Physical and Chemical Considerations of the Role of Firmly and Loosely Bound Fluoride in Caries Prevention

D.J. WHITE and G.H. NANCELLAS

The Procter & Gamble Co., 11511 Reed Hartman Highway, Cincinnati, Ohio 45241; and The State University of New York at Buffalo, Buffalo, New York 14214

Historically, there has been considerable debate concerning the roles of loosely bound (calcium fluoride) and firmly bound (fluorapatite) fluoride for caries prevention. Research now shows that fluorapatite (FAP) is a finite reaction product of enamel fluorapatite formation with or without CaF<sub>2</sub> formation, suggesting that CaF<sub>2</sub> can always be considered as a supplement to, rather than as a substitute for, FAP formation. In the presence of low levels of fluoride in the solution phase, the crystallization of hydroxyapatite is enhanced, while the corresponding dissolution is retarded. Fluoride in the bulk FAP or CaF<sub>2</sub> solid phase, in contrast, has limited impact on crystal growth or dissolution kinetics. Both FAP and CaF<sub>2</sub> can provide F<sup>-</sup> to the solution phase to enhance remineralization and retard demineralization of enamel HAP crystals. The FAP provides much of this F<sup>-</sup> under low pH conditions, while CaF<sub>2</sub> provides F<sup>-</sup> at neutral or lower pH. The reactivity of fluoride on sound and carious enamel differs significantly. Carious enamel acquires more fluoride, acquires it more quickly, and itself acts as a source of retained fluoride in comparison with the more limited reactivity of sound enamel. Overall, the most important question concerning fluoride reactivity relates to its efficiency in enhancing remineralization or retarding demineralization processes. This influence is not only by the reaction products, e.g., loosely or firmly bound fluoride, but also by the nature of the enamel substrate and frequency of application of the topical fluoridation agent. Inasmuch as the reactivity of bulk HAP is dominated by surface layers of FAP material, the debate over usefulness of various fluoride reaction products solely on a chemical level is no longer critical. Instead, all factors influencing the efficiency of a fluoridating regimen must be considered in the development of improved systems for caries prevention.


Introduction.

Fluoride has been proven to be an effective anti-caries agent when delivered in many forms and concentrations, including low levels of ionic fluoride in drinking water supplies, as well as a variety of fluoride compounds including monofluorophosphate, sodium and stannous fluoride in over-the-counter dentifrices, mouthrinses, and professionally applied (prescription) gels, varnishes, and lacquers (Nikiforuk, 1985; Wel, 1985; Mellberg and Ripa, 1983). The general effectiveness of fluoride under these varied conditions of application is intriguing, since the chemical reaction products and locations of fluoride reactivity within the dental tissues vary widely, depending upon the modality of treatment. This suggests that more than one type of effect of fluoride reactivity may be responsible for its anti-caries effects. Over the years, considerable research has been devoted to elucidating the most (clinically) beneficial, cost-effective, or efficient means of fluoride treatment for the prevention of caries.

One aspect of fluoride reactivity that has raised considerable controversy is the debate over the benefits of “firmly” vs. “loosely” bound fluoride in the prevention of caries. Historically, the adjectives “loose” and “firm” have served as alternative descriptions for calcium fluoride (hereafter CaF<sub>2</sub>) and fluorapatite (hereafter FAP) formation (ten Cate, 1984). These adjectives presumably originated from consideration of the solubility of these fluoridated minerals (with CaF<sub>2</sub> being more soluble than FAP) and from various retention studies of these fluoride products on tooth surfaces (Ogaard et al., 1983; Dijkstra, 1982; Dijkman et al., 1982; Larsen et al., 1981; Retief et al., 1980; Chow, 1977; Arends and Schulhof, 1975; Grön, 1973; Baud and Bang, 1970; Aasendahl et al., 1968; McCann, 1968a; Brudevold et al., 1967; Richardson, 1967; Mellberg et al., 1966; Brudevold et al., 1956).

Today, two schools of thought have emerged concerning the role of CaF<sub>2</sub> and FAP in caries prevention. One views firmly bound fluoride, in the form of FAP mineral, as most beneficial for caries efficacy, owing to its lower solubility and therefore presumably its increased retention within dental tissues. Studies discussing the principles behind the cariostatic effects of firmly bound (and in some cases loosely bound) fluoride include those of Mellberg and Ripa, 1983; Casalvics et al., 1975; Ericsson, 1977; Grön, 1973, 1977; and McCann, 1968b. In some instances, researchers in support of the importance of FAP mineralization have viewed loosely bound fluoride as an undesirably labile form of fluoride reactivity on the enamel (see, for example, McCann, 1968b). Overall, this view has held considerable support among researchers for many years, following McCann’s original studies on fluoride mineral solubility. Further research used in support of this concept came in the form of various studies showing a lack of correlation between enamel fluoride content and caries incidence reported in the early 1970’s (for an excellent review, see Mellberg, 1977). These studies were relevant to the CaF<sub>2</sub>/FAP question, since modalities to increase enamel fluoridation in many instances involved significant amounts of CaF<sub>2</sub> formation (Grön, 1977).

