Previous studies have suggested that a major part of the cariostatic activity of fluoride is a function of its concentration in the fluid environment of the teeth [Arends and ten Cate, 1981; Wefel and Harless, 1984; Arends and Christoffersen, 1990; Margolis and Moreno, 1990]. Sodium monofluorophosphate (NaMFP) and sodium fluoride (NaF), which are the most common sources of fluoride in commercial dentifrices, deliver fluoride to oral fluids by different mechanisms. Specifically, NaMFP requires a hydrolysis step to release fluoride to the mouth whereas NaF does not. This hydrolysis step has been postulated [Pearce and Dibdin, 1995] as a major reason for the observation that NaF dentifrices generally show higher salivary and whole plaque fluoride concentrations than NaMFP dentifrices [Duckworth et al., 1994]. In spite of these results, clinical studies have yielded somewhat ambiguous results.
about the relative anticaries efficacy of these ingredients [reviewed by Duckworth et al., 1994]. One explanation for this apparent contradiction is that the concentration of deposited fluoride in whole plaque and the concentration of fluoride in saliva does not reflect the concentration of fluoride in the fluid phase adjacent to the site of de- and remineralization in plaque, i.e. the plaque fluid. Only one previous study (on 6 subjects) has examined plaque fluid fluoride after NaF and NaMFP rinses [Ekstrand, 1997], and this study used a rather high fluoride concentration and did not measure the amount of fluoride in whole plaque or the amount of unhydrolyzed MFP. The purpose of this experiment was to measure the fluoride and MFP ion concentration in plaque fluid, whole plaque, and centrifuged saliva after NaF or NaMFP rinses whose concentration would expose the oral environment to similar levels of fluoride as obtained after dentifrice application, and to determine the amount of MFP remaining in these phases after the NaMFP rinse.

Materials and Methods

Subjects, Fluoride Administration and Sample Sites

The procedures used in this experiment [Vogel et al., 1990, 1992b, 1997] have been extensively described in recent publications and are only summarized here. Fluoride administration and the collection of plaque and saliva samples were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. Ten male and 2 female subjects, ranging in age from 27 to 56 years, with no dentures or unfilled cavities participated in this study. Each subject participated in all parts of the experimental regimen. They lived in an area with fluoridated water supplies and used F-containing dentifrices. Each subject was instructed to brush and floss their teeth thoroughly before a 48-hour plaque accumulation period, and to fast overnight before the samples were collected in the morning. Baseline plaque and saliva samples were collected between 8:30 and 9:30 a.m. followed by a 1-min 20 ml NaF or NaMFP rinse. Additional samples were then collected at 30, 60 and 120 min after rinsing.

The fluoride concentration of the rinses used in this study was 12 mmol/l which is equivalent to 228 ppm. (Although the proper SI unit for ppm fluoride is μg/g, the term ‘ppm’ is used in this manuscript to be in conformity to the common manner in which the fluoride content of these products is denoted in commercial dentifrices.) This concentration is near the maximum values observed by Bruun et al. [1987] in saliva-dentifrice samples obtained 30 s after a 1-min brushing period with 1,000 ppm (53 mmol/l) or 1,500 ppm (79 mmol/l) dentifrices. It also approximates the concentration found in current ‘over the counter’ rinse formulations. The NaFMP used in these experiments was obtained from Pfaltz and Bauer (Stamford, Conn., USA) and was found to contain a mass fraction of 3.5% free fluoride. The pHs of the NaMFP and NaF rinses were 6.1 and 6.4, respectively.

Supragingival plaque samples were recovered from the easily accessible buccal-interproximal surfaces of the molar and premolar teeth (excluding third molars). In plaque samples recovered before the rinse, two samples were recovered, one each from the upper and lower jaws, while after the rinse four plaque samples were obtained, one from each quadrant. Because previous studies have not shown any difference in concentration between left and right sites [Vogel et al., 1992a, 1992b], these results were averaged to obtain concentrations from upper and lower molar jaw sites. This procedure was repeated with a few subjects (2 in the NaMFP and 3 in the NaF experiments) for various reasons (insufficient plaque, obvious debris or contamination of the sample, equipment failure) and the results from these repeated experiments were averaged. At least 1 week separated the application of each rinse to the same subject.

