Turku sugar studies
II. Preliminary biochemical and general findings

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The application of a strict xylitol (X) diet maintained the plaque level approximately 50 per cent lower during the first year when compared with sucrose (S) of fructose (F) consumption. X-consumption decreased the specific activity of salivary invertase-like enzymes when compared to F- or S-consumption after the first 7—8 months' of diet. F-intake led to a lower enzyme activity than S-intake. Dextranase activity of the supernatant fluid of whole saliva was not changed by the consumption of either S, F or X, but a lower activity was observed in the plaque extracellular phase in the xylitol group when compared to other test groups. The amount of reducing sugars and the activity of aminopeptidase-like enzymes in the supernatant fluid of whole saliva were not changed significantly during the first 7—8 months' phase. The concentration of lactate in the plaque extracellular phase was noticeably lower in the X-group than in the two other groups, but the activity of β-galactosidase was elevated in this compartment in the X-group. The average monthly sugar intakes were: S, 2.4 kg; F, 2 . 3 kg; X, 1 . 6 kg. No differences in the weight of the test persons were observed

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The biochemical investigations of the Turku sugar studies form an entity divided into the following sections:

— Biochemistry of saliva and plaque
— Biochemistry of blood and urine
— Biochemistry of gingival exudate
— Biochemistry of certain oral microorganisms cultivated *in vitro*

The concept of the above biochemical studies was dual; to provide information about the chemical effects of the tested sugars in man, and in case significant differences between the groups were found, to elucidate the mechanism and bearing of the effects revealed. This communication presents certain 7—13 month intermediate results of the study of the biochemistry of saliva and plaque of the test persons. Certain observations pertinent to the formation of plaque and consumption of sugars are also provided. A detailed description will be presented separately, (*Mäkinen & Scheinin*, 1975).

MATERIALS AND METHODS

The clinical material of the present study has been described separately (*Scheinin*,

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Mäkinen & Ylitalo, 1974). The plaque and saliva samples were obtained when examining the test persons in random order at four of the six clinical registrations carried out during the first year of the trial. The participants were instructed not to brush their teeth on the preceding evening and the day of the examination, i.e. for 12—24 hours prior to collection of the samples. Dental plaque was quantitated through the Plaque Index, registered according to *Silness & Löe* (1964) and through determination of the plaque wet weight after collection as earlier described (*Scheinin & Mäkinen*, 1971, 1972). The collection of 10 ml of paraffin-stimulated saliva was carried out prior to brushing the teeth. The saliva and plaque samples of the test persons were analyzed for more than sixty different chemical compounds and more than 20 different enzyme activities. A detailed list and other pertinent information about the analyses carried out will be provided in the paper mentioned above.

Due to the complex nature of the study, all analyses, inspection periods and sample collections did not include exactly the same number of test persons. This resulted from occasional loss of samples, discontinuation, or disease of the test persons, etc. The number of test persons representing the material analyzed was, however, in most cases close to the original number of persons starting the trial. As to the biochemistry of the mouth, there were two main types of samples studied: 1) individual saliva samples and 2) pooled samples of saliva, plaque, or their various fractions. Plaque and saliva were divided and investigated according to the following classification:

— Supernatant fluid of whole mouth saliva (in a few cases uncentrifuged samples were analyzed: pH, Eh)
— Supernatant fluid of sonicated whole saliva sediment
— Supernatant fluid of the plaque (collected *in situ*) aqueous extract
— Supernatant fluid of sonicated plaque (collected *in situ*)

RESULTS

The most important biochemical and general findings available after 7 to 13 months of sugar diets will be briefly described below.

1. Plaque wet weight

Plaque wet weight of the test persons was determined four times during the first half of the two year study (Fig. 1). The plaque wet weight was almost the same in all test groups at the beginning of the study (baseline phase). As is always the case with the plaque wet weights, the standard deviations were noticeable. Fructose diet in combination with no oral hygiene seemed to maintain the same plaque amount as determined for the sucrose group, but xylitol diet reduced the wet weight of plaque by approximately 50 %.

