Effect of fluoride-containing alginates and gels on the acid resistance of demineralized human enamel

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A series of in vitro studies were carried out to determine the effect of commerically available alginate impression materials and gels on enamel solubility. This was performed by 4-min topical application of the tested products on partially demineralized enamel surfaces. The difference in the amounts of calcium and phosphorus dissolved in acetate buffer before and after topical treatment was considered a measure of the reduction in enamel solubility. All topically applied materials except APF-gel (Gelution®) exerted a considerable reduction in enamel solubility ranging between 41.4% and 61.5% in 0.2 M acetate buffer. Successive enamel solubility tests in weak acetate buffer (0.01 M) showed that Gelution was inferior to the other tested products. No simple relationship exists between the fluoride content of these products and their anti-solubility effect. □ *Dental materials; enamel solubility; F-gels*

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It is generally admitted that the efficiency of topical fluoride (F) treatment depends to a considerable extent on its ability to deposit F in the enamel and the extent to which it reduces enamel solubility (1–4). Although it is logical to assume that a decrease in solubility should be associated with enhanced resistance to caries, only limited evidence supports this view (5). This is attributed to the mode of F incorporation and distribution in enamel (3). Furthermore, experimental data are not always comparable with those collected in vivo (6). Nevertheless, the solubility tests did lead to the discovery of stannous fluoride (7) and have been very useful for monitoring the efficacy of F products (2, 8). It has recently been found that some commercially available alginate impression materials contain high concentrations of F and that part of this is readily transferred to the teeth and body fluids after impression-taking (9, 10). Although alginates are widely used in dental practice, no report concerning their effect on enamel solubility seems to exist.

In this study the enamel solubility test was carried out on partially demineralized human teeth exposed for brief periods to the testing agents. The purpose of the study was to evaluate in vitro the effect of alginate impression materials, F-gels, and alginate-based experimental formula (EF) on enamel solubility.

Materials and methods

Experiment A

Sixty-six intact premolars, extracted for orthodontic reasons, were cleaned with a rotating rubber cup and deionized water and degreased by briefly washing with acetone. This improved adhesion to the buccal surface of a round disc, 6 mm in diameter, made from Scotch tape (3M Scotch pressure-sensitive tape). The teeth were then covered with nail varnish and dried by air-blasting. The disc was subsequently removed, leaving behind a well-defined area of approximately 28.3 mm^2. Each tooth was dipped into a polystyrene cup containing 4 ml of a 0.2 M sodium acetate–acetic acid buffer at pH 4.0. The cups were agitated in a rotating shaker at 90 rpm at room temperature for two successive periods of 30 min each. This procedure removed the outermost enamel, which differs most among different teeth (5). Aliquots of the demineralization solutions were analyzed for F, calcium, and phosphorus. After these base-line etchings, the teeth were rinsed with deionized water and dried with tissue paper. Before

topical treatment, the teeth were exposed to a third decalcification run in a similar fashion for 20 min. The amount of calcium and phosphorus dissolved during this run was taken as the control value for that particular tooth.

Three commercial products of alginate powder (HI-Technical®, GC Dental Industrial Corp., Japan; Kerr®, Kerr Sybron Corp., USA; and Zelgan®, Amalgamated Dental, England) containing 0.44–1.87% F and EF containing 1% F (as NaF) were mixed with water in accordance with the manufacturer's instructions. The final F concentrations in the mixture were about 0.13–0.50% for the commercial products and 0.33% for the EF. The main components of the EF are as follows (% by weight): Potassium alginate, 12%; filler, 72%; calcium sulphate, 12%, details of which are described elsewhere (11). The pH values of the alginates were about neutral or higher. The F-gels tested in this study were Gel II® (Pacemaker Corp., Ore., USA), APF, orange flavor, 0.5% F, pH 4.5–5.0, and Gelution® (Unitek, Calif., USA), APF, 1.23% F, pH 3.0–3.5.

