Effect of in vitro Acidification on Plaque Fluid Composition With and Without a NaF or a Controlled-release Fluoride Rinse

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ABSTRACT

Plaque fluid ion concentration changes, especially fluoride, in response to the pH decrease associated with a cariogenic episode are important components of the caries process. A "controlled-release" (CR) fluoride rinse, based on the controlled release of fluoride in the presence of calcium, has been shown to form large fluoride reservoirs in resting plaque. In this study, the *in vitro* acid-induced release of fluoride, and other ions, was examined in 48-hour-fasted plaque fluid from subjects (n = 11) who received no rinse, or who used a 228-ppm CR or NaF fluoride rinse 1 hr before being sampled. After collection, the plaque was centrifuged to yield plaque fluid, acidified (0.1 μL of 0.5 mol/L HCl *per* milligram plaque), and then re-centrifuged before a second sample was obtained. Although previous studies indicated a higher plaque fluid fluoride after the new rinse relative to NaF, no statistically significant difference was observed here. Average fluoride release after acidification (average pH, 5.2) was statistically greater following the use of the CR rinse (153 μmol/L) compared with the NaF rinse (17 μmol/L). No fluoride release was seen in the no-rinse samples. The pH, free calcium, phosphate, acetate, propionate, and buffer capacity were not affected by the different amounts of fluoride deposited in the plaque. However, following acid addition, an increase in free calcium and phosphate was observed, which was also independent of the rinse. The large release of fluoride following acidification suggests that the new rinse may provide an improved cariostatic effect.

KEY WORDS: plaque fluid, rinses, fluoride, ions, acid.

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INTRODUCTION

Caries is strongly influenced by the concentrations of hydrogen, calcium, phosphate, and fluoride ions in the fluid environment of the teeth (Vogel *et al.*, 1990b, 1998; Margolis and Moreno, 1990). Although increases in calcium and phosphate ions can produce a cariostatic effect (Forward, 1994; Vogel *et al.*, 1998), a small increase in the fluid concentration of fluoride can have a profound anti-caries effect (ten Cate and Duijsters, 1983; Wefel and Harless, 1984; Margolis and Moreno, 1990). This observation has led to a heightened interest in labile fluoride reservoirs that could continue to release fluoride in the mouth after application. Although studies have indicated that absorbed fluoride (White *et al.*, 1994), perhaps mediated by calcium binding (Rose *et al.*, 1996), may be a significant fluoride source, most researchers have suggested that CaF_2 or "calcium-fluoride-like" deposits are the most important source of labile oral fluoride (Øgaard *et al.*, 1983; Arends and Christoffersen, 1990; Rølla and Saxegaard, 1990). A two-component rinse has been shown to enhance the oral deposition of CaF_2 (Chow and Takagi, 1991; Chow *et al.*, 1992). These rinses are referred to as "controlled-release" (CR) rinses because the rate of hydrolysis of the SiF_6^{2-} ion to free fluoride (contained in the "A" part of the CR rinse) is controlled by the pH, buffer capacity, and concentration of calcium (contained in the "B" part of the CR rinse). In theory, this control allows for greater penetration by rinse components into enamel or dentin pores, plaque, and mucosal tissue before the formation of CaF_2 deposits (Chow and Takagi, 1991; Chow *et al.*,1992; Vogel *et al.*, 1992a, 1997). In the case of a NaF rinse, deposition of CaF_2 during the period of rinse application is limited by the availability of calcium. Either 2 hrs (Vogel *et al.*, 1992a) or overnight (Vogel *et al.*, 1997) after these CR rinses are used, large increases in the plaque fluid, salivary, and especially whole plaque fluoride concentrations have been observed relative to a NaF rinse of the same total fluoride content.

