococcus mutans* in the plaque samples of the test persons in the Turku sugar studies. The lyophilized plaque samples were homogenized and incubated under anaerobic conditions. In addition, the pH-values were measured after incubation in culture media containing various sugars. The 1-year results showed a significantly lower incidence of *S. mutans* in the xylitol group in comparison to the fructose and sucrose groups. The pH-measurements carried out at the 4 and 12 month phases showed that only in the presence of xylitol the mean values did not fall below the pH-limit of 5.5. No evidence was obtained of the involvement of microorganisms capable of decomposing xylitol after one year of diet.

Key-words: Streptococcus mutans; dental plaque; xylitol; fructose; sucrose; sorbitol

F. Gehring, Department of Experimental Dentistry, University of Würzburg, 8700 Würzburg, Pleichertorstrasse 32, Germany

Specific extracellular polysaccharide-producing streptococci, for example, the varieties of *Streptococcus mutans*, have been considered to possess potential cariogenic characteristics. A comprehensive review, covering *i.a.* the taxonomy, epidemiology, and ecology of this species has recently been presented (*Fitzgerald et al.*, 1973). The potential cariogenic properties of *S. mutans* are strongly connected with a supply of sucrose as a source of energy. These microorganisms produce organic acids as well as intracellular and extracellular polysaccharides from sucrose. The latter substances, which most frequently represent glucans, function as a plaque matrix and have adhesive capacities holding bacteria on the surfaces of the teeth (*Gibbons & Banghart*, 1967; *Guggenheim*, 1970; *Newbrun*, 1972).

An association between *S. mutans* and active human caries has been repeatedly shown (*Krasse et al.*, 1968; *de Stoppelaar et al.*, 1969; *Jordan et al.*, 1969; *Littleton et al.*, 1969, 1970; *Duany et al.*, 1970; *Schamschula & Charlton*, 1971; *Woods*, 1971; *Ikeda et al.*, 1973; *Schamschula & Barnes*, 1970; *Kozlowski et al.*, 1973). In these studies *S. mutans* was isolated more frequently in individuals with high than low caries experience.

The growth and the quantitative

Received for publication, June 20, 1974.

occurrence of these germs in the plaque at the site of a potential carious lesion may thus provide information of the local conditions. The break of the relationship between a high sucrose consumption, and increased occurrence of *S. mutans* may over a prolonged period of time be reflected in a decrease of caries activity. Substitution of sucrose through suitable products may thus result in a decrease of *S. mutans*. On the other hand, a frequent supply of sucrose may lead to large numbers of these microorganisms, at least at defined areas on tooth surfaces.

The purpose of the present microbiological study was to evaluate the eventual changes in the number of *S. mutans* in dental plaque in relation to sucrose, fructose and xylitol consumption in man. In addition, the capability and adaptability of a mixed plaque flora to decompose various sugars was investigated in the trial.

MATERIAL AND METHODS

Colony count determinations of *S. mutans* were carried out to detect the quantitative changes in the plaque samples of the test persons divided into sucrose (S-), fructose (F-) and xylitol (X-) groups as reported for separately (*Scheinin, Mäkinen & Ylitalo*, 1974). Out of the initial 125 participants, plaque samples for microbiological analyses were available in the vast majority of cases. Only in a few instances, as seen in the results section, negligible deviations occurred.

The collection and treatment of the plaque samples was carried out as follows. Simultaneously with the clinical inspection and plaque collection for biochemical and microbiological analyses in Turku, special samples were obtained for the studies in Würzburg. The test persons refrained from all oral hygiene measures for 12—24 hours prior to the collection (overnight). The plaque samples in the first examination were collected from tooth surfaces where caries occurred or was likely to occur. In the second examination the samples were fractions of the total amount of plaque collected. The exact wet weight of the samples was determined (range approximately 0.5—1.5 mg). Each plaque sample was then suspended in 1.0 ml skim milk[1]); rehydration according the instruction given by the supplier in 10 ml centrifuge tubes. The mixtures were then sonicated for 5 sec with a MSE Ultrasonic Disintegrator[2]) in cold with a probe with an end diameter of 3 mm. The mixtures were then freeze-dried, the solid samples stored at −20 °C, and were later sent by air to Würzburg. The microbiological investigations were carried out within 8—10 weeks after the collection of the samples, as indicated in Fig. 1.

