

FLUORIDE RETENTION IN HUMAN ENAMEL AFTER A SINGLE PHOSPHORIC ACID AND MIXED PHOSPHORIC ACID/SnF₂ APPLICATION IN VITRO

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Summary—Pieces of enamel were treated with either an APF solution or a 1:1 mixture of APF/SnF₂ (APF: 1.23 per cent F⁻, 0.1 M H₃PO₄; and SnF₂: 2 per cent) for 4 min at 37°C followed by 30 min washing. There was an increase of 1640 parts/10⁶ in the fluoride concentration of APF-treated enamel. Most of the fluoride was, however, washed away within 24 h to a level of 230 parts/10⁶. After 7 × 24 h the F concentration was of the same order: 280 parts/10⁶. The initial increase in fluoride concentration was lower after a mixed topical application (1120 parts/10⁶) but after 24 h the fluoride concentration was 510 parts and after 7 × 24 h 370 parts/10⁶, although statistically not significant, higher than after a single APF-treatment. The higher F concentration may be due to the formation of more firmly-bound fluoride as a fluoridated hydroxyapatite. It is more probable that the CaF₂ layer is protected by insoluble Sn compounds.

INTRODUCTION

Topical applications of concentrated fluoride solutions are widely used in caries prevention. The solutions initially used were mainly of NaF and SnF₂. Brudevold *et al.* (1963) showed that there was higher F-uptake when NaF-solution was acidified with phosphoric acid (APF). Caslavská *et al.* (1971) showed that using NH₄F-solution increased initial F uptake further, as did acid pre-treatment (Aasenden, Brudevold and McCann, 1968). The disadvantage of a high initial F-uptake is, however, that only a few per cent is deposited as firmly-bound fluoride (Caslavská, Moreno and Brudevold, 1975a). The remainder is mainly present as CaF₂ which leaches out relatively quickly and may, in consequence, be of little use in caries reduction.

Shannon (1970) found that the application of APF and SnF₂ solutions sequentially produced greater enamel solubility reduction than when either was used separately. Kleinstub and Shannon (1974) showed that is true also with a freshly prepared mixture of APF and SnF₂ and suggested that CaF₂ was formed in both cases together with a tenacious layer of insoluble Sn-compounds. As most of the fluoride present as CaF₂ disappears rapidly after a single APF-treatment, we wondered whether the CaF₂ formed after a mixed application of APF/SnF₂ is protected by insoluble Sn-compounds and could therefore act as a fluoride reservoir. Our purpose here was to measure the amount of retained fluoride in enamel after washing. As pH fluctuations in the presence of fluoride increase the amount of fluoridated apatite (Duff, 1976; Duke and Forward, 1977), we investigated this aspect also.

MATERIALS AND METHODS

Incisor and canine teeth from subjects living in a non-fluoridated area were used. The teeth were stored

in a solution of 2 per cent cetrimide (Cetavlon) in tap water and lightly cleaned with pumice before treatment. A rectangular piece of known size was sawn at random from the buccal surface as a control. Cervical regions were excluded. The remainder of the tooth was cut longitudinally into two halves.

Treatment

Each series of 10 half teeth was fixed on sticky wax and dried for 1 min. The teeth then received either (1) a 4 min APF treatment at 37°C, or (2) a 4 min APF/SnF₂ treatment at 37°C. The composition of the APF was: total F 1.23 per cent; H₃PO₄ 0.1 M, pH = 3. The APF/SnF₂ mixture was prepared directly before use by mixing APF and a freshly prepared 2 per cent SnF₂ solution in a 1:1 ratio with a resultant pH of 2.1.

The tooth halves were dried for 0.5 min and subsequently washed for 3 × 10 min in 100 ml of tap water. After further washing for 24 h in tap water and twice-daily changed for 7 days in 100 ml of a solution containing 0.001 M Ca and 0.0025 M PO₄ at a pH of 5.5 (concentrations comparable with those in saliva, added to prevent dissolving of the enamel apatite), F was estimated in the outer layers of enamel.

In a second series of experiments, enamel was treated with a mixed APF/SnF₂ for 4 min at 37°C. Fluoride estimations of two enamel layers were carried out after a 24-h 1 M KOH-treatment preceded by either 0.5 h washing, 7 × 24 h storage at a constant pH of 6.5 or 7 × 24 h of cyclical pH fluctuations. One pH cycle was completed each day; the tooth halves were immersed in the washing solution for 5 min at pH = 3.5. This was followed by humid storage for 8 h at 37°C. This treatment was then repeated at pH = 8.0 and followed by 16 h humid storage.

Embedding procedure

Pieces of buccal enamel with a surface area of between 2.5 and 5 mm² were sawn from similar pos-

itions in each pair of tooth halves. The surface area was estimated from precision micrometer measurements. The experimental and control blocks were embedded in a fast-polymerising two-component poly-acrylate under 2 atmosphere pressure. When polymerization was complete, the embedded enamel pieces were washed for a few seconds in distilled water and alcohols. There was no measurable gap between the enamel and the polymer under a $\times 500$ magnification.