2. Plaque index

Fig. 2 shows the plaque indices recorded in the three test groups. In line with the values of plaque wet weight, the lowest plaque index recordings were made in the xylitol group.

3. Enzymes yielding reducing sugar (including invertase-like enzymes)

Fig. 3 shows the overall specific activity of enzymes in the supernatant fluid of whole mouth saliva, yielding reducing sugars in the presence of sucrose. This enzyme activity may be briefly called

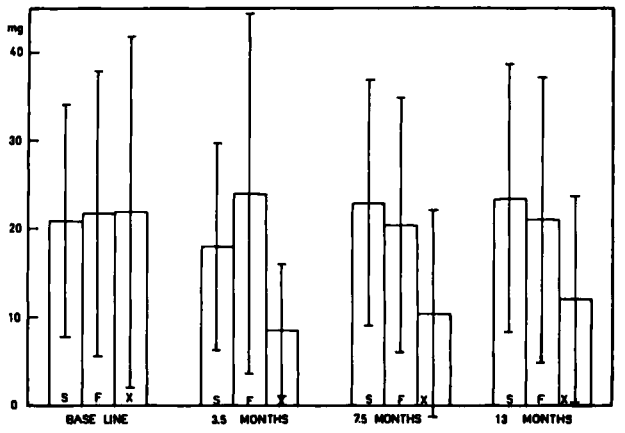

Fig. 1. The means and standard deviation of the plaque wet weight of the test persons during the first one year test period.

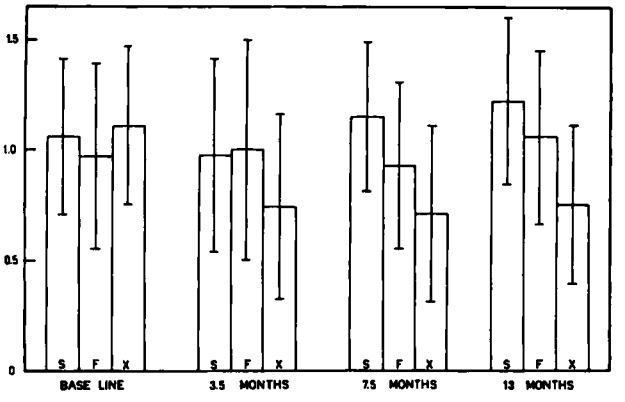

Fig. 2. The means and standard deviations of plaque indices of the test persons during the first one year test period.

invertase-like activity, although the crude samples studied with certainty also contained other enzymes attacking sucrose and liberating reducing sugars. It is not known to which extent these reducing sugars subsequently underwent other reactions, affecting the results obtained. However, the results can be considered as providing information about the sucrose-exploiting capacity of samples of oral fluid. In line with all earlier results (*Mäkinen & Scheinin*, 1971, 1972) obtained in this laboratory, the invertase-like activity was lowest in the supernatant fluid representing the xylitol group. No significant sex differences were detected. Due to the very high standard deviations which also seems to be a characteristic to this type of enzymes in saliva, no statistically significant differences between the groups were found. However, the lowering of the activity in the xylitol (and partially in the fructose) group was indicative when compared to the sucrose group.

When pooled plaque samples representing the three sugar groups were studied for the above mentioned enzyme activity, the following results were obtained. The extracellular water-soluble phase of plaque (collected *in situ*) exerted practically identical activity in the sucrose and fructose groups (Fig. 4). In the xylitol group the activity was only one fourth or one tenth (depending on the phase of the dietary regimen) of the values obtained for the two other groups. All

