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THE EFFECT OF VARIOUS SUGARS AND SUGAR MIXTURES ON THE ACTIVITY AND FORMATION OF ENZYMES OF DENTAL PLAQUE AND ORAL FLUID

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The effect of the consumption of certain sugars and sugar mixtures on the activity of certain enzymes of dental 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 activity of β-fructofuranosidase (invertase) of oral fluid and plaque extracellular compartment was lowered noticeably when replacing the dietary sucrose by other sugars and sugar mixtures. A decrease was observed in extracellular plaque dextranase in all other except the sucrose group.

The cells of a cariogenic streptococcus were found to become adapted to grow almost normally in xylitol and sorbitol (5 %) media, if the cells had been stored in the respective polyol media for 9 months, although signs of adaptation were observed earlier.

The previous paper (*Mäkinen & Scheinin*, 1971) of this series of investigations dealing with the effect of the consumption of various sugars on oral enzymes showed that the formation of some enzymes, particularly that of β-fructofuranosidase, or invertase, was affected by the sugar diet. The purpose of the present investigation was to verify these results and to extend the study of the effect of simple carbohydrates on oral enzymes to include several other enzymes than had been studied earlier. Furthermore, the aim was to provide information about the possible adaptability of a cariogenic streptococcus to utilize sorbitol and xylitol, and about the effects of sorbic acid and some deoxysugars on the growth of oral micro-organisms.

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MATERIALS AND METHODS

All the materials and methods were in principle the same as those used earlier (*Scheinin & Mäkinen*, 1971, 1972; *Mäkinen & Scheinin*, 1971). Clinical and chemical studies were performed prior to the start of the actual investigation (Period AO), at the end of sucrose period (Period A, five days) at the end of a normalization period (Period BO, four weeks), and at the end of the period of various sugars (Period B, five days). The diet consisted of sugar used as sweetener in coffee etc. and of five daily mouth washings. The dietary regime was not deliberately made strict because the aim was to show the effects of normal sugar amounts. The methods when studying the effects of sorbitol and xylitol on the growth of a cariogenic streptococcus (strain Ingbritt) were described elsewhere (*Mäkinen*, 1972). The effect of sorbic acid (2,4-hexadienoic acid; *trans-trans* configuration) and potassium sorbate (Fluka AG, Buchs SG, Switzerland) on the growth of the cariogenic streptococcus was carried out in principle in the same manner. This concerned also the study of the effects of 2-deoxy-D-ribose (Calbiochem Inc., Los Angeles, Caf., USA) and 2-deoxy-D-glucose (Mann Research Laboratories, New York, N.Y., USA) as well. The effect of sorbic acid and potassium sorbate on the acid production of a mixed culture of oral micro-organisms was studied in four different media: TSHGA medium adjusted to pH 6.8 and 5.0, and GSHT medium at the same pH values. The adjusting of the pH was made either with DL-lactic acid or sodium hydroxide. The composition of the media is given elsewhere (*Mäkinen*, 1968). Several 10 ml aliquots of the media were inoculated either with cells of the cariogenic streptococcus or with human oral fluid samples. The samples were obtained by paraffin stimulation and they were diluted with the respective medium in the ratio of 1:9 before inoculation. The change in the pH of the media was followed by the colour change of the cultures. The indicator added to the pH 7.0 media was methyl red and that added to the pH 5.0 media was bromcresol green. The turbidity of the cultures was measured with a Klett-Summerson colorimeter.

RESULTS

β-Fructofuranosidase (β-D-fructofuranoside fructohydrolase, invertase, EC 3.2.1.26)

β-Fructofuranosidase catalyzes the following reaction: A β-D-fructofuranoside + H_2O = an alcohol + D-fructose. The substrates of this enzyme include sucrose. The results presented in Fig. 1 show that the highest β-

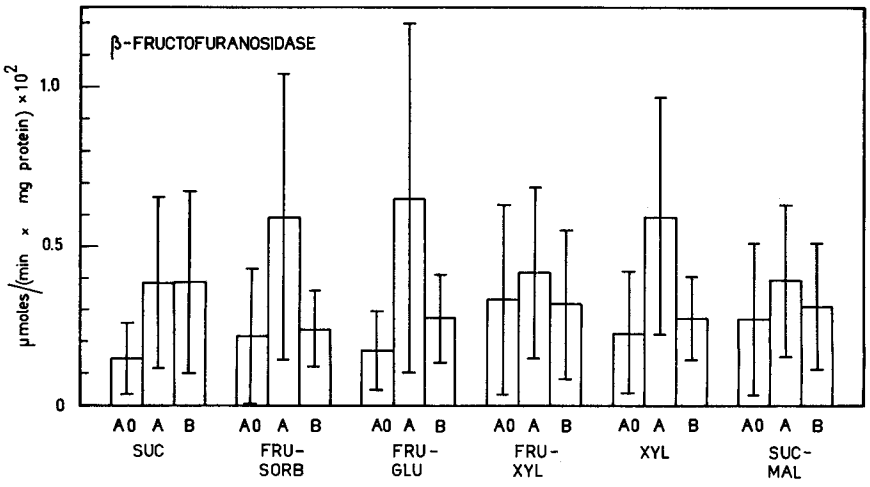

Fig. 1. The means and standard deviations of β-fructofuranosidase activities of centrifuged oral fluid determined at Period AO, Period A, and Period B for the different sugar groups. Comparison between Periods A and B in fructose-glucose group: *$p<0.05$; in xylitol group: **$p<0.01$; in fructose-sorbitol group: *$p<0.05$.

