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# Effect of a Water Rinse on 'Labile' Fluoride and Other Ions in Plaque and Saliva before and after Conventional and Experimental Fluoride Rinses

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**Key Words**
Fluoride · Labile ions · Plaque · Saliva

**Abstract**
Labile reservoirs are important in maintaining ion concentrations in oral fluids, especially after a fluoride dentifrice application, where a persistent increase in fluid fluoride can mitigate or reverse caries progression. In this study, the effect of experimental and conventional fluoride rinses on the in vitro and in vivo water-induced release of fluoride, calcium, phosphate, acetate and hydrogen ions from oral reservoirs was examined. At the start of each experiment, 13 subjects rinsed either with a conventional 228-ppm fluoride NaF rinse, a 228-ppm fluoride controlled-release rinse (CR rinse) or received no rinse. Sixty minutes later upper and lower molar plaque samples and 1-min saliva samples were collected. The subjects then rinsed with deionized water for 1 min, and 7 min later, a second set of samples was collected (in vivo study). Plaque fluid and clarified saliva were then recovered from samples by centrifugation, and the remaining plaque mass was sequentially extracted with water and acid to measure the water-extracted and total whole-plaque fluoride (in vitro study). All the samples were analyzed using microtechniques for pH, free calcium, phosphate, organic acids (plaque fluid) and fluoride (plaque fluid, centrifuged saliva and plaque extracts). Results showed that in vivo water rinsing decreased acetate and phosphate in plaque fluid, and fluoride in plaque fluid and saliva, but had no effect on plaque fluid pH. In vivo water rinsing, however, increased plaque fluid free calcium, apparently due to water-induced loss of calcium-binding ions. Water- or fluoride-rinse-induced changes in plaque fluid concentration were greater at the lower molar site, suggesting that rinse pooling may influence ion distribution. Before the water rinse, plaque fluid, saliva and whole-plaque total fluoride values were 1.7, 2 and 4 times higher after the CR rinse compared to the NaF rinse. Furthermore, the CR rinse deposited approximately 11 times more water-extracted fluoride compared to the NaF rinse, suggesting a 'more efficient' precipitation of 'labile' or 'loosely bound fluoride'. The results presented here, and in previous studies, suggest the possibility of formulating effective fluoride dentifrices with a lower fluoride content than is currently in use.



A very small increase in the fluid concentration of fluoride has been shown to have a profound effect on the de- and remineralization process. This has led to considerable attention being focused on the formation of 'labile' or 'loosely bound' fluoride reservoirs that can maintain the concentration of fluoride in plaque fluid and saliva after a conventional rinse or dentifrice application [ten Cate and Duijsters, 1983; Wefel and Harless, 1984; Margolis and

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Moreno, 1990; Vogel et al., 1992; White et al., 1994]. Such fluoride reservoirs can be distinguished from potential 'non labile' reservoirs (such as fluorapatite) whose insolubility or low rate of dissolution precludes them as fluoride sources except perhaps at very low pH [Vogel et al., 2000b]. Among the most important labile oral fluoride reservoirs are $CaF_2$ or phosphate-contaminated 'calcium-fluoride-like' deposits [Fejerskov et al., 1981; Øgaard et al., 1983; Rølla and Saxegaard, 1990; Arends and Christoffersen, 1990]. We have described a type of two-part 'controlled release' or A+B rinse for the enhanced oral deposition of labile fluoride by increasing the deposition of $CaF_2$ in oral tissue (to be consistent with previous reports this controlled-release formulation is referred to as a CR rinse in this study). The rinse consisted of two parts: part A contained $Na_2SiF_6$, part B $CaCl_2$ and sodium acetate. The rinses can be termed as 'controlled release' because the rate of hydrolysis of the $SiF_6^{2-}$ ion is controlled by the rate of pH rise and calcium concentration of these solutions when they are mixed. In theory this permits an in-depth penetration by the unhydrolyzed $SiF_6^{2-}$ and calcium ions into oral tissue before hydrolysis and subsequent formation of $CaF_2$ deposits [Chow and Takagi, 1991; Chow et al., 1992; Vogel et al., 1992, 1997, 2000b]. Large and persistent increases in salivary, plaque fluid and whole-plaque total fluoride are produced by the CR rinse, relative to a conventional NaF rinse [Chow and Takagi, 1991; Chow et al., 1992; Vogel et al., 1992, 1997]. However, although studies have shown a very large deposition of $CaF_2$ when the CR rinse was applied to enamel in vitro [Chow and Takagi, 1991] and X-ray analysis of the products after mixing these rinse components show only this mineral [Chow and Takagi, unpubl. data], recent studies have suggested that much of the labile fluoride in plaque is bound to bacteria via calcium bridges [Rose et al., 1996]. It is thus possible that much of the deposited fluoride found after a CR or a conventional F rinse is held in this manner. Furthermore, given the very high supersaturation of plaque fluid and saliva with respect to fluorapatite after a rinse, some of the applied rinse may be lost by precipitation of this phase.