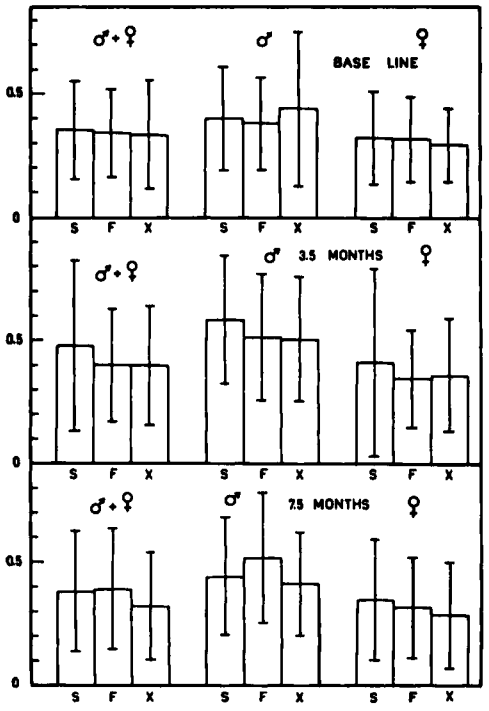

Fig. 3. The means and standard deviations of the overall specific activity [v, in μmoles/min × mg) × 10^{-2}] of invertase-like enzymes in the supernatant fluid of whole saliva (enzymes yielding reducing sugars from sucrose).

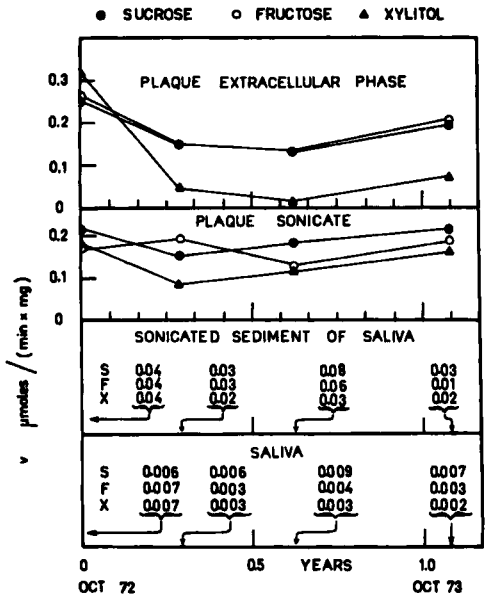

Fig. 4. The overall specific activity (v) of of invertase-like enzymes of pooled samples (enzymes yielding reducing sugars from sucrose).

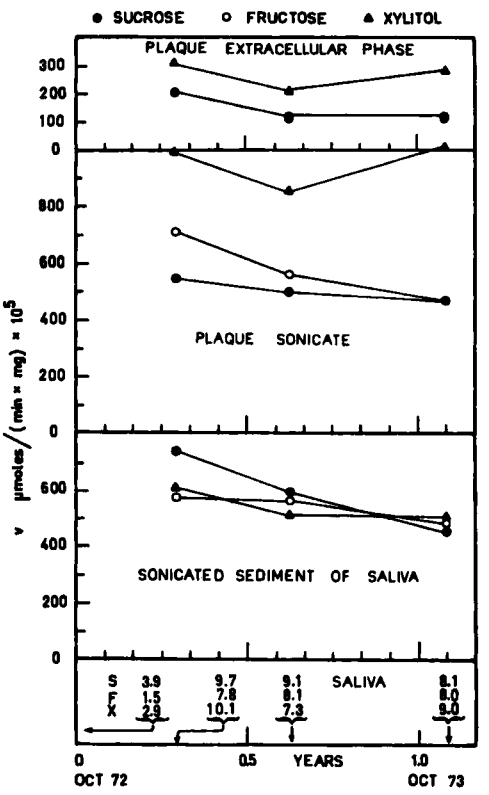

Fig. 5. The overall specific β-galactosidase activity (v) of pooled samples. Tested with p-nitrophenyl-β-D-galactopyranoside (*Mäkinen & Scheinin*, 1975b).

sonicated plaque or salivary sediment materials yielded more similar values for all three test groups. Preliminary results also indicated that xylitol diet almost totally removed from centrifuged whole mouth saliva an enzyme yielding reducing sugars from sucrose.

