Acta odont scand 1972 *30:* 235—257

Keywords:
Dietary carbohydrates
Dental plaque
Sucrose
Fructose
Glucose
Maltose
Sorbitol

From:
The Institute of Dentistry,
University of Turku,
Finland

EFFECT OF SUGARS AND SUGAR MIXTURES ON DENTAL PLAQUE

ARJE SCHEININ
KAUKO K. MÄKINEN

The effect of the consumption of certain sugars and sugar mixtures on the formation of plaque and on the chemical composition of plaque and oral fluid was investigated using 72 dental students divided in six sugar groups; I, sucrose; II, fructose-sorbitol, 1:1; III, fructose-glucose, 1:1; IV, fructose-xylitol, 1:1, V, xylitol; VI, sucrose-maltose, 9:1. The sugars and the sugar mixtures were consumed in coffee, tea and rolls in addition to five daily mouth washings. The xylitol-based IV and V particularly reduced the formation of plaque when compared to the values obtained during a preceding sucrose intake period. The mixture of fructose-xylitol was less plaque-producing than fructose-sorbitol. The deliberately mild dietary regime produced the most distinct changes in the rate of plaque formation and less significant or undetectable changes in its chemical composition.

In previous studies (*Scheinin & Mäkinen,* 1971; *Mäkinen & Scheinin,* 1971; *Paunio, Mäkinen & Scheinin,* 1972), conducted to explore the effects of certain simple carbohydrates on the formation and chemical composition of dental plaque and on the condition of the parodontium, as well as on the chemical composition of oral fluid, it was found that a short term sugar diet considerably affected the parameters thus investigated.

The findings thus obtained led to a new and more extended investigation where the effect of different sugar mixtures on the variables mentioned above was determined.

MATERIALS AND METHODS

Test groups and diet. 72 dental students took part in the study, each adhering to a dietary regime during the test periods. The test persons were divided into six groups. The number of students in each sugar group is shown in

Received for publication, December 15, 1971.

Table I.

The general arrangement of the study

Period	Group a 11 persons	Group b 11 persons	Group c 12 persons	Group d 13 persons	Group e 10 persons	Group f 15 persons
Period AO[1])	Normal diet	Normal diet	Normal diet	Normal diet	Normal diet	Normal diet
Period A[2]) (5 days)	Sucrose diet	Sucrose diet	Sucrose diet	Sucrose diet	Sucrose diet	Sucrose diet
Period BO (4 weeks)	Normal diet	Normal diet	Normal diet	Normal diet	Normal diet	Normal diet
Period B[2]) (5 days)	Sucrose diet	Fructose-Sorbitol diet (1:1)	Fructose-Glucose diet (1:1)	Fructose-Xylitol diet (1:1)	Xylitol diet	Sucrose-Maltose diet (9:1)

[1]) Normal diet and normal oral hygiene during the time preceding the whole study.

[2]) Periods A and B started immediately after the clinical inspection and sample collecting of Periods AO and BO, respectively.

Table I, which also presents the overall arrangement of the investigation. According to this, the whole experimental cycle was composed of four separate periods. Table I provides more detailed explanation of the test periods, but it may be mentioned that as an extension to the earlier study (*Scheinin & Mäkinen*, 1971; *Mäkinen & Scheinin*, 1971) the clinical inspection and chemical analyses on saliva and plaque material were also carried out during a period with normal diet and oral hygiene. This was designated as Period AO. After these measures, the sucrose period was started: all 72 test persons in the six groups were on a sucrose diet for 5 days. Clinical inspection and chemical analyses terminated this period (Period A). After a normalization period of approximately four weeks, a third clinical inspection and a control analysis of salivary β-fructofuranosidase activity were performed on the test persons, this period being designated as Period BO. Finally, the same test persons were put on another sugar diet; one group consumed still sucrose, whereas the other five groups were on a diet containing different sugar mixtures or xylitol (Table I). After the sugar diet of five days the whole investigation was terminated by the fourth clinical inspection and chemical analyses of plaque and saliva (Period B). The time schedule of the investigation is presented in Fig. 1.

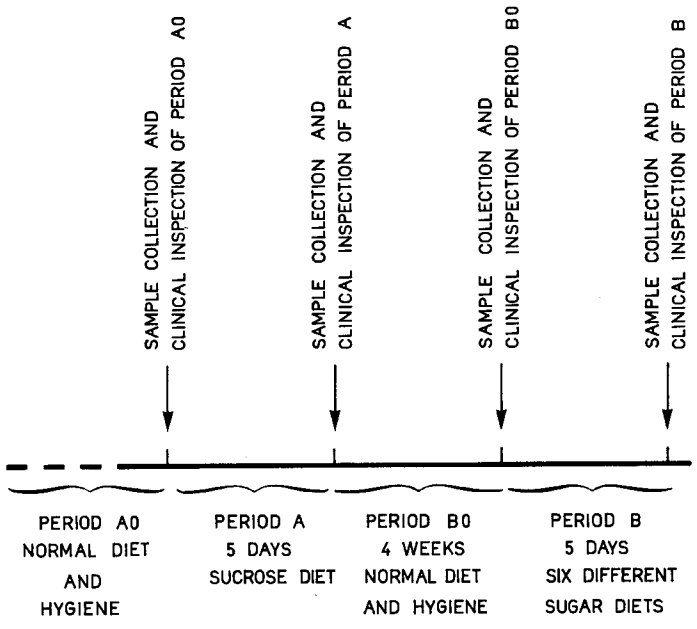

Fig. 1. Time schedule of the investigation. At the end of Period BO only plaque index and salivary β-fructofuranosidase activity was determined (cf. Figs. 2 and 3).

The total daily amount of the sugars or sugar mixtures used by each test person varied from 60 to 80 g. The total amount consisted of five daily mouth washings with a 10 % sugar solution, of sweet rolls (with sodium bicarbonate for raising the dough) which were baked with the respective sweetener, and of sufficient amounts of sugars to be used in coffee or tea (at least twice a day). The washings were performed with 100 ml solutions at a time for a period of one minute. The test persons were advised to wash their mouths with water after the sugar rinse. During the five days sugar periods altogether 25 rinses were performed. Otherwise the students were allowed to consume ordinary food, but as a sweetener they were instructed to use only that sugar distributed to the particular group. Hence the test persons in the xylitol group, for example, consumed also flour products, potatoes, etc.