Besides fluoride, increased intraoral concentrations of phosphate and particularly calcium have also been associated with a cariostatic effect [Ashley and Wilson, 1977a; Forward, 1994; Margolis and Moreno, 1994], and a number of treatment regimens have attempted to increase the concentration of these ions in saliva, and especially plaque, by the formation of labile oral reservoirs [Ashley and Wilson, 1977b; Rankine et al., 1989; Vogel et al., 1998, Pearce et al., 1984]. However, fluoride rinses, especially the calcium-containing CR rinse, may alter the amount of these ions or induce the formation of relatively non labile mineral phases, such as fluorapatite or hydroxyapatite. Furthermore, the concept of labile oral reservoirs as sources of calcium, phosphate or fluoride ions raises questions about the effect of the aqueous phase of an experimental rinse in studies in which plaque fluid ion concentrations are examined shortly after the rinse has been administered (for example the analysis of plaque fluid following a sucrose rinse). The purpose of this study was to examine: (1) the effect of a NaF or a CR rinse on the concentration of calcium, phosphate and fluoride in plaque fluid and the concentration of fluoride in whole plaque and saliva, (2) the effect of an in vivo water rinse on these ions, and (3) the amount of labile fluoride in plaque before and following the in vivo water rinse. Because of the different fluoride sources in plaque noted above, the term 'labile' with respect to fluoride must refer to the release of fluoride under a given set of conditions. As described below the amount of labile fluoride in the current study is inferred from an in vitro water extraction.

## Materials and Methods

The procedures used in this experiment have been extensively described in previous publications [Vogel et al., 1990, 1997, 2000b] and are only summarized here. The specific protocol followed in this experiment is illustrated in figure 1.

*Subjects, Fluoride Administration and Sample Sites*

Fluoride administration and the collection of samples were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. Eleven male and 2 female subjects (ages from 25 to 55 years) with no dentures or unfilled cavities participated in this study. All lived in a fluoridated water area and used F-containing dentifrices. During the experimental periods, they were frequently reminded to be meticulous in tooth-brushing and flossing and, as an additional measure, an electric toothbrush (Oral B Laboratories, Redwood, Calif., USA) was given to each subject to promote oral hygiene. Before each part of the experiment the subjects accumulated plaque for 48 h and fasted overnight before sample collection. In the morning, at about 8.30 a.m., subjects rinsed for 1 min with 20 ml of NaF or the CR rinse or they received no rinse. Plaque samples and saliva samples were then collected 1 h later (sample set 1, fig. 1). Immediately following the collection of the samples the subjects rinsed with 20 mL of water (in vivo water rinse) and 7 min later a second set of saliva and plaque samples was obtained (sample set 2, fig. 1). As described below, a third set of saliva samples was also obtained from some of the subjects to examine the effect of different methods of saliva collection.

*Rinse Composition and Mechanism of Action*

Part A of the two-part rinse used here contained 4 mmol/l $Na_2SiF_6$ (Aldrich, Milwaukee, Wisc., USA), while part B contained 20 mmol/l $CaCl_2$ and 50 mmol/l sodium acetate. After mixing parts A and B, the final rinse contained 12 mmol/l (i.e. 228 ppm) fluoride. The sodium