Chemical analysis

The first of two enamel layers was removed by holding the samples in a plastic vial containing 0.5 ml 0.5 M HClO_4 for 30 s. The surface was then washed in 1.5 ml H_2O and the two fractions combined. A second layer was then removed in the same way. The fluoride concentration in the acid solution was estimated by a modification of the gas-chromatographic method described by Fresen, Cox and Witter (1968): 0.5 ml solution F-unknown + 0.05 ml benzene-trimethylchlor-silan (TMCS) + 0.05 ml HCl 25 per cent were used. The following quantities were used in the extraction procedure originally described by Fresen *et al.*: 2 ml solution F-unknown + 1.0 ml benzene-TMCS + 1.0 ml HCl 25 per cent.

By accurate working in special conical plastic tubes, it was possible to estimate F concentrations as low as $0.002 \mu\text{g/ml}$. The modified method was tested in the range of 0.002 – $0.01 \mu\text{g F/ml}$ and 0.01 – $0.1 \mu\text{g F/ml}$. A linear relationship between F concentration and relative peak-length was demonstrated in both cases.

The calcium concentration was estimated spectrophotometrically as described by Gindler and King (1972) on 0.1–0.5 ml of the acid solution after neutralization with KOH. Phosphate was estimated by the method of Chen, Toribara and Huber (1956).

Calculation

The weight of dissolved enamel (W_e) was calculated from the phosphate concentration in $\mu\text{g/ml}$ assuming a phosphate percentage in enamel of 17 per cent.

$$W_e = 2 \times (\text{PO}_4^{3-}) \times \frac{100}{17}$$

The thickness of the corresponding enamel layer in μm was calculated from the enamel surface area (mm^2) and the enamel density (d) which was assumed to be 2.95.

$$d = \frac{W_e}{2.95 \times \text{surface area}}$$

The mean F percentage in each layer was estimated from the total amount of fluoride W_f and the corresponding enamel weight:

$$F_{\text{ppm}} = \frac{W_f}{W_e} \times 10^6$$

The F percentage of different samples should not be directly compared because of varying layer thickness. The F percentage at a standard depth is calculated by constructing an F gradient by linear interpolation, but this method is not entirely correct (Mühlemann, Schait and König, 1964). In our experiment, the F gradients were constructed on the assumption that

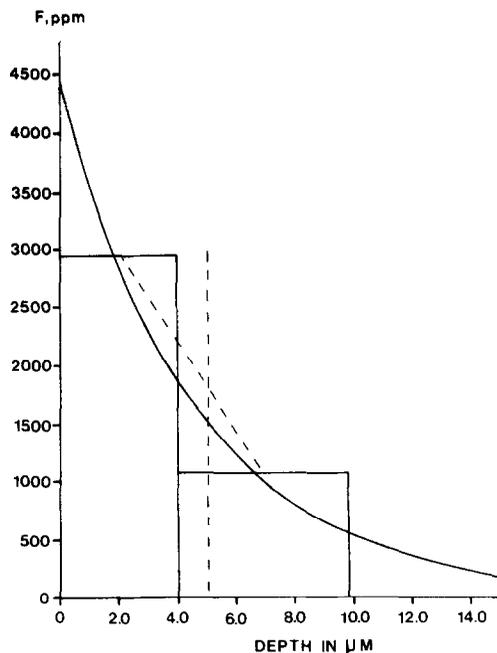


Fig. 1. The F gradient constructed from the F percentages of two enamel layers (—). The linear interpolation approach is given by the dotted line (---). Note the difference in the F percentage at $5 \mu\text{m}$ estimated by linear interpolation and the calculated F gradient.

they have an exponential path (Fig. 1). The F percentage at a depth of $5 \mu\text{m}$ was estimated by assessing the function:

$$F = F_0 \cdot e^{-kd}$$

which expresses the F percentage at an enamel depth d : F_0 being the F percentage at the enamel surface and k a constant. This function was estimated from the mean F percentages F_1 and F_2 of the first two enamel layers, d_1 and $d_2 \mu\text{m}$ thick respectively, by using 2 equations:

$$\int_0^{d_1} F_0 \cdot e^{-kd} = F_1 \cdot d_1 \quad (1)$$

$$\int_{d_1}^{d_2} F_0 \cdot e^{-kd} = F_2 \cdot (d_2 - d_1) \quad (2)$$

F_0 and k were first calculated using a computer program. The F function and the F percentage at $5 \mu\text{m}$ could then be estimated.

This method is only applicable for F percentages within the total etched depth. The F increase at a depth of $5 \mu\text{m}$ was estimated from the F percentage of the treated and control enamel samples using this technique.