The test persons were given a special form where they were asked to answer questions relevant for an evaluation of their suitability in the final material. The inquiries and interviews concerned all possible medication, the com-

PERIOD AO Normal diet and hygiene — Carried out to determine baseline clinical and chemical values of the participants

1. Clinical inspection. Plaque index. Interview. Delivery of instructions
2. Collecting of 10 oral fluid (paraffin stimulation)
3. Measurement of the pH of the oral fluid at 37°C (glass and calomel electrodes)
4. Lactobacillus count (1 ml was used)
5. Cooling of the sample to +4°C
6. Centrifugation (10 min at 12.000 rpm; 17.300×g) at +4°C

↓ Supernatant fluid

Assay of
7. Invertase
8. Dextranase
9. Lactic acid
10. Protein (F-C)

← Carried out for each sample immediately after centrifugation

11. Pooling of the samples into six pools representing the six sugar groups
12. Freezing and storing at —25°C

Assay of
13. Total carbohydrates (sugars) (Anthrone)
14. Total nitrogen

Carried out 1—2 months after collecting

↓ Pellet

→ Carried out for each sample immediately after centrifugation

7. All pellets were pooled to form six pools representing the six sugar groups
8. Freezing of the pools and storing at —25 C
9. Thawed after 2—6 weeks and suspended in cold (+4°C) water (14 ml per material of the whole sugar group)
10. Sonication (10 min; end diameter of the probe, 9 mm; 4 microns amplitude)
11. Centrifugation (10 min at 19.000 rpm; 43.500×g) The pellet was discarded

Assay of
12. Invertase
13. Dextranase
14. Protein (F-C)
15. Total nitrogen
16. RNA
17. DNA

(12.—17. carried out 2—6 weeks after collecting)

PERIOD A Sucrose diet — All participants were on sucrose diet of five days

Steps 1.—6. as above

↓ Supernatant fluid

Assay of
7. Invertase
8. Dextranase
9. Lactic acid
10. Protein (F-C)

← Carried out for each sample immediately after centrifugation

11. Freezing and storing at —25°C

↓ Pellet

7. Treated and analyzed exactly as above for period AO (i.e. the analyses were made on six pools representing the six sugar groups)

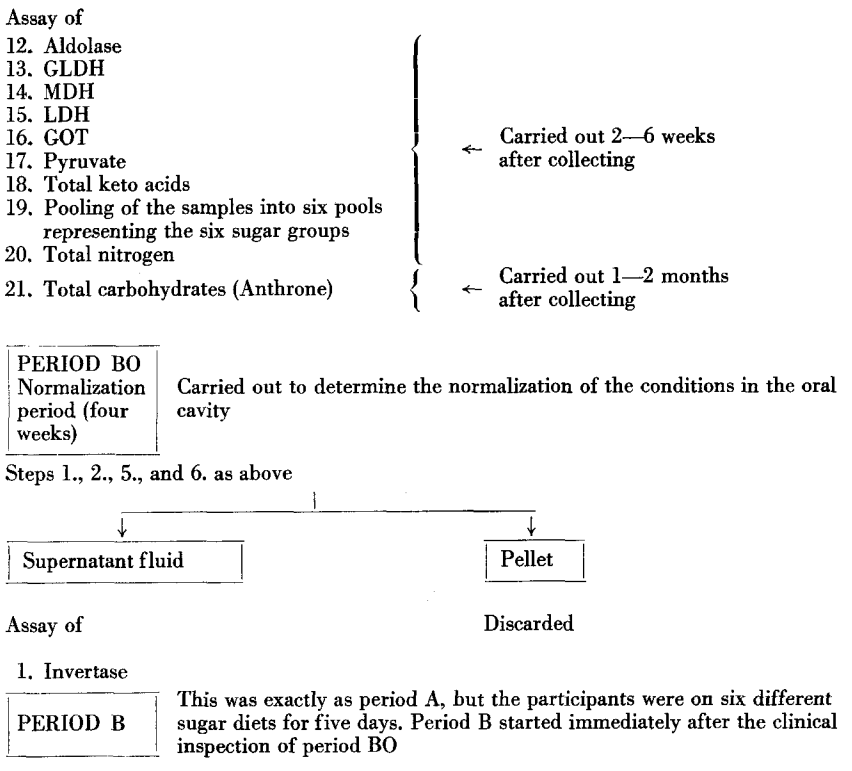

Fig. 2. The experimental procedure of the investigation concerning the study of oral fluid. F-C = Folin-Ciocalteu.

(If not otherwise stated, all centrifugations and sonications were performed at +4°C)

position of the meals, and in which way the predetermined regime was not possible to be followed. All answers were considered and in a few cases the contribution of a test person was rejected, actually on the basis of intake of antibiotics or traveling. Due to the participation of dental students only, the cooperation was very good.

During the actual test periods the students refrained from brushing their teeth. Tooth brushing was allowed immediately after Period A, during the »normalization» Period BO and after Period B. Brushing took place normally during the Period AO preceeding the actual test periods.

All other details concerning the general performance of the study are mentioned in Figs. 1—3 and Table I.

Preparation of samples and chemical assays. The preparation of the samples of plaque and oral fluid was in principle performed as earlier (*Schei-*

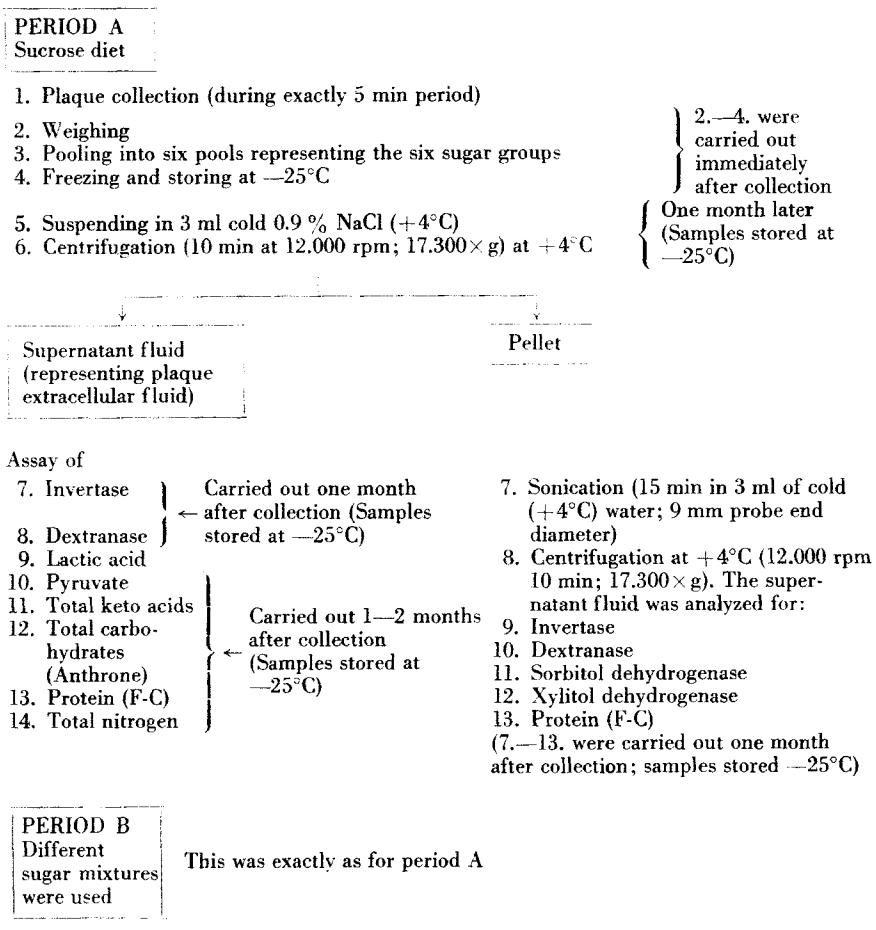

Fig. 3. The experimental procedure of the investigation concerning the study of plaque.