In recent years, however, there has been renewed interest in loosely bound fluoride as a reaction product of fluoridation to act as a potential “reservoir” or “depot” source of solution fluoride enhancing remineralization and retarding demineralization processes. This interest has been sparked in part by research demonstrating that fluoride in the solution, rather than in the bulk solid, phase has the most dramatic impact upon both remineralization and demineralization reactions of enamel minerals (Wong et al., 1987; Arends et al., 1984; ten Cate, 1979, 1984; ten Cate and Duijsters, 1983; Nelson et al., 1983; Silverstone, 1977). In addition, research now shows that topical which are applied frequently, such as daily-used dentifrices and mouthrinses, can provide renewed sources of fluoride in enamel and plaque, changing our perspective from studies of material formed from single high-concentration exposures at infrequent intervals (White, 1987a, 1988; Featherstone et al., 1986; Reintema et al., 1985; Stokey et al., 1985; Featherstone, 1983). Consistent with these mechanistic in vitro and in vivo studies, another school of thought has evolved which views CaF<sub>2</sub> itself as a potential reservoir source for solution ionic fluoride, bene-


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fitting both de- and remineralization (de Bruyn, 1987; Dijkman et al., 1985; Arends et al., 1984; Dijkman, 1982; Larsen et al., 1981; Fejerskov et al., 1981; Mellberg, 1977).

When these diverging views are considered, it is clear that a more detailed understanding of the conditions affecting "firmly" and "loosely" bound fluoride formation, dissolution, and conversion is necessary for the forms of fluoride reactivity most beneficial for caries prevention to be determined. (See Acta Odontol Scand 46: 325-397, 1988, for a recent symposium dealing with this subject.) This paper summarizes recent contributions to our knowledge of the chemical reactivity of fluoride and the location of its effects in assessments of the impact of firmly and loosely bound fluoride on caries processes. In addition, we describe some preliminary studies utilizing the constant composition mineralization technique (White et al., 1988b; de Rooij and Nancollas, 1984; Tomson and Nancollas, 1978) which examine the impact of topical fluoride on the de- and remineralization processes in artificially carious enamel.

Materials and methods.

Artificial caries lesions were prepared in lactic acid/polymer gels by use of methods described previously (White, 1987b). Lesions were soaked in 25 wt% water slurries of placebo (no added fluoride) or NaF (Advanced Formula Crest-1000 ppm F as NaF) dentifrices, respectively. After being rinsed in distilled water, the treated lesions were subjected to constant-composition de- or remineralization. For demineralization, the solution concentrations were 3.5 mmol/L calcium, 2.1 mmol/L phosphate, 0.05 mol/L lactic acid, pH 4.5, with the ion strength adjusted to 0.15 mol/L with NaCl. For remineralization, the supersaturated solutions contained 1.0 mmol/L calcium, 0.6 mmol/L phosphate, pH 7.0, with ion strength also adjusted to 0.15 mol/L. The rates of de- and remineralization were monitored by the measurement of titrant addition required to maintain constant solution composition (Tomson and Nancollas, 1978; White et al., 1988b; de Rooij and Nancollas, 1984).

Chemical reactivity of fluoride with apatite and enamel: The chemistry of loosely and firmly bound fluoride.

Previous reviews have carefully documented the main features of fluoride reactivity with apatite substrates in dental enamel (Arends et al., 1984; Moreno et al., 1977; Brown et al., 1977; Grøn, 1977). In this section, the chemical reactions of fluoride and the chemistry of the reaction products are supplemented with recent observations considered to be important toward addressing the question of the relative benefits of CaF2 and FAP in caries prevention.

Forms of fluoride reactivity.—The three principle forms of fluoride ion reactivity with apatite are illustrated in Eqs. 1-3:

Iso-ionic exchange of F− for OH− in apatite:

\[
Ca_{10}(PO_4)_6OH_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^- \tag{1}
\]

Crystal growth of fluorapatite from supersaturated solutions:

\[
10Ca^{2+} + 6PO_4^{3-} + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 \tag{2}
\]

Apatite dissolution with CaF2 formation:

\[
Ca_{10}(PO_4)_6OH_2 + 20F^- \rightarrow 10CaF_2 + 6PO_4^{3-} + 2OH^- \tag{3}
\]

As highlighted in previous reviews, these reactions can be strongly modified by the source of the fluoride (e.g., monofluorophosphate vs. inorganic fluoride), co-ions (e.g., stannous, zirconium, titanium, amine surfactants), and conditions of fluoride reactivity (e.g., pH, F concentration). Importantly, however, it can be generally said that reactions 1 and 2 take place with low fluoride levels in solution (such as between 0.01 and 10 ppm F) and are thus representative of the kind of chemistry anticipated from topical exposure to fluoridated water or elevated salivary levels of fluoride (from either systemic or topical sources).

