The hardening and softening of human tooth enamel

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Many studies[1] have shown that the hydroxyapatite in tooth enamel exists in dynamic equilibrium with its fluid environment. As one of the early stages in the formation of a carious lesion involves the demineralization of tooth enamel it is apparent that an agent which can reduce the rate of dissolution of hydroxyapatite may have some retarding effect on the progress of dental caries.

It is obvious that changes in the concentrations of calcium and orthophosphate ions in solution will alter the degree of over- or undersaturation of the solution at a given pH with respect to the solid hydroxyapatite phase. Thus the addition of calcium or orthophosphate ions to buffered acidic solutions can decrease the rate of demineralization of tooth enamel. This may be at least one of the mechanisms whereby salts containing these ions have been found[2] to inhibit dental caries when added to the diet of laboratory animals. In addition, we have described in previous papers[3,4] the inhibiting effect of various organic and condensed inorganic phosphate anions on the kinetics of dissolution of hydroxyapatite. The evidence indicated that these ions do not alter the equilibrium solubility of hydroxyapatite but retard the rate of dissolution of hydroxyapatite by a kink poisoning mechanism. This suggests at least one mechanism whereby these phosphates may inhibit dental caries, as they have been shown to do in both animal[2,5] and human[6] tests.

If retardation of the demineralization of tooth enamel can inhibit dental caries then it is also likely that the opposing reaction of the equilibrium, remineralization, can also influence the progress of a carious lesion. Koulourides, Cueto and Pigman[7] have shown that it is possible to reharden the surface of tooth enamel which has previously been softened by demineralization in an acidic buffered solution. The solutions which they used to reharden the enamel were metastable solutions, supersaturated with calcium ions and orthophosphate ions with respect to the solid hydroxyapatite phase. The evidence suggested that the rehardening was due to some remineralization process.

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Received for publication, October, 1967.

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(3) Brady, B. H. G., Napper, D. H., and Smythe, B. M.—The dissolution kinetics of hydroxyapatite. Nature, 212: 5057, 77-78 (Oct. 1) 1966.

(4) Napper, D. H., and Smythe, B. M.—The dissolution kinetics of hydroxyapatite in the presence of kink poisons. J. D. Res., 45: 6, 1775-1783 (Nov.-Dec.) 1966.

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(6) Harris, R., Schamschula, R. G., Gregory, G., Roots, Miriam, and Beveridge, J.—Observations on the cariostatic effect of calcium sucrose phosphate in a group of children aged 5-17 years: Preliminary report. Austral. D. J., 12: 2, 105-113 (Apr.) 1967.

(7) Koulourides, T., Cueto, H., and Pigman, W.—Rehardening of softened enamel surfaces of human teeth by solutions of calcium phosphates. Nature, 189: 4760, 226-227 (Jan. 21) 1961.

We have carried out experiments along similar lines and it is the purpose of this report to present these results.

Materials and methods

Knoop hardness scale

The technique for measuring the hardness of dental enamel is essentially identical with that used for testing the hardness of certain metallurgical specimens. Indeed, human dental enamel is comparable in hardness to that of a hard stainless steel.[9]

The hardness of enamel is related to the depth of penetration of an indenting diamond into the enamel surface for constant energy expenditure. The harder the enamel, the less is the distance of penetration. The indenting diamond is fashioned so that the area of indentation is proportional to the distance of penetration.

A Knoop diamond produces a diamond-shaped indentation with an axial ratio of about 7:1. The Knoop hardness number (KHN) is defined as the ratio of the applied load (in kilograms) to the area of indentation (in square millimetres) using a Knoop diamond.

Preparation of teeth

The teeth were predominantly posterior (both maxillary and mandibular) but about 20 per cent were anterior. Most of the posterior teeth were molars. The teeth were sectioned to obtain lingual, buccal, mesial, distal or labial surfaces. Previous workers have shown that different surfaces were of comparable hardness.[8][9] The teeth were stored in water with thymol crystals to reduce both dehydration and bacterial attack.

Each tooth section was mounted onto a polymethylmethacrylate block, the base of which had been machined flat. This mounting was achieved using a methacrylate glue (polymethylmethacrylate chips dissolved in chloroform).