fructofuranosidase activity of oral fluid at Period B was found in the sucrose group and that the enzyme activity determined at the end of the normal diet period (Period AO) was clearly lower in all test groups than the values determined at Period A, when all test persons had used large amounts of sucrose and neglected oral hygiene. Because the enzyme activity was calculated in liberated μmoles of reducing sugars per min and per mg protein (this concerns all enzyme activities of this study), it was evident that not only the total amount of molecules of β-fructofuranosidase was increased, but also its specific activity; i.e. its relative portion in the salivary protein population was increased considerably. For some reason the enzyme activity was rather low in the sucrose group during the whole study. However, it was the difference of the activity at different periods which was considered important. It is seen in Fig. 1 that in all sugar groups other than sucrose group, the activity of β-fructofuranosidase was lowered at Period B almost to the same level as at Period AO. This is an indication of an inability of the other sugars to induce the formation of β-fructofuranosidase in the conditions involved.

The individual variations in β-fructofuranosidase activity were rather large. The highest activity at Period AO was 0.015 and the lowest 0.004 μmoles/(min × mg protein). The same test person yielded the following values at Period A (after 5 days sucrose diet): 0.015 and 0.003 μmoles/(min × mg protein).

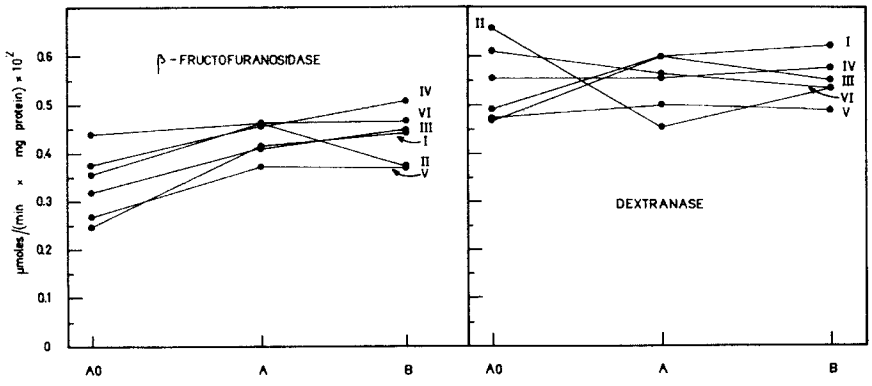

Fig. 2. Change of the activity of β-fructofuranosidase and dextranase of oral fluid sediment (sonicated and centrifuged as described earlier (*Scheinin & Mäkinen*, 1972) between Periods AO, A and B. The material studied was considered to represent easily removable soft plaque.

The β-fructofuranosidase assays revealed the fact that the consumption of sucrose and reduced oral hygiene resulted in an increase of the enzyme activity. The enzyme activity decreased in the xylitol (IV and V) and other groups (except for the sucrose group) in spite of reduced oral hygiene during Period B. Separate experiments in this laboratory have shown that the increase in β-fructofuranosidase activity in oral fluids takes place within a short period (within one hour) after sucrose consumption, and that after fructose or xylitol consumption no such increase could be detected. Hence the determination of β-fructofuranosidase activity of oral samples could be used to evaluate the cariogenicity of carbohydrates.

Fig. 2 shows the activity of β-fructofuranosidase in the supernatant fluid obtained by centrifuging the sonicated preparations of pooled oral fluid sediments. Hence this material represented sonicated soft plaque material which was detached from oral surfaces by paraffin chewing. The figure shows that the enzyme activity increased in all test groups when transferring from Period AO (normal diet and hygiene) to Period A (sucrose diet). After Period A noticeable changes took place only in the fructose-sorbitol group (group II), where the activity was decreased. The results of this type of experiment are difficult to evaluate, because it may be impossible to carry out the sonications of the pooled plaque samples in an exactly identical manner. However, it is to be noticed that the enzyme activity was not increased in the xylitol group when comparing the values determined at Periods A and B.

Fig. 3 shows the invertase activity in plaque supernatant fluid. This type of experiment demonstrates better than that in Fig. 2 the effect of the con-

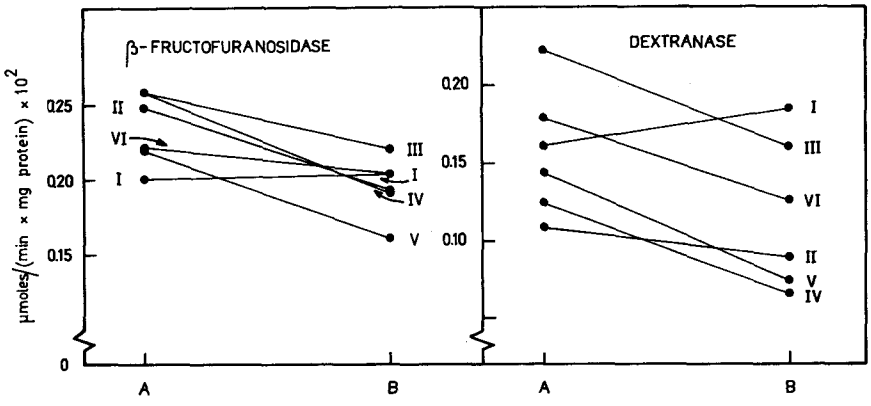

Fig. 3. Change of the activity of β-fructofuranosidase and dextranase of the supernatant fluid of plaque suspension between Periods A and B. The material studied was considered to represent water soluble and extracellular components of plaque matrix.