Although these results suggest an increased remineralization potential for the controlled-release formulation, recent studies suggest that the release of fluoride during a cariogenic challenge at the surfaces of the teeth may be especially beneficial (reviewed by White *et al.*, 1994). There is, therefore, an interest in determining if these large controlled-release-rinse-induced fluoride deposits would, at an acid pH, release greater amounts of fluoride into plaque fluid than a conventional rinse. The purpose of this study was to compare the fluoride release from a CR rinse and a conventional NaF fluoride rinse under acidic conditions produced by the *in vitro* addition of a small volume of concentrated HCl. Because the release of calcium and phosphate ions at low pH may also provide cariostatic benefits that should be synergistic with those of fluoride, the concentrations of these ions were also examined. We chose an *in vitro* acid addition to avoid confounding interactions when a sucrose rinse is used to induce an *in vitro* or *in vivo* pH decrease. These include fluoride inhibition of acid production, salivary clearance patterns that maintain high concentrations of sucrose and fluoride

at the same sites, and studies (Vogel *et al.*, unpublished data) that show that the water component of a sucrose rinse may induce the loss of plaque fluoride and other ions.

It is hypothesized that, after acidification of plaque to a cariogenic pH: (1) the difference in plaque fluid fluoride between the CR and NaF rinses will be increased due to the release of fluoride from the large plaque fluoride deposits induced by the CR rinse, (2) calcium and phosphate concentration will increase in plaque fluid following acidification, (3) neither rinse will influence the release of these ions, and (4) the buffer capacity will not be affected by differences in fluoride concentration induced by the rinses.

MATERIALS & METHODS

Subjects, Fluoride Administration, and Sample Sites

The procedures used in this experiment have been extensively described in recent publications (Vogel *et al.*, 1990a, 1992a,b, 1997, 1998) and are only summarized here. Fluoride administration and plaque collection were done with the informed consent of the subjects following protocols reviewed and approved by the appropriate institutional review boards. Ten males and two females ranging in age from 27 to 56 years, and with no dentures or unfilled cavities, participated in this study. However, one subject failed to complete the CR rinse part of the protocol and was removed from the study. All subjects lived in a fluoridated water area and habitually used F-containing dentifrices. Subjects were issued an electric toothbrush (Oral B Laboratories, Redwood, CA, USA) to ensure good oral hygiene, and were frequently reminded to be diligent in brushing and flossing their teeth. Before each experiment, subjects accumulated plaque for 48 hrs and fasted overnight before morning plaque sampling. About 8:30 am, subjects either rinsed for one min with 20 mL of NaF or CR rinse, or they received no rinse. Plaque samples were then collected 1 hr later. The rinse concentration, 12 mmol/L (*i.e.*, 228 ppm) fluoride, was chosen because it approximates current over-the-counter formulations and is near the maximum values observed in saliva samples obtained 30 sec after a one-minute brushing period with 1000 μg/g or 1500 μg/g (*i.e.*, ppm) fluoride dentifrices (Bruun *et al.*, 1984). Separate upper and lower jaw pools of supragingival plaque samples were recovered from the easily accessible buccal-interproximal surfaces of the molar and premolar teeth (excluding the third molars). At least 1 wk separated the application of each of the rinses, which were administered in a random order.

In a few of the experiments, in which no rinse was administered, we obtained a second set of samples and used them to test for the effect of water addition on plaque composition. Eight samples were thus treated. Because preliminary experiments had indicated a release of anions when plaque samples were stored for several hours, 12 no-rinse samples were stored at room temperature for about 3 hrs and then re-analyzed for phosphate, propionate, and acetate.

Rinse Composition and Mechanism of Action

The NaF rinse consisted of 12 mmol/L sodium fluoride in distilled water. The CR rinse consisted of two parts mixed in equal volumes before application: Part A contained 4 mmol/L Na_2SiF_6 (Aldrich, Milwaukee, WI, USA), while part B contained 20 mmol/L $CaCl_2$ and 50 mmol/L sodium acetate. It should be noted that previous CR formulations contained phosphate (Vogel *et al.*, 1992a, 1997) and are often referred to in the literature as "two-component", "two-solution" or A+B rinses.