A small area of enamel was then ground flat and parallel to the base of the mounting block by means of increasingly fine grits of silicon carbide and emery polishing paper (progressively 400 and 600A grit silicon carbide grinding paper, then 3/0 and 4/0 grit emery polishing paper). The final buffing was by a soft polishing cloth.

Test procedure

The Leitz Miniload hardness tester was employed to measure the original Knoop hardness of the flat, polished enamel surface. Three to five indentations only were made. The test load on the diamond was 200 g.

The enamel surface was then softened in 10^{-3} M acetate buffer at pH = 5·5 (10^{-3} M with respect to both potassium acetate and acetic acid) for four hours without stirring. After removal from the buffer, the teeth were washed and stored in double distilled water overnight (about 15 hours). The decrease in hardness of the enamel was *ca.* 100–150 Knoop hardness numbers. This large decrease was necessary in order to blanket the considerable variation in the hardness of the tooth surface (upwards of 50 Knoop hardness units), even over a small area.

Initially the indentations were placed in a row so that the rehardening indentations could be placed adjacent. However, the considerable variation in enamel hardness rendered this method rather suspect. Consequently an alternative procedure was adopted. This involved scattering the indentations over a suitable area of surface and placing the rehardening indentations between these original indentations. This procedure proved more satisfactory. The initial hardness thus involved about 20 measurements; subsequent hardness values during rehardening each involved four indentations scattered over the area.

Toothpaste experiments

Toothpastes were prepared containing 0, 5, 10 and 20 per cent calcium sucrose phosphate (CaSP). The same ingredients were used in all cases but their relative proportions were varied to produce pastes of satisfactory consistency. The abrasive was dicalcium phosphate which was present in amounts ranging from 50 per cent when no CaSP was present, to 10 per cent when 20 per cent CaSP was present. Water varied from about 20 per cent with no CaSP present, to about 60 per cent when 20 per cent CaSP was present. The other basic ingredients, the concentrations of which did not vary appreciably, were sorbitol, glycerol, sodium lauryl sulphate, ethyl parahydroxybenzoate, carrageenan, saccharin and flavouring.

[8] Caldwell, R. C., Muntz, M. L., Gilmore, R. W., and Pigman, W.—Microhardness studies of intact surface enamel. J. D. Res., 36: 5, 732-738 (Oct.) 1957.

[9] Newbrun, E., Timberlake, P., and Pigman, W.—Changes in microhardness of enamel following treatment with lactate buffer. J. D. Res., 38: 2, 293-300 (Mar.-Apr.) 1959.

sodium fluoride and stannous fluoride were used in some toothpastes where indicated.

The toothpastes were diluted with water and dispersed by a combination of mechanical and ultrasonic agitation. Dispersion factors were determined after teeth cleaning experiments: four subjects cleaned their teeth and the total volume of saliva and water which each used was measured. Dispersion factors from 1 g. of toothpaste with 2 ml. of saliva (1:2) to 1 g. of toothpastes with 10 ml. of saliva (1:10) were obtained. Studies on toothpaste rehardening were conducted at two different dispersions in distilled water, namely a dispersion of 1 g. of toothpaste with 5 ml. of water (1:5), and 1 g. in 1·25 ml. (1:1·25).

Measurements were also carried out on the toothpastes diluted with saliva at a dispersion of 1:5. The saliva was a composite sample collected from four subjects immediately before the experiments. The pH was usually about 6·5.

Further experiments on the ability of certain additives to reharden softened dental enamel when added to control toothpaste slurries have been made. These studies were performed at a level equivalent to a toothpaste containing 10 per cent by weight of the additive. The additives were dissolved into a slurry of the control toothpaste. The pH of the slurry was adjusted with one or two drops of 1N KOH where necessary.

The teeth to be rehardened were rotated at about 325 r.p.m. in the slurry; sedimentation of the slurry particles was prevented by concomitant mechanical agitation. At suitable intervals, the teeth were removed from the slurry, washed and their Knoop hardness determined. The indentations placed on the tooth surface prior to rehardening were also remeasured. No appreciable change in size was ever observed. Rehardening was carried out at ambient temperatures.