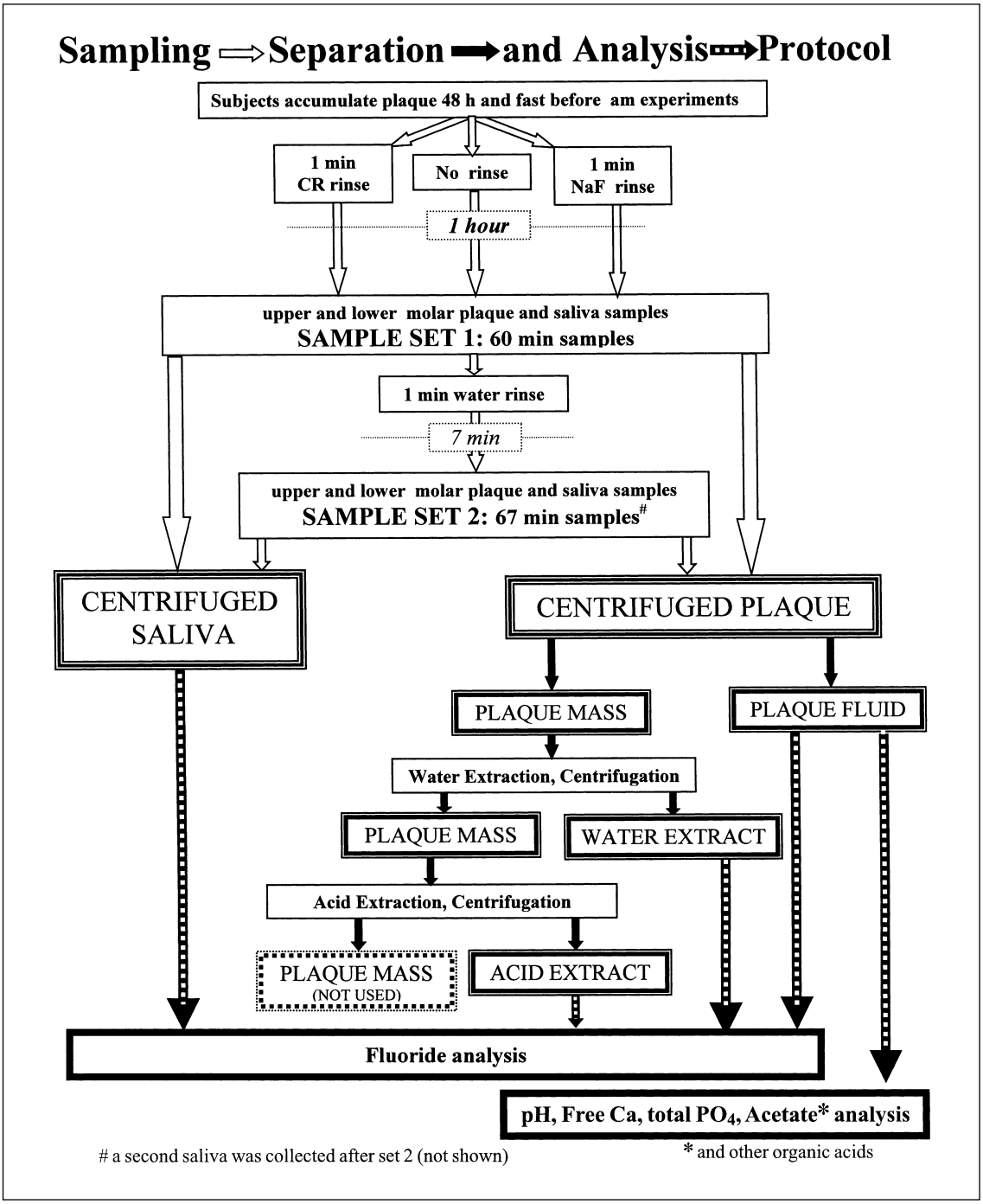


**Fig. 1.** Flow chart showing an overview of the experimental and analysis protocols. The type of the arrows denotes the following: the white arrows refer to the sampling procedure, the black arrows refer to the separation of the samples for analysis and the dotted arrows refer to the analysis.

fluoride rinse also contained 12 mmol/l. This concentration was chosen, because it approximates current over-the-counter formulations and because it is near the maximum concentration found in saliva-dentifrice samples obtained 30 s after a 1-min brushing period with conventional fluoride dentifrices [Bruun et al., 1984]. At least 1 week separated the application of each of the rinses, which were administered in a randomized crossover design, or before the collection of the no-rinse samples. Subjects used their normal fluoridated dentifrices in periods between plaque accumulations.

*Collection of Samples*

Separate upper and lower arch samples of plaque were recovered in plaque samplings 1 and 2 from the easily accessible supragingival buccal/interproximal surfaces of the molar and premolar teeth (excluding the third molars). As much as possible, these samples were recovered from the same regions in the two samplings, being careful on the first sampling to avoid unduly disturbing plaque from the adjacent areas. The method of plaque collection and sample preparation has been extensively described [Vogel et al., 1990, 1997, 1998] and is summarized as follows: collection of plaque with thin plastic strips, weighing and handling of samples under mineral oil to prevent evaporation, centrifugation of plaque to separate plaque fluid and whole-plaque solids in a sealed pipette tip, recovery of plaque fluid with mineral-oil-filled micro pipettes, expelling of the whole plaque residue into a micro centrifuge tube containing an extractant and dispersing of the sample into the extractant to promote recovery. A weighed 1-min saliva sample was collected, as previously described [Vogel et al., 1997, 1998], immediately following the plaque sampling by expectoration without prior swallowing. However, some studies indicated that the lack of saliva removal before collection of plaque samples may have increased the salivary flow rate [Vogel et al., 1998, 2000a] and thus altered salivary fluoride concentration. Therefore, a third saliva sample was taken from 10 of the 13 subjects immediately after the collection of sample set 2 in which subjects were instructed to remove as much saliva from their mouths as possible by swallowing before collection. These saliva samples are referred to as 'sample set 2a'.