Collection and Analysis of Samples

Before each sample was collected, a plastic plaque collection spatula and a mineral oil-filled microcentrifuge tube were weighed together on a microbalance [Vogel et al., 1997]. The subject was then asked to suck and swallow to remove as much saliva as possible, after which the spatula, held in hemostat, was used to collect the plaque sample. The spatula and sample were then quickly placed inside the oil-filled centrifuge tube (a procedure that prevents sample evaporation) and reweighed for determination of the sample weight. The centrifuge tube was then transported in an ice bath to a refrigerated centrifuge (Eppendorf 5402, Brinkman Instruments, Westbury, N.Y., USA) and spun 5 min (12,000 rpm) at 2 °C to expel the plaque from the spatula and to separate the fluid from the plaque solids. The spatula was withdrawn from the centrifuge tube, and small aliquots (5–15 nl) of the fluid phase of the plaque (i.e. the plaque fluid) were recovered with oil-filled capillary micropipettes [Vogel et al., 1997]. These plaque fluid samples were then diluted (1 part TISAB III, Orion Instruments, Cambridge, Mass., USA to 9 parts of sample) on the surface of an oil-covered ‘inverted’ fluoride electrode apparatus and analyzed for fluoride as previously described [Venkateswarlu and Vogel, 1996; Vogel et al., 1997]. It should be noted that most plaque samples yielded sufficient fluid so that two or three replicates of the same sample were analyzed. Except for dilute samples (below about 4 μmol/l), these replicates usually agreed within 1–2 mV, an error that is much lower than the biological variation of the samples.

Next, the tip of the centrifuge tube was cut, and the plaque residue centrifuged into a tube containing 3–10 μl 0.1 mol/l HClO4 for the room temperature extraction of the whole plaque fluoride, the extraction volume used being dependent on the concentration of F anticipated in the extract [Vogel et al., 1992b, 1997; Venkateswarlu and Vogel, 1996]. After 20 min, the extract was neutralized and buffered by adding an identical volume of 0.1 mol/l NaOH, containing a volume fraction of 20% of TISAB III and centrifuged to clarify the sample. Aliquots of the supernatant were then recovered with capillary micropipettes for fluoride analysis using the under-oil fluoride apparatus. It should be noted in regard to this procedure that (1) the removal of the small volume of plaque fluid does not significantly reduce the fluoride in the plaque residue (i.e. fluoride in the plaque fluid residue is thus nearly equal to whole plaque fluoride); (2) because of the low volumes used in these extractions, a slight correction to the extraction volume was made by assuming that plaque fluid is a volume fraction 35% of the plaque [Tatevossian and Gould, 1976], and (3) the room temperature hydrolysis of NaMFP in 0.1 mol/l HClO4 is slow (no measurable release of F was found from the 20-min acidification of a 100 μmol/l NaMFP solution). However, this acid is strong enough to recover nearly all the fluoride (samples re-extracted for 4 h with 1 mol/l HClO4 recovered only about an additional mass fraction of 15% fluoride). The pH produced by this acidification is also low enough to
inhibit the hydrolytic activity of phosphatase [Pearce and Jenkins, 1977].

Samples of unstimulated whole saliva were collected for 1 min immediately following plaque sampling by expectoration into a tube. An aliquot of this saliva was immediately centrifuged, diluted with TISAB III as described above, and transferred to the surface of the fluoride electrode apparatus for measurement of fluoride.

For samples containing NaMFP, the releasable fluoride in the samples (i.e. the total fluoride) was determined by measuring the F after the MFP in them was hydrolyzed. This was done [Duckworth et al., 1991] by adding a volume fraction of about 1% acid phosphatase (Sigma P1435-590 U/ml, Sigma Chemical, St. Louis, Mo., USA) and remeasuring the samples after 10–15 min. The amount of unhydrolyzed MFP in these samples was then obtained by subtracting the initial concentration from the final value.