In addition to these experiments on rehardening by rotation of softened teeth in a toothpaste dispersion, the use of an electric toothbrush was used to simulate the normal application of toothpastes. Toothbrushing was found to remove enamel from softened surfaces. Unsoftened tooth surfaces could be cleaned without loss of enamel. Consequently toothbrushing experiments have been confined to "superhardening" of unsoftened surfaces. The material was brushed onto the surface for two minute intervals and the increase in hardness after each application was measured.

Superhardening of unsoftened teeth was also measured by the normal tooth rotation method.

Rehardening with calcium sucrose phosphate solutions

To determine the effect of calcium sucrose phosphates in the absence of normal toothpaste ingredients, solutions of CaSP in distilled water were tested for their rehardening ability. These solutions were adjusted to pH = 7 by the addition of a small amount of hydrochloric acid. Softened teeth were rotated in the solution as in the case of toothpaste slurries.

Rate of softening of tooth enamel

Sections of teeth prepared as described above were immersed in the potassium acetate (10^{-3} M)/acetic acid (10^{-3} M) buffer at pH = 5·5 under unstirred conditions. When CaSP was added to the buffer the pH was adjusted to pH = 5·5 using the minimum quantity of 9N HCl. At hourly intervals the Knoop hardness of each tooth was measured. At least six teeth were studied and the mean results calculated.

Description of additives

Most of the additives used in these experiments have been described more fully in a previous paper.[10] A brief description follows:

Calcium sucrose phosphate (CaSP)

This is a normal production batch of a calcium sucrose phosphates-calcium orthophosphate complex association and corresponds to composition A in a published patent specification.[11] (Corresponds to CSRC-6—see [10]).

CaSP ("inorganic free")

This is a fraction prepared from the above product by the removal of the bulk of precipitable material as described in composition C in the above specification.[11]

K.SP ("inorganic free")

This is the potassium salt made from CaSP ("inorganic free") by ion exchange.

(10) Brady, B. H. G., Napper, D. H., and Smythe, B. M.—The effect of additives on the rate of dissolution of hydroxyapatite in unstirred acid buffers (submitted for publication).

(11) South African Patent Application No. 65/6046—Water soluble phosphate compositions and process for preparing. C.S.R. Co. Ltd., Sydney. Nov., 1965.

enamel. The presence of calcium sucrose phosphate (CaSP) in the toothpaste leads to significant rehardening of softened enamel, an effect which increases with increasing CaSP concentration. The addition of either sodium fluoride or stannous fluoride to these toothpastes is without significant effect on the rehardening due to CaSP. The result obtained with a commercially available stannous fluoride toothpaste is shown for comparison with toothpastes containing CaSP.

In Table 2 the rehardening obtained at different dispersions (or dilutions) of the toothpastes in water is shown. The maximum rehardening obtained is relatively insensitive to the dispersion factor. Despite a fourfold increase in slurry density the more concentrated solutions gave only marginally greater rehardening.

Table 3 shows the rehardening obtained with toothpastes containing 10 per cent CaSP dispersed in saliva instead of water. The results suggest that the rehardening ability of saliva *per se* is not significant. The rehardening by CaSP toothpastes in saliva was only marginally greater than in distilled water.

The effects of other additives added to a control toothpaste dispersed in water are shown in Table 4. The results indicate that calcium ions alone, phosphate ions alone and sucrose phosphate anions alone in distilled water do not significantly reharden softened dental enamel. However, when present together in a soluble form as in CaSP the combination of all three ions is effective. The so-called "inorganic free" CaSP rehardened to some extent. This particular CaSP contains about 0·2 per cent inorganic phosphorus compared with 2·8 per cent contained in CaSP.[20] The calcium sucrose phosphate (Toy product) contains much insoluble inorganic calcium phosphate but on a weight-for-weight basis less soluble calcium phosphate than CaSP. The rehardening ability of a toothpaste containing 20 per cent of this product was comparable with that of toothpaste containing 10 per cent CaSP. The toothpaste containing 10 per cent calcium glycerophosphate produced no significant rehardening.

In Table 5 the rehardening obtained with various additives added to a control toothpaste dispersed in saliva is shown. These results show that calcium ions and orthophosphate ions added to saliva do produce a significant rehardening, whereas sucrose phosphate anions do not. The rehardening produced by CaSP (fraction B) can be attributed to the calcium ions which it contains.