4. Other enzymes

In the middle of the trial the following intermediate results were available.

β-Galactosidase. The specific activity of β-galactosidase (p-nitrophenyl-β-D-galactopyranoside as the substrate) in the aquous plaque extract was almost doubled in the xylitol group when compared to the two other test groups (Fig. 5).

Practically the same situation was involved in the sonicated plaque material. However, in all material derived from saliva (the supernatant fluid and sonicated salivary sediment) the specific activities were practically the same in all test groups. This type of results were not achieved with the corresponding derivative of α-D-glucopyranoside.

Dextranase. The specific activity of dextranase in the extracellular aqueous phase of plaque was somewhat reduced in the xylitol group when compared to the activity calculated for the two other test groups (Fig. 6). The dextranase activity in the other test materials was more similar in all groups and the values were clearly lower than in plaque extracellular phase. It has to emphasized that in line with the assay of the invertase-like enzymes, even the determination of dextranase by the method used (assaying reducing sugars) may comprise the activity of certain other enzymes as well (possessing transferase activity, for example).

Fig. 7 shows the means and standard deviations of the dextranase activity of the supernatant fluid of whole saliva. There were no significant differences between the text groups.

Peroxidases. A separate paper of this series (No. XII, *Mäkinen, Tenovuo & Scheinin,* 1975) will provide information about the activity of oral peroxidases of the test persons. In this context it may be mentioned that there was an interesting phenomenon with lactoperoxidase, the very enzyme secreted from the salivary glands. Its activity was increased noticeably (from 4 to 10 fold) in the supernatant fluid of the whole mouth saliva in the xylitol group. No such increase was detected in the other test groups.

Aminopeptidases. The specific overall

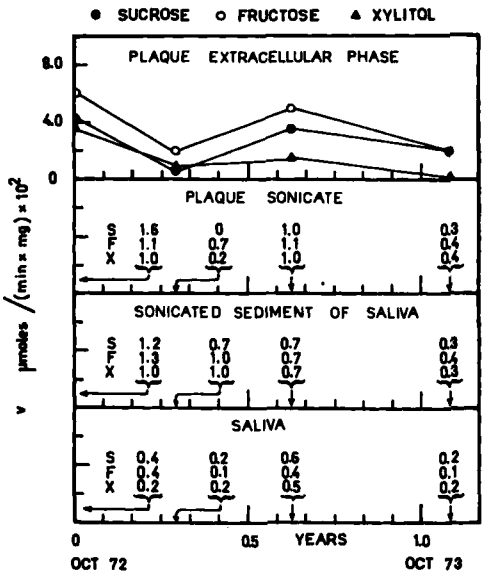

Fig. 6. The overall specific activity (ν) of dextranase-like enzymes of pooled samples.

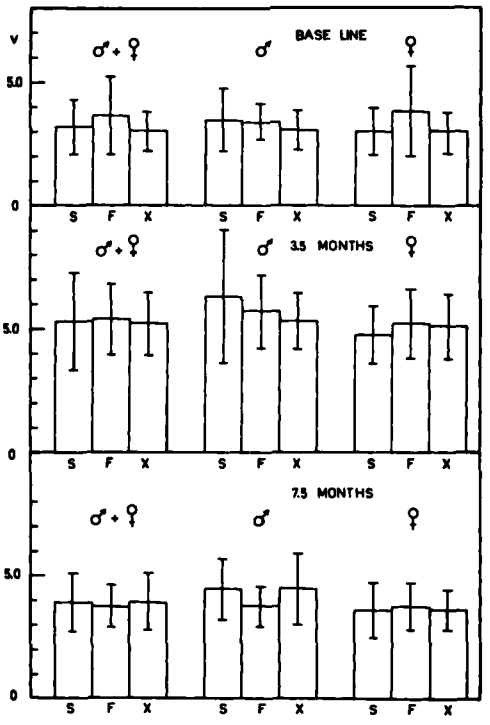

Fig. 7. The overall specific activity [ν, in μmoles/(min × mg) × 10^{-2}] of dextranase-like enzymes (yielding reducing sugars from commercial dextran, Mw. = 110000) of the supernatant fluid of whole saliva.

