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Distribution of Fluoride in Saliva and Plaque Fluid after a 0.048 mol/L NaF Rinse

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An ultramicro method has recently been described for measurement of plaque-fluid fluoride concentration (Vogel et al., 1990a). This method was used: (1) for exploration of the variation in fluoride concentration of plaque fluid collected from the same buccal tooth sites following a 0.048 mol/L NaF (0.2%) rinse, and (2) for examination of the distribution of fluoride in plaque fluid and saliva within one hour after this rinse. Results indicated an average coefficient of variation (CV) of 31% for plaque-fluid fluoride in triplicate samples recovered simultaneously from the buccal-proximal region of two teeth after the rinse. This was similar to the CV found for plaque-fluid fluoride from the same sites after separate administrations of the rinse. A strong linear correlation was found between salivary and plaque-fluid fluoride at 30 and 60 min after rinse administration, showing that plaque-fluid fluoride is influenced by the concentration of salivary fluoride after administration of this rinse. Plaque-fluid fluoride concentrations were higher than that in saliva at baseline, 30, and 60 min. Very large inter-site and intersubject variations in plaque-fluid distribution were observed, with the central incisors showing the slowest clearance. These variations suggest that an examination of plaque-fluid fluoride from specific tooth regions may be essential for understanding the effects of fluoride on the site-specificity of caries.

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Introduction.

Recent studies on the cariostatic action of fluoride have indicated the importance of fluoride in the fluid environment of the teeth (Arends and ten Cate, 1981; ten Cate and Duijsters, 1963; Margolis et al., 1986). Although numerous studies of salivary fluoride concentrations have been made, few measurements have been made on the extracellular phase of the plaque (e.g., plaque fluid, Tatevosian and Gould, 1976), and most studies have been made on pooled samples. However, local differences in fluoride concentration at the site of a cariogenic challenge (i.e., in the plaque fluid) are likely to be major factors governing caries etiology. Recently, Vogel et al. (1990a) described a micro-analytical method capable of measuring fluoride concentrations in very small volumes of plaque fluid. In the present study, this method was used: (1) for establishment of the distribution and reproducibility of plaque-fluid fluoride from the same tooth sites through repetitive examination of a few subjects within one h after a 0.048 mol/L NaF (0.2%) rinse, and (2) for study of the distribution of fluoride in plaque fluid and saliva after this rinse. The results of the first set of experiments were used in the selection of a more homogeneous set of sites to be included in Experiment 2.

Materials and methods.

Experimental design.—In all experiments, plaque samples were recovered from the buccal-proximal region (deep interproximal areas were avoided) of two adjacent teeth. In Experiment 1, the coefficients of variation of plaque-fluid fluoride concentrations in samples from molar and incisor teeth sites (16/15, 11/21, 25/26, 46/45, 41/31, 35/36) were measured before the rinse, and at 10, 20, 30, and 60 min following the rinse. During these experiments, all of the whole saliva was collected in five-minute fractions, beginning five min before the administration of the fluoride rinse and ending at 65 min after the rinse. Experiment 1-A measured the variability of fluoride concentrations in three plaque-fluid samples recovered simultaneously from the same sites on subject L over the course of three rinses. Experiment 1-B provided: (1) information about the variability of plaque-fluid fluoride concentrations in replicate experiments and (2) a profile of plaque-fluid fluoride in two subjects (L and H) having very different average salivary flow rates (Subject L, 0.45 ± 0.12 mL/min, n = 91; Subject H, 0.96 ± 0.20 mL/min, n = 65 (± refers to the standard deviation, and n is the number of determinations)). Six experiments were performed on these subjects, with the same collection sites and times as in Experiment 1-A.

The results of experiment 1-B (below) suggested that plaque-fluid fluoride clearance from the central incisor sites was quite variable between subjects. Therefore, in Experiment 2, only molar sites (16/15, 25/26, 46/45, 35/36) were used. Plaque-fluid and salivary fluoride concentrations were examined in four additional individuals (Subjects A, B, C, and D) at 30 and 60 min after fluoride administration. The average flow rates of these subjects fell within a narrow range: Subject A, 0.64 ± 0.20 mL/min, n = 79; Subject B, 0.68 ± 0.19 mL/min, n = 65; Subject C, 0.77 ± 0.17 mL/min, n = 14; and Subject D, 0.52 ± 0.08 mL/min, n = 38. Five-minute salivary samples were obtained immediately before administration of the rinse and before and after plaque collection. This experiment was repeated several times with each subject, although in some of the experiments salivary samples were not obtained.