In Table 6 the results of allowing toothpaste dispersions in water to stand for 0, 2 and 5 hours before commencing enamel rehardening experiments are shown. These show that toothpastes containing CaSP retain their rehardening ability over 5 hours, whereas a commercial stannous fluoride toothpaste showed a significant decrease in rehardening ability after 2 hours standing.

Rehardening studies carried out at a 1:5 dispersion factor in water on two toothpastes containing CaSP after six months aging are shown in Table 7. These results suggest that these toothpastes retain their ability to reharden tooth enamel over long periods of time.

TABLE 2

Effect of dispersion factor on rehardening

Dispersion medium = water

pH = 6·5–7·0

Toothpaste	Dispersion factor	Hardness after softening (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN)				Average increase (KHN)
				Time (minutes)				
				30	60	90	120	
20% CaSP + 0·1% NaF	1 : 1·25	214	117	42	49	52	44	47
As above	1 : 5	212	126	29	32	38	41	35
10% CaSP	1 : 1·25	225	114	21	26	20	22	22
10% CaSP	1 : 5	213	126	16	21	26	22	21
5% CaSP	1 : 1·25	211	146	18	14	21	31	21
5% CaSP	1 : 5	219	128	9	9	9	15	11
A commercial stannous fluoride toothpaste	1 : 1·25	212	114	17	17	21	23	20
	1 : 5	221	104	24	21	20	14	20

TABLE 3

Effect of saliva as dispersion medium
Dispersion factor = 1 : 5 g./ml.
pH = 6·5–7·0

Toothpaste	Dispersion medium	Hardness after softening (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN) Time (minutes) 30	60	90	120	Average increase (KHN)
Control	Saliva	220	115	9	7	6	7	7
Control	Water	206	133	4	7	2	6	5
10% CaSP	Saliva	197	114	31	36	32	36	34
10% CaSP	Water	213	126	16	21	26	22 *	21
10% CaSP + 0·1% NaF	Saliva	211	114	36	17	39	39	33
10% CaSP + 0·1% NaF	Water	215	116	28	27	31	26	28
10% CaSP + 0·4% SnF_2 ..	Saliva	174	173	25	17	23	14	21
10% CaSP + 0·4% SnF_2 ..	Water	220	144	20	25	18	23	21

TABLE 4

Effect of certain additives in toothpastes
Dispersion factor = 1 : 5 g./ml.
Dispersion medium = water

Additive	Hardness after softening (KHN)	Average decrease in hardness (KHN)	pH	Average increase in hardness (KHN) Time (minutes) 30	60	90	120	Average increase (KHN)
None	206	133	6·7	4	7	2	6	5
10% CaSP	213	126	6·8	16	21	26	22	21
10% $Ca(NO_3)_2$	201	121	6·8	4	−1	6	6	4
10% Na_2HPO_4* ..	188	129	6·5	5	6	3	6	5
10% K_2SP ("inorganic free")	133	112	6·8	6	2	3	5	4
10% CaSP ("inorganic free")	199	127	7·0	20	9	16	19	16
20% CaSP (Toy product)	183	150	5·7	22	15	31	32	25
10% CaSP (fraction B) ..	208	133	7·0	3	10	10	10	8
10% Calcium glycero-phosphate	219	90	7·0	23	−1	−5	−10	2

* A mixture of 2 parts Na_2HPO_4 and 1 part KH_2PO_4.

TABLE 5

Rehardening efficiency of additives in toothpastes in saliva
Dispersion factor = 1 : 5 g./ml.

	Hardness after softening (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN) Time (minutes) 30	60	90	120	Average increase (KHN)
None	220	115	9	7	6	7	7
10% CaSP	197	114	31	36	32	36	34
10% Na_2HPO_4*	163	158	14	20	8	14	14
10% K_2SP	190	136	0	4	0	4	2
10% $Ca(NO_3)_2$	231	117	19	16	23	16	18
10% CaSP (fraction B) ..	208	93	10	10	25	20	16

* See Table 4.