Plaque analysis was carried out for material obtained from period A and period B. For details cf. Table II.

nin & Mäkinen, 1971). The whole research plan including the treatment and analyses of the samples is presented in Figs. 2 and 3. The chemical methods are additionally listed in Table II. All reagents were the same as mentioned earlier (*Scheinin & Mäkinen*, 1971). Due to the simultaneous use of six laboratory technicians and one chairside assistant, most of the chemical assays were carried out within a short period, the bulk of them during the same day the samples were collected. Figs. 2 and 3. give the conditions where cold storage of the samples before analysis was necessary. The

Table II.

Chemical and biochemical assay methods used in the study

Enzyme or compound to be assayed	Method	Reference
Invertase (β-Fructofuranosidase)	The 3,5-dinitrosalisylic acid method	*Hostettler, Borel & Devel* (1951); *Scheinin & Mäkinen* (1971)
Dextranase	In principle as for invertase, but replacing sucrose with a 5 % aqueous solution of dextran (Dextran T70, Mw < 70.000, light scattering; Pharmacia Fine Chemicals, Upsala, Sweden)	As above
Aldolase	The spectrophotometric assay of Warburg (with enzymatic auxiliary and indicator reactions). Fructose 1,6-diphosphate was used as substrate	p. 728*)
Glutamate dehydrogenase	The spectrophotometric assay using 2-oxoglutarate and NADH	p. 752*)
Malate dehydrogenase	The spectrophotometric assay using L-malate and NADP	p. 757*)
Lactate dehydrogenase	The spectrophotometric assay using lactate and NADP	p. 736*)
Glutamate oxalate transaminase	The colorimetric method using 2,4-dinitrophenylhydrazine	p. 842*)
Sorbitol dehydrogenase (SDH)	The spectrophotometric method using fructose as substrate	p. 761*)
Xylitol dehydrogenase (XDH)	In principle as for SDH, but replacing fructose with xylylose	
Pyruvate	Spectrophotometrically with LDH and NADPH	p. 253*)
»Total keto acids»	A colorimetric assay using 2,4-dinitrophenylhydrazine	*Friedemann & Haugen* (1943); *Mäkinen* (1968)
Lactic acid	The spectrophotometric method using LDH and NADP	p. 266*)
Protein	Folin-Ciocalteu (Bovine serum albumin as standard)	*Layne* (1963)

Nitrogen	Micro-Kjeldahl (ammonium sulphate as standard)	
RNA	Orcinol method (D-ribose as standard)	*Bial* (1902); *Mejbaum* (1939)
DNA	Diphenylamine method (2-desoxy-D-ribose as standard)	*Burton* (1956)
»Total sugars»	Anthrone method (glucose as standard)	*Dreywood* (1946); *Morris* (1948)

*) The page numbers refer to: *Bergmeyer, H.* (1963) *Loc. cit.*

lactobacillus count was made on Rogosa agar and the visual inspection of the colonies was performed after three days growth.

The data obtained at various test periods were treated numerically for significance by the *t*-test (95 per cent confidence), after having applied the *F*-test.

RESULTS

Formation of plaque

The clinical condition (as plaque index, Pl I) and salivary β-fructofuranosidase activity were determined four times, and most other chemical or enzyme assays three times during the investigation. This was performed in order to determine the baseline condition and characteristics of the test persons. Due to better oral hygiene during the Period AO, the plaque index and the amount of various organic compounds and the enzyme activity in oral fluid and plaque increased considerably when transferring from Periods AO and BO to Periods A and B, respectively. In Period BO the plaque index was as low as in Period AO. The plaque index value was not found to measure the variations in plaque development as well as the determination of the plaque fresh weight. However, also the development of the plaque index during the periods of reduced oral hygiene showed that least plaque was developed in the xylitol group (Fig. 4). The results showed that the Period BO of four weeks was long enough to »normalize» the conditions in the oral cavity after the sucrose period (Period A).

The correlation between plaque index and plaque fresh weight was approximately the same as in the previous study (*Scheinin & Mäkinen*, 1971). The plaque index and plaque fresh weight of the test persons determined at

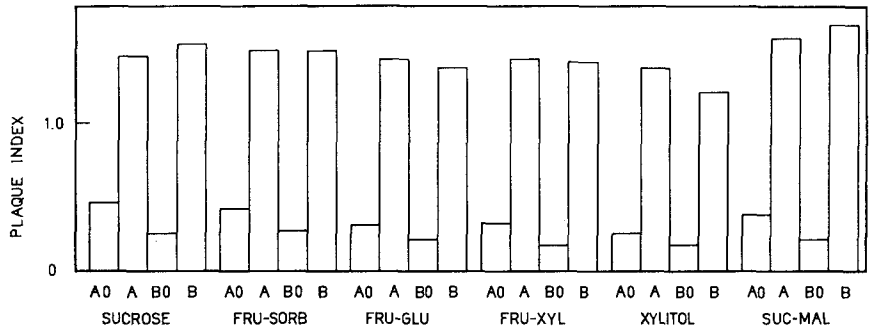

Fig. 4 The medians of plaque indexes in the different sugar groups determined at Periods AO, A, BO, and B.

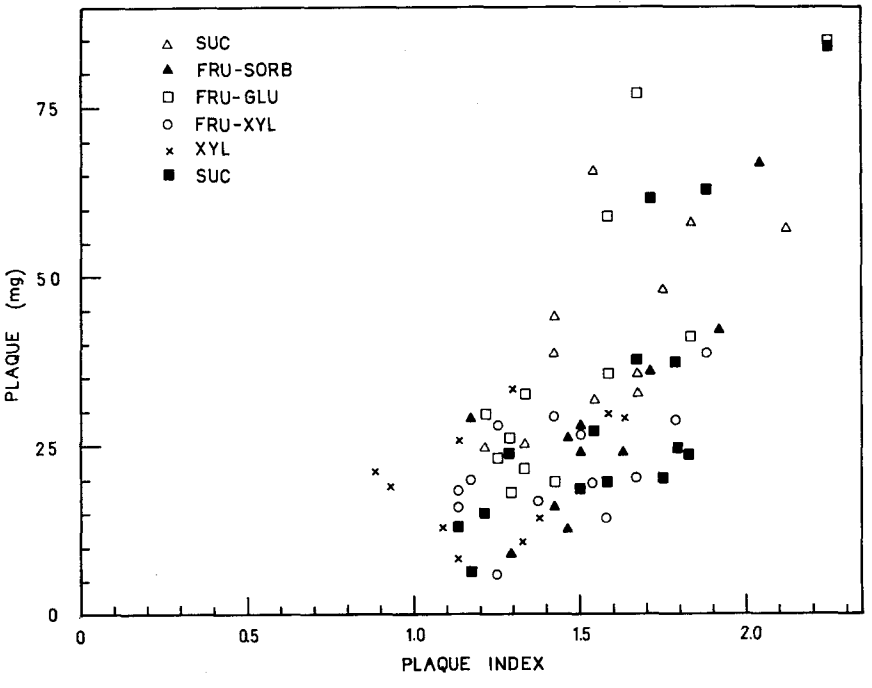

Fig. 5. Correlation between plaque index and the fresh weight of plaque.

the end of Period B (the period of six different sugar diets) are presented in Fig. 5. The values recorded both for the xylitol and fructose-xylitol groups were generally located in the lower left part of the figure, whereas in the other test groups the values were located oabve or at the right side of the xylitol values. Fig. 6. shows the individual changes as well as the fact that