sumption of various carbohydrates on β-fructofuranosidase activity. The plaque supernatant fluid in this case merely represented the water (containing 0.9 % NaCl) with which the plaque material was extracted. The Fig. clearly shows that only in groups where sucrose had been consumed in large amounts at Period B (i.e. in groups I and VI, representing sucrose alone and sucrose-maltose), the activity of β-fructofuranosidase was almost the same as at Period A (when all test persons consumed sucrose). The decrease in the enzyme activity was most noticeable in groups where xylitol had been consumed. Hence in those test groups where the actual substrate of the enzyme was consumed in high amounts, the activity of β-fructofuranosidase was high.

Dextranase (α-1,6-glucan 6-glucanohydrolase, EC 3.2.1.11)

Dextranase catalyzes the hydrolysis of α-1,6-glucan links. All of the saliva and plaque samples investigated exerted dextranase activity which did not vary to the same extent as that of β-fructofuranosidase (Fig. 4). No statistically significant correlation was found between the enzyme activity and the type of carbohydrate consumed. It can be noticed, however, that the relative decrease of enzyme activity when transferring from Periods AO or A to Period B was not so large in the sucrose group as in the other groups. Fig. 2 shows the dextranase activity in the supernatant fluid of sonicated prepara-

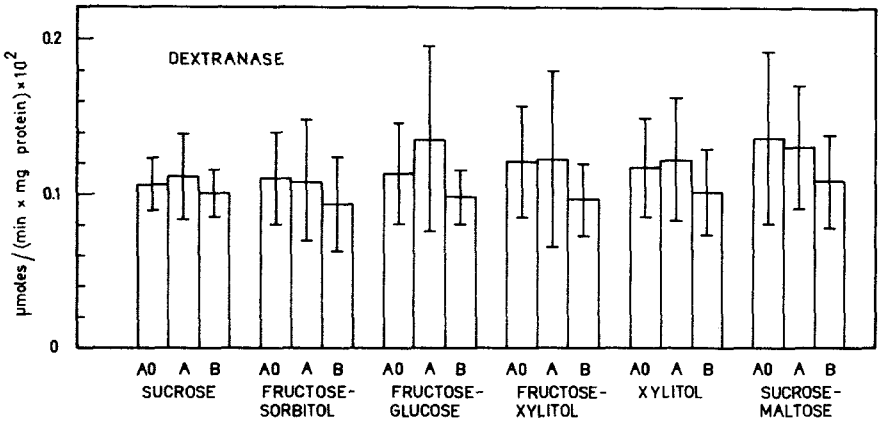

Fig. 4. The means and standard deviations of dextranase activities of centrifuged oral fluid determined at Period AO, Period A, and Period B. Comparison between Periods A and B: in fructose-glucose group: *$p<0.05$; in xylitol group: °$p<0.1$; in sucrose-maltose group: °$p<0.1$.

tions of pooled oral fluid sediments. These values did not reveal any correlation between the enzyme activity and sugars, but Fig. 3 shows clearly that only in the sucrose group (group I) was the dextranase activity increased when transferring from Period A to Period B (in this group sucrose was consumed during the whole investigation). The material studied was the supernatant fluid of aqueous plaque suspension. The strongest decrease in dextranase activity had taken place in those test groups where xylitol had been consumed, although a considerable effect was observed also in fructose-glucose and sucrose-maltose groups. It has been found that maltose inhibits the synthesis of extracellular polysaccharides (cf. *Knuuttila & Mäkinen*, 1972).

Aldolase (fructose-1,6-diphosphate D-glyceraldehyde-3-phosphatelyase, EC 4.1.2.13)

This enzyme catalyzes the following reaction:

Fructose 1,6-diphosphate = dihydroxyacetone phosphate + glyceraldehyde 3-phosphate

The enzyme also acts on ketose monophosphates. This enzyme is different from another aldolase, ketose 1-phosphate aldehyde-lyase (EC 4.1.2.7) which has a wide specificity. It is possible, however, that both types of

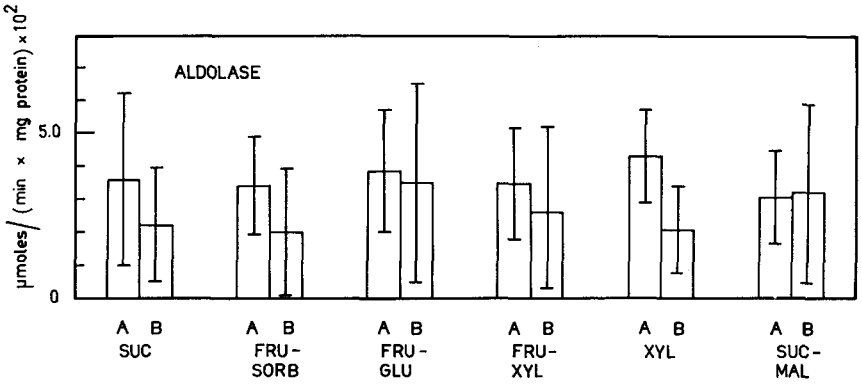

Fig. 5. The means and standard deviations of aldolase activities of centrifuged oral fluid determined at Period A and Period B. Comparison between Periods A and B: in fructose-sorbitol group: *$p<0.05$; in xylitol group: **$p<0.01$.