The alginate mix was loaded onto a plastic tray and the APF gels onto a disposable foam tray. The teeth were then dipped into the trays for 4 min, followed by rinsing under running water and careful brushing with a soft toothbrush around the windows to remove any remnants of the applied materials. Each tooth was then agitated in deionized water (100 ml) for 1 min and submerged in 5 ml of an inorganic solution simulating the saliva. The solution contained 1.5 mM calcium as $CaCl_2$, 5 mM phosphorus as KH_2PO_4 and Na_2HPO_4, 15 mM bicarbonate as $KHCO_3$, 0.15 mM magnesium as $MgCl_2$, 10 mM NaCl, and 0.15 mM citric acid (12). The pH was adjusted to 7.0, and the F concentration was less than 0.02 ppm. The teeth submerged in artificial saliva were divided into two groups. In one group the teeth were shaken for 30 min, whereas in the other group the shaking was continued for 24 h at room temperature. The teeth were then washed, dried, and demineralized in 4.0 ml of 0.2 M acetate buffer, pH 4.0, for an additional 20 min. Thus two demineralized values were available from each tooth, the first representing the control (pretreatment) and the second the posttreatment value. The difference in the amounts of calcium and phosphorus dissolved before and after treatment—that is, between the third and fourth layers—was considered a measure of the reduction in enamel solubility achieved by the treatment procedure (Table 1).

Experiment B

Twenty-two premolars were selected and prepared as described above. In this experiment a weak acetate buffer (0.01 M, pH 4.0) was used to evaluate the resistance of F-treated teeth to a successive dissolution sequence. Before topical F treatment, a baseline etching was carried out for 30 min to remove the outermost enamel, followed by 15 min demineralization to assess the control values. Gel II, Gelution, and EF were applied topically for 4 min in the same manner as in experiment A. Six demineralization runs of 15 min each were carried out successively. The reduction in enamel solubility for each run was calculated from the difference between the control and posttreatment value (Table 1).

Analysis

The F concentration was measured by means of fluoride-ion-sensitive electrodes (ORION model 96–09 and ORION model 801 digital pH/mV meter). Aliquots (0.9 ml) of acetate buffer were neutralized with 0.015 ml of 4 M sodium hydroxide and mixed with 10% by volume acetate buffer (7.5 M, pH 5.2) containing 2% CDTA. The final pH of the samples was approximately 5.0. F standards were prepared in the same manner.

The calcium concentration was measured in solutions containing 1% lanthanum chloride by means of an atomic absorption spectrophotometer (Pye Unicam SP 190).

The phosphorus concentration was determined spectrophotometrically (Beckman model 24) by the malachite green method (13).

From the amount of calcium and phosphorus dissolved, the weight and thickness of the enamel layers removed after each demineralization were calculated as previously described (10). An F distribution curve was prepared by plotting the resulting mean F