*Measurement of Labile Fluoride*

As noted above, an important property of labile or loosely bound fluoride reservoirs is their ability to release fluoride into the oral environment over a prolonged period. Thus the term 'labile', with respect to fluoride, refers to the release of fluoride under a given set of conditions. In the current experiment the amount of labile fluoride in plaque was assessed by examining the in vitro extraction of plaque by water [Vogel et al., 1997]. The water extractant volume was calculated (Chemist, Micromath, Salt Lake City, Utah, USA) to meet criteria based on the calcium, phosphate and fluoride content of plaque determined in previous experiments [Vogel et al., 2000a, b]: (1) all the fluoride held in the known potential labile pools in plaque ($CaF_2$ and fluoride held in 'calcium bridges') should be theoretically liberated, (2) none of the potential non-labile fraction of plaque fluid fluoride, specifically fluorapatite, should be theoretically liberated, (3) the fluoride in the sample must not be outside the analytical range of the fluoride electrode, but should be as dilute as possible to avoid the possibility of re-precipitation of fluorapatite when mineralization inhibitors in plaque fluid are diluted [Venkateswarlu and Vogel, 1996] and finally (4), to keep the comparison unbiased, the same volume of extractant for the CR and NaF samples should be employed. Given the very different characteristics of the labile and non-labile reservoirs examined, these conditions are relatively easy to choose: a mass-to-volume ratio of 3 μl/mg for water in the no-rinse samples and 17 μl/mg for the post-fluoride-rinse samples (either CR or NaF) were found to fit these criteria. Using these volumes of water, a preliminary experiment with fluoride rinse subjects demonstrated that the fluoride concentration in the extract reached an equilibrium value in about 20 min and was stable for at least 3 h; therefore a 1-hour extraction time was employed. However, these experiments also found that when fluoride-rinsed samples were re-extracted with the same volumes, nearly the same concentration of fluoride was present in the second extraction, suggesting that appreciable amounts of fluoride remained after a single extraction. Thus, although the water-extracted fluoride measured under the conditions of this experiment can be taken as a measure of the amount of labile fluoride in plaque, the values do not represent the entire amount of labile fluoride that may be released under neutral conditions. In practice, because the masses of plaque recovered in these experiments were reasonably uniform (as shown below the average plaque masses for the experimental groups are similar), and because of the apparent insensitivity of the extraction to small changes in the extraction volume found in these preliminary experiments, a constant volume of 5 μl was used for the water extraction in the no-rinse samples and 25 μl for the CR or NaF rinse plaque samples (equivalent to recovery of 1.5 mg of plaque). Following the water extraction a similar extraction was done with 1 mol/L $HClO_4$ to measure the amount of total fluoride in the samples [Vogel et al., 1997].

*Analysis Methods*

All saliva samples were immediately clarified by centrifugation after collection and then diluted 9 parts clarified supernatant with 1 part TISAB III (Orion, Cambridge, Mass., USA). The water extracts of plaque were buffered by adding them to an identical volume of a 20% (volume fraction) of TISAB III. However, with the acid extract, the TISAB solution also contained 1 mol/l NaOH to neutralize the sample. A micro transfer pipette [Vogel et al., 1990] was used to remove as much water as possible after the water extraction and to dilute the small volumes employed in the no-rinse samples with TISAB. Because of the low volumes employed in these latter samples, a slight correction to the extraction volume was made by assuming that plaque has a mass fraction of 35% fluid [Tatevossian and Gould, 1976]. Aliquots of the TISAB-diluted saliva and whole-plaque extracts were analyzed using an oil-covered inverted fluoride electrode apparatus [Vogel et al., 1997]. Plaque fluid samples recovered from the samples before and after water rinsing (sets 1 and 2, fig. 1) were split into two aliquots. The first aliquot was also deposited on the surface of the inverted fluoride apparatus and analyzed for calcium activities and pH using ion-selective microelectrodes [Vogel et al., 1990, 2000b]. The free calcium was calculated, from the calcium activities using the Davies modification of the extended Debye-Hückel equation and an approximate ionic strength for plaque fluid of 150 mmol/l [Carey and Vogel, 2000]. However, as described below, a somewhat lower ionic strength may be appropriate in the set 2 samples. Subfractions of these plaque fluid samples were then diluted, 9 parts sample with 1 part TISAB III, on the surface of the fluoride electrode apparatus [Vogel et al., 1997] for the determination of fluoride. The second plaque fluid aliquot was used for the determination of phosphate and acetate by capillary electrophoresis [Vogel et al., 2000b]. It should be noted that lactate and some other organic acids were also measured in these samples but, with regard to lactate, the amount was usually too small to quantitate, while the behavior of the other organic acids (primarily propionate) was qualitatively similar to acetate and is not shown in the data tables.