Collection of Samples

Before plaque was sampled, plastic collection spatulas and mineral-oil-filled microcentrifuge tubes were weighed together on a microbalance (Vogel *et al.*, 1997). The oil in the centrifuge tube was water-saturated and equilibrated with a gas mixture having volume fractions of 5% CO_2 and 95% N_2 for the prevention of sample evaporation and pH changes associated with CO_2 loss (Vogel *et al.*, 1990b). Next, each subject was asked to suck and then swallow to remove as much saliva as possible, after which the collection spatula, held with a hemostat, was used to collect the sample. The spatula and sample were then placed inside the oil-filled centrifuge tube that was re-weighed to determine the sample weight. The tube, spatula, and sample were then centrifuged (14,000 rpm and 2°C for 4 min) to expel the plaque from the spatula and to separate the fluid from the plaque solids. Next, the spatula was withdrawn, and small aliquots of the fluid phase of plaque (*i.e.*, the plaque fluid) were recovered with oil-filled capillary micropipettes (Vogel *et al.*, 1990b, 1992b). It should be noted that the removal of a small volume of plaque fluid does not significantly reduce the fluoride in the plaque residue (Vogel *et al.*, 1997). Next, the tip of the centrifuge tube was cut, and the plaque centrifuged into oil-filled 200-μL PCR tubes (Rainin Instrument Co., Emeryville, CA, USA) containing about 0.1 μL of 0.5 mol/L HCl *per* milligram plaque. The volume of acid was delivered to the PCR tube by means of a calibrated miniature oil-filled Lange-Levy-type micropipette which was prepared by use of a pipette puller (Narishige, Seacliff, NY, USA). After 4 min, a time period sufficient for the acid to diffuse throughout the rather flat plaque residue, the sample was re-centrifuged for 2 min, and a sample of acidified plaque fluid was recovered (total exposure to acid, about 12 min). Sixty-six samples were thus collected and acidified. A similar procedure was used in the water addition experiments.

Several points should be noted with regard to this procedure. First, the volume and concentration of acid were chosen to: (1) obtain a pH between 4 and 5 based on the published buffer capacity of whole plaque (Shellis and Dibdin, 1988), (2) prevent excessive dilution of the sample [a dilution of about volume fraction 30% could be anticipated based on plaque being about a mass fraction of about 35% fluid (Tatevossian and Gould, 1976)], and (3) avoid acid strong enough to rupture cells before dilution into plaque fluid. The acid used here was 1/2 of the concentration used by others in the determination of plaque buffer capacity (Shellis and Dibdin, 1988). Second, the PCR tubes were used in this extraction because: (1) their conical aspect permitted the plaque and acid to form a thin rounded cake that would encourage a rapid diffusion of the acid thoughout the cell mass, and (2) their shape was conical enough to permit an aliquot of fluid to be recovered after re-centrifugation. Third, samples were kept fairly large (average mass = 3.3 mg) so that adequate amounts of fluid could be recovered from these tubes. However, since the acid was added in whole volume increments, but the plaque weight was not controllable, the ratio of acid to plaque is only approximate. Thus, the magnitude of the pH values obtained is not of biological significance.

Analysis Methods

Aliquots of plaque fluid recovered before and after acid additions were split into two parts (A and B). Part A was deposited on the surface of an oil-covered, inverted fluoride electrode apparatus and

analyzed for calcium and pH by means of microelectrodes (Vogel *et al.*, 1987, 1990b). However, one important improvement in these methods should be noted: A movable glass tube filled with water was pressed onto the calcium electrode body (Fig. 1) to aid in placement of the microelectrode in mineral oil without causing the loss of the water barrier in the tip of the electrode. This water barrier prevents contamination of the calcium ion exchange fluid by mineral oil or proteinaceous substances, which has proved to be the major problem in the use of calcium electrodes in oral fluids (Vogel *et al.*, 1987). Aliquots of part A were then diluted, 9 parts sample with 1 part TISAB III (Orion, Cambridge, MA, USA), on the surface of the electrode for the determination of fluoride concentration (Vogel *et al.*, 1992b, 1997). Part B was placed on the surface of a mineral-oil-filled 50-mm disposable Petri dish. Aliquots of these samples were then diluted for the determination of phosphate and organic acids by capillary electrophoresis. Because the capillary electrophoresis method used in this laboratory is described for the first time in this report, it is described in more detail.