Table 1. Experimental procedures used with alginates and gels

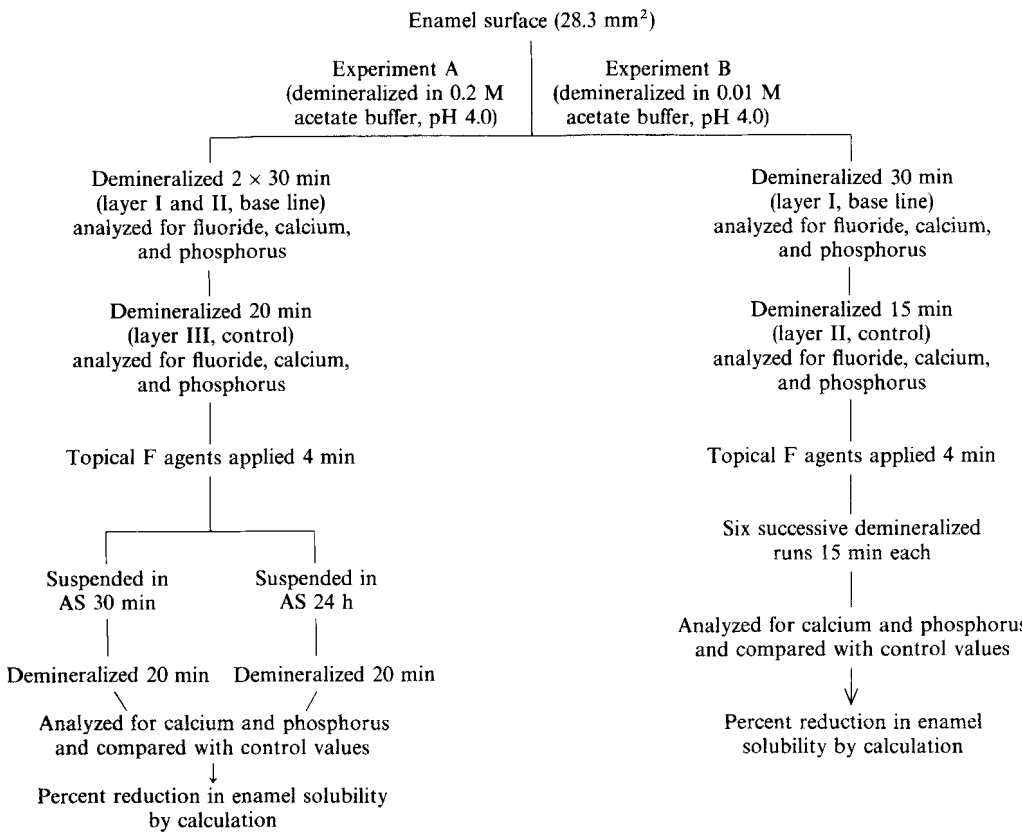

Table 2. Enamel dissolution data before F topical applications

Experiment	Layer no.	Acetate buffer exposure time (min)	No. of teeth	Mean weight of enamel (μg), $\bar{X} \pm SD$	Mean layer thickness (μm), $\bar{X} \pm SD$	Fluoride weight (μg), $\bar{X} \pm SD$	Fluoride concentrations (ppm), $\bar{X} \pm SD$
A	I	30	35	132 ± 26	1.59 ± 0.31	0.176 ± 0.054	1307 ± 376
	II	30	35	164 ± 28	1.96 ± 0.34	0.159 ± 0.056	971 ± 363
	III	20	66	161 ± 22	1.93 ± 0.26	0.084 ± 0.028	551 ± 179
B	I	30	22	89 ± 9	1.06 ± 0.10	—	—
	II	15	22	53 ± 4	0.64 ± 0.06	—	—

concentration for each enamel layer against half the thickness of the layer plus the total thickness of the preceding layers. Thus, the mean F concentration was located at the center of each layer analyzed.

To determine the reliability of the F analysis, known amounts of F (0–0.875 μg) were added to the demineralized solutions containing the sampled enamel. The recovery ranged from 98–103% (CV = 3.8–6.0%, $n = 18$).

A statistical analysis for significant differences in the reduction of enamel solubility was performed with the t test.

Results

Experiment A

Table 2A shows the average amounts of F and enamel dissolved in a 0.2 M acetate buffer

after three consecutive demineralizations. The data obtained from the first and second demineralization were used solely to study the F distribution and the enamel depth removed, whereas the third layer served as the control value for the subsequent demineralization that followed topical application. The results indicate that the cumulative thickness of the enamel layers removed before topical treatment was about 5.5 μm. The first layer removed from the surface enamel was thinner than the second for an identical etching period. The rate of enamel dissolution (μg/min) for successive layers showed that dissolution of the first layer was 24.2% and 83.0% less than that of the second and third layers, respectively.