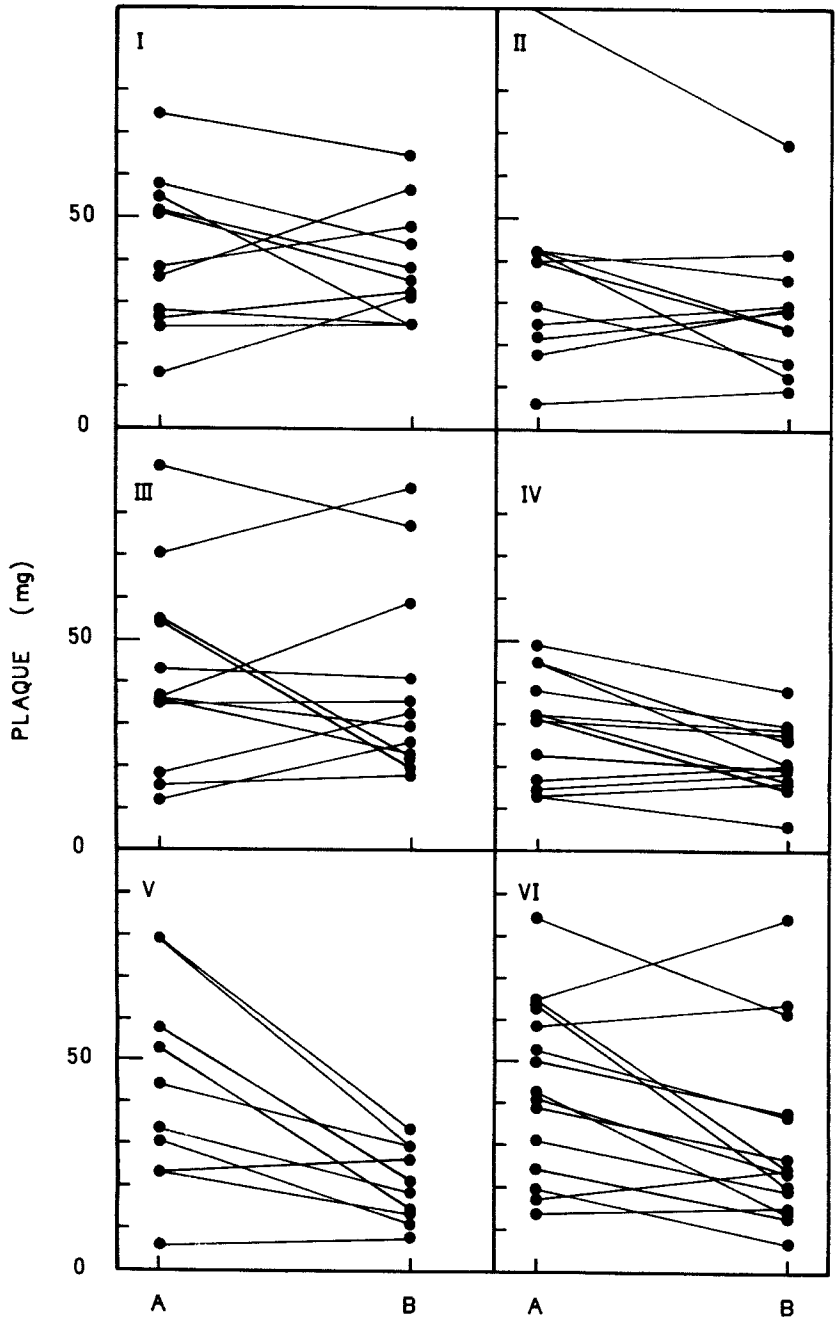

Fig. 6. Individual changes in plaque fresh weight recorded at the end of Periods A (sucrose diet) and Period B (six different sugar diets). Sugar groups: I, sucrose; II, fructose-sorbitol; III, fructose-glucose; IV, fructose-xylitol; V, xylitol; VI, sucrose-maltose.

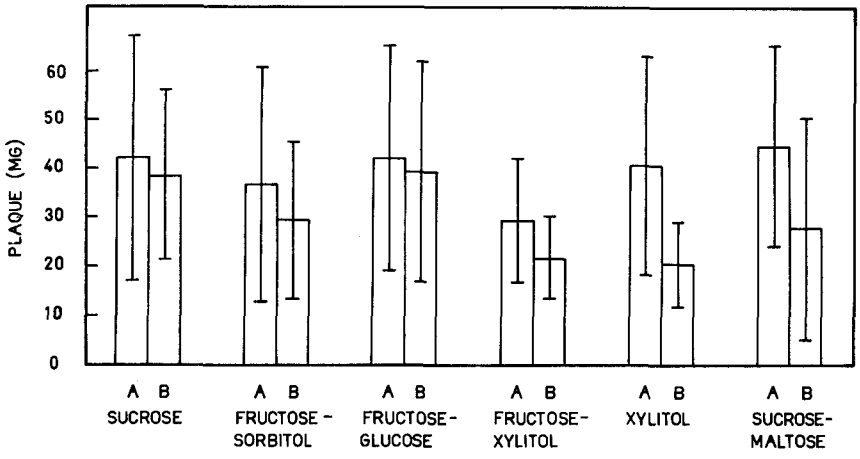

Fig. 7. The means and standard deviations of plaque fresh weight in different sugar groups determined at the end of Period A and Period B. Comparison between various sugar groups at Period B:
between sucrose and xylitol: $^{**}p<0.01$;
between fructose-sorbitol and xylitol: $^{\circ}p<0.1$;
between sucrose and fructose-sorbitol; $^{**}p<0.01$;
between sucrose and sucrose-maltose: $^{*}p<0.05$;
between sucrose and fructose-xylitol; $^{**}p<0.01$.
Comparison between Periods A and B:
in fructose-xylitol group: $^{**}p<0.01$; in xylitol group: $^{**}p<0.01$.

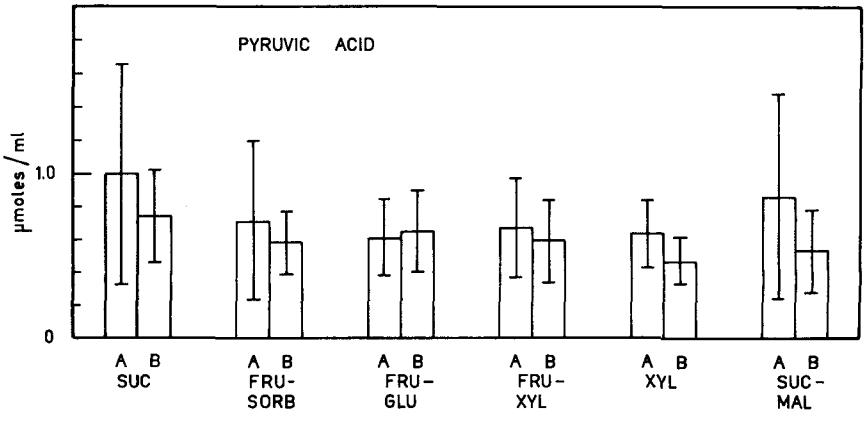

Fig. 8. The means and standard deviations of the amount of pyruvic acid in centrifuged oral fluid in different sugar groups at the end of Period A and Period B.

