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ELEVATION OF PLAQUE SODIUM CONTENT AND pH THROUGH A BICARBONATE-PHOSPHATE ADDITION TO SUCROSE

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INTRODUCTION

Earlier studies have shown that the low pH which can be found in human dental plaque during ingestion of pieces of solid sucrose (*Luoma*, 1964) can be elevated by alkali carbonate-phosphate combinations which are added to supplement 3—5 % of the sugar (*Luoma, Hurskainen & Isokangas*, 1964a; *Luoma & Luoma*, 1968). Furthermore, the combinations comprising primarily sodium bicarbonate and a small amount of alkali orthophosphate have been shown to possess caries reducing capabilities when added to the sucrose component of cariogenic diets of rats (*Luoma et al.*, 1968; *Luoma et al.*, 1970a). The present study was conducted in order to check the pH elevating property in vivo of a combination $NaHCO_3 + KH_2PO_4$ (mole ratio 9.82/1, respectively, *Luoma & Luoma*, 1968), hitherto tested for this property in vitro and which combination was found the most effective among the bicarbonate containing combinations in the abovementioned animal experiments. Furthermore, to obtain more information on the mechanism of action of this combination, the in vivo diffusion of cations, especially sodium, into and out of the plaque was observed, so also the alkali cation content and other properties of the saliva in association with ingestion of the above additive in the sucrose.

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MATERIALS AND METHODS

The experimental and control tablets were prepared according to the previous procedure (*Luoma & Luoma*, 1968) with the exception that the breaking hardness was lower than earlier, about 1 8 kg/cm². Third year dental students served as test subjects. They were advised to behave as before (*Luoma et al.*, 1970b) both before and during the plaque growth. To increase the amount of the plaque grown during the 3 day period used, ten sucrose tablets were consumed daily between meals keeping approximately 2 h intervals.

The plaque was collected from 38 subjects altogether in two sessions from each, following previous procedures (*Luoma & Luoma*, 1968). The sample was diluted by shaking in 0.75 ml of cold distilled water and the pH was read. For the determination of total sodium and potassium by flame photometry, the sample suspensions from 14 subjects were digested with 0.5 ml of concentrated H_2SO_4 in Kjeldahl digestion units, made to volume and aliquots from it were then used for the alkali determinations as well as for the total nitrogen assay. This as well as the flame photometry were performed according to previous adaptations (*Luoma et al.*, 1970b, 1971) of published methods.

The suspended material of plaques of 20 of the remaining 24 subjects was centrifuged down at 2,250 g for 15 min in a refrigerated centrifuge and then resuspended, washed and centrifuged as above. The washing water was made to volume and cleared by microfiltration using filters with 0.3 μ pore size. The extracellular potassium was determined from the filtrate and the total nitrogen from the washed and digested plaque material. The intended determination of the sodium content of the same fluid was not successful owing to the extra sodium that was found exceptionally to be liberated from one batch of the microfilters used.

On each of the sessions, a control plaque sample was taken at first. Then the subjects consumed 5 experimental or control tablets during 15 minutes at constant speed. The second plaque sample was taken during the three last minutes of this period. If solid residues of the tablets were left, they were now removed. The third sample was taken 10 min after the termination of the ingestion.

For the observations of the changes in the salivary properties, the samples were collected under paraffin into ice cold measuring cylinders as more exactly described in the legend of Fig. 2. Each subject arrived for each session in separate days. No smoking or food was allowed within 3 h before the session. 250 ml of water was drunk 10 min before the first sample. The rate of secretion, the pH and buffer effect were determined as previously (*Luoma*

Table I.

Total sodium and potassium content per total nitrogen (µg/mg N) found in samples of unwashed dental plaque in association with ingestion of solid sucrose and sucrose with a $NaHCO_3 + KH_2PO_4$ additive. Average values ($\bar{x}$) and standard errors (S.E.) of 14 subjects participating in both experiments.

	Sucrose				Sucrose with $NaHCO_3 + KH_2PO_4$			
	Na		K.		Na		K.	
	$\bar{x}$	S.E.	x	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Before ingestion (B)	25.9	2.3	150	10	18.5	1.8	158	12
During ingestion (D)	28.4	3.0	153	11	42.6	4.7	159	10
After ingestion (A)	25.7	2.6	168	11	23.2	2.2	162	12
P- value; D—B	<0.1		<0.2		<0.001			
» A—B	—		<0.05		<0.1		<0.2	

& *Luoma*, 1968) and the sodium and potassium content were determined by flame photometry after precipitation of the mucoids with HCl, filtration and a 50—100 fold dilution with distilled water.