TABLE 6

Effect of delay on rehardening
Dispersion factor = 1 : 5
Dispersion medium = water

Toothpaste	Waiting time (hrs.)	Hardness after softening (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN) Time (minutes) 30	60	90	120	Average increase (KHN)
20% CaSP	0	214	121	13	27	31	—	24
20% CaSP	2	219	124	20	10	25	23	20
20% CaSP	5	219	121	19	32	21	26	25
10% CaSP	0	213	126	16	21	26	22	21
10% CaSP	2	209	134	17	18	20	18	18
10% CaSP	5	211	134	21	24	22	21	22
A commercial stannous fluoride toothpaste	0	221	104	24	21	20	14	20
A commercial stannous fluoride toothpaste	2	208	123	0	+1	—3	—4	—2
A commercial stannous fluoride toothpaste	5	208	124	—1	+5	—3	+2	1

TABLE 7

Effect of storage on rehardening ability

Toothpaste	Time (months)	Initial hardness (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN) Time (minutes) 30	60	90	Average increase (KHN)
20% CaSP	0	214	120	13	27	31	24
20% CaSP	6	227	120	27	29	29	32
10% CaSP	0	213	126	16	21	26	21
10% CaSP	6	224	127	24	24	30	26

TABLE 8

Superhardening of dental enamel by brushing

Material	Initial hardness (KHN)	Average increase in hardness (KHN) Time (minutes) 2	4	6	Average increase (KHN)
Water	359	2	6	—8	0
20% CaSP solution (pH=7·0)	323	20	10	24	18
1% CaSP solution (pH=7·0)	312	7	9	10	9
20% CaSP toothpaste (dispersion factor = 1 : 5)	313	42	31	42	38

Rates of rehardening with toothpastes

The above results deal with the total amount of rehardening of softened dental enamel. The following results measure the rate of this rehardening obtained with three toothpastes dispersed in water (1:5). At least four separate teeth were studied to ascertain the rehardening rate of each toothpaste. The results are shown in Fig. 1. It will be seen that the rate of rehardening under these conditions is a rapid process, being virtually complete in five minutes.

TABLE 9

Superhardening of dental enamel by rotation

Toothpaste	Initial hardness (KHN)	Average increase in hardness (KHN)				Average increase (KHN)
		Time (minutes)				
		30	60	90	120	
20% CaSP	296	21	25	36	30	28
A commercial stannous fluoride toothpaste ..	304	20	36	41	42	35

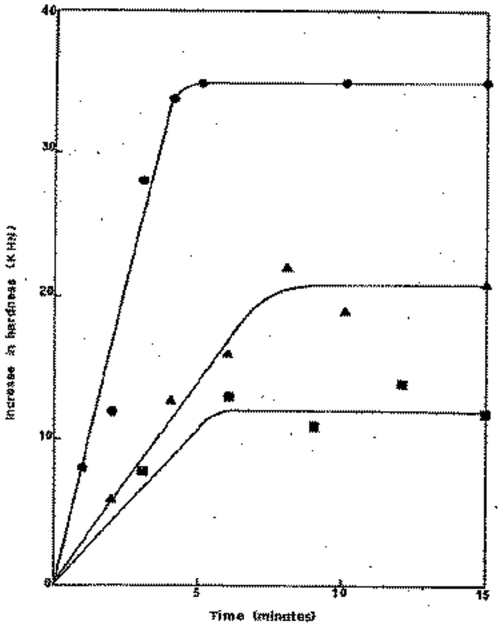

Fig. 1.—Rate of rehardening of tooth enamel. Toothpastes containing: ● 20% CaSP, ▲ 10% CaSP, ■ 5% CaSP.

Superhardening with toothpastes

In Table 8 the results of brushing unsoftened enamel surfaces with various materials are shown. The increase in hardness was measured at two-minute intervals. These results show that CaSP in solution and in toothpastes can increase the hardness of unsoftened tooth enamel. The increase in hardness is comparable with the values obtained by rotating softened teeth in the previous experiments.

The superhardening of unsoftened teeth has also been measured by the normal tooth rotation method for a toothpaste containing 20 per cent CaSP and a commercial stannous fluoride toothpaste both at a dilution of 1:5. The results shown in Table 9 show the comparable efficacies of these two toothpastes in superhardening.