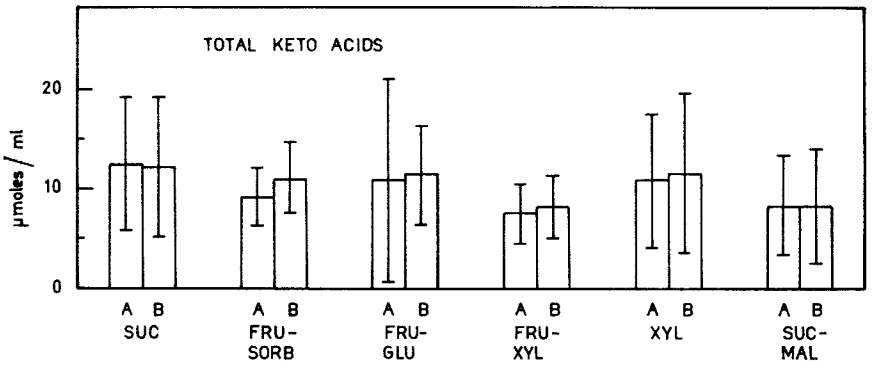

Fig. 9. The means and standard deviations of the amount of »total keto acids» in centrifuged oral fluid in different sugar groups at the end of Period A and Period B.

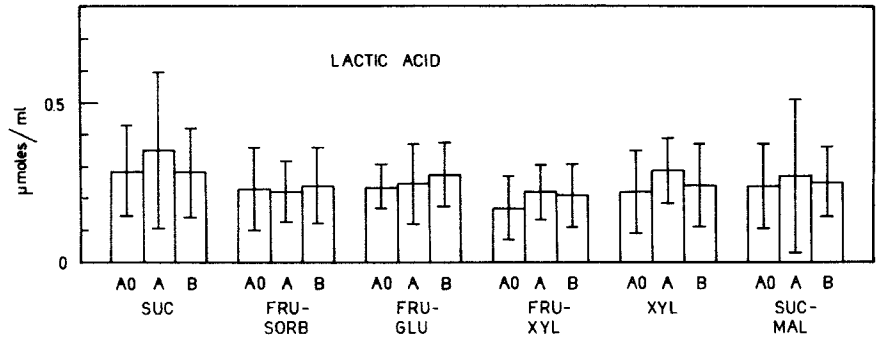

Fig. 10. The means and standard deviations of the amount of lactic acid in centrifuged oral fluid at the end of Period A0, Period A, and Period B.

also in the sucrose-maltose group the values recorded at the end of Period B were generally lower than at Period A. In the three first test groups the change from A to B was expressed by an approximately equal number of increasing and decreasing values. The mean values and standard errors calculated for the plaque fresh weight values are shown in Fig. 7. The lowering of the plaque weight was statistically significant in the three last sugar groups (see legend to Fig. 7).

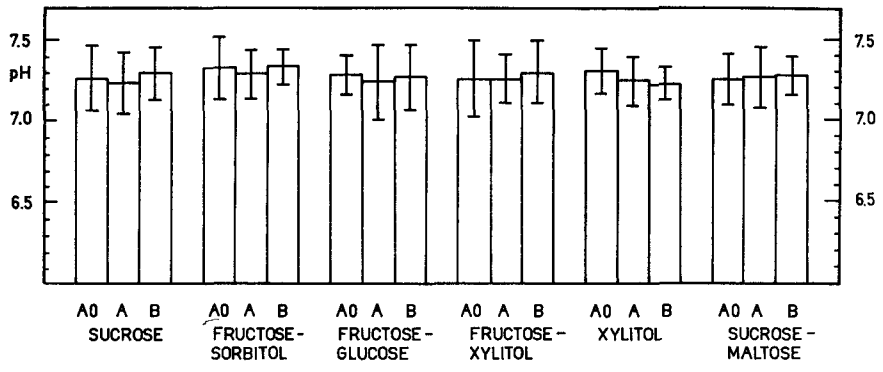

Fig. 11. The means and standard deviations of pH of oral fluid in different sugar groups at the end of Period AO, Period A, and Period B. The measurement took place immediately after obtaining exactly 10 ml paraffin stimulated oral fluid from the test person.

Formation of organic acids

The amount of pyruvic acid in centrifuged oral fluid was approximately one tenth of the amount of »total keto acids» (Figs. 8 and 9). No statistically significant correlation between the type of carbohydrate consumed and the amount of pyruvic acid or total keto acids was found, although it may be mentioned that only in fructose-glucose group the amount of pyruvic acid had not decreased when transferring from Period A to Period B. This could be explained by the suitability of this sugar mixture for fermentation via several pathways available for oral micro-organisms. The amount of lactic acid of centrifuged oral fluid (Fig. 10) varied in this investigation almost to the same degree as earlier (*Scheinin & Mäkinen*, 1971). The amount of lactic acid expressed in μmoles per ml oral fluid (centrifuged) was approximately the same or somewhat lower than the amount of pyruvic acid, an indication of a close metabolic relationship between these compounds.

There were no pronounced differences in the amount of lactate between Period A and Period B in the different sugar groups. However, an inspection of Figs. 8 and 10 may still give an explanation why the fructose-glucose mixture did not prove to be effective in reducing plaque amount. Only in this sugar group the amount of pyruvate and lactate increased when transferring from Period A to Period B. The increases were not statistically significant, but in all other sugar groups the values were decreased from Period A to Period B. Hence a fructose-glucose mixture would be comparatively good energy source for oral mixed cultures. In the fructose-sorbitol group there was also a slight increase in lactic acid when transferring from Period A to Period B.

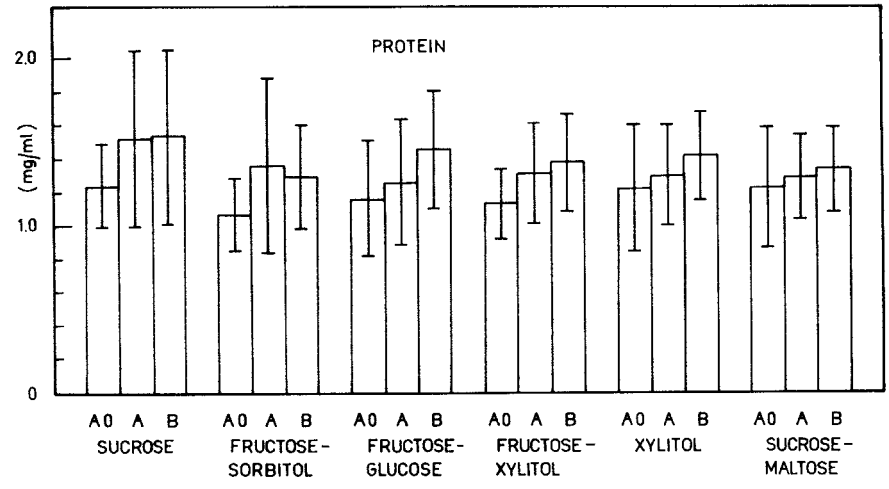

Fig. 12. The means and standard deviations of the amount of protein (Folin-Ciocalteu) in centrifuged oral fluid on different sugar groups at the end of Period AO, Period A and Period B.