When mean enamel F concentrations for each of the three pretreatment layers were plotted against the depth of the center of each layer on a linear graph, a nearly straight line was obtained (Fig. 1). The results also show that the F concentration was invariably highest in the outermost layer of the enamel and decreased by as much as of 25.7% in the second layer and 57.8% in the third layer. The results of the enamel solubility test in experiment A are presented in Fig. 2. In all instances, with the exception of the Gelution group, there was a significant ($0.005 < P < 0.05$) reduction in enamel solubility which ranged from 41.4% to 61.5% for teeth treated with alginates or Gel II. The corresponding result for the Gelution group was 18.5%. In alginates and Gel II groups the average amount of calcium dissolved from the control and post-treatment (layers III and IV) etchings were 62.6 ± 6.63 and 31.8 ± 7.56 μg, respectively ($\bar{X} \pm SD$). The corresponding phosphorus data were 30.0 ± 4.53 and 14.4 ± 2.92 μg ($\bar{X} \pm SD$).

Extending the washing time of F-treated

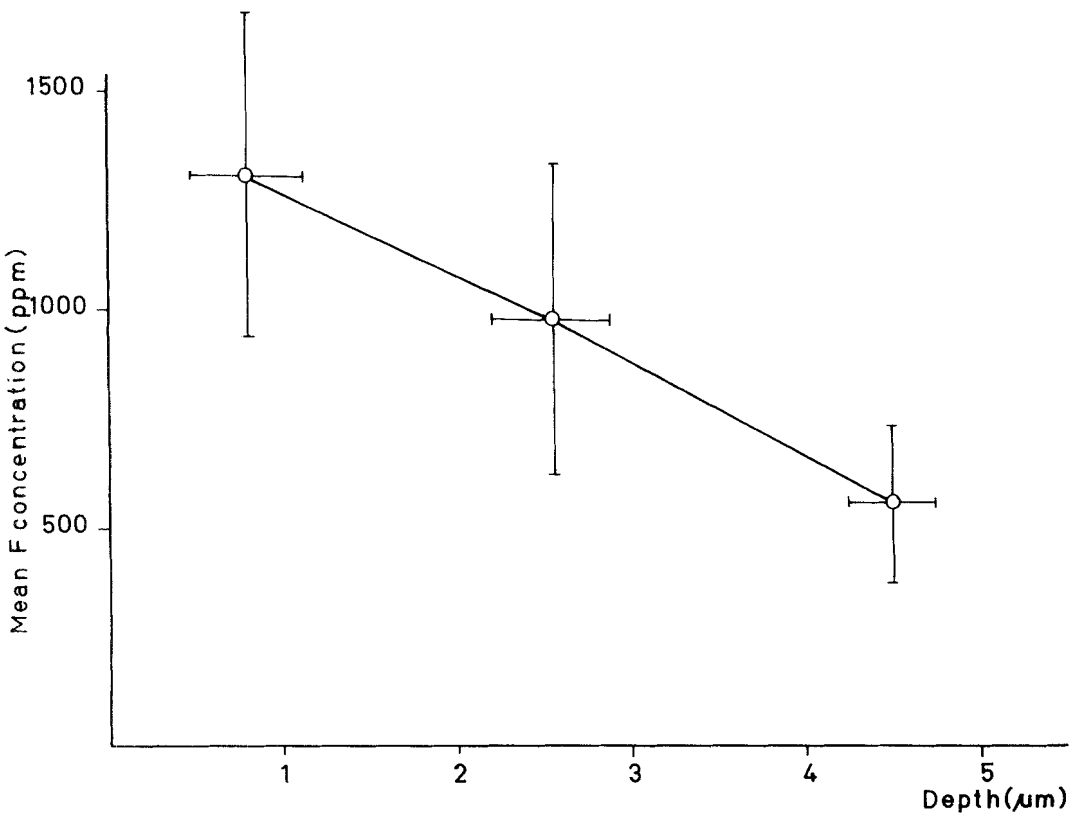

Fig. 1. Mean fluoride concentration (ppm) versus midpoints of successive enamel layers (μm) for teeth exposed to 0.2 M acetate buffer (pH 4.0). The bars represent standard deviations.