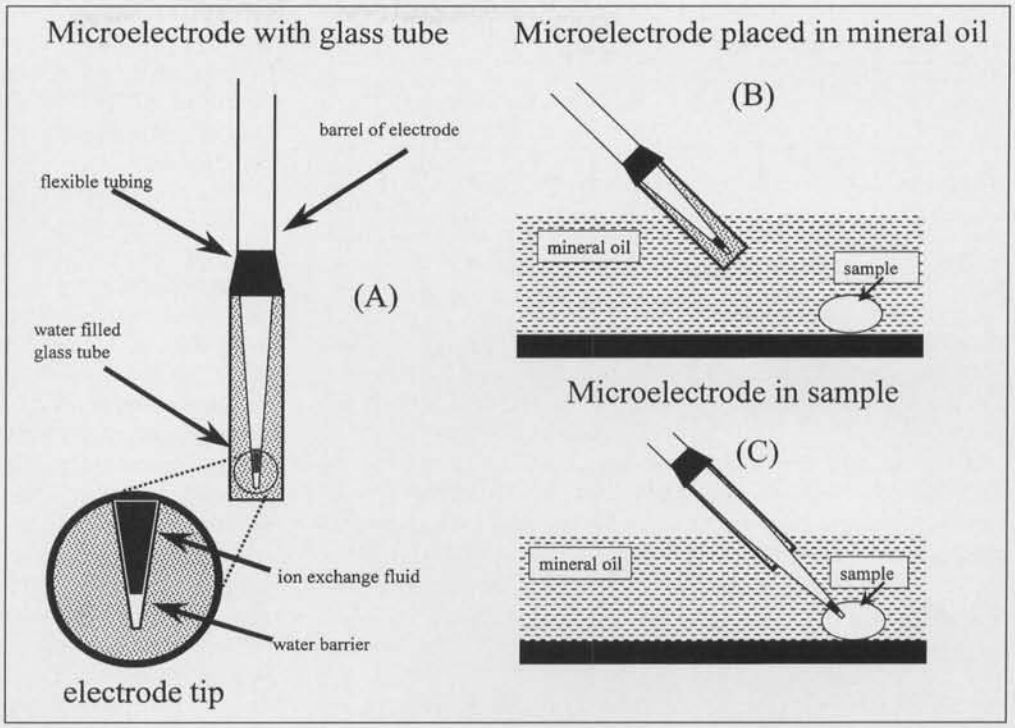

Figure 1. Method for insertion of calcium or other liquid-filled microelectrodes into mineral-oil-covered samples without damaging the ion-selective fluid. (A) A microelectrode (Vogel *et al.*, 1987) is prepared with a water barrier at the tip (A, detail of tip). Next, a glass tube (about 1 cm long) is pulled over the back of the electrode. The tube, held in place by a tubing cone formed from heat-pulled polyethylene tubing, is filled by insertion of the tip of the electrode in water. The glass tube is then pulled and withdrawn over the tip in the same manner as a syringe is filled. (B) After being filled, the tube is positioned over the tip, connected to a manipulator and electrometer (Vogel *et al.*, 1987), and inserted into mineral oil near the samples. (C) Finally, the glass barrel is pulled back to expose the tip (without removal of the electrode from the oil), and the tip is manipulated into contact with the samples (C). The water held in the electrode tip not only prevents mineral oil from contaminating the organic ion-sensitive fluid, but also tends to prevent damage to the electrode from hydrophobic components in plaque.