In terms of ion exchange, research has recently corroborated that this process readily takes place with some concomitant HPO_4^{2-} loss from the surface layer of apatite crystallites (Lin et al., 1981; White et al., 1988a). In terms of crystal growth of FAP, the principal effect of fluoride is to increase the thermodynamic driving force for apatite mineralization (Moreno et al., 1977; Brown et al., 1977), thereby increasing the overall growth rate of mineral, although when these reactions are considered, it must be remembered that different solid solution fluorohydroxylapatitic phases can form, depending upon the solution fluoride level. In general, the increased rate is typically found to be directly proportional to the increase in fluoride concentration, but is more correctly correlated with increased supersaturation. In some cases (Amjad and Nancollas, 1979; Meyer and Nancollas, 1972), fluoride at lower levels (i.e., from 5.3–53 μmol/L) has been found to retard mineralization processes (although above these concentrations, the anticipated rate enhancement was observed). This effect remains unexplained at present but may involve kinetic inhibition of precursor phase formation by the fluoride, or effects of growth morphology of FAP onto HAP substrates of different crystallographic habit. Most importantly, comparison of constant-composition mineralization studies demonstrates that the crystal growth of HAP onto FAP (bulk phase) is not demonstrably faster than growth onto pure HAP material (Amjad et al., 1981; Koutsoukos et al., 1980), again confirming that it is mineralization in the presence of solution fluoride that is a key to the observed enhanced mineralization rates. Fig. 1 shows the constant-composition crystal growth of hydroxyapatite in solutions with various initial fluoride contents ranging from 7.95–53 μmol/L (i.e., 0.15–1.0 ppm F), in which the large increases in crystallization rate are clearly observed with increasing fluoride concentration. The chemical reactions in Eqs. 1 and 2 result in fluoride incorporation that, in a traditional sense, would be described as firmly bound, since it is part of the apatitic structure. As the fluoride concentration in solution is raised, additional chemical reactions begin to dominate the mineral fluoridation process, including significant amounts of calcium fluoride formation. In general, fluoride concentrations in the range from 5.3 to 53 mmol/L (100–1,000 ppm, i.e., those encountered with fluoride topicalics, such as professional gels and varnishes or over-the-counter toothpastes and mouthrinses) are required to produce significant amounts of CaF2 as a reaction product. This is illustrated in Fig. 2, showing the uptake isotherms of fluoride onto hydroxyapatite and dental enamel (White et al., 1988a). Solution chemical analyses suggest that the fluoridation process shown in Fig. 2 can be described by ion exchange/crystal growth of FAP in the region up to and including the plateau of the curves, while fluoridation at higher concentrations causes the formation of calcium-fluoride-like precipitates. As also shown in Fig. 2, the reaction profiles are strongly dependent upon pH, with CaF2 formation occurring with fluoride treatments in the range of 500–1000 ppm fluoride (i.e., 26.3–52.6 mmol/L) at neutral pH, but at only 10–100 ppm fluoride at lower pH.

In the aforementioned study, the highly specific ^19F Magic Angle Spinning-NMR method also elucidated some key fea-
Fig. 1.—Effect of various levels of solution fluoride (from NaF) on constant composition crystal growth of hydroxyapatite at pH = 7.4, ionic strength = 0.15, Ca/P = 1.67, [Ca] = 1.1 mmol/L. 0 ppm F; △ 0.15 ppm F; □ 0.4 ppm F; ◊ 1 ppm F (1 ppm F = 52.6 μmol/L).

Fig. 2.—F uptake isotherms on hydroxyapatite and enamel substrates under various conditions of solution treatment (from White et al., 1988a). (Top) Fluoride adsorption at pH 7.0: △, ○, synthetic HAP; ○, human dental enamel; X, data from Lin et al. (1981). (Bottom) Comparison of F uptake at pH 7.0 and 4.5: △, ▲, synthetic HAP; ○, X, data from Lin et al. (1981).

Theories of apatite fluoridation that are worth noting relative to the question of loosely vs. firmly bound fluoride. The 19F MAS-NMR technique is a highly selective spectroscopic method which can unambiguously probe the local environment of fluoride (Yesinowski et al., 1983; Yesinowski and Mobley, 1983). By use of the Hahn-Spin-Echo technique, it is possible to distinguish between small amounts of surface-formed FAP and CaF₂ in solid samples. In analyzing the samples from the apatite fluoridation experiments in Fig. 2, we noted that FAP-FHAP formation accompanied reactions in all regions of the uptake isotherms, including those in which significant calcium fluoride formation occurred (White et al., 1988a). Thus, loosely bound fluoride cannot be considered as a substitute for, but only as a supplement to, FAP formation.

Reactivity of F-apatite reaction products. Firmly bound fluoride—The formation of FAP and substituted FHAP affects mineral reactivity in several ways. First, we must recognize that the formation of only a few layers of the mineral can dominate the chemical reactivity of the apatitic phase (Brown et al., 1977; Arends et al., 1984). This explains why F-treated apatite or enamel is affected so greatly at fluoride contents of 1–2000 ppm [i.e., 52.6–105.2 mmol/L], well below the levels, 37,000 ppm, in pure FAP (ten Cate, 1984]). Overall, the formation of FAP or FHAP layers has the most dominant effect on the solubility product, crystallinity, and acid resistance of the minerals formed. The thermodynamic Ksp of FAP is two orders of magnitude lower than that of HAP (7.1 × 10⁻⁴⁴ for pure FAP, as opposed to 2.35 × 10⁻⁹⁰ for pure HAP [Amjad et al., 1981; Brown et al., 1977]). As Brown has pointed out, these differences alone, while real and measurable, are not sufficient to account for the dramatic effects of fluoride on the acid resistance of enamel and apatite (Brown et al., 1977). Instead, it is currently believed that it is the influence of fluoride released from the solid during dissolution (or the effect of ambient fluoride in the demineralization fluid) which is most active in suppressing the dissolution of apatite. It has been shown, for example, that plaque fluid is supersaturated with respect to both HAP (Moreno and Margolis, 1988) and FAP (Carey et al., 1986). Clinical evidence supporting the importance of ambient solution fluoride on caries resistance has been provided by research demonstrating that the caries activity of residents of fluoridated areas increases significantly following discontinuation of fluoridation (Way, 1964; Lemke et al., 1970).

The effect of adsorbed and solution fluoride on apatite dissolution in a closed system is illustrated in Fig. 3, from recent studies by White and McClanahan (1988), which are in agreement with the findings of Wong et al. (1987) and Nelson et al. (1983). Overall, bound fluoride in the form of FAP or FHAP is only minimally released under neutral pH conditions (McCann, 1988b). As a result, the greatest influence of firmly bound fluoride is on the acid resistance of mineral phases to dissolution under acidogenic conditions.