RESULTS

The sodium and potassium content of the unwashed plaque before, during and after ingestion of solid sugar as well as sugar with the 4 per cent additive of the $NaHCO_3 + KH_2PO_4$ combination is shown in Table I. The ingestion of sugar alone (with accompanied increase in salivary sodium, see also Fig. 2) did not significantly alter the sodium content of the plaque but during ingestion of sugar with the additive the plaque sodium was more than doubled as compared with the respective mean before ingestion. The small increase of sodium after sugar with the additive was not significant. The total potassium of plaque was increased significantly after sucrose ingestion. The potassium in the phosphate component of the additive did not modify the potassium content of the plaque.

Table II shows the amount of potassium found in the plaque washing water per amount of total nitrogen of the washed plaque of the respective sample in association with ingestion of sucrose and sucrose with the additive. The potassium was increased significantly during ingestion of the additive and to some degree also after ingestion. The sucrose alone did not alter these potassium values significantly.

Table II.

The extracellular potassium of dental plaque per total nitrogen of the washed material of the respective samples (μg/mg N) and modification by ingestion of sucrose and sucrose with a $NaHCO_3 + KH_2PO_4$ combination. Means ($\bar{x}$) and standard errors (S.E.) of 20 subjects, each participating in both experiments

Sample	Sucrose		Sucrose with $NaHCO_3 + KH_2PO_4$	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Before ingestion (B)	178	11	157	10
During ingestion (D)	203	13	191	17
After ingestion (A)	194	12	185	14
P- value; D—B	<0.1		<0.01	
» ; A—B	<0.2		<0.02	

The results of the pH measurements are given in Table III. While the mean pH in the first samples of both sessions was almost the same, the significantly ($P<0.001$) decreased pH during sucrose ingestion was almost completely compensated by the additive. Even after ingestion the additive caused a slight but significant ($P<0.02$) elevation of the pH from the respective mean produced by sucrose alone.

The relationship between the magnitude of the plaque pH value during ingestion of sugar alone and the respective pH compensation exerted by the salt combination additive (obtained from the results given in Table III)

Table III.

Plaque pH before, during and after ingestion of solid sucrose and sucrose with a $NaHCO_3 + KH_2PO_4$ addition. Means ($\bar{x}$) and standard deviations (S.E.) and ranges of 38 subjects, each participating in both experiments

Sample	Sucrose		Sucrose with $NaHCO_3 + KH_2PO_4$	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.
Before ingestion	6.65	0.26	6.68	0.27
	(6.04)	(7.09)	(6.12)	(7.50)
During ingestion	5.98	0.37	6.56	0.33
	(5.00)	(6.48)	(5.85)	(7.30)
After ingestion	5.95	0.51	6.09	0.46
	(5.20)	(7.07)	(5.32)	(7.20)

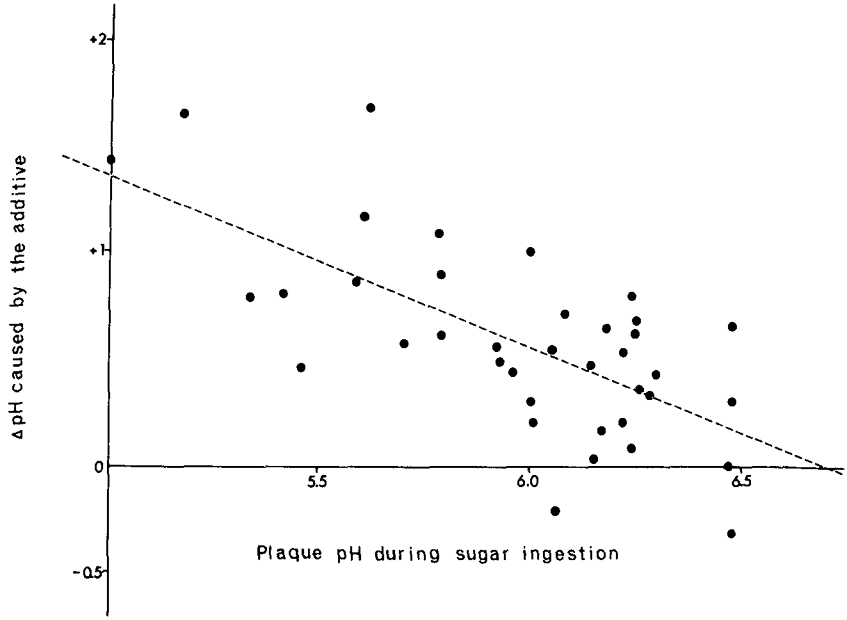

Fig. 1. The relationship between the plaque pH value during ingestion of sucrose and the magnitude of the pH compensation obtained by the use of the $NaHCO_3+KH_2PO_4$ additive in the sucrose.

is shown in Fig. 1. It appears that the lower was the pH during ingestion of sugar alone, the greater was the pH correction caused by the additive in the same subject. This negative correlation was significant ($r=-0.678$, $P<0.01$).