In the present study, the term »anaerobic» implies that the incubation for the colony count determinations was carried out in BBL GasPak-jars[3]) under anaerobic conditions.

In order to find out whether a selection and adaptation of microorganisms capable of decomposing xylitol took place, the changes of the pH-values in culture media containing 1 % xylitol, sorbitol, sucrose or fructose were measured after inoculation with the mixed plaque flora of the individuals in the three sugar groups. Further comparison was carried out with corresponding sugar-free culture media as controls. The pH of the media was determined at + 25° C with glass and calomel electrodes.

[1]) Difco Laboratories, Detroit, Michigan, U.S.A.
[2]) Measuring & Scientific Equipment Ltd., London, England.
[3]) Baltimore Biological Laboratories, Baltimore, Md., U.S.A.

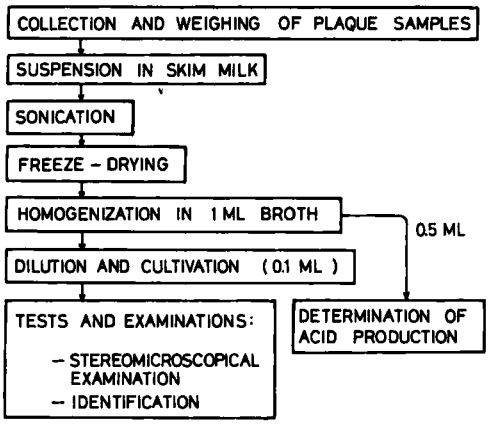

Fig. 1. A scheme of the overall performance of the microbiological tests. The homogenization was performed in a solution containing 1.0 ml Bacto-NTH-Thioglycollate broth (Difco) and 2.0 ml saline. The dilution ranges were from 10^{-1} to 10^{-4}. The cultivations were performed for 48 h in anaerobic jars at 37° C on phenol red agar base (Difco) containing 5% sucrose. The identification comprised the following tests: Degradation of mannitol and sorbitol, and the growth in 4% NaCl and 40% sucrose. The 0.5 ml aliquots were used in tests in which the pH of the medium containing either sorbitol, xylitol, sucrose, or fructose was determined after incubation for 1 or 7 days (when compared to a control medium). Other details occur in the text.

RESULTS

The first microbiological evaluation was carried out on plaque samples collected approximately 4 months after commencement of the respective sugar diets. The incidence of growth of *S. mutans* in the three sugar groups is shown in Table I. Application of the χ^2-test proved the observed differences to be significant at the 5 % level.

In the F- and S-groups growth occurred in about half of the cases. In the X-group merely one fourth of the test persons displayed the presence of *S. mutans*.

The absolute and relative frequencies of appearance of *S. mutans* after one year of the respective diets is shown in Table II. When applying the χ^2-test the difference was not significant, in contrast to the findings in Table I.

The difference in the occurrence of *S. mutans* between the first and second examination was further reflected in the

Table I. *Incidence of anaerobic S. mutans after 4 months of sugar diets*

	Xylitol		Fructose		Sucrose		Total	
	No.	%	No.	%	No.	%	No.	%
Growth	13	25	17	44.7	17	48.6	47	37.6
No growth	39	75	21	55.3	18	51.4	78	62.4
Total	52	100	38	100	35	100	125	100

$\chi^2 = 6.32$ $P < 0.05$

Table II. *Incidence of anaerobic S. mutans after 12 months of sugar diets*

	Xylitol		Fructose		Sucrose		Total	
	No.	%	No.	%	No.	%	No.	%
Growth	11	21.6	9	24.3	11	31.4	31	38.2
No growth	40	78.4	28	75.7	24	68.6	92	61.8
Total	51	100	37	100	35	100	123	100

$\chi^2 = 1.09$ P = n.s.

Table III. *Occurrence of anaerobic S. mutans in plaque samples obtained after 4 and 12 months of sugar diets*

	1st sample only	2nd sample only	in both samples
Xylitol	7	5	6
Fructose	11	3	6
Sucrose	9	3	8

number of persons exhibiting growth in the first, but not in the second trial, and vice versa. Table III gives the number of the test persons in the sugar groups with regard to the presence of *S. mutans* in the first, second or both examinations.