Loosely bound calcium fluoride.—The most notable aspect of CaF₂ reactivity is its increased gravimetric solubility relative to that of FAP and FHAP in fluids simulating the oral environment. By use of a Ksp of 3.47 × 10⁻¹¹ moPL⁻³ (Shyu and Nancollas, 1980), calculations confirm that this phase would ordinarily be undersaturated in saliva or plaque fluids. Thus,
CaF$_2$ as a reaction product on enamel (apatic) would be expected to dissolve rapidly in the oral environment, as McCann pointed out some 20 years ago (McCann, 1968b; Brudevold et al., 1956). In recent years, however, researchers have observed significant retention of fluoride in the form of “CaF$_2$-like globules” in vivo both on sound enamel and within cavities enamel following topical exposures to fluoride (Bruun et al., 1983; Larsen et al., 1981; DePaola et al., 1978; Øgaard et al., 1983; Dijkman, 1982; de Bruyn, 1987; Arends et al., 1984). Researchers have also shown that the solubilization of CaF$_2$ in the oral solution is probably more complex than in in vitro studies, with phosphate, pyrophosphate, and salivary macromolecules acting as strong inhibitors of calcium fluoride dissolution (Lagerlöf et al., 1988; Kanaya et al., 1983; Chandler et al., 1982; Rölla and Øgaard, 1986). It has been speculated that the formation of surface phases of calcium-phosphate-fluoride might act as a pH-triggered fluoride source, providing high levels of solution F$^-$ for suppression of mineral dissolution. In terms of mineral growth, CaF$_2$ has been shown to promote the nucleation and mineralization of FHAP in a closed constant-composition system (Koutsoukos, 1980); however, since the CaF$_2$ formed in situ is usually derived from, or at least occurs on, an apatic substrate, it is more likely that the acceleration of apatic growth via reservoir availability of F$^-$ from dissolving CaF$_2$ would be more important.

**Fig. 3—Effect of adsorbed and solution fluoride on apatite dissolution** (0.10 M, lactic acid, pH = 4.5) from White and McClishan (1988). *Non-treated control; Δ 0.5-ppm F pre-treated onto HAP; □ 1-ppm F in demineralization solution.*

Benefits observed for fluoride substitution in the firmly bound form. At neutral pH, however, the ambient F$^-$ level in equilibrium with FAP or FHAP is expected to be quite small (McCann, 1968a). Since the F$^-$ level in solution strongly affects the growth rate of apatite, firmly bound fluoride probably provides limited reservoir F$^-$ for increasing the ambient salivary mineralization capacity. The reservoir capacity of loosely bound CaF$_2$, in contrast, would be not significantly influenced by solution pH (neglecting the formation of surface PO$_4$ layers). At equilibrium, a CaF$_2$ solid could provide a high ambient solution level of fluoride, much greater than from firmly bound FAP. This fluoride could, of course, act to enhance remineralization processes in sub-surface carious or surface-softened carious enamel. Thus, while both FAP and CaF$_2$ can be pictured as reducing demineralization through the release of fluoride into acidic undersaturated demineralization solutions, CaF$_2$ would be expected to exert a greater effect on the remineralization rate of adjacent minerals through release of fluoride at neutral pH as well.

**Fluoride reactivity in sound and carious enamel.** Over the last decade, research in a direction different from that discussed above has also contributed significantly to our understanding of firmly and loosely bound fluoride effects. This involves the *site* of fluoride action in the tooth substrate—more precisely, sound vs. carious enamel. In this section, we suggest that carious enamel itself acts as a retention site for fluoride, which is retained quite strongly regardless of the source of the fluoridation reaction, and which can be differentiated from from sound enamel in terms of its effect on caries.

**Reaction of F$^-$ in sound enamel.**—Sound enamel acquires fluoride through both systemic and topical routes (Nikiforuk, 1985; Mellberg and Ripa, 1983). In general, topical reactions of fluoride with sound enamel are restricted to the outer - 10 μm of the teeth due to diffusion limitations (Arends et al., 1984). At the low concentrations in saliva and systemically, it would seem likely that fluoride forms mostly fluorapatitic material in the surface enamel. Studies have found the crystallites in surface enamel to be larger in diameter than in subsurface (sound) enamel, reflecting the effects of FAP formation on crystallinity (Arends et al., 1983). Although the formation of fluoridated mineral at the enamel surface might be expected to impart significant acid resistance to the mineral, studies have shown limited association between sound enamel fluoride levels and caries resistance (Mellberg, 1977; Weatherell et al., 1983). This could be due to the limited depth where enhanced fluoridation occurs in this tissue. In general, significant enhancement of fluoridation of sound enamel requires reaction conditions conducive to calcium fluoride formation (such as those encountered in APF or high-concentration neutral NaF gels). While systems forming calcium fluoride on the exterior of sound enamel have shown clinical efficacy, a limitation to their reactivity and presumably efficacy for caries prevention is the loss of fluoride from the enamel with time (Ericsson, 1977; Mellberg and Ripa, 1983). As already mentioned, the cause of fluoride loss has been mostly attributed to the increased solubility of CaF$_2$; however, the rapid loss is probably also affected by the accessibility of the outer enamel surface to abrasion and chemical attack by various foods and beverages (Schamshuka et al., 1979). Most importantly, sound enamel would appear to have a limited capacity for fluoride incorporation and thus a limited capacity (in terms of duration of time that elevated fluoride could be provided) to act as a reservoir fluoride source. The fluoride that remains in the tissue (long-term) near the surface is firmly bound and, following the loss of all labile CaF$_2$, not likely to act as a reservoir source for...
oral tissues. This narrow fluoride layer does not apparently act to limit caries susceptibility of the teeth, as described recently by Weatherell et al. (1983). Reactions with agents which increase sound enamel fluoride would work best if performed frequently, so that reservoir levels of fluoride within the mouth can be maintained.