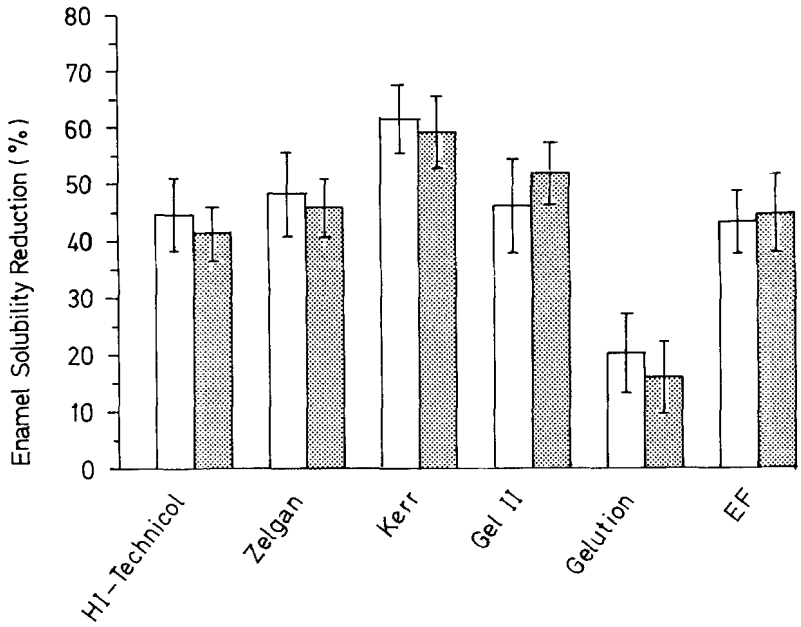

Fig. 2. Effect of topical application of various F-agents on enamel solubility. The open columns represent results based on calcium data, and the closed columns represent the results based on phosphorus data. The vertical bar in the center of each column gives the standard deviation.

teeth in artificial saliva from 30 min to 24 h resulted in the disappearance of the protective effect of treatment, giving a reduction in enamel solubility of $-7.2\% \pm 5.72$ ($\bar{X} \pm$ SD). The mean amount of F which leached from the treated teeth after 30 min of washing in artificial saliva was as follows (μg F/5 ml): Gelution, 0.40; EF, 0.43; HI-Technicol, 0.46; Zelgan, 1.28; Kerr, 1.88; and Gel II, 4.49.

Experiment B

Table 2B represents the enamel and F dissolved in a 0.01 M acetate buffer. The rate of enamel dissolution in the outermost layer was 33.0% less than that found in 0.2 M acetate buffer.

The data for the enamel solubility test in experiment B are shown in Fig. 3 and represent the successive solubility of enamel after treatment with EF, Gelution, and Gel II. Again, both calcium and phosphorus estimates were used to calculate the extent of enamel protection. Gel II and EF provided about the same antisolubility tendency throughout the six demineralizing runs, whereas in the Gelution group the protective action was reduced to about 50% by the sixth run.

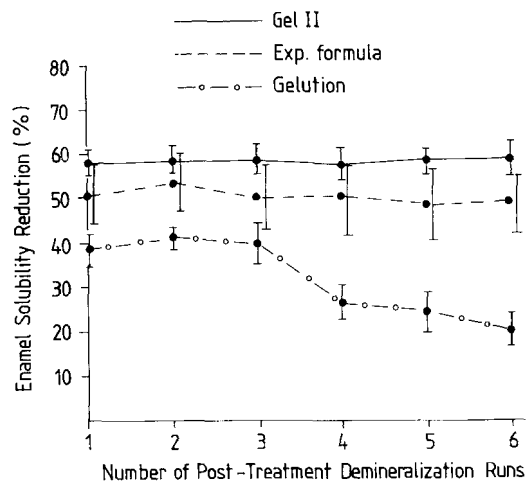

Fig. 3. Effect of F-gels and experimental formula (EF) on successive enamel solubility tests. The bars represent standard deviations.