**Table 1.** Saliva flow and fluoride concentration 60 min after a 1-min CR, NaF rinse or a no-rinse 'control' (sample set 1), 7 min after a subsequent water rinse (sample set 2)

| Rinse | Sample set | Salivary flow g/min | Fluoride μmol/l |
|---|---|---|---|
| None | 1 | 1.65 ± 0.68 | 3.7 ± 1.9 |
| | 2 | 1.44 ± 0.42 | 3.1 ± 2.4 |
| | 2a | 0.36 ± 0.20 | 1.9 ± 0.4 |
| NaF | 1 | 1.56 ± 0.58 | 13.8 ± 8.3 |
| | 2 | 1.44 ± 0.48 | 8.3 ± 4.9 |
| | 2a | 0.44 ± 0.23 | 8.5 ± 5.0 |
| CR | 1 | 1.45 ± 0.39 | 22 ± 16 |
| | 2 | 1.55 ± 0.39 | 16.1 ± 9.2 |
| | 2a | 0.55 ± 0.48 | 14 ± 11 |

Sample set 1 and 2 were taken immediately following plaque collection but without clearing saliva from the mouth before collection. Sample set 2a was taken 9 min after the water rinse with saliva being cleared by swallowing before collection.
The ± refers to the standard deviation, n = 13.

**Table 2.** Multiple comparisons of marginal means for the salivary data of table 1 using treatment and sample set as fixed variables

| Fixed variable | Salivary flow g/min | Fluoride μmol/l |
|---|---|---|
| Treatment | | |
| NaF – no rinse | 0.028 | 7.4* |
| CR – no rinse | 0.055 | 14.8* |
| CR – NaF | 0.027 | 7.4* |
| Sample set | | |
| 1–2 | 0.06 | 4.4* |
| 1–2a | 1.1* | 5.9* |
| 2–2a | 1.0* | 1.5 |

Statistical comparisons made using the Newman-Keuls multiple-comparison test. The figures are the overall effect of each fixed variable without regard to the other variable. Factors showing a difference at the 0.05 significance level are marked with an asterisk (*).

Because of the relatively large volume of the samples, and hence higher accuracy, the electrode determination of free calcium and pH measurements were performed only once. The estimated standard uncertainty of these measurements is less than 0.2 pH units and less than a mass fraction of 7% for free calcium [Vogel et al., 1990]. Because small diluted or buffered aliquots were used for fluoride (fluoride electrode) and acetate or phosphate measurements (capillary electrophoresis), these analyses were performed in triplicate to increase the accuracy. The estimated standard uncertainty of the replicates ranged from mass fraction 3 to 10% depending on the type and concentration of the ion [Vogel et al., 2000b].

*Statistical Methods*

Statistical analyses were done with a commercial statistical package (Texasoft, Cedar Hill Tex., USA) using a 2- or 3- way ANOVA with treatment (CR, NaF, no rinse), sample set (1 = before water rinse, 2 = after water rinse) and site (upper molar site, lower molar site; plaque and plaque fluid only) as factors. The Newman-Keuls multiple-comparison test was used to examine the effect of the individual variables, and a significance level of $p < 0.05$ was used in all statistical tests. As in similar studies, a very large range of fluoride values were found, especially in the plaque data, which was usually due to one or two 'outliers' in the data set [Duckworth et al., 1994; Vogel et al., 1997, 2000b]. However, elimination of the outliers using criteria previously described [Vogel et al., 1997] did not change the conclusions of this study.

## Results

The average salivary and plaque values are given in tables 1 and 3. The ± in these tables refers to the standard deviation, which in this paper is used as a measure of the standard uncertainty. Since no significant interaction between the variables (rinse, site and set) was found, the independent examination of the effect of individual variables could be done (tables 2 and 4).

*Saliva*

Except where noted, the data presented below refer to table 2.

*Treatment Effects.* The CR rinse produced a significantly higher salivary fluoride concentration with both rinses demonstrating an increased fluoride concentration compared to the no-rinse samples. None of the treatments influenced salivary flow.

*Water Rinse Effect.* Water rinsing significantly reduced the salivary fluoride concentration. These reductions were 40, 26 and 17% (mass fractions) for the NaF, CR and the no-rinse sample respectively (table 1). The salivary flow was similar in all the samples before and after water rinse (sample sets 1 and 2), but, as in previous studies [Vogel et al., 1998, 2000a], the lack of expectoration before sample collection produced a very high flow rate. Although expec-