The capillary electrophoresis instrument was a Waters Quanta 4000 (Waters Corp, Milford, MA, USA) with a 50-cm column of 375 μm o.d. x 75 μm inner diameter operating at 30 kV with the detector at positive polarity. Quantification was done by indirect detection at 254 nm, with 5 mmol/L sorbic acid (Sigma Chemical Co., St. Louis, MO, USA) as the displaced ion in the running buffer. This buffer was prepared daily with de-ionized, de-gassed water (from a 50 mmol/L sorbate stock solution that was neutralized to pH 5.7 with LiOH). Additionally, the diluted buffer contained 0.6 mmol/L tetradecyltrimethylammonium bromide (TTAB) to reverse the electrosmotic flow (Sigma T-4762, prepared from a 300 mmol/L stock) and 3.0 mmol/L pH 5.7 MES [2-(N-Morpholino)ethanesulfonic acid] buffer to control the pH and hence the separation of relevant peaks (Sigma M-5287, prepared from a 750 mmol/L stock, neutralized to pH 5.7 with LiOH). Samples and standards were both diluted with the same capillary "to contain" micropipette (0.01 μL approximate volume) into 5 μL of diluent delivered to a PCR tube by a P10 Pipetman micropipette (Ranin, Woburn, MA, USA), thus obviating the need for calibration of the micropipette (Vogel *et al.*, 1990b, 1997). The diluent was de-ionized water containing 0.1 mmol/L pentane sulfonic acid as a internal standard (used to compensate for small shifts in peak location, thereby ensuring proper peak identification). Sampling was done by means of a 30 s hydrostatic injection directly from the PCR tubes, which were covered with Parafilm "M" (American National Can, Greenwich, CT, USA) to prevent evaporation. Prior to each injection, the capillary was given a six-second pre-wash with 0.1 mol/L HCl in isopropanol (to remove traces of mineral oil and to condition the column), followed by a two-minute wash with the running buffer. Peaks occurred between 2 min and 3 min from the application of voltage (current about 11 μA). The minimum detectable concentration is about 0.0025 mmol/L after dilution (equivalent to 1 mmol/L in the 0.01 μL of sample). It should be noted that several organic acids were detected by this system that are not reported here, notably lactate. However, in these fasted plaque samples, the concentrations of these ions were usually near the detection limit and were not quantified. Because of the large size of the samples, and hence higher accuracy, free calcium and pH measurements were performed only once. Because smaller aliquots were used for the anions (including fluoride), these analyses were performed in triplicate for increased accuracy. The estimated standard uncertainty of the replicates ranged from mass fraction 3% to 10%, depending on the type and concentration of the ion.

Calculation of the ion activity products (IAP) for dicalcium phosphate dihydrate (DCPD), hydroxyapaptite (HAp), and fluorapatite (FAp) from these analytical data was performed as previously described (Vogel *et al.*, 1997). The solubility products (Ksp) for these minerals at 25°C are $-\log(Ksp)_{DCPD} = 6.58$ (Gregory *et al.*, 1970), $-\log(Ksp)_{HAp} = 58.5$ (McDowell *et al.*, 1977), $-\log(Ksp)_{FAp} = 60.5$ (Moreno *et al.*, 1977). Although

Table 1. Effect of Water Addition (0.1 μL/mg) on Baseline (*i.e.*, no-rinse) Plaque Fluid Composition (n = 8 samples)

Treatment	pH	Free Ca mmol/L	Phosphate mmol/L
Before water	6.96 ± 0.19[a]	0.68 ± 0.21[a]	14.6 ± 4.3[a]
After water	6.95 ± 0.26	0.73 ± 0.13	13.8 ± 3.1

[a] Mean ± standard deviation.

the solubility product of pure CaF_2 is $-\log(Ksp)_{CaF_2} = 10.4$ (McCann, 1968), it has been found that calcium fluoride formed in the presence of phosphate can exhibit ion activity products of about $-\log(Ksp)_{CaF_2} = 10$ (Christoffersen *et al.*, 1988, 1995). The solubility product for tooth enamel calculated, assuming that its stoichiometry is similar to that of HAp, has been found to be highly variable, ranging from $-\log (IAP)_{HAp} = 52.1$ to nearly as high as that of HAp after repeated extraction with acid (Patel and Brown, 1975). However, the single value of 54.3 has been frequently cited (Margolis *et al.*, 1988, 1993; Margolis and Moreno, 1992).