Discussion

We have raised the possibility as to whether alginate could be used as a vehicle for topical F application (10). The motive behind this is that alginate has the advantage of being economical and time-saving, since it enables

the entire mouth to be treated during a single application. Although these advantages might also be attained by using F-gels, several studies have reported that the risk of systemic absorption of F from topically applied alginate is far less than from commercial F-gels (14–17).

In the present study the solubility tests were carried out on partially demineralized enamel to simulate the primary and most significant stage in the initiation of dental caries. Moreover, the pH of acetate buffer (4.0) used was the same as that of interproximal plaque found following rinsing with 10% sucrose (18). The experimental teeth were immersed in artificial saliva to enable the unreacted or loosely bound F to wash out, as may occur in the oral environment (5, 19).

The current findings showed that the F distribution in enamel followed a linear gradient curve from the enamel surface inward (Fig. 1)—that is, dissimilar to the well-known steep F gradient obtained in enamel biopsied by grinding or by strong acid etching (e.g. 20, 21). This may indicate that part of the F dissolved in acetate buffer was refixed to the demineralized enamel surface. Evidence supporting this phenomenon has previously been reported (22, 23). It might, therefore, be concluded that the F dissolved in the acidic buffer did not represent the original F concentration in the enamel layer biopsied.

The results of experiment A indicate that topical application of alginates and Gel II exerts a significant reduction in enamel solubility ($0.005 < P < 0.05$), whereas the protective effect of Gelution was not significant. This outcome was obtained from teeth exposed for 30 min to artificial saliva after receiving the F treatment. Prolonged washing in artificial saliva (24 h) resulted in the disappearance of the protective effect of tested F-agents. This is inconsistent with the observation that the major part of F deposited on the tooth from a single F application is washed away as unreacted F and CaF_2, within 24 h after application (1, 24, 25). It has been reported that the whole or artificial salivas are undersaturated with regard to CaF_2 (26, 27) and that 7 ppm F is required to prevent dissolution of CaF_2 in saliva (27). This concentration is well above the highest F level obtained in the present saliva samples. Many clinical trials have shown that a significant caries inhibition is achieved after topical treatments in which large amounts of CaF_2 are believed to have formed (3, 28). This could partly be attributed to the physical nature of the CaF_2 reaction product formed on the enamel surface. In fact, it has been reported that topical application of different F salts form precipitates of CaF_2 that differ in appearance and adhesiveness (8, 29).

The successive enamel solubility tests in experiment B showed that Gel II and EF offer a persistent enamel protection during all demineralization runs, whereas teeth treated with Gelution showed a definite decline in their protection after the third run (Fig. 3).

It is interesting that Gelution (1.23% F), which contained more than twice as much F as Gel II and four times more than EF, provided the weakest enamel protection. It may therefore be suggested that no simple correlation exists between the F content of the tested products and their antisolubility effect. Evidence supporting this concept was reported by Shannon & Edmonds (30), who showed that several dilutions of APF solution (1.23% F) did not lead to any significant loss in its solubility reduction effect.

The results of the present study indicate that the use of alginate-based materials as a vehicle for topical application of F offers a valuable means of increasing the enamel resistance against acid demineralization. Furthermore, it is questionable whether the high F content of some of the available gels is really necessary, considering that it may also expose the body to high F levels through swallowing. Animal experiments are planned, to assess the caries-preventive effect of EF before such a material can be recommended for routine clinical use.