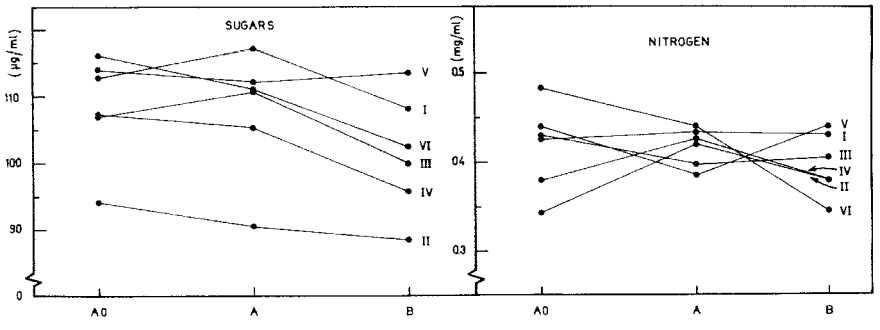

Fig. 13. Change of the amount of »total sugars» (determined with the anthrone method) and total nitrogen (Micro-Kjeldahl) in pooled samples of centrifuged oral fluid between three stages of the investigation. Sugar groups: I, sucrose: II, fructose-sorbitol; III, fructose-glucose; IV, fructose-xylitol; V, xylitol; VI, sucrose-maltose: All solid circles represent 10—15 persons.

Salivary pH

The pH values determined immediately after the collection of the oral fluid are presented in Fig. 11. As expected, no substantial changes of the pH values were observed in any of the sugar groups when transferring from Period A to Period B.

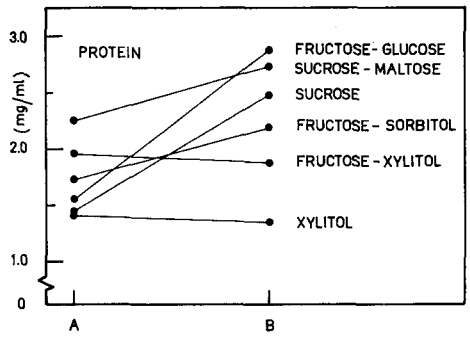

Fig. 14. Change of the amount of protein (Folin-Ciocalteu) in pooled samples of plaque between Periods A and B. The pooled plaque samples were sonicated and the supernatant fluid resulting was analyzed.

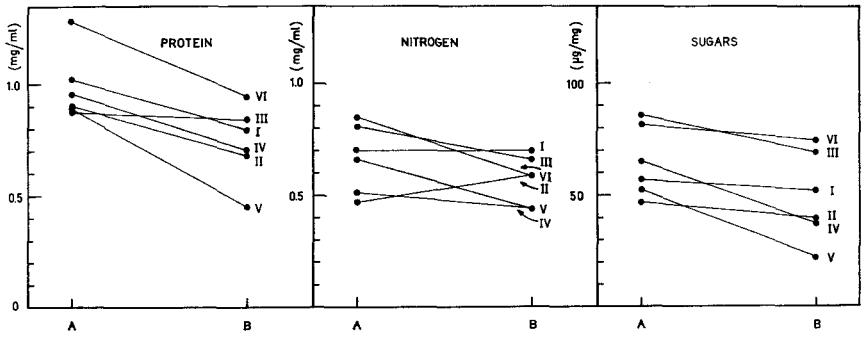

Fig. 15. Change of the amount of protein (Folin-Ciocalteu), total nitrogen (Micro-Kjeldahl), and »total sugars» (anthrone) in pooled plaque water extracts between Periods A and B. The plaque material in each sugar group was pooled and suspended in cold water containing 0.9 % NaCl (see Fig. 3). The suspension was centrifuged and the clear supernatant fluid was analyzed.

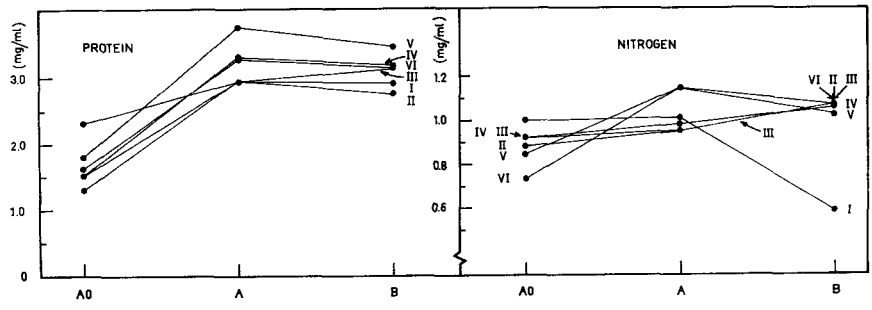

Fig. 16. Change of the amount of protein (Folin-Ciocalteu) and total nitrogen (Micro-Kjeldahl) in pooled sediment material obtained after centrifuging oral fluid samples between three stages of the investigation. The sediment material was suspended in cold water containing 0.9 % NaCl (see Fig. 2). The suspension was conicated and the clear supernatant fluid was analyzed.

Protein and total nitrogen

The protein and total nitrogen assays yielded results presented in Figs. 12—13 for the supernatant fluid of oral fluid, in Fig. 14 for the supernatant fluid of sonicated plaque, in Fig. 15 for the supernatant fluid of plaque, and in Fig. 16 for the supernatant fluid of pooled and sonicated oral fluid sediment (representing easily removable soft plaque). All the protein values increased in oral fluid (Fig. 12) and in its sonicated sediment preparation (Fig. 16) when transferring from Period AO to Period A. There was no clear correlation between the protein and nitrogen values. This may be due to the unspecificity of the colorimetric protein assay and to the fact that in the total nitrogen assays evidently all nitrogen containing compounds were involved. In Fig. 14 (sonicated plaque) the highest increase in protein took place in glucose-fructose group. In Fig. 15 this group displayed almost identical values at Periods A and B. In Fig. 14 the xylitol groups (IV and V) showed a slight decrease when transferring from Period A to Period B and the same groups were the most decreasing in Fig. 15. Figs 14 and 15 show that when the amount of proteins in plaque water extract decreased (Fig. 15), the amount of protein in the insoluble compartment, representing intact microbial cells etc., increased (Fig. 14), except for groups IV and V. There was in general least protein and total nitrogen at Period B in the xylitol groups.

»Sugars»

The »sugar» determinations are presented in Fig. 13 for the supernatant fluid of oral fluid and in Fig. 15 for the supernatant fluid of plaque. In both cases the values were decreasing when transferring from Period A to Period B (except for xylitol in Fig. 13). In Fig. 15 it is evident that the least decrease in the sugar amount between Period A and B had taken place in the sucrose group (group I). It is known that this method to assay carbohydrates is not specific. Several compounds give coloured complexes with sulphuric acid, but the sensitivity of the method merited its exploitation.