enzymes were involved in the aldolase assays of this study, due to the use of crude enzyme preparations. The aldolase activity varied considerably among the test persons (Fig. 5). The activity was in four test groups (sucrose, fructose-sorbitol, fructose-xylitol, and xylitol) noticeably lower at Period B than at Period A. In fructose-glucose and sucrose-maltose groups no change in the enzyme activity was observed. It is perhaps important to observe that aldolase activity was highest at Period B in the fructose-glucose group. In the previous study (*Mäkinen & Scheinin*, 1971) aldolase activity was highest in the fructose group. It may be questioned whether this (although slight) increase in aldolase activity in fructose groups could be explained by the ready two-step phosphorylation of fructose to form fructose 1,6-diphosphate, the substrate of aldolase. In groups II (fructose-sorbitol) and IV (fructose-xylitol) the lower aldolase activity at Period B could be explained by the presence of the polyols in the diet. For some reason, however, the aldolase activity was low at Period B also in sucrose group.

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27)

Lactate dehydrogenase catalyzes the following reaction:
D-Lactate + NAD = pyruvate + reduced NAD

The activity of lactate dehydrogenase (LDH) varied considerably in oral fluid, but not to the same extent as that of β-fructofuranosidase. Inspection of the results shown in Fig. 6 may provide the following explanation: In

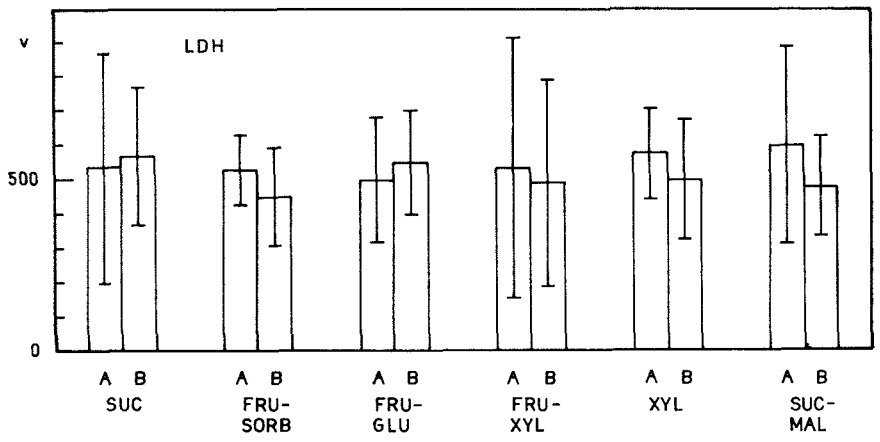

Fig. 6. The means and standard deviations of lactate dehydrogenase activities of centrifuged oral fluid determined at Period A and Period B. The activity is expressed in LDH units.

groups I (sucrose) and III (fructose-glucose) the slight increase in the LDH activity would be due to the better fermentability of sucrose and fructose-glucose mixtures by oral mixed flora than of other sugars used. In other test groups the polyols or maltose would hinder the formation of LDH or the growth of the micro-organisms being effective formers of LDH.

Glutamate dehydrogenase (L-glutamate: NAD oxidoreductase (deaminating), EC 1.4.1.2)

Glutamate dehydrogenase catalyzes the following reaction:
L-Glutamate + H_2O + NAD = 2-oxyglutarate + NH_3 + reduced NAD

The activity of this enzyme (GLDH) was studied with the reverse reaction, i.e. using 2-oxoglutarate and NADH as substrates. The results shown in Fig. 7 do not provide any clear correlation between the enzyme activity and the type of carbohydrate consumed. There was a considerable increase in the enzyme activity in the fructose-glucose group when transferring from Period A to Period B. However, the matebolic relationship between these two hexoses and a particular intermediate in the citric acid cycle is not necessarily very close. Therefore it is difficult to readily provide an explanation of the result, but the finding certainly reflects the ability of a mixture of the two sugars to promote the growth of such micro-organisms which would form more GLDH in the conditions involved. The present results