Reactions of fluoride in carious enamel.—In contrast to sound enamel, enamel softened or made initially carious by acid is extremely reactive with fluoride. The increased porosity and surface area within the enamel allow for greater penetration and increased specific surface area for overall reaction in the caries lesions (Weatherell et al., 1977; Silverstone, 1977). Studies have consistently reported greater fluoride content for carious enamel directly adjacent to sound enamel (Sakkab et al., 1984; Little et al., 1962; Little and Steadman, 1966). As with synthetic apatite and sound enamel mineral, the carious enamel-fluoride reactions are strongly affected by the fluoride source and level. Importantly, however, the additional effects of the diffusion into the subsurface areas strongly influence the reactivity of the acquired fluoride, most importantly affecting the retention of the fluoride. Numerous denture case studies (experiments in which artificially carious or softened enamel are placed in intra-oral appliances), for example, have demonstrated that fluoride is rapidly taken up into carious enamel over periods of 2-4 weeks in situ. Fluoridation occurs readily from both water supplies and diet, but it is increased dramatically by the daily use of fluoridated dentifrices and mouthrinses (Reimtsema et al., 1985; Stookey et al., 1985; Bowman et al., 1988a-d; Mellberg and Chomicki, 1983, 1985). The acquired fluoride has been shown to accelerate remineralization significantly (White, 1987a, 1988; Mellberg et al., 1985; White et al., 1985; Corpron et al., 1986; Featherstone et al., 1982).

In addition, studies show that lesions treated with fluoride remain significantly resistant to secondary acid attack, i.e., developing so-called “acquired acid resistance” (White et al., 1988c; Koulourides, 1982; Featherstone et al., 1988; White and Featherstone, 1987; Koulourides and Housh, 1983). Critically, the carious enamel, despite high levels of fluoride uptake, does not lose significant amounts of fluoride during demineralization or salivary equilibration. In a previous study, Clarkson et al. (1981) demonstrated fluoride redistribution rather than loss from sound enamel during demineralization. Koulourides and Housh (1983) showed that acid-softened enamel, rehardened by fluoridation, acquired significant secondary resistance to acid attack, again without losing significant amounts of fluoride. This was referred to as “acquired acid resistance”, and it was suggested that this reactivity was critical for the ability of fluoride to arrest decay. In our laboratories, we have subjected artificial caries lesions to pH-cycling conditions similar to those of ten Cate and Duijsters (1982) and ten Cate and Simons (1986), and have assessed the effects of fluoridated dentifrices on remineralization and secondary acid resistance in vitro (White, 1987a, 1988, 1989). Recent test results, shown in the Table, illustrate that, in addition to increased resistance, caries lesions do not lose significant amounts of fluoride during demineralization (White, 1989). Cumulatively, the results of these varied studies suggest that the physical substrate for fluoride reactivity—that is, carious vs. sound enamel—is as important a consideration as direct chemical aspects in descriptions of the cariostatic effects of various forms of fluoride. To a certain extent, the caries lesion can be considered as a significant source of “firmly retained” fluoride. The increased fluoride within the lesion body may serve as an important source of intra-lesion solution fluoride, preventing further acid solubilization of enamel minerals in deeper layers. Consistent with this, several recent in vivo studies have shown a direct correlation between lesion fluoridation, remineralization efficiency, and clinical cariostatic action for fluoridated dentifrices (Bowman et al., 1988a-c; Lu et al., 1987; Sakkab et al., 1984).

Effects of topical fluoride on remineralization and demineralization rates of carious enamel.

Having reviewed some of the general concepts concerning fluoride reactivity, it is instructive that we consider some recent experimental work highlighting the effects of fluoride on carious lesion reactivity. Figs. 4 and 5 show the effects of NaF dentifrice treatment on the reactivity of artificially carious enamel during constant-composition de- and remineralization conditions. In the experiments (see “Materials and methods” for details), artificial caries lesions were prepared in lactic acid gels, and the lesions were treated with slurries of 1100 ppm (57.9 mmol/L) NaF dentifrices. Following brief rinsing, the lesions were placed in de- or remineralization solutions, and the rates of reactivity were monitored under constant-composition conditions. Results clearly showed that NaF topical treatment effected significant increases in remineralization rate and decreases in demineralization rate. Interestingly, analysis of solutions for fluoride demonstrated very little fluoride loss from the carious enamel under either de- or remineralizing conditions. (See paper by ten Cate and Duijsters, 1982; only after many cycles was there significant loss of fluoride from enamel lesions. This demonstrates that the uptake capacity of lesions is apparently very high!)

Discussion.

The debate concerning the benefits of loosely vs. firmly bound fluoride originated from the discovery that the formation of CaF₂ deposits formed on sound dental enamel, following treatments with APF or high-concentration fluoride, were lost with time due to dissolution of more soluble CaF₂ into the oral fluids. From this research, and the theoretical and experimental studies of McCann and co-workers, many researchers concluded that the less-soluble FAP was the most ideal form of fluoride incorporation, and that treatment modalities forming this phase were to be preferred for maximum caries protection from fluoride. These conclusions did not take into account several fundamental factors which have been elucidated more

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Post-AAR ΔZ (from micro-radiography)</th>
<th>F Uptake (µg/cm²) Post-cycling</th>
<th>F Uptake (µg/cm²) Post-AAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Dentifrice</td>
<td>3024 (1043)</td>
<td>1.51 (0.23)</td>
<td>1.61 (0.65)**</td>
</tr>
<tr>
<td>NaF Dentifrice</td>
<td>719 (530)</td>
<td>13.95 (5.68)</td>
<td>15.62 (8.00)**</td>
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(Δ) SD.