The test of McNemar (*Bradley*, 1968) was applied in order to check for possible significant changes between the two determinations in the distribution of the test persons with respect to the incidence of *S. mutans*, and showed that no significant changes had occurred. The somewhat surprising result was further elucidated with regard to the frequencies of occurrence of *S. mutans* in the first and/or the second trial (Table IV). The percentages of persons in the F- and S-group harbouring *S. mutans* were practically equal. When pooling these two groups and applying the exact test of Fisher (*Bradley*, 1968), the value of $\alpha = 0.021$ was obtained, demonstrating a significant difference in the proportion of persons with *S. mutans* between the X-group and the pooled F- and S-groups. The Fisher-test gave $\alpha = 0.037$ when comparing the X- and S-groups, and $\alpha = 0.062$ between the X- and F-groups. The former value was clearly significant, whereas the latter was close to the usual margin of 0.05. The two circle diagrams (Fig. 2) describe the corresponding situation between the X-group and the pooled F- and S-groups.

Table V. *Occurrence of S. mutans in 1st and 2nd plaque samples*

	1st sample	2nd sample
Xylitol	25.0%	21.6%
Fructose	44.7%	24.3%
Sucrose	48.6%	31.4%

A further analysis of the results showed a decline from the first to the second examination with regard to the number of persons exhibiting the presence of *S. mutans* (Table V). Nevertheless the intermediate 1-year results showed a significantly lower incidence of *S. mutans* in the X-group in comparison to the F- and S-groups.

In addition, also quantitative determinations of *S. mutans* were carried out on the plaque samples. Table VI shows the results of anaerobic colony counts of *S. mutans*, and the total mixed counts in units of 10^5 per mg plaque wet weight. The standard deviations of the colony counts varied highly. For this reason a logarithmic transformation of the obtained data was applied. A comparison between the sugar groups (Table VI) showed that the

Table IV. *The incidence of S. mutans in 1st and/or 2nd samples*

	Xylitol		Fructose		Sucrose		Total	
	No.	%	No.	%	No.	%	No.	%
Growth	18	35	20	54	20	57	58	47
No growth	33	65	17	46	15	43	65	53
Total	51	100	37	100	35	100	125	100

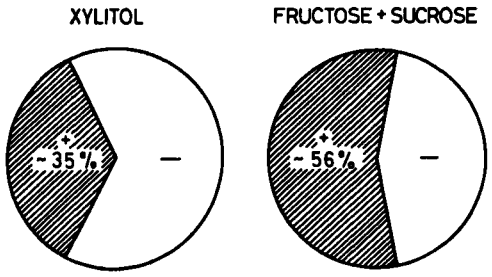

Fig. 2. Distribution of persons in the xylitol group and the pooled fructose and sucrose groups exhibiting growth of *S. mutans* in the first and/or second examination.

approximate confidence intervals for *S. mutans* in the first trial were disjoint in the X- and F-groups. There was also a significant difference between the X- and S-groups, as further proved by applying the Mann-Whitney U-test (*Bradley*, 1968) between the X- and F-groups ($\alpha = 0.0046$), X- and S-groups ($\alpha = 0.073$), and for the total colony count between the X- and S-groups ($\alpha = 0.0042$). A similar situation occurred at the second examination with regard to the total colony count.

The ability of the mixed plaque microbiota to ferment various carbohydrates

An analysis of the eventual adaptation or mutation of the plaque flora to degradate xylitol through acid production was considered to be of importance, particularly in view of the long duration of the study. Information was obtained through pH-measurements in culture media, as described in the material and methods section. The culture tubes containing the various sugars and the sugar-free controls were inoculated with samples of the mixed plaque flora obtained from the test persons. The changes in the pH-values were measured after the 1st and 7th day of inoculation. Table VII shows the means and standard deviations in the three sugar groups after approximately 4 months after the onset of the respective diets. It was thus found that only in the presence of xylitol and in the controls pH values above 6 were recorded on the 7th day. All other corresponding values, measured in the presence of either sorbitol, sucrose or fructose, were between pH 4 and 5.