Baseline concentrations of plaque-fluid fluoride for all subjects except Subject C were determined in separate experiments where the conditions were the same as those used for the NaF administration, with distilled water substituted for the fluoride rinse.

Subjects and fluoride administration.—Subjects consisted of three females and three males, aged 24 to 46, who lived in an area where the water is fluoridated. Subjects chosen had no decayed or filled teeth at the collection sites. Forty-eight h before each experiment, the subjects thoroughly flossed and brushed their teeth. The toothpastes used contained fluoride; however, the fluoride baseline (given below) was similar to that in recent experiments (Vogel et al.,...
1992) in which plaque removal was done with a non-fluoride dentifrice. Subjects refrained from further flossing and brushing before the experiment. The experiments started in the morning, with the subjects instructed not to eat or drink (except water) before the experiment. Each subject rinsed with 20 mL of an aqueous solution of 0.048 mol/L NaF for one min. This large volume was chosen to minimize any asymmetry in the initial distribution of the rinse. The subjects were instructed to keep their heads still and tilted forward at a slight angle, so that saliva flowed toward the front of the dorsum of the tongue. Although the choice of head position may have influenced fluoride distribution and clearance at the different sites, a standardized head position was considered essential.

Saliva and plaque-fluid collection and analysis.—Saliva was collected by a fine polyethylene tube connected to a slight vacuum that aspirated saliva from the dorsum of the tongue into a pre-weighed test tube. The collection tube was inserted so that saliva could be constantly collected while the mouth remained nearly closed. Each five-minute saliva sample was weighed for the determination of salivary flow and immediately diluted [nine parts sample with one part total-ionic-strength adjusting buffer (TISAB) containing CDTA (Oliveby et al., 1990)]. The samples were analyzed in triplicate with a standard fluoride electrode. Additional measurements of flow rate were done for Experiment 2.

During plaque collection, the saliva collection tube was placed at the gingival margin of the tooth for removal of excess saliva from the site. Plaque samples were collected in under two min with a plastic point cut from plastic cover slips (Fisher Scientific, Pittsburgh, PA) and held in a small hemostat. Immediately after plaque collection, each plastic point with sample was placed in a mineral-oil-filled microcentrifuge tube, prepared by pulling a point on the end of a glass tube (6 mm OD by 5 mm ID). This tube was then inserted into a cut-off 500-μL plastic microcentrifuge tube and centrifuged at 12,000 g and 5°C for 15 min. Recovery of the plaque-fluid was done with microcapillaries filled with mineral oil (Vogel et al., 1990a). These microcapillaries were stored until fluoride analysis (1 to 2 h) under an atmosphere of water-saturated 5% CO2/95% N2 gas. Each analysis was done in triplicate, after dilution of the samples with TISAB, by the inverted fluoride electrode technique (Vogel et al., 1990a).

Statistical methods.—The various sites were compared by use of a one-way ANOVA and a Newman-Keuls multiple comparison. Where appropriate, Student t or paired-difference tests were used. These comparisons, and analysis of regression, were performed with commercially available computer programs Instat (Graphpad Software, Inc., San Diego, CA) and Kwikstat (Texasoft, Cedar Hill, TX).

Results.

In Experiment 1-A, the average coefficient of variation (CV) for the triplicate samples simultaneously obtained from various sites and times after rinse administration in Subject L was 31 ± 17%, n = 23. The CV did not appear to be related to the site of collection or the time the sample was obtained. The average CV of the plaque-fluid fluoride concentration of samples recovered from the same site in different experiments (Experiment 1-B) was 32 ± 15%, n = 24, and 46 ± 23%, n = 24, for Subjects L and H, respectively. The average CV of the salivary fluoride concentration was 31.6 ± 7.2%, n = 14, and 37.4 ± 6.8%, n = 14, for these subjects.

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plaque-fluid (μmol/L)</th>
<th>Saliva (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.3 ± 2.1 15&quot;</td>
<td>2.9 ± 1.0 6</td>
</tr>
<tr>
<td>B</td>
<td>15.7 ± 2.7 16</td>
<td>1.1 ± 0.20 6</td>
</tr>
<tr>
<td>C</td>
<td>30.4 ± 8.4 21&quot;</td>
<td>0.90 ± 0.00 1</td>
</tr>
<tr>
<td>D</td>
<td>16.0 ± 4.4 15</td>
<td>1.3 ± 0.44 3</td>
</tr>
<tr>
<td>H</td>
<td>16.0 ± 4.9 11</td>
<td>1.9 ± 0.54 5</td>
</tr>
<tr>
<td>L</td>
<td>17.0 ± 5.5 15</td>
<td>0.87 ± 0.23 6</td>
</tr>
<tr>
<td>average</td>
<td>16.5 ± 0.76 5</td>
<td>1.48 ± 0.76 6</td>
</tr>
</tbody>
</table>

*Data = Average ± SD, number of samples.