Several points should be noted in regard to the measurement of MFP in plaque, plaque fluid and saliva. (1) Preliminary studies found that the addition of this phosphatase solution released 96% of the fluoride from a 1,000 µmol/l NaMFP standard in 5 min insuring that the hydrolysis was essentially complete under the conditions employed here. (2) Hydrolysis is inhibited by the low temperatures used during the centrifugation to obtain the fluid phase of plaque and saliva. (3) In agreement with other studies [Pearce and Jenkins, 1977], preliminary experiments also demonstrated that the rate of hydrolysis of MFP is slow in the centrifuged saliva and plaque fluid obtained after centrifugation. However, in spite of these observations, a small amount of MFP hydrolysis doubtlessly occurred prior to the determination of MFP content. For this reason, although the amount of total fluoride is not affected, the amounts of free fluoride and MFP reported here are, respectively, somewhat of an overestimate and underestimate of the amount of these ions at the time of sample collection. This small error, however, does not affect the conclusions of this study.

Statistical Methods

To normalize the data, fluoride concentrations in the various samples were logarithmically transformed prior to analysis [Bulman and Osborn, 1989]. Comparisons of the fluoride levels associated with the rinses at each time point were made using factorial analysis of covariance (ANCOVA) [Zero et al., 1992], which provided a comparison of the baseline-adjusted means across sites (upper and lower molar), treatments (NaF and NaMFP rinses), and site by treatment interactions. It is noted here that no significant interactions were indicated in any analysis. All statistical tests of hypotheses were two-sided and employed a level of significance of α = 0.05.

Results

The results of this study are shown in table 1 and figures 1–3. These results are comparable to those obtained previously using similar methodologies [Vogel et al., 1992b, 1997] and rinses. Baseline values obtained for whole plaque were also within the range of values summarized by Duckworth et al. [1987]. As in previous studies [Vogel et al., 1992b, 1997, Duckworth et al., 1994], relatively high standard deviations were observed especially in the whole plaque values after fluoride administration.

<table>
<thead>
<tr>
<th>Rinse type</th>
<th>Time</th>
<th>Plaque fluid F</th>
<th>Whole plaque F</th>
<th>Centrifuged saliva F</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF 0</td>
<td>11.8±5.6</td>
<td>1.9±1.6</td>
<td>3.8±1.5</td>
<td></td>
</tr>
<tr>
<td>MFP 30</td>
<td>99±73</td>
<td>10.7±7.9</td>
<td>23±11</td>
<td></td>
</tr>
<tr>
<td>NaF 30</td>
<td>148±77b</td>
<td>23±17b</td>
<td>39±22</td>
<td></td>
</tr>
<tr>
<td>MFP 60</td>
<td>31±21</td>
<td>10.1±8.8</td>
<td>6.7±2.2</td>
<td></td>
</tr>
<tr>
<td>NaF 60</td>
<td>43±25b</td>
<td>21±20b</td>
<td>11.2±4.5b</td>
<td></td>
</tr>
<tr>
<td>MFP 120</td>
<td>16.6±7.5</td>
<td>5.8±5.5</td>
<td>3.7±0.8</td>
<td></td>
</tr>
<tr>
<td>NaF 120</td>
<td>25±13b</td>
<td>11.6±12.3b</td>
<td>3.7±0.6</td>
<td></td>
</tr>
</tbody>
</table>

Statistical comparisons performed on log-transformed data using subject and site as blocking factor (n = 12). Since no interaction between site and rinse was found, the data here are presented for the upper and lower sites combined. See fig. 1 and 2 for individual site data. Figures are presented ± standard deviation.