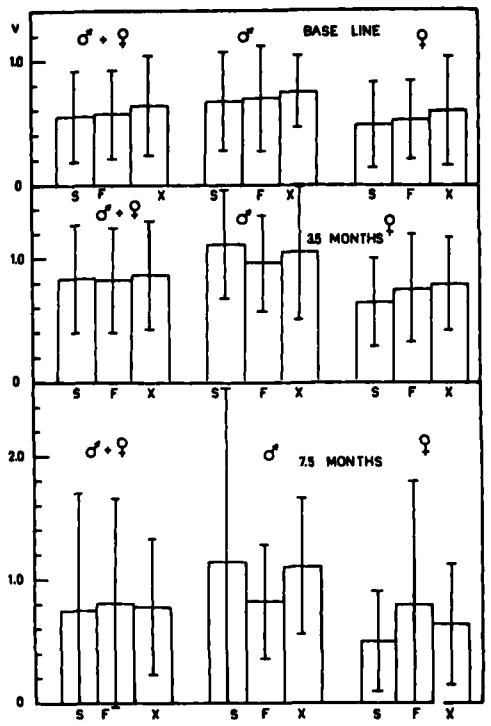

Fig. 8. The overall specific activity [v, in μmoles/(min × mg) × 10] of aminopeptidase-like enzymes acting on *N*-L-alanyl-2-naphthylamine, of the supernatant fluid of whole saliva.

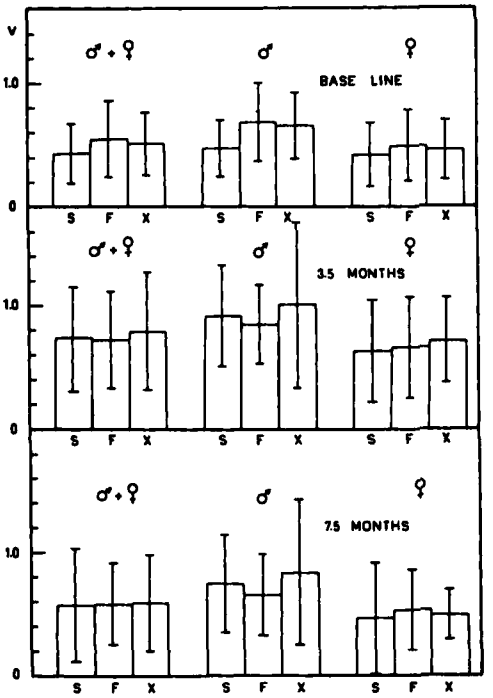

Fig. 9. The overall specific activity [v, in μmoles/(min × mg) × 10] of aminopeptidase-like enzymes acting on *N*-L-arginyl-2-naphthylamine, of the supernatant fluid of whole saliva.

aminopeptidase activity was determined with three *N-L*-aminoacyl-2-naphthylamines using individual samples of centrifuged whole mouth saliva. The results are given in Figs. 8—10 which indicate that there were no significant differences between the sugar groups up to the 7.5 month phase. There was a trend, however, the values of the male test persons being higher than those of the females.

5. *Chemical compounds*

Of the chemical compounds assayed in various samples of oral origin, lactate seemed to undergo interesting concentration changes depending on the sugar group. The most pronounced changes occurred in the extracellular aqueous