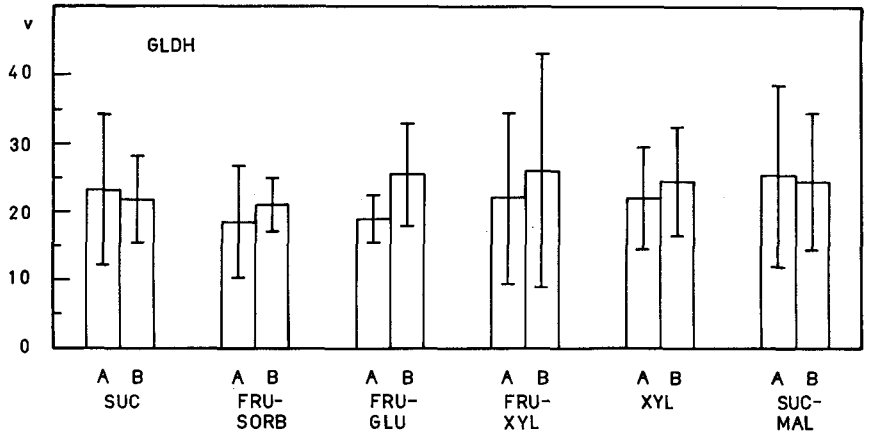

Fig. 7. The means and standard deviations of glutamate dehydrogenase activities of centrifuged oral fluid determined at Period A and Period B. Comparison between the periods in fructose-glucose group: **$p<0.01$. The activity is expressed in mU/ml.

showed that if fructose and glucose occurred chemically linked to each other (groups I and VI) the activity of GLDH was almost identical at Periods A and B.

Malate dehydrogenase (L-malate: NADP oxidoreductase (decarboxylating), EC 1.1.1.40)

This enzyme catalyzes the following reaction:
L-Malate + NADP = pyruvate + CO_2 + reduced NADP

Fig. 8 shows the means and the standard deviations of the activity of malate dehydrogenase (MDH) of oral fluid. There was no apparent correlation between the enzyme activity and the type of carbohydrate consumed, but the lowest MDH activities were observed in xylitol group, where the difference between Periods A and B was greatest.

Reactions involving oxidation of sorbitol or xylitol

Several attempts were made to determine sorbitol dehydrogenase (SDH) ans xylitol dehydrogenase (XDH) activity using the forward or backward reactions, and also $NADP^+$, NAD^+ and the corresponding reduced forms.

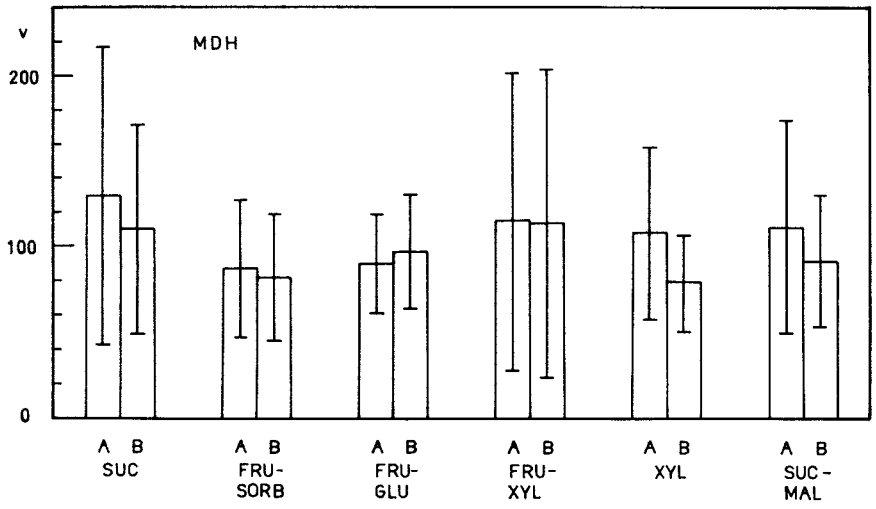

Fig. 8. The means and standard deviations of malate dehydrogenase activities of centrifuged oral fluid determined at Period A and Period B. Comparison in the xylitol group between Periods A and B: °$p<0.1$.

Only rather low activity of sorbitol dehydrogenase was occasionally obtained in oral fluid samples. The xylitol dehydrogenase activity was in most cases nil. These results may indicate the inability of human whole saliva and plaque to effectively utilize xylitol and sorbitol through the reactions described. According to *Gehring* (1968) no sorbitol dehydrogenase activity could be shown in plaque streptococci.

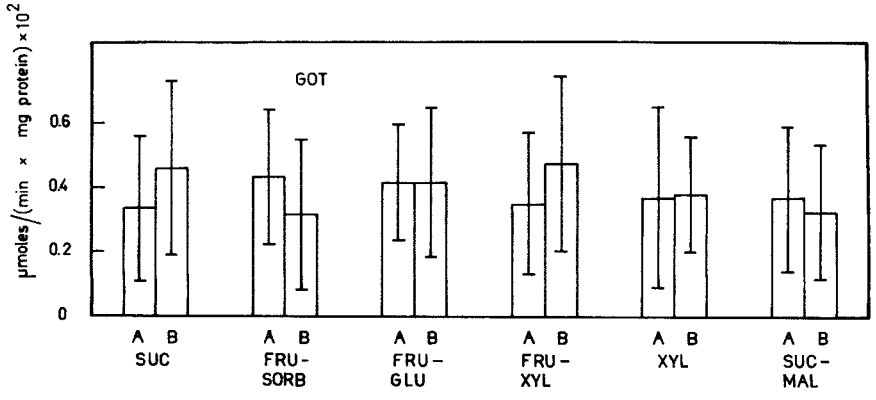

Fig. 9. The means and standard deviations of aspartate aminotransferase activities of centrifuged oral fluid determined at Period A and Period B.