Rehardening by solutions of calcium sugar phosphates

The usual tooth rotation procedure has been used to measure the rehardening properties of calcium sucrose phosphate and calcium glucose phosphate solutions in distilled water. The results in Table 10 show that aqueous solutions

TABLE 10

Rehardening by aqueous solutions of calcium sugar phosphates

Solution	Initial hardness (KHN)	Average decrease in hardness (KHN)	Average increase in hardness (KHN)				Average increase (KHN)
			Time (minutes)				
			30	60	90	120	
20% CaSP	213	100	30	28	36	37	33
10% CaSP	214	97	24	26	22	25	24
5% CaSP	231	91	20	21	24	25	23
2% CaSP	223	106	13	20	18	18	17
1% CaSP	229	112	18	19	23	24	21
20% Calcium glucose phosphate..	219	108	13	7	16	24	15
2% Calcium glucose phosphate..	261	61	11	20	19	26	19
10% CaSP (Neuberg product)	217	121	11	10	10	8	10

of CaSP reharden softened tooth enamel comparably with toothpastes containing CaSP. Calcium glucose phosphate also rehardened but to a lesser extent at higher concentrations than CaSP. CaSP (Neuberg product), which is essentially free of organic phosphate, possessed only marginal, if any, rehardening ability.

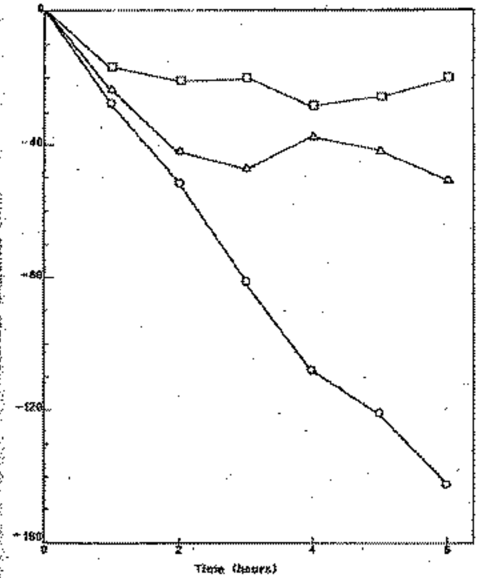

Fig. 2.—Rate of softening of tooth enamel (pH 5·5): ◯ No CaSP, △ 0·05% CaSP, □ 0·5% CaSP.

Rate of softening of tooth enamel

In a previous paper[10] it was shown that CaSP inhibits the rate of dissolution of synthetic hydroxyapatite in acid buffers under unstirred conditions. It would therefore be expected that CaSP would retard the rate of softening of human tooth enamel in acid buffer solutions.

Figure 2 shows that rate of softening of tooth enamel in the normal acid buffer used in this work. The softening rate is about 24 KHN per hour which is similar to that measured by others.[9] In the presence of both 0·05 per cent and 0·5 per cent CaSP the softening rate was markedly reduced.

The rates of softening of teeth, which were softened and then rehardened by a toothpaste containing 20 per cent CaSP, were compared with teeth softened to the same Knoop hardness as these rehardened teeth. As shown in Fig. 3 there was little significant difference in the rates of softening of the teeth, indicating that teeth rehardened with CaSP do not possess any enhanced or reduced susceptibility to acid attack.

Discussion

Before discussing the results of the present study it is desirable to consider briefly some of the previous work on the remineralization of tooth enamel. Some of the data obtained with synthetic solutions containing calcium and orthophosphate ions are summarized in Table 11.

Pigman, Cueto and Baugh[12] found that in their rehardening solutions containing 1·5 $\times$ 10^{-3}M Ca and 0·9 $\times$ 10^{-3}M P (i.e., at an ion