*These values were obtained by a different procedure and are not included in average.
An ANOVA test on the plaque-fluid fluoride concentration at each time period (Fig. 1) showed that there were significant differences between some sites for the two subjects used in Experiment 1-B \((p < 0.05)\). However, a paired-difference test found no difference between plaque-fluid fluoride from the left and right molars within either subject. These sites were combined for the plots of fluoride clearance shown in Fig. 2. This Fig. indicates that fluoride concentrations at the molar sites of Subject L were higher than at the corresponding sites in Subject H. Except for the 10-minute sample, these differences were statistically significant \((p < 0.05)\). In contrast, fluoride concentrations at the upper incisor sites were not different between these subjects. However, this site showed a statistically higher fluoride concentration than the other sites \((p < 0.05,\) Newman-Keuls multiple comparison) in both subjects. The lower incisor of Subject L (Fig. 2, left) had initially less fluoride than the other sites, but the clearance of fluoride was very slow from this site and subject.

An ANOVA showed that none of the baseline plaque-fluid fluoride concentrations at each of the six collection sites was significantly different in the six subjects used in Experiment 2. The

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td><strong>MEAN (± SD) AND NUMBER OF SAMPLES FOR MOLAR PLAQUE-FLUID AND SALIVARY FLUORIDE CONCENTRATIONS</strong> (^{(a)}) (µmol/L) AFTER A 0.048 mol/L NaF RINSE</td>
</tr>
<tr>
<td>30 min</td>
</tr>
<tr>
<td>subject</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>H</td>
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<tr>
<td>average</td>
</tr>
</tbody>
</table>

*Salivary fluoride concentration* \(^{(a)}\) refers to the salivary fraction taken just before the plaque collection.

\(^{(b)}\) Significantly higher than the salivary fluoride fraction at both 30 and 60 min \((p < 0.01,\) paired-difference test).

\(^{(c)}\) Significantly higher than in upper molars at 30 min \((p < 0.05,\) paired-difference test).

\(^{(d)}\) Linearly correlated \((p < 0.01)\) with saliva concentration \(r = -0.934\) (upper sites), \(r = -0.936\) (lower sites).

\(^{(e)}\) Linearly correlated \((p < 0.001)\) with saliva concentration \(r = -0.974\) (upper sites), \(r = -0.986\) (lower sites).
average plaque-fluid baseline fluoride concentration (Table 1) was significantly greater (p < 0.001) than the average baseline salivary concentration. A paired-difference test found no difference between plaque-fluid fluoride from the left and right molars within these subjects, and these data have been combined in Table 2. Calculated as paired data (e.g., 15/16 vs. 45/46, 12 pairs) for each subject, the 30-minute plaque-fluid fluoride concentrations from the lower molar sites were statistically (p < 0.05) higher than the fluoride concentrations from the upper molar sites. However, at 60 min this paired difference was not significant. The 30- and 60-minute plaque-fluid fluoride concentrations were statistically greater than the concentration of fluoride in the salivary fraction taken just before plaque collection (calculated as paired data). The correlations of the linear regressions of the molar plaque-fluid vs. salivary fluoride data were highly significant (Table 2).

**Discussion.**

The average salivary baseline concentration shown in Table 1 (1.48 ± 0.76), although numerically higher, is not statistically different from the average value reported by Oliveby et al. (1990) from 410 samples from children (0.87 ± 1.0 μmol/L) in a 1.2 ppm-fluoride area. A value of about 1 μmol/L has been suggested by Dawes and Weatherell (1990) for basal salivary fluoride. The baseline plaque-fluid fluoride concentrations shown in Table 1 are similar to recent values obtained in our laboratory from molar sites: 14.7 ± 4.0 μmol/L, n = 28 and 12.7 ± 2.8, n = 28 (Vogel et al., 1992). They are, however, higher than those previously reported by this laboratory: 4.9 ± 2.7, n = 53 (Carey et al., 1986). The reason for the lower value in the previous study is not apparent, although it may be related to a more careful conditioning of the electrode in the current study (Vogel et al., 1990a) and the use of an acetic acid TISAB in the earlier study. However, a preliminary experiment did not show any difference in baseline plaque-fluid fluoride between these two TISABS. Apparently, only two studies have been made on plaque-fluid fluoride (Tatevossian, 1978; Moreno and Margolis, 1988). Both studies found baseline values of 2 to 5 μmol/L fluoride; however, no TISAB was added to the samples in these studies, preventing a comparison with the results obtained here.