The table and the figures show, as do other studies with similar rinses [Zero et al., 1988; Vogel et al., 1992b], that salivary fluoride concentrations were very close to baseline by 120 min while the whole plaque and plaque fluid fluoride concentrations remained elevated. This elevation was especially large in the case of whole plaque NaF, which was about 6 times the baseline values. At almost all time periods, the lower molar sites have a greater plaque fluid fluoride than the upper molar sites (fig. 1), but the difference was only significant at 60 min. However, in whole plaque (fig. 2), the upper site values were found to be significantly higher than the lower site values.

Average plaque fluid fluoride concentrations after the NaF rinse were significantly higher than after the NaMFP rinse for all time periods (table 1). Similarly, except for the near-baseline 120-min values, the salivary fluoride was also greater after the NaF rinse, but the difference was only significant at 60 min. A rather large amount of MFP remained unhydrolyzed in the plaque fluid and salivary samples (fig. 1, 3) so that the total fluoride following the NaMFP rinse (i.e. the sum of the free fluoride and the unhydrolyzed MFP) was significantly lower than the NaF total fluoride only in the 120-min plaque fluid samples. The mole percentage of the total plaque fluid fluoride that remained as MFP (calculated from the data of fig. 1) was, averaged for both upper and lower sites, 28, 20 and 14% at the 30-, 60- and 120-min times, while the corresponding percentages for

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saliva (calculated from fig. 3) were 34, 26 and 20%. It should be noted, however, that the 120-min percentages shown in these figures are, because of the small concentration, only an estimate. Similarly, the whole plaque fluoride values were also significantly greater after the NaF rinse (fig. 2), but, unlike plaque fluid and saliva, less than 10% of the whole plaque fluoride remained as MFP 30 min after the NaMFP rinse.

**Discussion**

Figure 1 confirms the observation that fluoride concentrations in the plaque fluid are greater at lower molar sites [Vogel et al., 1992a; Ekstrand, 1997]. However, the reason for the opposite distribution seen in the whole plaque fluoride (fig. 2) is not apparent. As in other studies [Vogel et al., 1992a, 1992b, 1997; Ekstrand, 1997], plaque fluid fluoride greatly exceeds salivary fluoride (table 1); furthermore, the plaque fluid/salivary fluoride concentration ratio was not constant. For example, for the NaF lower molar site data, this ratio was 7.9 at 120 min, while for NaMFP the corresponding ratio was 4.6. The ratio of whole plaque to sali-
vary fluoride was also quite variable: using the same 120min lower site samples as above, the ratio of whole plaque to saliva fluoride was 2.8 for NaF and 1.3 for NaMFP. This result suggests that in kinetic experiments in which several types of fluoride compounds are compared, salivary fluoride is a relatively poor predictor of whole plaque and plaque fluid fluoride.

The high concentration of fluoride in plaque fluid relative to saliva suggests the existence of large labile fluoride reservoirs in plaque. Since, as calculated from the free fluid volume of plaque (above) and the data of figures 1 and 2, the total plaque fluid fluoride was only about a mass fraction ≈ 3% of the total plaque fluoride, the release of even a small amount of fluoride from these reservoirs could maintain an elevated level of plaque fluid fluoride. Although the nature of this binding is unknown, it may include binding to cellular anion retention sites by cation bridging, notably calcium [Rose et al., 1996]. Another probable source is CaF₂ or ‘CaF₂-like’ deposits [Arends and Christoffersen, 1990] which have been proposed as the source of labile fluoride reservoirs found on clean and plaque-covered enamel after in vitro and in vivo fluoride application [Hellwig et al., 1987; Bruun and Givskov, 1993; Rølla and Ekstrand, 1996]. Specifically, assuming a resting plaque fluid free calcium of about ≈ 1 mmol/l [Carey et al., 1986; Margolis and Moreno, 1994], a fluoride concentration less than 16% of

**Fig. 2.** Data on fluoride and unhydrolyzed MFP in the whole plaque residues, same samples as in figure 1. See there for a description of the symbols and table 1 for a statistical comparison of the rinses.
the applied fluoride rinse concentration would begin to precipitate CaF$_2$ (calculated using Chemist, Micro Math, Salt Lake City, Utah, USA).