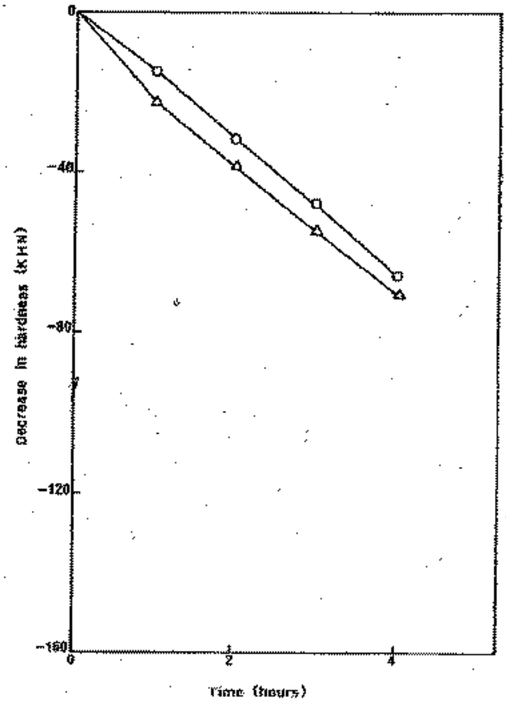

Fig. 3.—Rate of softening of rehardened tooth enamel: ◯ Control, △ Previously rehardened with CaSP.

product of 1·35 $\times$ 10^{-6}) at pH = 7·4, they obtained slow precipitation of a solid phase in the absence of any tooth surface. Solomons and Neuman[13] found that their calcifying solutions at a pH of 7·4, a constant ionic strength of 0·16 at 37° C., and containing

(12) Pigman, W., Cueto, H., and Baugh, D.—Conditions affecting the rehardening of softened enamel. J. D. Res., 43: Supp. 6, 1187-1195 (Nov.-Dec.) 1964.

(13) Solomons, C. C., and Neuman, W. F.—On the mechanisms of decalcification: the remineralization of dentin. J. Biol. Chem., 235: 8, 2502-2506 (Aug.) 1960.

the dicalcium phosphate abrasive and CaSP in the rehardening slurries with the different toothpaste formulations, which do not occur at different dispersions of the same toothpaste.

It is of interest in this connection that the dilution of a toothpaste containing CaSP does not result in any deterioration of the rehardening properties with time (see Table 6). On the other hand the dilution of a commercial toothpaste containing stannous fluoride causes a rapid deterioration of rehardening properties with time. This is probably due to the precipitation of Sn^{++} and F^{-} ions in the dispersion. The storage of a toothpaste containing CaSP for periods of six months also has no effect on the rehardening property of the toothpaste (see Table 7).

In relation to the normal use of a toothpaste containing CaSP it is pertinent that the rehardening characteristics displayed by such a toothpaste on softened tooth enamel are also observed when the toothpaste is brushed on intact tooth enamel which has not been previously softened. The brushing of such a tooth for a period as short as 2 minutes resulted in a significant increase in the hardness of the enamel surface (see Table 8).

The data obtained in this present work thus suggest that the use of toothpastes containing a complex association of calcium sugar phosphates and inorganic phosphates, which remains soluble in aqueous solutions, has potential as a means of inhibiting dental caries by remineralizing areas of tooth enamel which could otherwise develop into carious lesions.

A trial[23] to determine whether this was so was carried out in Melbourne, Australia. This trial involved over 1,000 children. Half were given a conventional toothpaste containing dicalcium phosphate as the abrasive and the other half the same toothpaste containing 5 per cent CaSP (equivalent to CSRC-6). The use of this toothpaste was not supervised and there were indications that after two years many children were not using the supplied toothpastes. Nevertheless, dental examinations showed a significant reduction in dental caries in certain teeth of the children in the group using the CaSP toothpaste.

Although further more carefully conducted human trials are required in order to determine the efficacy of such toothpastes, the indications are that the principle of remineralization of tooth enamel is worthy of further study as a means of reducing dental caries in man.

Summary

Experiments are described which show that the surface of human tooth enamel, which has previously been softened by partial demineralization in an acidic buffered solution, can be rehardened by immersion in solutions containing excess calcium and phosphate ions.

Solutions containing high concentrations of calcium and phosphate ions can be obtained in the presence of sugar phosphates. These solutions reharden softened tooth enamel in minutes compared to hours required by metastable solutions which contain lower concentrations of these ions in the absence of sugar phosphates.

The application of these findings to toothpastes is briefly discussed.