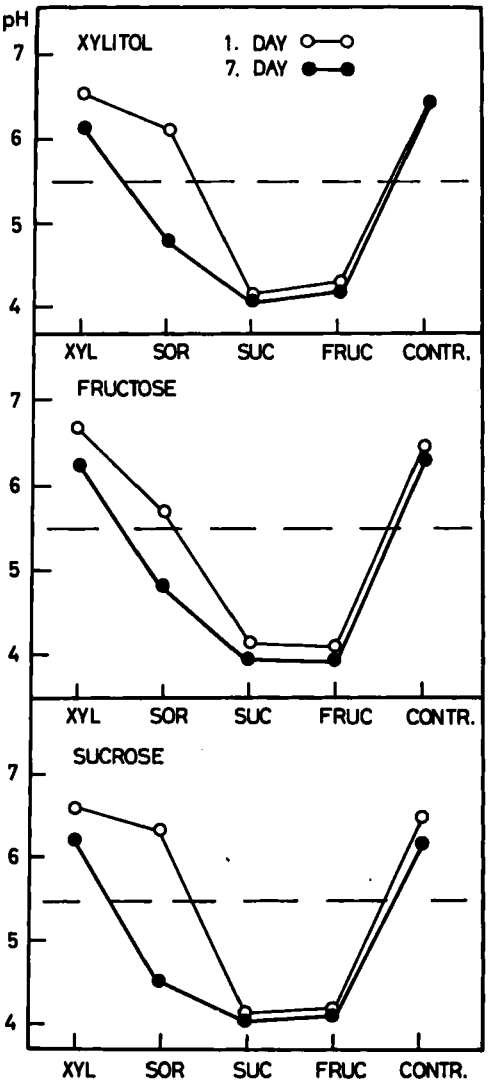

Fig. 3. Mean pH values after incubation for 1 and 7 days in the presence of 1% xylitol, sorbitol, sucrose or fructose. Inoculation with individual plaque samples after 12 months of sugar diets.

Table VI. *Anaerobic colony counts of S. mutans and total plaque microbiota in units of 10^5/mg plaque after 4 and 12 months of sugar diets*

			S. mutans				Total colony count			
			1st samples	n	2nd samples	n	1st samples	n	2nd samples	n
Xylitol	Md		0.00008	52	0.00004	51	19.25	52	22.73	51
	95% interval	Min.	0.00002		0.00001		12.35		14.98	
		Max.	0.00029		0.00017		30.01		34.49	
Fructose	Md		0.00233	38	0.00005	37	19.94	38	12.66	37
	95% interval	Min.	0.00034		0.00001		12.74		12.65	
		Max.	0.01560		0.00025		31.19		23.49	
Sucrose	Md		0.00155	35	0.00013	35	18.13	35	9.26	35
	95% interval	Min.	0.00018		0.00002		9.44		5.35	
		Max.	0.01279		0.00077		34.81		16.00	
Kruskal-Wallis-test			P = 0.0191		n.s.		n.s.		P = 0.043	

Table VII. *Mean pH values after incubation for 1 and 7 days in the presence of various carbohydrates. The broths were inoculated with the mixed microbiota of the individual plaque samples obtained after 4 months of sugar diets*

Sugar added	Day	Xylitol group n = 52		Fructose group n = 38		Sucrose group n = 35	
		$\bar{x}$	S.D.x	$\bar{x}$	$S.D._f$	$\bar{x}$	$S.D._s$
Xylitol	1	6.74	0.16	6.78	0.27	6.79	0.19
	7	6.28	0.50	6.42	0.50	6.19	0.53
Sorbitol	1	6.34	0.58	6.24	0.64	6.29	0.59
	7	4.87	0.64	4.74	0.75	4.89	0.78
Sucrose	1	4.33	0.17	4.24	0.12	4.32	0.28
	7	4.27	0.14	4.24	0.21	4.23	0.16
Fructose	1	4.42	0.22	4.28	0.23	4.33	0.33
	7	4.37	0.18	4.22	0.21	4.31	0.18
Control	1	6.67	0.42	6.75	0.13	6.76	0.18
	7	6.51	0.62	6.47	0.33	6.54	0.53

In the second series of examination after approximately 12 months of diet, the results of these pH measurements were almost the same. The results are shown in Fig. 3 with regard to the mean pH values on the 1st and 7th day of inoculation. The lowest pH levels were again registered in the presence of sucrose and fructose, whereas with sorbitol the values were below pH 5 after 7 days of inoculation. The mean pH value measured in the presence of xylitol was clearly above pH 6, but on the other hand, a few individual plaque samples yielded pH values below 5.5. The number of these test persons and their sugar groups are given in Table VIII. The persons giving these lower pH values in the xylitol group at the 1st and 2nd examination were not the same, and the number of cases capable of reducing the pH value below 5.5 in the presence of xylitol was smaller in the second than in the first examination. On the basis of these experiments there was thus no

Table VIII. *Distribution of individual plaque samples yielding values below pH 5.5 after 7 days of incubation in the presence of 1% xylitol*

	Sample	
	1st	2nd
Xylitol group	3	2
Fructose group	1	1
Sucrose group	3	—

evidence of the adaptation of micro-organisms to decompose xylitol in the conditions involved.