**Table 3.** Plaque and plaque fluid composition of samples from upper and lower molar sites 60 min after a 1-min CR, NaF rinse or no rinse (sample set 1) and 7 min after a subsequent water rinse (sample set 2)

| Rinse and sample set | Plaque mass mg | Plaque fluid | | | | | Whole-plaque fluoride | |
|---|---|---|---|---|---|---|---|---|
| | | pH | free calcium mmol/l | fluoride μmol/l | acetate mmol/l | phosphate mmol/l | total extractable F ppm | water-extracted F ppm |
| Upper Molar sites | | | | | | | | |
| None | | | | | | | | |
| 1 | 1.15±0.15 | 7.02±0.18 | 0.66±0.254 | 8.6±2.2 | 25±10 | 14.8±4.4 | 8.6±10 | 0.43±0.19 |
| 2 | 1.64±0.44 | 7.02±0.14 | 0.83±0.33 | 6.1±1.3 | 21.2±8.3 | 13.1±3.6 | 5.5±5.2 | 0.36±0.31 |
| NaF | | | | | | | | |
| 1 | 1.55±0.32 | 6.94±0.30 | 0.63±0.34 | 31±22 | 22.8±6.7 | 14.7±4.8 | 38±38 | 3.6±1.8 |
| 2 | 1.79±0.65 | 6.92±0.19 | 0.86±0.29 | 14.5±7.7 | 17.2±5.4 | 11.6±2.3 | 29±36 | 3.2±2.7 |
| CR | | | | | | | | |
| 1 | 1.39±0.54 | 6.89±0.22 | 0.63±0.24 | 58±49 | 18.4±8.5 | 13.8±4.7 | 142±128 | 43±39 |
| 2 | 1.66±0.73 | 6.88±0.21 | 0.78±0.16 | 44±30 | 20±10 | 11.4±2.5 | 136±126 | 31±28 |
| Lower molar sites | | | | | | | | |
| None | | | | | | | | |
| 1 | 1.40±0.42 | 6.83±0.32 | 0.83±0.26 | 7.0±2.2 | 23.2±6.4 | 12.5±1.9 | 6.2±7.7 | 0.49±0.30 |
| 2 | 1.54±0.20 | 6.83±0.29 | 1.04±0.38 | 5.8±3.9 | 17.2±7.5 | 9.2±2.8 | 5.5±5.3 | 0.38±0.14 |
| NaF | | | | | | | | |
| 1 | 1.74±0.65 | 6.80±0.39 | 0.75±0.36 | 48±32 | 17.2±6.1 | 12.8±4.0 | 42±43 | 5.0±4.0 |
| 2 | 1.94±0.77 | 6.81±0.25 | 0.92±0.25 | 20±11 | 14.7±5.0 | 9.3±2.7 | 42±46 | 4.5±3.0 |
| CR | | | | | | | | |
| 1 | 1.53±0.41 | 6.80±0.18 | 0.80±0.36 | 115±114 | 18.9±7.9 | 12.8±2.3 | 157±146 | 55±39 |
| 2 | 1.51±0.53 | 6.74±0.20 | 1.04±0.20 | 53±24 | 14.7±4.9 | 8.5±1.8 | 107±81 | 48±33 |

The water-extracted fluoride is taken as a measure of the amount of labile fluoride. Total fluoride = water + acid extracted. The ± refers to the standard deviation, n = 13. pH was calculated as the average of the individual pH values rather than the negative log of the average hydrogen ion concentration [Margolis et al., 1988]. Calcium: based on the decrease in acetate and phosphate there appears to be a water-induced loss of ionic strength in the set 2 samples (see text). This suggests that the set 2 free calcium concentrations should be decreased by 4–8 % (see text).

toration significantly reduced the flow (sample set 2 – 2a), only a small effect was seen on the fluoride concentration in the post-fluoride-rinse group.

*Plaque and Plaque Fluid*

Except where noted, the data presented below refer to table 4.

In several instances, an effect on plaque mass is noted in the data. However, the numerical difference in all cases appears too small to affect the results, and in any case, the amount collected, which does not include the total amount available, is without biological significance.

*Treatment Effects.* The CR rinse produced a significantly higher plaque fluid, whole-plaque total and water-extracted fluoride compared to the NaF rinse. The plaque fluid fluoride from the NaF rinse was, however, significantly higher than the no-rinse samples. Neither rinse produced an effect on pH, phosphate or free calcium. Acetate was, however, decreased in both the CR and NaF group relative to the no-rinse group.

*Water Rinse Effect.* The in vivo water rinsing (sample set 1 – sample set 2) produced a significant loss of fluoride from plaque fluid and a nonsignificant decrease in whole-plaque total and water-extractable fluoride. Although this rinsing produced no effect on plaque fluid pH, it did induce a significant increase in the plaque fluid free calcium and a decrease in plaque fluid phosphate and acetate.