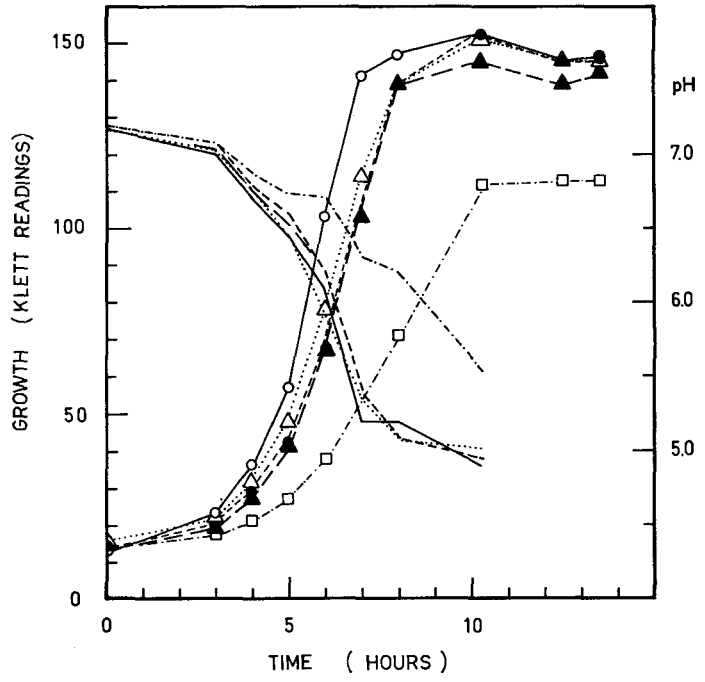

Fig. 10. Adaptation of the cells of a cariogenic streptococcus (*Streptococcus mutans*, strain Ingbritt) to utilize polyols. The growth was followed in different culture media: o—o, growth in normal Trypticase Soy Broth, normal cells (stored in normal solid Trypicase Soy Broth); △ . . . △, growth of »xylitol cells» in xylitol-Trypticase Soy Broth (5 % xylitol); ● - - - ●, growth of »sorbitol cells» in sorbitol-Trypticase Soy Broth (5 % sorbitol); ▲- - - ▲, growth of normal cells in sorbitol-Trypticase Soy Broth (5 % sorbitol); □ - - - □, growth of normal cells in xylitol-Trypticase Soy Broth (5 % xylitol). Normal cells denote cells stored in solid Trypticase Soy Broth with monthly transfers into the same solid media. Xylitol and sorbitol cells denote to cells stored 9 months in Trypticase Soy Broth to which was added 5 % xylitol or sorbitol, respectively. Descending curves: pH of the growth media; ascending curves; growth.

Aspartate aminotransferase (L-aspartate:2-oxoglytarate aminotransferase, EC 2.6.1.2)

This transaminase catalyzes the following reaction:
L-Aspartate + 2-oxoglutarate = oxaloacetate + L-glutamate

This reaction was studied in the forward direction. Fig. 9 shows the results. There was no apparent correlation between the enzyme activity and the type of sugar consumed. This result was thus similar to that obtained in the previous investigation (*Mäkinen & Scheinin*, 1971).

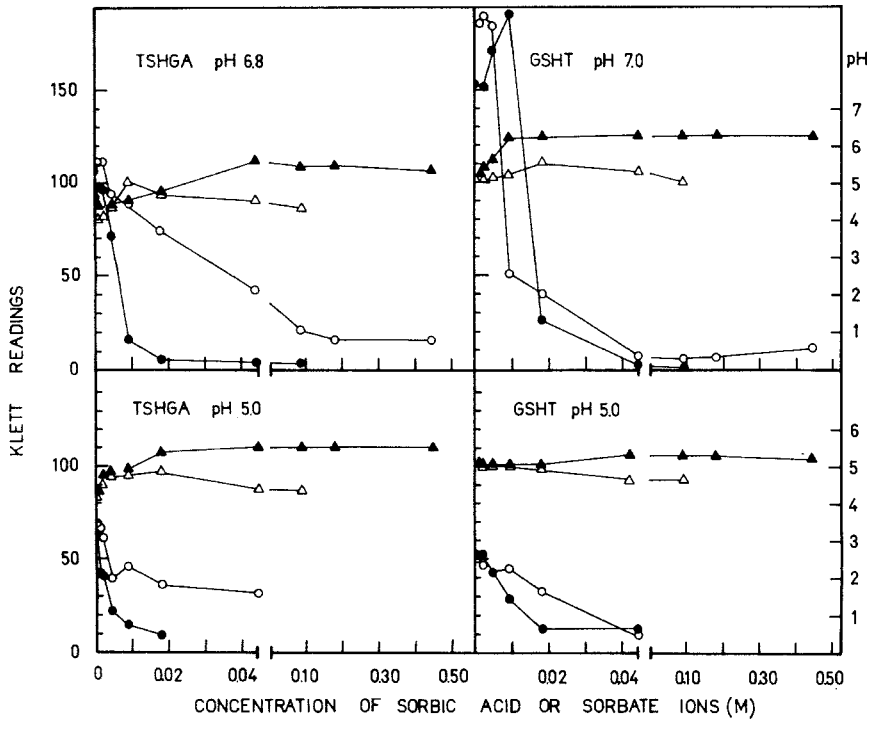

Fig. 11. Growth of cells (17 hours) of oral mixed cultures in four different media containing varying amounts of sorbic acid or potassium sorbate. ●—●, growth in orbic acid medium; O—O, growth in potassium sorbate medium; △ — △, pH in sorbic acid medium; ▲ — ▲, pH in potassium sorbate medium. The initial pH of all media was adjusted to the value indicated after the dissolution of sorbic acid and sorbate. The micro-organisms grown in the conditions were mainly lactobacilli and streptococci.