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References

1. Brudevold F, McCann HG, Nilsson R, Richardson B, Coklica V. The chemistry of caries inhibition. Prob-

lems and challenges in topical treatments. J Dent Res 1967;46:37–45.
2. Ericsson Y. Reduction of the solubility of dental surfaces by fluoride-containing polishing pastes and tooth pastes. Sven Tandläk Tidskr 1963;56:127–142.
3. Grøn P. Chemistry of topical fluorides. Caries Res 1977;11(suppl 1):172–191.
4. König KG, Marthaler TM, Mühlemann HR. Interrelation between enamel solubility reduction and caries inhibition. Helv Odont Acta 1958;2:34–39.
5. Brudevold F, McCann HG. Enamel solubility tests and their significance in regard to dental caries. Ann N Y Acad Sci 1968;153:20–51.
6. Brown WE, Gregory TM, Chow LC. Effects of fluoride on enamel solubility and cariostasis. Caries Res 1977;11(suppl 1):118–136.
7. Muhler JC. The development of stannous fluoride as an anticariogenic agent for topical application by means of a dentifrice. J Indiana Dent Assoc 1955;34:9–18.
8. Cooley WE. Applied research in the development of anti-caries dentifrices. J Chem Ed 1970;47:177–182.
9. Hattab F, Frostell G. The release of fluoride from alginate impression materials. Community Dent Oral Epidemiol 1978;6:273–274.
10. Hattab F, Frostell G. The release of fluoride from two products of alginate impression materials. Acta Odontol Scand 1980;38:385–395.
11. Hesselgren SG. Method of applying preventive and therapeutic agents. US Patent 1976;3:964, 164.
12. Ericsson Y. Unpublished formula.
13. Hattab F, Lindén LÅ. Micro-determination of phosphate in enamel biopsy samples using the malachite green method. Acta Odontol Scand 1984;42:85–91.
14. Ekstrand J. Koch G, Lindgren LE, Petersson LG. Pharmacokinetics of fluoride gels in children and adults. Caries Res 1981;15:213–220.
15. Hattab F. Absorption of fluoride following inhalation and ingestion of alginate impression materials. Pharmacol Ther Dent 1981;6:79–86.
16. Le Compte EJ, Whitford GM. The biological availability of fluoride from alginate impression and APF gel application in children. J Dent Res 1981;60:776–780.
17. Owen D, Morris M, Adir J, Bakker V. Monitoring ingestion and urinary excretion of topical fluoride. IADR 1979;58:Abstract 1256.
18. Shields WF, Mühlemann HR. Simultaneous pH and fluoride telemetry from the oral cavity. Helv Odont Acta 1975;19:18–26.
19. Stookey GK. Enamel solubility studies with a fluoride-containing prophylactic cup. JASPD 1977;7: 22–26.
20. Brudevold F, Gardner DE, Smith FA. The distribution of fluoride in human enamel. J Dent Res 1956;35:420–429.
21. Weatherell JA, Hargreaves JA. The fluoride content of surface enamel from permanent and deciduous teeth. Adv Fluor Res 1966;4:181–191.
22. Clarkson BH, Wefel JS, Silverstone LM. Redistribution of enamel fluoride during white spot lesion formation: an in vitro study on human dental enamel. Caries Res 1981;15:158–165.
23. Ericsson Y. Influences of sodium chloride on fluoride reactions with dental tissues. Caries Res 1982;16:287–297.
24. Mellberg JR, Laakso PV, Nicholson CR. The acquisition and loss of fluoride by topically fluoridated human tooth enamel. Arch Oral Biol 1966;11:1213–1220.
25. Richardson B. Fixation of topically applied fluoride in enamel. J Dent Res 1967;46:87–91.
26. Birkeland JM. The effect of pH on the interaction of fluoride and salivary ions. Caries Res 1973;7:11–18.
27. McCann HG. The solubility of fluorapatite and its relationship to that of calcium fluoride. Arch Oral Biol 1968;13:987–1001.
28. Brudevold F, Naujkos R. Caries preventive fluoride treatment of the individual. Caries Res 1978;12 (suppl 1):52–64.
29. Mühlemann HR. The cariostatic effects of amine fluoride. Die Quintessenz 1967;18:Ref. 3192. Issues 5–8.
30. Shannon IL, Edmonds EJ. Enamel solubility reduction by acidulated phosphate-fluoride (APF) treatment. Community Dent Oral Epidemiol 1978;6:12–16.

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