Although the CV's observed in the plaque-fluid experiments were similar to the CV calculated from replicate salivary fluoride determinations, plaque-fluid fluoride concentrations from the same site sometimes differed by as much as 100%, while the maximum variations in the salivary fluoride were about 40%. Since the CV obtained in simultaneous sampling from the same site after a rinse (Experiment 1-A) was similar to the CV obtained in samples obtained on different days (Experiment 1-B), inter-experimental variables such as changes in the amount of plaque growth at a site do not appear to contribute to the large coefficient of variation found in the experiments reported here.

Plaque-fluid fluoride concentrations were much higher than salivary fluoride concentrations in salivary samples obtained prior to fluoride administration and at 30 and 60 min (Tables 1 and 2). The high level of fluoride in plaque-fluid relative to that in saliva found in this and other recent studies (Vogel et al., 1992) may be due to: (1) a slow removal of ions from the plaque (Vogel et al., 1990b), attributed to the thinness of the salivary film (Collins and Dawes, 1987) or (2) the release of fluoride from sources of fluoride in plaque (Ophaug et al., 1987) such as calcium fluoride (Rolla and Saxegaard, 1990). However, fluorapatite is an unlikely source of plaque-fluid fluoride due to its insolubility under physiological conditions (McCann, 1968). Although the oral mucous has been suggested as a source of fluoride for plaque (Yao and Orr, 1970; Tatevossian, 1978), the decreasing fluoride concentration gradient from plaque fluid to saliva shows that fluoride cannot diffuse from the soft tissue through the saliva and into the plaque. In spite of the high levels of plaque-fluid fluoride relative to salivary fluoride, plaque-fluid fluoride was very strongly correlated with salivary fluoride concentration (Table 2). Since plaque, with its small surface area, is not a likely source of fluoride for the saliva (Zero et al., 1990), this correlation suggests that plaque-fluid fluoride concentration is strongly influenced by the concentration of salivary fluoride after a NaF rinse. This correlation was not observed in recent studies where a rinse designed to precipitate large amounts of labile fluoride was administered (Vogel et al., 1992).

The distribution of fluoride at various oral sites after a NaF rinse was studied, in a limited number of subjects, by Weatherell and co-workers with two complementary techniques: (1) an absorption study, where fluoride was absorbed by dentin splints was measured (Weatherell et al., 1989); and (2) a salivary distribution study, where the fluoride was examined by the taking of salivary samples with paper points (Weatherell et al., 1986). Although the study reported here measured a quite different parameter, some similarities are apparent: (a) Experiment 1-B (two subjects) showed that the upper incisor site maintained a much higher concentration of fluoride than the other sites (Figs. 1 and 2). This is the same site found by Lecomte and Dawes (1987) to show a slow salivary clearance of KCl from an agarose gel. (b) Experiment 2 (six subjects) showed that the upper molars had a lower fluoride exposure than the lower molars at 30 min (Table 2). Some differences in these experiments were also apparent: Unlike the data of Weatherell et al. (1988), which suggest that lower incisors receive a lower fluoride exposure than the lower molars, the plaque-fluid fluoride clearance from the lower incisor plaque fluid was variable in the two subjects examined in Experiment 1-B (Fig. 2).

Although the specific patterns of fluoride distribution and clearance seen in the study reported here and the studies noted above need to be confirmed with a larger number of subjects, large inter-site and inter-subject plaque-fluid fluoride variations appear to be observed after a fluoride rinse. These variations may be ascribed to: (1) variation in fluoride binding and release within the plaque, (2) the site-specificity of salivary clearance in the oral cavity (Lecomte and Dawes, 1987), and (3) the anatomical and physiological characteristics of the individual mouth (Weatherell et al., 1988, 1986).

Numerous studies have shown that the rate of enamel lesion progression and remission can be influenced by the fluoride concentration in the fluid environment of the teeth (Koulourides et al., 1974; Arends and ten Cate, 1981; ten Cate and Duijsters, 1983; Wefel and Harless, 1984; Margolis et al., 1986; Øgaard et al., 1986). Since caries is a site-specific disease, the large variations in inter-site plaque-fluid fluoride observed in these experiments should be considered in any assessment of the cariostatic effects of fluoride (Dawes and Weatherell, 1990). Studies on the intra-oral kinetics of fluoride with the methodology described here should prove valuable in devising treatments that deliver a maximum fluoride benefit.

**REFERENCES**