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[23] Lilienthal, B., Gregory, G., and Wood, B.—Private communication.

organic phosphates can act as kink poisons in the dissolution of hydroxyapatite and it is therefore likely that these ions can inhibit the crystallization of hydroxyapatite at low supersaturations. Organic phosphates are present in saliva[19] and if the Ca^{++} and $HPO_4^{=}$ levels and pH of saliva are low, remineralization could well be inhibited. It is also interesting that Howard and his co-workers[18] have recently isolated, from urine and serum, peptides which are effective inhibitors of crystallization of hydroxyapatite.

Shannon[20] has shown that the inorganic phosphate level in parotid fluid is inversely related to caries status in man. It is therefore likely that the balance between the degree of supersaturation of calcium and phosphate in saliva, the effects of pH variations and the levels of inhibitors of calcium phosphate deposition, is important in governing the remineralization of tooth enamel *in vivo*.

If metastable solutions containing high Ca^{++} and $HPO_4^{=}$ concentrations could be obtained, it appeared likely to us that these would have greater utility in the field of enamel remineralization than unstable solutions containing only low levels of Ca^{++} and $HPO_4^{=}$. This led to the present work to determine whether the property of sugar phosphates of solubilizing large concentrations of inorganic calcium phosphates would be of value in the remineralization of tooth enamel.

The results summarized in Table 10 show that solutions containing as low as 1 per cent calcium sucrose phosphate (CaSP CSRC-6) produce significant rehardening of softened tooth enamel in a matter of minutes. CaSP (CSRC-6) contains 12·5 per cent Ca and 2·8 per cent inorganic P. Thus a 1 per cent solution contains 31×10^{-3}M Ca and 9×10^{-3}M P (an ion product of 279×10^{-6}) and is highly supersaturated with respect even to dicalcium phosphate in this pH range, 6·5–7·0. It is therefore not surprising that these solutions will reharden softened tooth enamel very rapidly due to the formation of a solid calcium phosphate phase.

The marginal rehardening obtained with a 10 per cent solution of CaSP (Neuberg product) which contains 8·1 per cent Ca and 0·05 per cent inorganic P is likewise due to the presence of Ca^{++} and $HPO_4^{=}$ ions in solution, but under these conditions the lower concentration of $HPO_4^{=}$ and the presence of a large excess of sucrose phosphate anions reduces the degree of rehardening.

From Table 4 it is clear that in a toothpaste slurry in *water* the presence of either Ca^{++}, $HPO_4^{=}$ or the sucrose phosphate anion alone does not produce significant rehardening. However, when all three ions are present together in sufficient concentration significant rehardening occurs even at pH values as low as 5·7. When these ions are present alone in a toothpaste slurry in *saliva* (see Table 5), the additional concentration of Ca^{++} or $HPO_4^{=}$ (over the concentrations of Ca^{++} and $HPO_4^{=}$ present in the saliva) results in significant rehardening. However, the addition of the sucrose phosphate anion alone, as would be expected, does not produce rehardening. It is therefore not surprising that calcium sucrose phosphate, containing appreciable quantities of inorganic phosphate which is capable of remaining in solution even at high pH values, when added to toothpastes can lead to high concentrations of Ca^{++} and $HPO_4^{=}$ in solutions which are capable of rapidly remineralizing previously softened tooth enamel (see Table 1). The influence of F^{-} on this remineralization in these highly supersaturated solutions is of no significance, in contrast to its effect in synthetic solutions containing comparatively low supersaturations of Ca^{++} and $HPO_4^{=}$.

Likewise the addition of saliva in the presence of these high concentrations of Ca^{++} and $HPO_4^{=}$ is without significant effect (see Table 3).

The effect of different concentrations of CaSP, brought about both by changes in the level of CaSP in the toothpaste and by the degree of dispersion of the toothpaste in water, is not quite as clear. While the effect of different degrees of dispersion of a toothpaste in water or saliva is without appreciable effect on rehardening (see Tables 2 and 3), the effect of changing the concentration of CaSP in the toothpaste is significant (see Table 1). Not only is the degree of rehardening increased by increasing the concentration of CaSP in the toothpaste, but the rate of rehardening is also significantly increased (Fig. 1). These differences may be due to the varying proportions of

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(20) Shannon, I. L.—Parotid fluid flow rate, parotid fluid, and serum inorganic phosphate concentrations as related to dental caries status in man. J. D. Res., 43: Supp. 6, 1029-1038 (Nov.-Dec.) 1964.