Mean values of the whole test material

Table I presents the mean values and standard deviations of the enzyme activities determined at Period AO and Period A, when all test persons were on a similar diet. The Table shows that the dextranase activity in the whole material was relatively constant when transferring from Period AO to Period A. Changes in dextranase activity appeared at Period B when different sugar diets were used (Figs. 3 and 4). The activity of β-fructofuranosidase increased considerable from Period AO to Period A. In centrifuged oral fluid the increase was greater than in its sediment. β-Fructofuranosidase activity was also determined at Period BO to show if this normalization period

Table I.

Means and standard deviations of some enzyme activities of human dental plaque and oral fluid of 72 test persons at Periods AO and A where the whole test group was on a similar diet. The detailed method in obtaining the samples has been described elsewhere (Scheinin & Mäkinen, 1971, 1972)

Enzyme	Source	Period AO Normal diet and oral hygiene		Period A Sucrose diet and reduced hygiene		Dimensions
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	
β-Fructofuranosidase	Centrifuged oral fluid	0.25	0.25	0.54	0.42	μmoles/(min × mg protein) (× 10^2)
Dextranase	—»—	0.12	0.03	0.13	0.04	—»—
GOT	—»—			0.38	0.22	—»—
LDH	—»—			563	253	LDH-units[4,5])
GLDH	—»—			23.14	17.50	mU/ml[4])
MDH	—»—			106.8	59.0	—»—
Aldolase	—»—			3.61	1.74	μmoles/(min × mg protein) (× 10^2)
SDH	—»—	Very low activity		Very low activity		
XDH	—»—	Practically nil		Practically nil		
β-Fructofuranosidase	Sonicated preparation of oral fluid sediment[1])	0.33	0.07	0.43	0.03	—»—
Dextranase	—»—	0.54	0.08	0.54	0.05	—»—
β-Fructofuranosidase	Centrifuged plaque suspension[2])			0.23	0.02	—»—
Dextranase	—»—			0.16	0.04	—»—
β-Fructofuranosidase	Sonicated preparation of plaque[3])			0.60	0.06	—»—
Dextranase	—»—			0.09	0.01	—»—

[1]) The sediment was obtained by centrifuging oral fluid collected by paraffin stimulation. The sediment was sonicated and the supernatant fluid was analyzed.

[2]) Plaque (excavated *in situ*) was suspended in cold 0.9 % NaCl and the mixture was centrifuged. The supernatant fluid was analyzed.

[3]) Refers to plaque excavated *in situ*. The pellets of 2) were sonicated and the supernatant fluid resulting was analyzed.

[4]) See references indicated in the previous paper (*Scheinin & Mäkinen*, 1972), concerning the explantation of the enzyme units.

[5]) These values are about ten times higher than those reported earlier for LDH (*Mäkinen & Scheinin*, 1971). This was now found to be due to properties of the NADP earlier used. Consequently, these earlier LDH activities should be multiplied by a factor of ten to obtain the true activities.

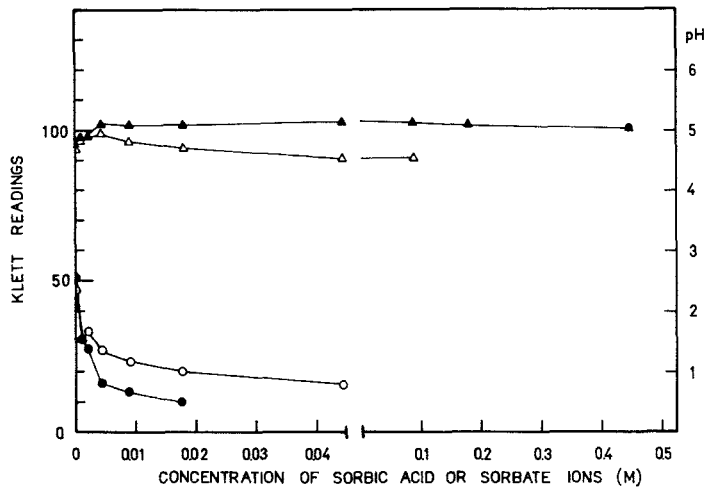

Fig. 12. Growth of the cells (17 hours) of a cariogenic streptococcus (*Streptococcus mutans*, strain Ingbritt) in Trypticase Soy Broth containing varying amounts of sorbic acid (●—●) or potassium sorbate (O—O), △ — △, pH in sorbic acid medium, ▲ — ▲, pH in potassium sorbate medium.

had decreased the activity. At this period the activity was indeed 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 previous paper showed that also the plaque index values were approximately the same at Periods AO and BO.