The results of the saliva study are given in Fig. 2. The rate of secretion and the pH were nor significantly altered by the additive as compared with the values obtained in association with sucrose alone. The increases in both the sodium content and buffer effect during ingestion of sucrose plus the additive were about fourfold as compared with the respective sucrose values. The significantly ($P<0.05$) decreased potassium content during sucrose ingestion was compensated by the additive.

DISCUSSION

The appreciable pH decrease of plaque during sugar ingestion confirmed the earlier findings (*Luoma*, 1964; *Luoma & Luoma*, 1967, 1968; *Luoma et al.*, 1970b), so also pH compensation obtained by the use of combinations of the same type.

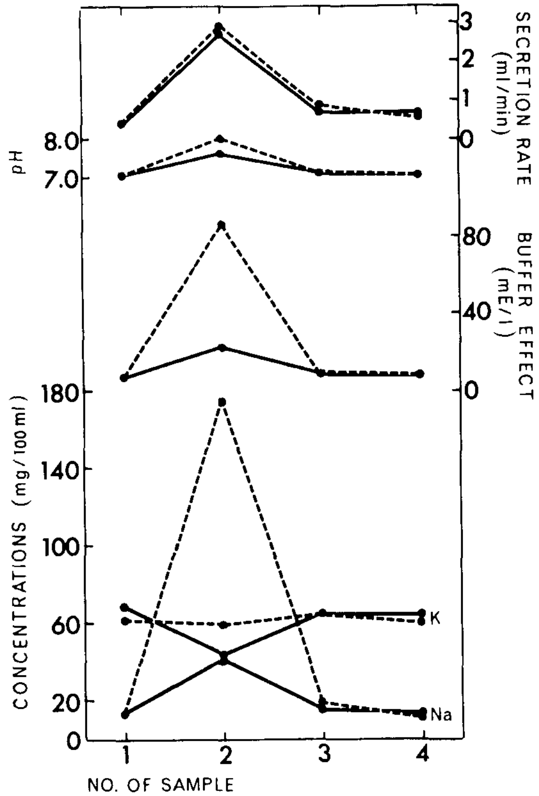

Fig. 2. The rate of saliva secretion, pH, buffer effect (titration from initial pH to pH 5.0) and the concentrations of sodium and potassium before, during and after ingestion od sucrose (*solid lines*) and sucrose with the $NaHCO_3+KH_2PO_4$ additive (dashed lines). Averages of 7 subjects, each participating in both experiments.

Sample 1. Resting saliva collected during 10 minutes.
» 2. Sample collected during the last 3 minutes of a 15 min period of ingestion of sucrose or sucrose with the additive.
» 3. Resting saliva collected 5—15 min after the ingestion.
» 4. Resting saliva collected 20—30 min after the ingestion.

Perhaps most significant in the present results was the dependence of the pH compensation, exerted by the additive, on the plaque pH during ingestion. This would suggest that if the buffering capacity of the present combinations does have any significance in the reduction of human caries, these combinations would provide their best protection against caries that is occurring underneath plaques indicating low pH values in the presence of sugar without any additives. The relation of the buffer capacity of the present combinations to the pK value of the bicarbonate has been pointed out earlier

(*Luoma & Luoma*, 1968). In an other previous work (*Luoma et al.*, 1970a) tihe present bicarbonatephosphate combination was weaker than a combina ton of alkali phosphates in reducing rat caries. If a rat's plaque is generally-less acid than that of man, as the published data suggest, the weaker anti-caries effect of this combination than that of phosphates, having higher pK value, could be understood on the basis of the present findings.

Since the time of the sucrose effect on the plaque was rather short compared with the slow growth rate of the plaque, the nitrogen basis can be considered constant within the period used.

The high content of sodium in the plaque during ingestion of sugar with the additive and the rapid depletion after ingestion suggests that the sodium penetrates freely the microbial membranes as usually is the case. In fact the first author's recent unpublished findings indicate that sodium does penetrate easily the membranes of nongrowing cariogenic streptococci. The present finding agree with in vitro results of *Dobbs* (1932) and *Bruckner* (1948) showing the penetration of sodium bicarbonate through natural and artificial plaques. With regard to the potassium, the relatively much increased extracellular content (Table II) in association with ingestion of the sugar plus additive must be originating in part from the potassium phosphate of this combination. In the same table, the non significant trend of the potassium to increase during ingestion of sucrose alone was the reverse to what was expected. Earlier data (*Luoma*, 1969) revealed an increase in both the potassium and phosphorus content of fermenting cariogenic streptococci which must cause a corresponding reduction in the extracellular potassium. The dead or dying cells in the plaque might easily release their potassium, and if their percentage in the whole population of the plaque was high, their influence could mask the net influx occurring in the functioning cells.