** Differences between post-cycling and post-AAR non-significant at p < 0.05. From White (1989).
clearly in recent years concerning the ability of fluoride to prevent decay.

First, research now clearly shows that FAP is a reaction product of enamel/apatite fluoridation with or without CaF₂ formation (White et al., 1988a). Thus, the question of whether CaF₂ is “better” than FAP formation is moot, since both are formed together, in agreement with the theoretical arguments of Nelson and Higuchi (1970).

Second, fluoride in solution has a greater influence on both de- and remineralization than in the form of either bulk pre-
cipitate. In the absence of significant levels of solution fluoride, FAP does not grow significantly more rapidly than HAP, and under “sink conditions” (without F allowed to build up), FAP dissolves in a fashion similar to that of HAP. Obviously, both FAP and CaF₂ can provide F to the solution phase. However, the two reaction products differ in that FAP provides solution fluoride principally under demineralization conditions, since its solubility is too low to provide much fluoride at neutral pH, while CaF₂ provides elevated solution fluoride levels at both neutral and lower pH conditions.

Third, the reactivity of fluoride on sound and carious enamel differs significantly. Carious enamel is much more reactive with fluoride than sound enamel, rapidly acquiring greater amounts of total fluoride. The acquired fluoride strongly enhances both remineralization and demineralization resistance. Interestingly, the fluoride in carious enamel is not readily lost during “remin” or “demin” periods, suggesting that the added complications of diffusion processes in carious enamel result in the lesion serving as its own “retention source” for fluoride. Overall, the reaction products within carious enamel may include either firmly bound FAP or more loosely bound CaF₂. In general, the most important question concerning any fluoride reactivity in carious or sound enamel relates to its efficiency in enhancing remineralization or retarding demineralization processes of the tooth mineral, as well as the time periods where this enhancement can be realized.

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Dr. Frank replied to questions from Dr. Hefferren and Dr. Marthaler that in root canal lesions they had observed bacteria in the cementum and in the tubules of the dentin. In their observations the destruction was abrupt, without an obvious gradient of demineralization. One tubule can be filled with bacteria, and the adjacent one could be empty. Dr. Øgaard suggested that the early stage of enamel caries was surface softening, and Dr. Frank replied that they had observed small surface defects directly related to contact with bacteria, and that these small defects connected with the main subsurface defects. Dr. Silverstone stated that the initial diffusion is fairly evenly distributed through the surface and that even though it is called the intact surface layer, it is at least 20 to 50 times more porous than sound enamel.

Dr. ten Cate asked Dr. LeGeros what evidence there was for the formation of brushite (DCPD) inside the caries lesion. She replied that Drs. Moreno and Margolis had shown that DCPD should theoretically form in the caries lesion. She stated that in biological systems in the presence of fluoride, DCPD should transform to apatite. In reply to Dr. Robinson, who asked where magnesium is in the enamel crystals, Dr. LeGeros said that in synthetic systems small amounts of magnesium in solution are incorporated into apatite, and this is reflected in lower crystallinity and higher dissolution rates. She concluded that magnesium was incorporated into enamel and dentin crystals to a limited extent and that more carbonate permitted more magnesium to be incorporated. Replying to a question by Dr. Øgaard, Dr. LeGeros said that shark enamel contained about 1.8% carbonate compared with about 3% in human enamel.

Otherwise shark enamel was essentially fluorapatite. Dr. LeGeros stated that in studies where they treated shark and human enamel with acid phosphate solution, no DCPD formed on the shark enamel, whereas it did on the human material. When artificial caries-like lesions were made in shark enamel, they progressed more slowly and repaired more rapidly than in human enamel.

Dr. Larsen responded to a comment from Dr. ten Cate that in vivo caries progresses more slowly than in vitro by stating that his experimental conditions were quite different from those in the mouth. In the mouth the plaque fluid volume in contact with the tooth is very small, and in vitro the solution volume was usually relatively large. In vivo this small volume holds the calcium, phosphate, and fluoride, thus normally preventing enamel dissolution to some extent. Dr. Rella asked about the relative roles in the caries process of fluoride in the crystal and in the solution. Dr. Larsen pointed out that human enamel normally had relatively low fluoride concentrations in the 1000-2000 ppm range, that this was far below the 3.9% F in fluorapatite, and that this set the limit for the fluoride effect, being largely from fluoride in the solution surrounding the crystals. Dr. Cüress questioned the concept of resistance of the surface enamel. Dr. Larsen responded that there certainly was a resistance of the surface enamel to acid when the tooth had been in the mouth for some time, but that he could not define any one factor that imparted this resistance. Dr. Koulouridis suggested that the resistance of the tooth to acid attack was a combination of factors, including incorporation of organic materials (protein) into the arrested lesion, and that this organic material plus fluoride contributed to the observed resistance to caries. Dr. Ingram agreed that there was experimental evidence to support this view. Dr. Gedalia and Dr. Bawden asked whether sufficient fluoride was released from dissolving enamel to have a dissolution-retarding effect by then being present in the fluid surrounding the tooth crystals. Dr. Ingram was of the opinion that this was correct and that the initial solubility of the mineral was not as important as the solubility of the subsequent mineral surfaces formed by the action of the fluoride during demineralization and subsequent remineralization.