DISCUSSION

The observations indicated differing results at the 1st and 2nd examination concerning the qualitative and quantitative occurrence of *S. mutans* in the plaque samples of the three sugar groups. This discrepancy seemed most likely to have been due to a difference in the method of plaque sampling. In the first examination plaque was collected specifically for the present study, from locations where caries was present or likely to occur. In the second examination the plaque samples represented aliquots of the total amount of plaque collected for other analyses as well. The second samples were thus deliberately obtained without attention to the location of plaque.

Shklair et al. (1974) in a study of the distribution of *S. mutans* on the teeth of caries-free individuals (DMFT = 0) and a control group (DMFT = 1—27) of naval recruits, reported that in the control group the percentage of occurrence was higher than in the caries-free group. The authors also noted that in determining whether *S. mutans* may or may not be found, the differences seemed probably to have been due to the different methods of sampling on approximal surfaces, to the number of sites sampled, and possibly to age differences of the test persons.

Ikeda & Sandham (1971) found that in children the pits and fissures were the major sources of *S. mutans;* fewer germs of this species were found on the buccal and none on the approximal surfaces. In a further study *Ikeda et al.* (1973) showed that during the 12 subsequent months the mean number of *S. mutans* increased on all aforementioned surfaces, but not in all individuals. Wide fluctuations were noted in many persons over a period of time. It also appears from the present Tables III—VI that variations in the incidence of *S. mutans* occurred between individuals, between the sugar groups, and also between the two examinations.

Gibbons (1973) pointed out the unusual ecology of *S. mutans* which colonizes in a localized manner, and which is not quickly transmitted from one tooth surface to another. *Littleton et al.* (1970) found *S. mutans* in all carious lesions sampled, but recovered the organism only occasionally from the plaque of intact teeth. *Shklair et al.* (1972) showed that the prevalence and isolation-frequency of *S. mutans* decreased when the plaque samples were obtained from sites distant from a carious lesion. *Duany et al.* (1972) found no significant differences in the prevalence of *S. mutans* on molar surfaces of caries-free and caries-active students. These authors indicated that the differences in relative numbers, rather than the presence of *S. mutans* alone, may be a critical determinant in the initiation of caries at a specific tooth surface.

The microbiological data of the present study showed a lower occurrence of *S. mutans* in the plaque samples of the test persons after one year of xylitol consumption in contrast to the sucrose and fructose groups. The results also emphasize,

fundamentally, the complex ecological system of potentially cariogenic plaque in relation to the consumption of sucrose, fructose and xylitol. The present findings should obviously be considered in view of the corresponding clinical, radiographical, biochemical and microbiological findings (*Scheinin, Mäkinen & Ylitalo*, 1974; *Mäkinen & Scheinin*, 1974; *Larmas, Mäkinen & Scheinin*, 1974). In view of the intermediate nature of these findings, the present microbiological investigations being extended to cover a 2-year period, no detailed analysis between the occurrence of *S. mutans* and the incidence of dental caries is now carried out.

Recently, the cariogenicity of fructose, sucrose, sorbitol and xylitol in chocolate was tested in rats (*Karle & Gehring*, 1974a, 1974b). The sucrose diets exerted the highest and the xylitol diets the lowest caries increment rate. The plaque samples of the »sucrose-rats» harboured abundant *S. mutans* in contrast to the »xylitol-rats» in which these germs were not detected.

The in vitro-studies on the pH-changes in various culture media with the mixed plaque microbiota yields no appreciable acid production from xylitol, in contrast to sucrose, fructose or sorbitol (Table VII, Fig. 3). Investigations with experimental animals have demonstrated the presence of oral microorganisms, which are capable of degrading xylitol to acids at a very low rate, as compared with other low-molecular carbohydrates (*Gehring*, 1974a, 1974b). Animal experiments on the cariogenicity of xylitol have hitherto shown that this polyol was less cariogenic than sorbitol, although sorbitol may also display rather low cariogenic properties (*Mühlemann et al.*, 1970; *Karle & Büttner*, 1971; *Grunberg et al.*, 1973; *Karle & Gehring*, 1974a).