*Effect of Site.* Plaque fluid pH was similar at the upper and lower molar sites. Plaque fluid calcium was, however, significantly higher at the lower molars, while plaque fluid acetate and phosphate were significantly lower at this site. The difference in plaque fluid fluoride between the upper and lower molar sites fell just short of significance in the ANOVA analysis. However, a few pairs of upper and lower molar samples obtained at the same time contained very large amounts of plaque fluid fluoride. When a paired dif-

**Table 4.** Multiple comparisons of marginal means for the plaque data of Table 3 using treatment, site, and sample set as fixed variables (see table 2 for notation)

| Fixed variable | Plaque fluid | | | | | | Whole-plaque fluoride | |
|---|---|---|---|---|---|---|---|---|
| | plaque mass mg | pH | free calcium mmol/l | fluoride μmol/l | acetate mmol/l | phosphate mmol/l | total extractable F ppm | water-extracted F ppm |
| Treatment | | | | | | | | |
| NaF – no rinse | 0.31* | –0.060 | –0.044 | 22* | –4.0* | –0.30 | 31 | –3.7 |
| CR – no rinse | 0.092 | –0.098 | –0.035 | 61* | –3.5* | –0.64 | 129* | 43* |
| CR – NaF | –0.22* | –0.038 | 0.010 | 39* | 0.54 | –0.34 | 98* | 40* |
| Sample set | | | | | | | | |
| 1–2 | –0.22* | 0.16 | – 0.19*[1] | 24* | 2.8* | 3.0* | 13 | 3.5 |
| Site | | | | | | | | |
| upper – lower | –0.091 | 0.14 | –0.16* | –16[2] | 2.7* | 2.2* | –0.53 | –5.7 |

Statistical comparisons were made using the Newman-Keuls multiple-comparison test, except where noted. The figures represent the overall effect of each fixed variable without regard to the other variable. Factors showing a difference at the 0.05 significance level by this test are marked with an asterisk (*).

[1] A water-induced loss of ionic strength in the set 2 samples, as noted in the footnotes to table 3, suggests that the sample set 1–2 free calcium values should be reduced to between –0.16 and –0.12. This difference is still statistically significant.

[2] Lower sites significantly higher than the upper site ($p < 0.001$) by the paired difference test (see text).

ference test was used (which minimizes the influence of such pairs), the lower sites were found to be significantly higher in fluoride than the upper sites ($p < 0.001$).

## Discussion

The salivary fluoride concentrations and flow rates (sample set 1, table 1) are very similar to those observed in previous studies [Vogel et al., 1992, 1998, 2000a]. Swallowing before sample collection (sample set 2) reduced the salivary flow rates to values that are more consistent with literature data for unstimulated salivary flow [Dawes and Macpherson, 1992]. However, comparing the 2 and 2a data sets (table 2), it can be seen that the swallowing before collection did not affect the measured fluoride in the saliva samples. As in other studies, the CR treatment produced a statistically significant increase in salivary fluoride compared to the NaF rinse (tables 1 and 2). Water rinsing decreased the salivary fluoride concentration by the same amount (about 6 μmol/l) in the two fluoride rinses resulting in NaF salivary values that were about 2.5 times baseline while the CR values remained about 5 times baseline.

All the no-rinse plaque fluid values shown in table 3 are consistent with values previously obtained in this and in other laboratories [Carey et al., 1986; Margolis et al., 1993; Margolis and Moreno, 1994; Vogel et al., 1997, 1998, 2000a, b]. The no-rinse whole-plaque total fluoride values are, however, higher than previously obtained [Vogel et al., 1992, 1997]. The similarity in pH values of all the plaque fluid samples (tables 3 and 4) shows that higher concentrations of fluoride, calcium and acetate in the CR rinse did not influence the resting plaque pH in the 1-hour post-fluoride-rinse samples (sample set 1) and that the high buffer capacity of plaque [Shellis and Dibdin,1988] overcame any effect of water rinsing (sample set 2). Similarly, no effect on pH was seen between the upper and lower molar sites as a result of water rinsing in these resting plaque samples.

The concentrations of the calcium and phosphate in the pre-water-rinse plaque fluid samples (table 3, sample set 1) are also very similar in spite of the calcium in the CR rinse. This result is in agreement with recent studies [Vogel et al., 2000a] showing no increase in plaque fluid calcium and phosphate about 15 min after use of a calcium-phosphate-containing gum, relative to a control gum. The significant decrease in the concentrations of plaque fluid phosphate and acetate after a water rinse (7-min sample, table 4) reflects the lack of any apparent equilibrium mechanism by which these concentrations could be maintained during the increase in ion diffusion induced by water rinsing. Given the low concentration of anions in saliva relative to plaque, a similar increase in diffusion can account for the decrease in plaque fluid phosphate seen when salivary flow was increased during chewing of sugarless gum [Vogel et al.,

1998]. The general decrease in the concentration of acetate and phosphate at the lower site before the water rinse (tables 3 and 4) is perhaps also a consequence of increased salivary clearance induced by lower jaw salivary pooling. It should be noted that the lower molar samples collected in the current study include a significant amount of plaque from anterior molar areas so that this mechanism is not in conflict with studies that have shown a reduced salivary clearance in the lower posterior region of the mouth [Weatherell et al., 1988, 1989].