The rate of release of fluoride by hydrolysis of MFP is pH-dependent and has been shown to occur most rapidly between 7.2 and 8.6 in saliva [Pearce and Jenkins, 1977]. However, it is unlikely that a specific pH effect would be a factor in the values presented here (other than the effect of the natural pH of the plaque and saliva samples), given the similarity of the pHs of these rinses and their low buffer capacities. Because NaF does not require a hydrolysis step to release fluoride, larger amounts of such labile fluoride sources as CaF$_2$ or cellular cation-bound fluoride may form in plaque, salivary precipitates and oral mucosa [Zero et al., 1992] following this rinse. No similar mechanism for retaining the MFP ion is apparent, and in fact the amount of whole plaque MFP shown in figure 2 is similar to the amount of this ion calculated by assuming that all of the MFP is held in the plaque fluid. This enhanced deposition and retention may be the reason for the higher amount of fluoride following the NaF rinse found in this and in previous studies [Duckworth et al., 1994; Ekstrand, 1997]. However, reviews of the clinical literature comparing the anticaries efficacy of dentifrices containing these ingredients have varied from those that suggest clinically equivalent effects [Proskin, 1993; Volpe et al., 1993; Mellberg, 1991] to those which suggest that a small advantage is provided by NaF [Johnson, 1993; Stookey et al., 1993]. Among the mechanistic reasons that have been postulated to explain these apparently discrepant results are (1) a specific effect of the MFP ion to reduce enamel solubility [Grönetal et al., 1971; Ingram, 1972; Duff, 1983], and (2) a synergistic effect of the MFP ion with calcium and phosphate. With regard to the first mechanism, the persistent concentration of the MFP ion found in the fluid phase of plaque and saliva (fig. 1, 3) is relevant and it is noteworthy that when the total fluoride content of the NaMFP samples is considered, only the post-rinse NaF 120-min plaque fluid values are statistically greater than the corresponding NaMFP values. Although the second mechanism is usually considered with respect to the calcium and phosphate content of the dentifrice abrasive [Mellberg, 1983; DePaola, 1993; Sullivan et al., 1997], a more important consideration may be the interaction between MFP and the high concentrations of calcium and phosphate present in the oral environment and more particularly where this interaction occurs. Specifically, following a NaMFP rinse, the interaction of these ions with the fluoride ion, which causes the formation of CaF$_2$ or fluorapatite precipitates, would only occur after MFP hydrolysis within the plaque or in the enamel pores where such mineral phases would be of maximum benefit. On the other hand, following a NaF rinse, the immediate reaction of these ions with fluoride would cause such minerals to form primarily on the surface of the plaque or enamel [Rolla and Saxegaard, 1990] where they would be lost more rapidly, or where they could occlude the surface of a remineralizing lesion [Silverstone, 1977]. Here again the high levels of MFP found in plaque fluid (fig. 1) are relevant, and in agreement with these measurements, a recent diffusion-hydrolysis model for MFP in plaque [Pearce and Dibdin, 1995] pre-

Oral Fluoride after NaMFP or NaF Rinse
dicts an appreciable concentration of MFP within plaque after rinsing. This study also shows fluoride levels from MFP hydrolysis that exceed the concentrations (noted above) for the formation of CaF₂.

The fact that the higher oral concentrations of fluoride seen in this study are not reflected in greater clinical anticaries efficacy suggests that the modes of action of NaF and NaMFP rinses and dentifrices are not being fully described by current caries models, that these measurements may not fully reflect the concentrations of ions at the surface of the enamel which are relevant to the de- and remineralization process, or that these increases in oral fluoride simply do not produce significant differences in clinical studies comparing these ingredients. In any case, the analytical techniques employed in these experiments can produce a more complete knowledge of the anticaries mechanisms of NaF and NaMFP dentifrices and should prove useful in the development of more effective treatment modalities.

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References


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