Replying to a question by Dr. Rella, Dr. Nancollas confirmed that phosphate in solution slows down the formation of calcium fluoride (CaF₂), but that phosphate also adsorbs to the surface of CaF₂, markedly increasing the residence time of calcium-fluoride-like material in the mouth. Dr. Nancollas clarified the role of fluoride in crystal growth, stating that generally fluoride enhances calcium phosphate crystal growth, but that at some very low F concentrations there could be a decrease in growth rate. Dr. Ingram commented that at all the concentrations they had tested, F enhanced crystal growth. In response to Dr. Weatherell and Dr. Margolis, Dr. Nancollas confirmed that consideration of all phases in the plaque fluid was important, especially fluorapatite and calcium fluoride.

Replying to a question by Dr. Silverstone, Dr. Chow advised that laboratory, animal, and human in situ studies had been done which showed increased uptake of fluoride resulting from his two-step DCPD/F treatment system. Dr. Chow responded to Dr. Nancollas that, yes, there could be catastrophic nucleation and growth of crystals on the surface rather than in areas where the mineral is really needed, but SEM examination in their studies had shown that this did not happen. Dr. Arends asked whether it was possible to form DCPD in vivo in the presence of pellicle and saliva. Dr. Chow advised that they had not attempted to detect DCPD after in vivo experiments, but that the enamel F content 30 days after treatment was 1500 ppm higher in the outer 4 µm of enamel in the DCPD/F group compared with the non-DCPD group.

Following Dr. Arends’ presentation, Dr. Nancollas commented that partial conversion of crystal surfaces from hydroxyapatite to fluoridated apatite would alter the solubility and hence the driving force for dissolution. Dr. Arends agreed and responded that at present we do not know whether the adsorbing fluoride is forming a monomolecular layer of fluorapatite or whether it is a partially fluoridated apatite. However, this “adsorbed” fluoride markedly altered the properties of the apatite. Dr. Nancollas suggested that experiments of the same type as those presented by Dr. Arends should be done at pH values below 5.0. Dr. Arends answered questions from Dr. Chow regarding the relative importance of F in the crystal, adsorbed onto the surface, or in solution. He stated that experimental conditions could be chosen so that if the total surface of a crystal of any apatite was covered by the equivalent of a monomolecular layer of fluorapatite, then the dissolution behavior of the crystal would be the same as 100% pure fluorapatite. Dr. Chow pointed out that if enamel crystals dissolved to produce 3 mmol/L calcium in solution, there would need to be 10,000 ppm F in the solid to produce a concentration of 1 ppm F in solution. In reply to Dr. Stamm, Dr. Arends stated that it was not possible to see a monomolecular layer of fluorapatite around a crystal, but that we had to rely on calculations following appropriate experiments.

Dr. Margolis replied to Dr. Ingram regarding experiments
with demineralizing solutions which incorporate calcium and phosphate. He agreed that experiments where the PO₄/Ca ratio was greater than 1.0, as it was in saliva and plaque fluid, should be done, since most studies reported to date have used Ca/P ratios closer to those of hydroxyapatite. Dr. Margolis pointed out, however, that the degree of saturation was probably more important than the ratios.

In response to Dr. Tatevosian, Dr. Margolis confirmed that x-ray diffraction has been used by several authors to demonstrate the presence of mineral in plaque.

In response to Dr. Silverstone, Dr. ten Cate stated that in some circumstances, fluoride can stimulate the formation of a mineral deposit in the surface layer of a caries lesion, arresting it, but blocking complete subsurface remineralization. That is, fluoride is effective in preventing caries, but it does not always (for example, in the above circumstances) enhance remineralization. Dr. Bawden asked for clarification of the mechanism of fluoride action with respect to frequent low-concentration applications vs. high-concentration treatments every six months. Dr. ten Cate replied that there was experimental evidence to show that daily low levels of fluoride exposure markedly shifted the balance from demineralization to remineralization. Consequently, more fluoride can sometimes be given with no better result. With high-concentration treatments, a fluoride reservoir can be formed that interferes with the dissolution processes at the enamel surface over time. Referring to Dr. Ingram, Dr. ten Cate confirmed that he had reported experiments that showed that the uptake capacity of demineralized enamel for fluoride is very high. It took many pH cycles before significant F⁻ was found in the demineralized solution. Perhaps fluoride is redistributed during demineralization to sites which are at risk at that time.

In response to a question from Dr. Nancollas, Dr. Featherstone pointed out that the dissolution conditions in his apatite experiments were such that the mineral loss was essentially linear for 50 min, making the calculation of initial dissolution rates straightforward. However, when apatites with higher carbonate content were used, the dissolution was more rapid, with linearity being lost sooner. Dr. Ingram asked about the effect of specific surface area on the dissolution rates of the apatites containing 0.1 and 3.5% carbonate. Dr. Featherstone replied that these were pressed pellets incorporating polyethylene as a filler and that the surfaces were abraded prior to dissolution. This minimized the effect of particle size. Consequently, the carbonate content then dictated the dissolution rate. The overriding effect on the dissolution rates, however, was that of fluoride in solution in the acid buffer, with the reduction in rate being proportional to the logarithm of the fluoride concentration in solution. Referring to Dr. LeGeros, Dr. Featherstone confirmed that carbonate was lost from the carbonatedapatite during dissolution in the weak acid buffer, and that the fluoride was taken up onto the apatite.

In reply to a plea from Dr. Weatherell for investigators to add a dye to their etching solution when doing surface-etch fluoride analyses, Dr. Wofel stated that many of the studies referred to by him had used the microdrill sampling method, which overcame the problem of spreading of the etchant in the porous tissue. Dr. ten Cate asked whether there was a conversion factor that would allow fluoride concentrations found in vitro to be translated into effective in vivo treatments. Dr. Wofel replied that there was no one factor, since this depended on the laboratory model, as well as on the intra-oral model used. Contributing items were the clearance by saliva, how much fluoride remains in the mouth, and for how long. Dr. Wofel suggested that future experiments should concentrate on plaque chemistry, plaque fluid, actual sites of caries attack, clearance, and retention. Some of these questions need to be answered before better products can be made.