The higher concentration of calcium at the lower site, which has also been observed in other studies [Vogel et al., unpubl. studies], and the increase in calcium following the water rinse may be a consequence of the decrease in calcium-binding anions noted above. Accordingly, lower jaw pooling of the water rinse would also be expected to affect preferentially the lower molar sites, and indeed the loss of phosphate and acetate and the corresponding increase in calcium do appear to be enhanced at these sites (table 3). It is important to note in this regard that the decrease in acetate and phosphate suggests a water-rinse-induced decrease in ionic strength (table 3, footnote) in the set 2 samples which would, through an effect on the calculation of activity coefficients [Carey and Vogel, 2000], somewhat decrease the free calcium of the set 2 samples. However, the increase in free calcium of the set 2 samples remains significant even with a fairly large decrease in ionic strength (table 4, footnote). More importantly, the statistical interaction between site and sample set was not significant for any of these individual ions and the correlation between acetate or phosphate and free calcium was found to be modest. However, acetate and phosphate appear to account for about half the calcium binding observed in plaque fluid [Margolis and Moreno, 1994], and a very large amount of calcium appears to be bound to bacteria in plaque [Rose et al., 1993]. Finally it is noteworthy that, although the CR rinse contained acetate at about the same concentration as plaque fluid, both the CR and NaF actually decreased plaque fluid acetate relative to the no-rinse samples. The reason for this loss is unclear but it has been observed in other studies [Vogel et al., unpubl. data].

The post-rinse fluoride results for the sample set 1 plaque fluid and whole-plaque fluoride samples (table 3) were similar to previous values [Vogel et al., 1992, 2000b] and showed a significantly higher concentration after the CR rinse compared to the NaF rinse (a relative concentration increase of about 200 and 400%, respectively). Water rinsing significantly reduced the amount of plaque fluid fluoride (sample set 1 – 2, table 4) to about 2.5 times baseline in the case of NaF and to about 7 times baseline with CR (table 3), the decrease being too large, by comparison to previous studies [Vogel et al., 1992], to be accounted for solely by salivary clearance between the collection of the set 1 and 2 samples. As in most previous measurements, a higher concentration of plaque fluid fluoride was found at the lower molar sites following application of either rinse. Water rinsing, as in the case of phosphate and calcium, primarily affected the lower site post-fluoride-rinse plaque fluid samples, suggesting that pooling in the lower jaw of both the fluoride and the water rinse may be an important determinant of fluoride distribution.

The CR rinse appears to deposit 11 times more water-extracted fluoride compared to the NaF rinse (table 3, sample set 1). Although, as noted in the Methods section, the amount of the whole plaque fluoride shown as water extracted in table 3 is less than the total amount of fluoride that can be extracted under neutral conditions, this increase in water-extracted fluoride is consistent with the higher plaque fluid fluoride seen with this rinse (tables 3 and 4) and with the mechanism of enhanced deposition of labile fluoride in oral tissue describe above for this rinse. Finally, similar recent studies [Vogel et al., unpubl. data] using a similar methodology have shown a very large formation of water-extracted fluoride in the oral mucosa following the CR rinse suggesting that deposition of labile fluoride in oral tissue may be responsible for the elevated salivary fluoride observed after this rinse. This study suggests that water rinsing and the pooling of saliva, water or a fluoride rinse in the lower jaw may be important determinants of the concentration and distribution of fluoride and other ions in the oral environment. This study also shows that the inclusion of high concentrations of calcium and acetate in oral rinses has no substantive effect on the free concentration of these ions in resting plaque fluid. Finally, this study demonstrates that, since the total fluoride contents of the rinses are the same, the two-component CR rinse produces a ‘more efficient’ precipitation of water-extractable (both in vivo and in vitro) fluoride than a conventional NaF rinse. Although the nature of these loosely bound fluoride or labile plaque reservoirs is not fully understood, their increased formation appears to be responsible for the persistent increase in plaque fluid and salivary fluoride observed after these rinses in this and in previous studies. Finally, the results presented here, together with recent studies showing that plaque recovered after the new CR rinse released a very large amount of fluoride when acidified to a ‘critical pH’ [Vogel et al., 2000b], suggest the possibility that a CR low-fluoride dentifrice could be manufactured that is as effective as current over-the-counter formulations.

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## Disclaimer

Certain commercial materials and equipment are identified in this paper to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or the American Dental Association Health Foundation or that the material or the equipment identified is necessarily the best available for the purpose.

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