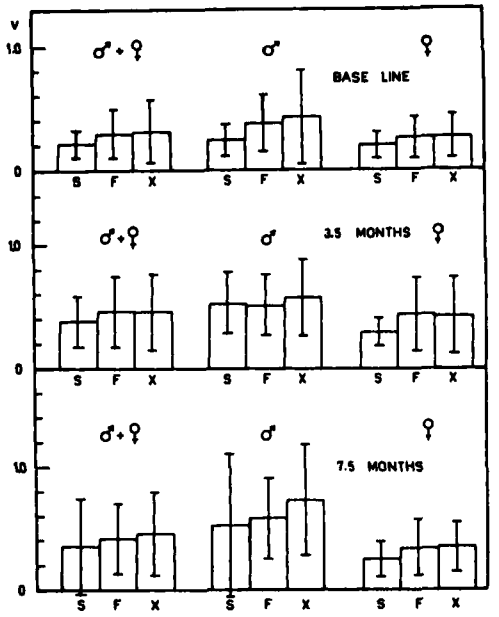

Fig. 10. The overall specific activity [v, in μmoles/(min × mg) × 10] of aminopeptidase-like enzymes (also comprising specific iminopeptidases) acting on *N*-L-prolyl-2-naphthylamine, of the supernatant fluid of whole saliva.

phase of plaque of the xylitol group: the concentration of lactate was reduced (by approximately 60 per cent) when compared to the other test groups. The reduction was thus higher than the 50 per cent decrease in plaque wet weight allowed to assume, *i.e.* the amount of lactate per mg plaque wet weight was lowered.

The preliminary findings indicated that pyruvate formed the major part of the keto acid pool in all samples studied. Pyruvate and total keto acids were not shown to undergo the changes found with lactate. The preliminary findings also indicate that the amount of RNA and total carbohydrates (anthrone method) in the plaque extracellular phase were reduced when compared to other groups, and that this reduction was practically directly proportional to the reduction of plaque wet weight. This concerns the concentrations of proteins as well. The amount of reducing sugars (Fig. 11) decreased in the xylitol group.

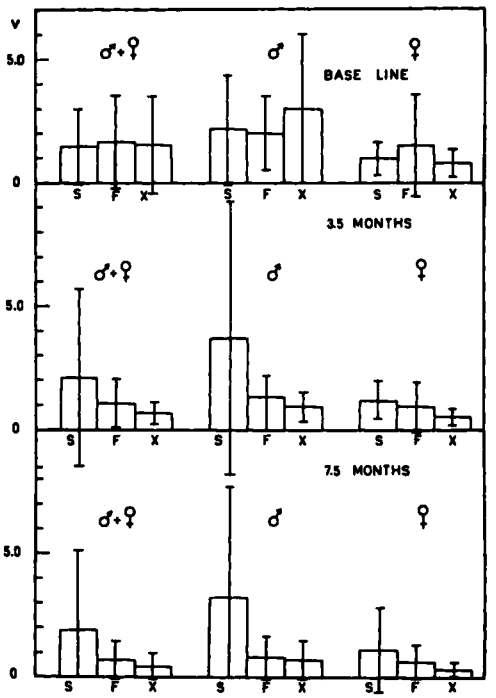

Fig. 11. The means and standard deviations of reducing sugars in the supernatant fluid of human whole saliva. The results are given in μmoles/ml (as glucose; 4.5-dinitrosalicylic acid method).

6. Consumption of sugars

By exploting the dairy and other control measures (described in more details later) it was possible to obtain a rather reliable estimation on the daily, weekly, monthly and annual consumption of the three sugars (Fig. 12). There was an initial and expected increase in the consumption of all three sugars. The curves passed through a maximum at Christmas 1972. After this peak the sugars were used in gradually lower quantities.

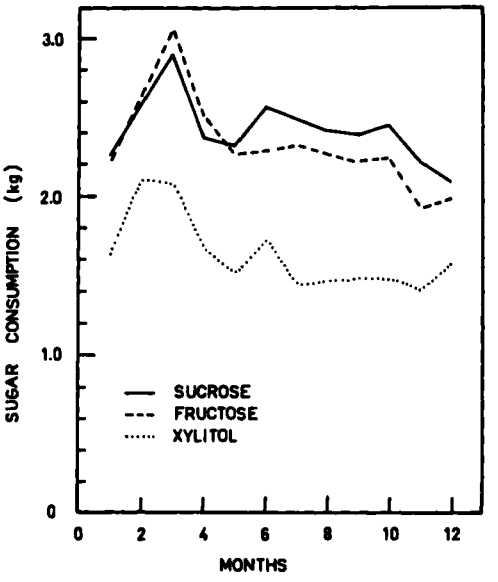

Fig. 12. Mean consumption per person of sucrose, fructose and xylitol during the first test year.