Replying to a question by Dr. ten Cate about the intra-oral experiments with shark enamel in human mouths, Dr. Øgaard stated that the pH was probably very low under the bands, explaining why the mineral loss was relatively large even with the high fluoride content. In reply to Dr. Weatherell who asked what the differences were between human and shark enamel, Dr. Øgaard stated that the carbonate content was lower and that the fluoride content was essentially that of fluorapatite. Dr. LeGeros had earlier stated that the shark enamel she had used contained 1.8% carbonate. Dr. Geddes questioned the structural differences. Dr. Rapa reminded the audience that Gwinnett and Rapa published structural information on shark enamel 20 years ago, and that pore size, crystal orientation, and subsurface characteristics were very different from those of human enamel, perhaps helping to explain some of Dr. Øgaard's observations. Dr. Øgaard agreed that there was no question that shark enamel was completely different from human enamel, but that his experiments were just to show that even mineral containing high fluoride dissolved to some extent. Dr. Øgaard replied to a question by Dr. Stamm that the experiments were done with a unique panel of five or six subjects. He further stated that in their experiments they had not analyzed any extracted human teeth for the presence of calcium fluoride, although they hypothesized that it was present. Only microangiographic assessments had been made. In reply to questions from Dr. Bowen and Dr. Weatherell regarding proof that the globules observed in the in vitro experiments were calcium fluoride, it was agreed that these should be called calcium-fluoride-like material. The exact nature of the material will be dependent on the conditions of formation, such as pH. Dr. Christofersten, for example, reported last year that when phosphate was incorporated, the resulting crystals were much less soluble. Dr. Rølla commented that several investigators had confirmed calcium-fluoride-like material by electron diffraction and x-ray diffraction.

In response to a question by Dr. Ericsson, Dr. Arens suggested that fluoride concentrations for in vivo efficacy may be considerably higher than those found to be optimum under controlled laboratory experiments. Dr. ten Cate emphasized that a "log"-type dose response to fluoride has been shown in vitro and during in situ experiments. In vitro experiments with dentifrices have indicated that 300 ppm was sufficient, but similar in situ models showed that this concentration was definitely not optimum. No one had addressed the question as to which source of fluoride — say, NaF, MFP, or amine fluoride — was the best.

Dr. Whitford suggested that since carbonate and magnesium were implicated in the caries process, we need to know the physiological variables that determine the incorporation of these ions into the tooth crystals. Dr. LeGeros replied that little was known, but predicted that the carbonate:phosphate ratio in the micro-environment of the developing crystal would determine the carbonate content, and that this would also be influenced by the presence of fluoride. Dr. Robinson pointed out that fluorotic enamel contains more magnesium than normal enamel. We still do not know where the magnesium really is; the magnesium location may be a way of distinguishing between normal and fluorotic enamel. The magnesium does not render the fluorotic enamel more soluble. These points need investigating.

Discussion regarding the level of fluoride needed in oral fluids to enhance remineralization and/or inhibit demineralization was considerable. Dr. Ekstrand pointed out that values ranging from 0.1 to 30 ppm in the oral fluids had been de-
scribed during this meeting. He asked whether a "therapeutic level" could be established for maximum effect on the remineralization process. He emphasized that the doses currently in use had been derived empirically and were not based on dose-effect relationships. Dr. ten Cate replied that the concentration of fluoride needed for remineralization in specific circumstances will also depend on the concentrations of calcium and phosphate present. We need more information about plaque fluid conditions before a definite answer can be given.

Dr. Featherstone agreed that a critical factor was the degree of supersaturation with respect to fluorapatite at a particular site; therefore, it was difficult to come up with a "therapeutic level" based purely on inorganic chemistry considerations. He went on to state that we must remember that caries is a plaque-generated disease, the acid generated is different from person to person, as is the base produced, and the saliva factors. We are dealing with big extremes. There is no "magic number", but we may be able to develop a range of numbers that will help. Empirically, if orthodontic patients rinse daily with a 225-ppm-fluoride mouthrinse and use a fluoride dentifrice, caries around the brackets can be completely prevented.

To illustrate the value of the mechanistic studies, Dr. Featherstone referred to a recently published study (Hardwick et al.) where fluoride in drinking water was shown to be effective in teenagers when a previously unfluoridated city was fluoridated. That study is a good example of fluoride acting through the various "topical" mechanisms, as described at this meeting. Dr. Dawes added emphasis to the "topical" effect of water fluoridation by stating that although we say the optimum concentration is only 1 ppm, this value corresponds to 50 times the concentration in saliva. Dr. Ingram referred to results where they had seen a doubling of fluoride concentrations in saliva between non-fluoridated and fluoridated water communities when the subjects were not using other sources of fluorides. The apparently low levels of fluoride in saliva, when elevated by fluoride in the drinking water, or by fluoride-containing products, are available for uptake by the apatitic and are enough to encourage crystal growth.

Dr. Silverstone emphasized some points in summing up the discussions. Even with the recently reported reductions in caries prevalence, there remains a group of approximately 20% of the population who have much of the caries. The most effective anti-caries regimens are those that employ low levels of fluoride on a regular basis, rather than previous regimens that aimed at high fluoride doses to sound enamel. Fluoride uptake by very early lesions is very important as part of the action of fluoride and as one of the measures of product efficacy.