The results of the present paper can also be examined in light of experiments on the adaptability of oral microorganisms to utilize xylitol, or to tolerate its presence in the culture medium *in vitro*. The first report dealing with these phenomena (*Mäkinen*, 1972) claimed that after four or five months continuous presence of xylitol, the cells of *S. mutans* (Ingbritt) became capable of utilizing xylitol. This argument was presented on the basis of the lowering of the pH and on the increase of turbidity of the growth media after the »adaptive» period involved. The subsequent experiments, however, provided more detailed information about the actual reactions. The possibility suggested (*Mäkinen*, 1972, 1974) about an adaptation to utilize xylitol has likely turned out to be incorrect.

Recent experiments indicate that the cells of *S. mutans* grow identically in the presence of xylitol as the only added carbohydrate, and without any added carbohydrate at all (*Knuuttila & Mäkinen*, 1975a). Radioactive C14-xylitol (universally labelled) was not incorporated into the cells of either »adapted» or »non-adapted» cells of *S. mutans*. The true adaptation of the cells involved synthesis of increased amounts of extracellular proteolytic enzymes which utilized the peptides and proteins of the medium as substrates for energy-requiring reactions of the cells. The action of these extracellular proteinases rendered the growth of the cells and the lowering of the external pH possible, thus explaining the results obtained originally (*Mäkinen*, 1972). Consequently, the sucrose- or glucose-exploiting organism was converted to a protein-using type (*Knuuttila & Mäkinen*, 1975b). Xylitol behavied in all these experiments as an inert compound. Similar findings were recently obtained with certain strains of oral Candida (*Mäkinen, Oja-*

notko & Vidgren, 1975). Under the experimental conditions of the present study there are no indications of an adaptation or mutation of the plaque microbiota to degrade xylitol through acid production.

REFERENCES

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

Duany, L. F., Zinner, D. D. & Jablon, J. M. 1970. Identification of types of potentially cariogenic streptococci on human tooth surfaces by the fluorescent antibody technique. J. Dent. Res. 49, 1527

Duany, L. F., Jablon, J. M. & Zinner, D. D. 1972. Epidemiologic studies of caries-free and caries-active students: I. Prevalence of potentially cariogenic streptococci. J. Dent. Res. 51, 723

Fitzgerald, R. J. Coykendall, A. L., Shklair, I. L., Gibbons, R. J., Tanzer, J. M., Brown, A. I., Cowman, R. A. & Jordan, H. V. 1973. Streptococcus mutans and dental caries. DHEW Pub. No. (NIH) 74—286

Gehring, F. 1974a. Mikrobiologische Aspekte zur Kariogenität von Zuckern und Zuckeraustauschstoffen. Dtsch. zahnärztl. Z. 29, 769

Gehring, F. 1974b. Microbiological aspects in cariogenicity of sugars and sugar substitutes. Program and abstracts, 52nd General Meeting. Internat. Assoc. for Dent. Res., 270

Gibbons, R. J. & Banghart, S. B. 1967. Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. Arch. Oral Biol. 12, 11

Gibbons, R. J. 1973. Ecology of Streptococcus mutans. In: Streptococcus mutans and dental caries. DHEW Pub. No. (NIH) 74—286

Grunberg, E., Beskid, G. & Brin, M. 1973. Xylitol and dental caries. Efficacy of xylitol in reducing dental caries in rats. Internat. J. Vit. Nutr. Res. 43, 227

Guggenheim, B. 1970. Extracellular polysaccharides and microbial plaque. Int. dent. J. 20, 657

Ikeda, T. & Sandham, H. J. 1971. Prevalence of Streptococcus mutans on various tooth surfaces in negro children. Arch. Oral Biol. 16, 1237

Ikeda, T., Sandham, H. J. & Bradley, E. L., Jr. 1973. Changes in Streptococcus mutans and lactobacilli in plaque in relation to the initiation of dental caries in Negro children. Arch. Oral Biol. 18, 555

Jordan, H. V., Englander, H. R. & Lim, S. 1969. Potentially cariogenic streptococci in selected population groups in the western hemisphere. J. Am. Dent. Assoc. 78, 1331

Karle, E. & Büttner, W. 1971. Kariesbefall im Tierversuch nach Verabreichung von Sorbit, Xylit, Lycasin und Calciumsaccharosephosphat. Dtsch. zahnärztl. Z. 26, 1097