Since earlier observations indicate, that acid production by cariogenic streptococci is dependent primarily on the absolute amount of available potassium (*Luoma*, 1969) and that eccessive sodium does not markedly alter this activity, the pH correction obtained here seems to be largely due to the extra buffering provided by the additive. The effect on the cell metabolism through altered cation content might be small.

The present results concerning saliva also confirm the previous findings (*Luoma, Hurskainen & Isokangas*, 1964b, *Luoma & Luoma*, 1968) indicating decreased salivary potassium during sucrose ingestion. Furthermore, additions to sucrose which contain alkali carbonates and phosphates elevate the buffering action and the alkali cation content of saliva only during the ingestion period and that within a few minutes after the ingestion, the saliva shows the preingestion values. The slight pH correction in the plaque obtained after ingestion in the present study as well as a somewhat greater correction obtained earlier (*Luoma & Luoma*, 1968) must be due to residues of the additives which had diffused into the plaque during the ingestion period.

The results obtained support the belief that some protection of human caries is expectable when the bicarbonate-phosphate combinations of the present type are used as additives of ingested sucrose or of sucrose constituents of food products.

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SUMMARY

The sodium content of the dental plaque was doubled during ingestion of sucrose with a 4 % addition of a $NaHCO_3 + KH_2PO_4$ combination (mole ratio 9.82/1, respectively). Ten minutes after the ingestion it was almost at the same level as before ingestion.

Plaque pH decreased on the average by 0.67 unit during ingestion of solid sucrose for 15 min and it was still at about the same level 10 min after ingestion. The decrease was fully compensated during ingestion, when the sucrose contained the above additive. There was a significant negative correlation between the plaque pH during ingestion of the sucrose alone and the magnitude of the pH compensation exerted by the salt combination when added to the sucrose. A slight pH compensation was obtained by the same additive 10 minutes after the ingestion. Marked increases were noted in the salivary buffer effect and its sodium content during ingestion if the sucrose with the additive.

RÉSUMÉ

ÉLÉVATION DE LA TENEUR EN SODIUM ET DU pH DE LA PLAQUE PAR ADDITION DE BICARBONATE-PHOSPHATE AU SUCROSE

La teneur en sodium de la plaque dentaire a doublé pendant l'ingestion de sucrose additionné de 4 % d'une combinaison de $NaHCO_3 + KH_2PO_4$ (rapport moléculaire respectif 9,82/1). Dix minutes après l'ingestion, elle avait retrouvé à peu près le même niveau qu'avant l'ingestion.

Le pH de la plaque diminuait en moyenne de 0,67 unités pendant l'ingestion de sucrose solide durant 15 minutes et il était encore à peu près au même niveau 10 minutes après l'ingestion. Cette diminution était entièrement compensée pendant l'ingestion lorsque le sucrose contenait l'additif ci-dessus. Il existait une corrélation négative significative entre le pH de la plaque pendant l'ingestion de sucrose seul et l'amplitude de la compensation exercée sur le pH par la combinaison saline en addition au sucrose. Une légère compensation du pH a été obtenue au moyen du même additif 10 minutes après l'ingestion. Des augmentations notables ont été constatées en ce qui concerne l'effet tampon de la salive et sa teneur en sodium pendant l'ingestion du sucrose contenant l'additif.

ZUSAMMENFASSUNG

DIE ERHÖHUNG DES NATRIUMGEHALTS UND DES pH-WERTES DER ZAHNPLAQUE DURCH EINE BIKARBONAT-PHOSPHAT ZUSATZ ZU SACCHAROSE

Der Natriumgehalt der Zahnplaque wurde während der Einnahme von Saccharose mit einer 4-prozentigen Zusatz der Kombination $NaHCO+_3$ KH_2PO_3 (Molrelation 9.82/1) verdoppelt. Zehn Minuten nach der Einnahme war er wieder auf beinahe demselben Niveau wie vor der Einnahme.

Das pH- Wert der Plaque sank im durschschnitt 0.67 Einheit während der Einnahme von Saccharose für 15 Minuten und es war noch 10 Minuten nach der Einnahme auf beinahe derselben Niveau. Die pH-Senkung wurde vollständig verhindert während der Einnahme wenn die Saccharose den obengenannten Zusatz enthielt. Eine signifikante negative Korrelation wurde zwischen dem pH- Wert während der Einnahme und der Grösse der von dem Zusatz verursachten pH- Kompensation gefunden. Derselbe Zusatz verursachte eine kleinere pH- Kompensation 10 Minuten nach der Einnahme. Der Natriumgehalt und der Puffer-effekt des Speichels waren beträchtlich erhebt während der Einnahme von Saccharose mit dem Zusatz.

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