Adaptability of Str. mutans to use polyols

Fig. 10 shows the results from an experiment where the ability of the cells of a cariogenic streptococcus (Ingbritt) to become adapted to utilize polyols was demonstrated. The cells were stored for 9 months, with monthly transfers, in solid media containing 5 % sorbitol or xylitol. After the 9 months period these »sorbitol and xylitol cells» grew in an almost normal manner. This »normality» included the decrease of the pH of the medium and the increase of the number of the cells. Cells which were not previously stored in polyol media (»normal cells») were not able to grow normally. Xylitol produced the strongest inhibitory effect in the growth of normal cells.

Effect of sorbic acid and sorbate ions on the growth of Str. mutans and oral mixed cultures

Fig. 11 shows that the ability of sorbate ions and sorbic acid to inhibit the growth of mixed oral cultures (obtained from normal paraffin stimulated oral fluid) took place at rather low molar concentrations. Total growth inhibition took place, however, at rather high concentrations. Separate experiments showed that concentrations lower than approximately 1 mM in the growth medium were innocuous to the micro-organims involved. The same was true for the growth of a cariogenic streptococcus (Fig. 12). Sorbic acid is a week acid (pK = 4.76 at 25°C) and a considerable portion of it occurred as undissociated molecules in the conditions involved.

When the effect of certain low concentrations of 2-deoxy-D-ribose and 2-deoxy-D-glucose on the growth of the same streptococcus was studied, the former compound did not inhibit at all at the highest concentration used (0.015 M). 2-Deoxy-D-glucose caused a reduction in the number of cells after 17 hours growth by 13 % at the concentration of 0.012 M. Certain sugars, especially 2-deoxy-D-glucose can block inflammation produced by dextran or ovomucoid in the rat (*Goth*, 1968).

DISCUSSION

Due to the complex composition of oral cultures, oral fluid and plaque, it is obvious that very many enzymes will be found in the oral cavity. Due to cell death, a fraction of the intracellular microbial enzymes is continuously being liberated into the extracellular compartment. It is perhaps important to show not only their presence, but also their function in health and disease. The present investigation used a dietary regime which was deliberately made mild. While the oral mixed microflora, diet, glands, epithelial cells etc. furnish the oral cavity with a versatile enzyme composition, it was interesting against this background to observe certain changes occur in the enzyme pattern, as evoked by the sugar diet. The specific activity of β-fructofuranosidase (invertase) was highest in those sugar groups where sucrose had been consumed, a result also obtained earlier (*Mäkinen & Scheinin*, 1971). This enzyme has gained only little attention in dentistry, although, for example, yeast invertase has been known and investigated over a hundred years and has been the basis for certain landmarks in biochemistry. It is suggested that the determination of the activity of β-fructofuranosidase should be used as a caries test and to detect patient cooperation. It is generally maintained

that the cleavage of the energetic glucose/fructose bond in the sucrose molecule is of major importance in caries. This bond can be used particularly by streptococci to build up dextrane. Against this background the determination of oral β-fructofuranosidase activity may be important.

It was anticipated that the rather innocuous effect of low amounts of sorbic acid, potassium sorbate and the deoxysugars on the growth of the micro-organisms studied would be observed. The quantities needed for effective growth inhibition may perhaps be considered high from a dental point of view. It is possible that sorbic acid affects the growth of yeast. Sorbic acid is claimed to be a fungicide and a fungistatic compound. *Deák & Novák* (1968) found that the inhibitory effect of sorbate is stronger under anaerobic than under aerobic conditions. The antibacterial propreties have been described, for example, by *Becker & Roeder* (1957).

The cells of the cariogenic streptococcus were found to become adapted to grow almost in a normal way in the presence of a 5 % addition of xylitol and sorbitol to the basal, glucose containing medium (Fig. 10). This »normality» included the increase of the number of the cells and the drop in the pH of the medium. It should be noted, however, that the present results don't indicate that xylitol would be utilized by the bacterial cells to form acids. Consequently, it is not known whether the mixed culture of the oral cavity would, as an entity, be able to undergo such a forced adaptation.

The adaptation has been mentioned earlier (*Mäkinen*, 1972), and also *Schokley, Randels & Dodd* (1956) have stated that sorbitol can be shown to be attacked by an adaptine enzyme or enzymes. The adaptation described in this and in an earlier paper (*Mäkinen*, 1972) took place 4 or 5 months after the first »contact» of the cells with the polyols. Also *Frostell* (1964) and *Gülzow* (1968) have come to the conclusion that acid production from sorbitol may increase with time in the oral cavity and that sorbitol will be broken down in the mouth. *König & Guggenheim* (1968) have shown that several cariogenic streptococci cleave sorbitol and mannitol.

The four papers of this series of investigations (*Scheinin & Mäkinen*, 1971, 1972; *Mäkinen & Scheinin*, 1971, and the present paper) revealed the final major result that fructose and xylitol were considered less cariogenic than the other carbohydrates studied. When comparing sorbitol and xylitol, the latter polyol was seen to more effectively retard the growth of oral micro-organisms and to produce less such compounds and enzymes whose appearance is usually related to high cariogenicity. When additionally taking into account the pronounced sweetness of xylitol, this polyol could be used as a sweetener in various products.

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