Karle, E. & Gehring, F. 1974. The effect of some sugar substitutes to caries incidence and plaque flora of the rat. Abstracts of Research Reports, 11th Annual Meeting, IADR-CED, 2

Karle, E. & Gehring, F. 1974a. Wirkung der Zuckeraustauschstoffe Fruktose, Sorbit und Xylit auf Kariesbefall und Plaqueflora der Ratte. Dtsch. zahnärztl. Z. (in press)

Kozlowski, G. G., Shklair, I. L., Keene, H. J. & Levine, J. A. 1973. Prevalence of Streptococcus mutans and association with dental caries in children. Program und Abstracts, 51st General Meeting. Internat. Assoc. for Dent. Res. No 552

Knuuttila, M. L. E. & Mäkinen, K. K. 1975a. The effect of xylitol on the growth and metabolism of *Streptococcus mutans*. Caries Res. In press

Knuuttila, M. L. E. & Mäkinen, K. K. 1975b. Induction of extracellular proteinases in *Streptococcus mutans* by xylitol. (To be published)

Krasse, B., Jordan, H. V., Edwardsson, S., Svensson, I. & Trell, L. 1968. The occurrence of certain 'caries-inducing' streptococci in human dental plaque material. Arch. Oral Biol. 13, 911

Larmas, M., Mäkinen, K. K. & Scheinin, A. 1974. Turku sugar studies. III. An intermediate report on the effect of sucrose, fructose and xylitol diets on the numbers of salivary lactobacilli, candida and streptococci. Acta Odont. Scand. 32, 423—433

Littleton, N. W., Kakehashi, S. & Fitzgerald, R. J. 1969. Study of differences in the occurrence of dental caries in caucasian and Negro children. J. Dent. Res. 49, 742

Littleton, N. W., Kakehashi, S. & Fitzgerald, R. J. 1970. Recovery of specific 'caries-inducing' streptococci from carious lesions in the teeth of children. Arch. Oral Biol. 15. 461

Mühlemann, H. R., Regolati, B. & Marthaler, I. M. 1970. The effect on rat fissure caries of xylitol and sorbitol. Helvet. Odont. Acta 14, 48

Mäkinen, K. K. 1972. Enzyme dynamics of a cariogenic streptococcus: The effect of xylitol and sorbitol. J. Dent. Res. 51, 403—408

Mäkinen, K. K. 1974. Sugars and the formation of dental plaque, in *Sugars in nutrition.* Ed. by Sipple, H. L. & McNutt, K. W., Academic Press, Inc., New York, pp 645—687

Mäkinen, K. K. & Scheinin, A. 1974. Turku sugar studies. II. Preliminary biochemical and general findings. Acta Odont. Scand. 32, 413—421

Mäkinen, K. K., Ojanotko, A. & Vidgren, H. 1975. (To be published)

Newbrun, E. 1972. Extracellular polysaccharides synthesized by glucosyltransferases of oral streptococci. Caries Res. 6. 132

Schamschula, R. G. & Barnes, D. E. 1970. A study of the streptococcal flora of plaque in caries-free and caries-active primitive peoples. Aust. Dent. J. 15, 377

Schamschula, R. G. & Charlton, G. 1971. A study of caries etiology in New South Wales school children. I. The streptococcal flora of plaque and caries prevalence. Aust. dent. J. 16, 77

Scheinin, A., Mäkinen, K. K. & Ylitalo, K. 1974. Turku sugar studies. I. An intermediate report on the effect of sucrose. fructose and xylitol diets on the caries incidence in man. Acta Odont. Scand. 32, 383—412

Shklair, I. L., Keene, H. J. & Simonson, L. G. 1972. Distribution and frequency of Streptococcus mutans in caries-active individuals. J. Dent. Res. 51, 882

Shklair, I. L., Keene, H. J. & Cullen, P. 1974. The distribution of Streptococcus mutans on the teeth of two groups of naval recruits. Arch. Oral Biol. 19, 199

de Stoppelaar, J. D., van Houte, J. & Backer Dirks, O. 1969. The relationship between extra-cellular polysaccharide producing streptococci and smooth surface caries in 13-year old children, Caries Res. 3, 190

Woods, R. 1971. A dental caries susceptibility test based on the occurrence of *Streptococcus mutans* in plaque material. Aust. Dent. J. 16, 116—121