RESULTS

All the available CaF_2 in APF-treated enamel was washed away within 24 h (Table 1). The remaining F increase, present as a firmly-bound insoluble fluoride compound, was about 250 parts/ 10^6 after 24 h washing and remained at this level even after 7 days.

The initial F uptake of APF/ SnF_2 -treated enamel was much lower than that which occurred after an APF-application, but after washing for 24 h nearly

Table 1. Difference in F concentration compared with the control values at a depth of 5 μm after different washing periods, in enamel treated with APF and APF/SnF₂ at 37°C for 4 min

Washing period (h)	APF	APF/SnF ₂	Difference
$\frac{1}{2}$	1640 (540)	1120 (840)	-520 $p \leq 0.1$
24	230 (230)	510 (350)	+280 $p = 0.1$
7 \times 24	280 (260)	370 (210)	+90 n.s.

Standard deviations given in brackets.

n.s. = not significant.

half was still present. Washing for 7 days resulted in a further decrease in the F-content, demonstrating that 24 h washing did not remove all soluble fluoride compounds. There was no significant increase in firmly-bound fluoride in APF/SnF₂-treated enamel either after storage for 1 week at a constant pH or with pH fluctuations (Table 2). In both, the amount of fluoride deposited was similar to that found after an APF/SnF₂ treatment followed by a 24-h KOH-extraction.

DISCUSSION

The F percentages were calculated on the assumption that the F gradient follows an exponential path (Fig. 1) based on Mühlemann's (1961) observation that the shape of the F gradient is concave. A single exponential function was chosen to approximate the F gradient. The F-values calculated with linear interpolation tend to be some 20 per cent higher.

Our results show that the CaF₂ layer is retained for longer after an APF/SnF₂ treatment than after a single APF treatment and can, in consequence, serve as a temporary fluoride reservoir. We have assumed that the soluble fluoride compounds formed after an APF/SnF₂ treatment consist solely of CaF₂, as the Sn compounds formed are insoluble (Wei, 1975). Our results agree with experiments using electronmicroscopy after either a sequential APF/SnF₂ or an APF treatment, both followed by 24-h washing (Wei, 1975). As the formation of a fluoridated apatite (FHAP) occurs quite slowly, the continued presence of CaF₂ may be beneficial (Mühlemann, 1967).

A few per cent only of the fluoride initially present after an APF treatment is firmly bound (FHAP) and appears to be independent of the amount of CaF₂ initially present, whereas the presence of CaF₂ for a longer period results in a slow increase in the percent-

age of FHAP (Caslavská, Moreno and Brudevold, 1975b). The bulk of the CaF₂ would be quickly washed away unless protected, and is therefore of little value. We therefore followed the F applications with $\frac{1}{2}$ h washing. The F increases recorded after APF treatment are, in consequence, lower than the values of the unwashed samples of Wei and Schulz (1975).

The roles played by FHAP and CaF₂ in caries protection are complicated. Caslavská *et al.* (1975a) suggested that the effectiveness of a topical treatment depends on the amount of fluoride incorporated as FHAP. Clinical investigations by Wellock and Brudevold (1963) showed that there is a greater caries reduction after an APF treatment than after NaF and this may be caused by the CaF₂ layer. However, Wei and Schulz (1975) found, *in vivo*, that a high initial F level (20,000 parts/10⁶), probably caused by CaF₂ formed after an acid pre-treatment and APF application, decreased to a value below that of the original untreated level after 3 months, showing that the long-term effects of techniques producing high initial F uptake are not necessarily beneficial.

The concentration of FHAP we recorded after APF treatment followed by 24-h washing is similar to that reported by Caslavská *et al.* (1975b). The amount of firmly-bound fluoride, formed after an APF/SnF₂ treatment is, however, higher and probably consists mainly of FHAP, although stannous-fluoride phosphate compounds and protected CaF₂ may also be present.

We used pH fluctuations and humid storage after APF/SnF₂ treatment to simulate the *in vivo* situation. There was, however, no increase in the amount of firmly-bound fluoride. We expected slow dissolution of CaF₂ to result in the formation of more FHAP, especially in conditions of fluctuating pH, as Duff (1976) demonstrated for APF-treated enamel. One reason for this negative result might be that a protective layer of Sn compounds could inhibit diffusion.

APF/SnF₂ treatments have, until now, been tested mainly on the basis of enamel solubility reductions. Our results also demonstrate that CaF₂ formed after mixed APF/SnF₂ applications may be protected by insoluble Sn compounds, it therefore washes out more slowly.

Table 2. Difference in F concentration compared with the control values at a depth of 5 μm after different procedures. Enamel treated with APF/SnF₂ at 37°C for 4 min

Treatment	APF/SnF ₂
KOH	370 (330)
pH fluctuation (3.5-8.0) + KOH	440 (350)
constant pH = 6.5 + KOH	410 (400)

Standard deviations given in brackets.

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