DNA and RNA

Fig. 17 shows the results obtained in the nucleic acid assays. The transfer from Period AO to Period A naturally resulted in a considerable increase in the amount of nucleic acids in the supernatant fluid of pooled and sonicated

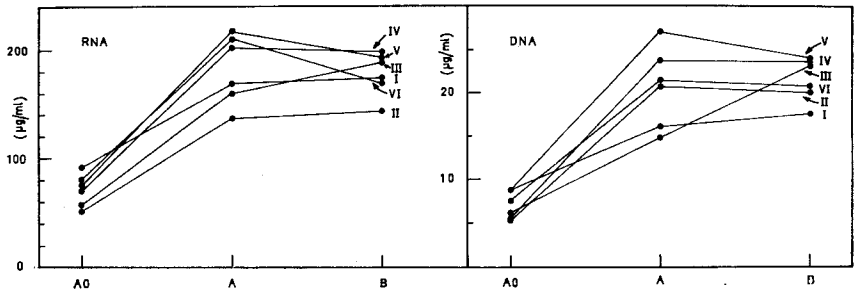

Fig. 17. Change of the amount of RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) in pooled sediment material after centrifuging oral fluid samples obtained at three stages of the investigation. The sediment material was suspended in cold water containing 0.9 % NaCl (see Fig. 2). The suspensions were sonicated and the clear supernatant fluid was analyzed.

preparations of oral fluid sediment (material representing easily removable soft plaque). The transfer from Period A to Period B did not yield such large changes except in the fructose-glucose group (DNA) and in the sucrose-maltose group (RNA).

Lactobacillus count

Due to the use of dental students in the study, it was evident that there were only few individuals with high caries activity. Therefore, the lactobacillus counts generally yielded low values especially at Period AO, but a few reasonably low values were recorded at Periods A and B as well. The mean lactobacillus count obtained for all participants at Periods AO and A were 7800±9500, and 32800±29200, respectively. There was no statistically significant correlation between the type of sugar consumed and the lactobacillus index. Neither was a statistically significant correlation found between the lactobacillus count and plaque fresh weight: high counts were recorded for persons with only moderate amount of plaque (20—30 mg) and large plaque fresh weights (up to 100 mg) were obtained with persons with very low counts. Table III shows that only in the sucrose group at Period B no zero lactobacillus index values were recorded, an indication that sucrose had promoted the growth of lactobacilli. Table IV shows that the number of cases where Candida was detected increased at the end of the investigation. In three cases streptococci were found to grow on the Rogosa agar (2500—7500 cells/ml). There was a statistically significant cor-

Table III.

Occurrence of positive and zero lactobacillus index values at Period B

Sugar group	Number of zero values	Number of positive values	Total
Sucrose	0	11	11
Fructose-sorbitol	7	4	11
Fructose-glucose	4	8	12
Fructose-xylitol	3	10	13
Xylitol	5	5	10
Sucrose-maltose	2	13	15

Table IV.

Candida index values at different periods of the study (cells per ml saliva)

Period AO	Period A (two cases)	Period B (four cases)
No cases	50 (sucrose)	2500 (xylitol)
	250 (sucrose)	500 (suc-mal)
		2600 (fru-xyl)
		100 (fru-glu)

relation between the lactobacillus count and salivary β-fructofuranosidase activity: high counts were more frequently accompanied by high than low enzyme activity (for β-fructofuranosidase activity, see *Mäkinen & Scheinin*, 1972).

Mean values of the whole test material

Table V presents the mean values and standard deviations of the chemical and other assays carried out with 72 test persons at Period AO and Period A, when all persons were on a similar diet. The plaque index was also determined at Period BO to show if this normalization period had led to a decrease of the index to the level of Period AO. The index was indeed seen to have decreased to the level of Period AO, an indication that a »normalization» in the oral conditions had taken place before Period B was started. The next paper will show that also the activity of β-fructofuranosidase was approxi-

Table V

Means and standard deviations of some constituents of human dental plaque and oral fluid of 72 test persons at Periods A and AO, where the whole test group was on a similar diet. The detailed method in obtaining the samples has been described in the Materials and Methods section and in a previous paper (Scheinin & Mäkinen, 1971)

Constituent or property	Source	Period AO Normal diet and oral hygiene		Period A Sucrose diet and reduced hygiene		Dimensions
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	
Pyruvate	Centrifuged oral fluid			0,69	0.42	μmoles/ml
Total keto acids	Centrifuged oral fluid			10.78	8.38	μmoles/ml
Lactate	Centrifuged oral fluid	0.25	0.17	0.29	0.21	μmoles/ml
Protein	Centrifuged oral fluid	1.17	0.29	1.34	0.38	mg/ml[5])
Lactobacillus count	Oral fluid	7800	9500	32800	29200	cells/ml
pH	Oral fluid	7.28	0.18	7.25	0.18	
Plaque	Excavated *in situ*[1])			39.5	20.2	mg
Plaque index	Clinical inspection	0.37	0.22	1.47	0.27	
DNA	Sonicated preparation of oral fluid sediment[2])	6.92	0.16	20.68	0.46	μg/ml
RNA	Sonicated preparation of oral fluid sediment[2])	71.13	1.50	182.60	3.22	μg/ml
Total nitrogen	Sonicated preparation of oral fluid sediment[2])	0.89	0.10	1.04	0.10	mg/ml
Sugars	Centrifuged oral fluid	108.50	7.95	107.72	9.23	μg/ml
Protein	Sonicated preparation of oral fluid sediment[2])	1.67	0.35	3.17	0.32	mg/ml
Protein	Centrifuged plaque suspension[3])			0.99	0.15	mg/ml
Protein	Sonicated preparation of plaque[4])			1.72	0.33	mg/ml
Total nitrogen	Centrifuged plaque suspension[3])			0.66	0.15	mg/ml
Sugars	Centrifuged plaque suspension[3])			64.67	1.52	μg/ml[5])
Lactate	Centrifuged plaque suspension[3])			0.77	0.35	μmoles/ml

[1]) Collected from four surfaces of six teeth during 5 minute per test person.
[2]) The sediment was obtained by centrifuging oral fluid collected with paraffin stimulation. The sediment was sonicated and the supernatant fluid was analyzed.
[3]) Plaque (referring to »collected *in situ*») was suspended in cold 0.9 % NaCl and the mixture was centrifuged. The supernatant fluid was analyzed.
[4]) Refers to plaque »collected *in situ*». The pellets of 3) were sonicated and the supernatant fluid resulting was analyzed.
[5]) Almost three times as high values were obtained from uncentrifuged oral fluid.

mately the same at Periods AO and BO. The means and standard deviations of the enzyme assays are shown in the next paper (*Mäkinen & Scheinin*, 1972).

DISCUSSION

The results obtained in this investigation were basically the same as in the previous one (*Scheinin & Mäkinen*, 1971). The use of mixtures of various carbohydrates in the present study evidently led to less profound differences in the amount of plaque and its constituents than earlier, when only one sugar compound was used in a particular test group. One carbohydrate, xylitol, which even in the present investigation was used alone in one group, generally yielded the most profound differences in several analyses between the test periods. It can be stated that the dietary regime of the present study was made deliberately mild in the sense that all test persons were allowed to consume ordinary meals. One could expect rather convincing differences in the amount of plaque etc., if an even slightly more strict regime would be achievable in reality. It is, however, evident that people always would consume a wide variety of different carbohydrates in their meals, although certain particular sugars, other than sucrose could form the main constituent. Hence the dietary regime used was evidently realistic also in this sense. The present investigation showed that the exploitation of a restricted regime will result in interesting findings concerning plaque formation and its chemical composition.

One difficulty impossible to abolish in this type of study is the fact that a huge number of individual chemical determinations and velocity measurements would have to be carried out on fresh samples as soon as possible after collection. In this investigation those parameters which, on the basis of the previous study, were considered important to determine immediately after the collection of the samples were, for example, plaque fresh weight, some enzyme activities and organic acids. All these are listed in Figs. 2—3. In cases where the sample had to be stored prior to the assays, they were analysed after an equal time of storage, so that comparison between different periods and test groups would be possible.