7. Weight of test persons

One year consumption of sucrose, fructose or xylitol did not cause significant changes in the weight of the test persons.

DISCUSSION

According to the results of the middle of the Turku sugar studies the most important effects of the three diets involved on the biochemical properties of plaque and saliva were as follows:

- A decrease of the invertase-like activity in the plaque extracellular phase in the xylitol group
- An increase of the β-galactosidase activity in the plaque extracellular phase in the xylitol group
- A decrease in the concentration of lactate in the plaque extracellular phase in the xylitol group
- An increase in the peroxidase activity of the supernatant fluid of saliva in the xylitol group

It seems natural that some, if not all, of the phenomena mentioned above represent mere consequences of more important primary reactions to the different diets. In many cases the chemical changes found in saliva and plaque may be pure outcomes of changes in the plaque microbial flora. The first three reactions mentioned above are most likely results of this type of oral changes. The rather strong increase of the peroxidase activity in the supernatant fluid of whole mouth saliva may be explained. however, on other grounds. Although the main attention is often directed to the observation of chemical changes, the phenomenon that a great part of the chemical compounds and enzyme activities in saliva and plaque was not found to undergo any changes at all, is of equal importance.

The present long-term study verified the previous finding of the short-term experiments about the lower invertase-like enzyme activity in saliva and plaque of persons consuming xylitol, when compared to the intake of sucrose (*Mäkinen & Scheinin*, 1971, 1972). Based on the present results, this situation prevailed at least 7—8 months. It is likely that these enzyme changes are related to variations in the growth of the bacterial flora; the invertase-like activity may stem from bacteria in the oral cavity.

The finding that the extracellular phase of plaque displayed elevated activity of β-galactosidase, may be important in view of the claims that his type of enzymes are most likely derived from oral microorganisms only (*cf. Leach & Melville*, 1970). The importance of oral glycosidases in general may be reflected in the growth and evolution of plaque (for example, in the breakdown of salivary glycoproteins). However, the increased peroxidase activity of saliva may be a more decisive finding, because there are claims that the salivary lactoperoxidase may be involved in the antibacterial action of saliva (*Hoogendoorn & Moorer*, 1973; *Hugoson et al.*, 1974; *Koch, Eklund & Hoogendoorn*, 1973; *Morrison & Steele*, 1968).

The above results and the finding that the aminopeptidase activity of the supernatant fluid of whole mouth saliva remained practically unchanged and that there were no significant differences between the sugar groups, indicates a selectivity of the sugar effect: some enzymes appear in higher amounts (or exert higher specific activity), whereas some are not affected or they may be inhibited. The idea with the aminopeptidase assays was to elucidate the possible correlation between the occurrence of *Streptococcus mutans* (which is known to be capable of producing inducible extracellular proteinases and aminopeptidases in the presence of xylitol; *Knuuttila & Mäkinen*, 1975a, b) and the

enzyme activity. No such correlation was found. However, the aminopeptidase activity in the xylitol group was to a certain extent higher than in the other groups. The significance of this trend will be separately dealt with (*Mäkinen & Scheinin*, 1975).

One of the net results of the chemical and microbiological reactions caused by the consumption of xylitol was a rather strong reduction in the quantity of plaque. This peculiar effect of xylitol thus prevailed at least during the first half of the two year trial and reinforced the earlier suggestion about the plaque-inhibiting qualities of xylitol (*cf. Scheinin & Mäkinen*, 1971, 1972; *Mäkinen*, 1974).

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