The individual variations in the lactobacillus index were quite high (Table III). Standard deviations of equal degree have been obtain elsewhere (*Lehnert*, 1968). It is assumed that a more strict regime could result in a positive correlation. The micro-organisms appearing at Period AO on the Rogosa agar were almost exclusively lactobacilli, but at Periods A and B streptococci and Candida were found in a few saliva samples.

The number of publications dealing with the effect of polyols on the growth of oral micro-organisms and on plaque pH and related subjects has been increasing. Most of the polyols studied with regard to their »dental» effects, like sorbitol, are slowly fermented by oral micro-organisms (*Grubb*, 1945; *Shaw*, 1954; *Shockley, Randels & Dodd*, 1956; *Crowley, Harner, Bennet & Jay*, 1956). Several papers have shown more or less directly the low-cariogenicity of sorbitol (for example, *Mühlemann*, 1969; *Mühlemann*, 1970; *Graf*, 1969; *Larje & Larson*, 1970; *Dallmeier, Bestmann & Kröncke*, 1970).

The first paper of this series of investigations (*Scheinin & Mäkinen*, 1971) also suggested that fructose and xylitol would be less cariogenic than sucrose. There are numerous studies where this quality of fructose has been evoked. *Charlton, Fitzgerald* and *Keyes* (1971) have determined the hydrogen ion activity in dental plaques of hamsters during metabolism of various sugars. Fructose was fermented at a slightly lower rate than sucrose or glucose by the plaques.

The present study revealed that xylitol and several sugar mixtures now investigated were less cariogenic than sucrose. It may be said that of all the clinical and chemical determinations performed in the present study, the amount of plaque fresh weight during a period of sugar intake and reduced oral hygiene can be considered to be most closely related to the caries activity. A significant drop in plaque fresh weight was observed also in the sucrose-maltose group (9:1). The reason the include this sugar mixture lays in the finding that maltose inhibited the synthesis of extracellular polysaccharides by a cariogenic streptococcus (*Knuuttila & Mäkinen*, 1972). In this study the sucrose-maltose mixture proved that it was able to reduce the plaque amount.

The present paper showed that of the six different diets those containing xylitol were seen to be less cariogenic than the other diets, comprising either sucrose alone, a sucrose-maltose mixture, or mixtures containing either fructose and glucose, or fructose and sorbitol.

Acknowledgements. The authors wish to thank the following persons for their interest and skilled assistance: Mrs. Irma Rintanen, Mrs. Leila Saarinen, Miss Leena Lehto, Mrs. Paula Lehtimäki, Miss Rauni Suominen, Mrs. Raili Turta, and Mrs. Christina Tuominen.

This investigation was in part financially supported by the Finnish Medical Council. The authors wish to extend their sincere thanks to Suomen Sokeri Oy (Finnish Sugar Company), for generously provided help and financial support through the Finnish Dental Society.

REFERENCES

Bergmeyer, H., 1963: Methods in enzymatic analysis. In Bergmeyer, H. U. (ed.) Academic Press, New York.

Bial, M., 1902: Die Diagnose der Pentosurie. Deut. Med. Wochsch. *28:* 253.

Burton, K., 1956: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. *62:* 315.

Charlton, G., D. B. Fitzgerald & P. H. Keyes, 1971: Hydrogen ion activity in dental plaques of hamsters during metabolism of sucrose, glucose and fructose. Archs. oral Biol. *16:* 655.

Crowley, M. C., V. Harner, A. S. Bennet & P. Jay, 1956: Comparative fermentability of sorbitol, glucose and glycerol by common oral micro-organisms. JADA *52:* 148.

Dallmeier, E., H.-J. Bestmann & A. Kröncke, 1970: Über den Abbau von Glucose und Sorbit durch Plaques-Streptokokken. Dtsch. zahnärtzl. Z. *25:* 887.

Friedemann, T. & G. E. Haugen, 1943: Pyruvic acid. II. The determination of keto acids in blood and urine. J. biol. Chem. *147:* 415.

Graf, H., 1969: Telemetric des pH der Interdentalplaque. Schweiz. Mschr. Zahnheilk. *79:* 146.

Grubb, T. C., 1945: Studies on the fermentation of sorbitol by oral micro-organisms. J. dent. Res. *24:* 31.

Hostettler, F., E. Borel & H. Deuel, 1951: Über die Reduktion der 3,5-Dinitrosalicylsäure durch Zucker. Helv. chim. Acta *34:* 2132.

Knuuttila, M. L. E. & K. K. Mäkinen, 1972: The effect of maltose on the polysaccharide synthesis by a cariogenic streptococcus. J. dent. Res. *51:*674.

Larje, O. & R. H. Larson, 1970: Reduction of dental caries in rats by intermittent feeding with sucrose substitutes. Archs. oral Biol. *15:* 805.

Layne, E., 1963: Spectrophotometric and turbidimetric methods for measuring proteins. In »Methods in enzymology» (ed. by S. P. Colowick and N.O. Kaplan) third printing. Academic Press, N.Y. p. 447.

Lehnert, S., 1968: Biochemische und mikrobiologische Untersuchungen über Korrelationen zwischen Speichel, Zahnbelägen und Karies. Carl Hanser Verlag, München, p. 78.

Mejbaum, W. Z., 1939: Über die Bestimmung kleiner Pentosemengen insbesondere in Derivaten der Adenylsäure. Hoppe-Seylers Z. Physiol. Chem. *258:* 117.

Morris, D. L., 1948: Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science *107:* 254.

Mäkinen, K. K., 1968: Studies on oral enzymes. VII. Pyridoxal-5-phosphate dependent cleavage of L-cystine and related compounds by enzyme preparations from human oral cavity and certain micro-organisms. Acta odont. scand. *26:* 443.

Mäkinen, K. K. & A. Scheinin, 1971: The effect of the consumption of various sugars on the activity of plaque salivary enzymes. Int. Dent. J. *21:*331.

Mühlemann, H. R., 1969: Zuckerfreie, zahnschönende und nichtkariogene Bonbons und Süssigkeiten. Schweiz. Mschr. Zahnheilk. *79:* 117.

Mühlemann, H. R., B. Regolati & T. M. Marthaler, 1970: The effect on rat fissure caries of xylitol and sorbitol. Helv. odont. Acta *14:* 48.

Paunio, K. U., K. K. Mäkinen & A. Scheinin, 1972: Aminopeptidase B in human gingival exudate as affected by reduced oral hygiene and sugar diet. Acta odont. scand. *29:* 583.

Scheinin, A. & K. K. Mäkinen, 1971: The effect of various sugars on the formation and chemical composition of dental plaqure. Int. Dent. J. *21:* 302.

Shaw, D. E., 1954: The *in vivo* fermentability of sugars and polyhydric alcohols in carious lesions. J. dent. Res. *33:* 717.

Shockley, T. E., C. I. Randels & M. C. Dodd, 1956: The fermentation of sorbitol by certain acidogenic micro-organisms. J. dent. Res. *35:* 233.

Address
Institute of Dentistry,
University of Turku,
Lemminkäisenkatu 2,
SF-20520 Turku 52, Finland