Statistical Methods

Descriptive statistics were calculated for all variables measured for each group and for upper and lower arches separately. Multiple regression analysis was used to determine the effects on plaque fluoride level of the independent variables which were measured both before and after the addition of acid. Interactions among variables were also assessed. A logarithmic transformation of the plaque fluoride level was used prior to an analysis of variance test of the null hypothesis that there is no difference in plaque fluoride levels between the two rinses. When the assumption of equal variances was violated, the Welch ANOVA test was used to determine statistically significant differences between the groups (Welch, 1951). All tests of the null hypothesis were two-sided and based on a level of significance (alpha) of 0.05. One no-rinse group sample was lost during the study, and thus 10 subjects were used in the statistical analysis. The data tables and figures, however, show the values for 11 subjects. Additionally, two values found in the CR rinse data (one fluoride and one calcium value from different samples) were very large and appear to be outliers (Grubbs and Beck, 1972). These data were not omitted from the analysis, but the effect of their omission on the average values is noted in the footnote to the tables. In the text and tables, ± refers to the standard deviation, which in this paper is used as a measure of the standard uncertainty.

RESULTS

The addition of 0.1 μL/mg distilled water to plaque (Table 1) had only a minimal effect on pH and calcium and phosphate concentrations in plaque fluid. However, the 12 samples that were stored for about 3 hrs had the following mean increases in the mass fraction of anion concentrations: 87% ± 38%, 15% ± 17%, and 173% ± 107% for acetate, phosphate, and propionate, respectively.

No significant differences in plaque fluid composition, except for the fluoride concentrations, were produced by the different fluoride rinses before acidification (Table 2). Both rinses produced plaque fluid fluoride concentrations that were statistically greater than those at the no-rinse baseline. Although a larger mean plaque fluid fluoride level was observed with the CR rinse compared with the NaF rinse, the difference was not statistically significant. Except for phosphate, which was significantly higher at the upper molar sites, no other significant site-related concentration differences were found.

The mean plaque fluid fluoride values among the NaF, CR, and no-rinse groups (Table 2) after acidification were all found to be statistically different from each other. In terms of acid-induced fluoride release (Fig. 2), a large release is seen with the CR rinse plaque samples (average releases from upper and lower sites, 153 μmol/L), while only a small increase is seen with the NaF samples (average release, 17 μmol/L). Plaque acidification produced similar pH values (about 5.2) in each experimental group (Table 2) and resulted in a similar increase in the free calcium (61 of 66 samples) and phosphate (all samples). However, these increases were not correlated with the post-acidification pH. The number of micromoles of H^+ ions added *per* gram of plaque *vs.* observed pH curves (Fig. 3) was similar among all experimental groups. Because the initial pH values were similar (Table 2), and because most of the buffer capacity is below pH 6 (Shellis and Dibdin, 1988), the quantity plotted in Fig. 3 is essentially the integrated whole plaque buffer capacity required to reach the final pH.

All plaque samples were supersaturated with respect to enamel, HAp, FAp, and, except for one no-rinse sample, DCPD, before the acid addition (Table 2). After acidification, only 2 of the no-rinse samples, with very low pH values (3.69 and 3.81), became undersaturated with respect to FAp, so that, on average, all samples remained highly supersaturated with respect to this mineral. Most of the samples became undersaturated after the acid addition with respect to both enamel and DCPD (Table 2). Specifically, 57 out of the 66 acidified samples (those with pH < 5.4) had $-\log IAP_{enamel}$ values > 54.3, and 53 of the samples had $-\log IAP_{DCPD} > 6.6$. All but one of the CR-rinse plaque-fluid samples were undersaturated with respect to CaF_2 before acidification. After acidification (Fig. 4), about half of the CR-rinse samples were either supersaturated or slightly undersaturated with respect to this mineral (*i.e.*, in this Fig., are either above or below the dotted line, respectively). In contrast, all but one of the acidified NaF samples remained undersaturated with respect to calcium fluoride. With respect to the calcium fluoride ion activity products observed in the presence of phosphate described above (solid line, Fig. 4), two of the CR-rinse post-acidified samples appear to be slightly supersaturated. The CaF_2 saturation of the no-rinse samples was unaffected by acidification (Table 2) and, therefore, is not shown in Fig. 4.

DISCUSSION

The constancy of the plaque fluid pH, free calcium, and phosphate values (Table 1) after the addition of distilled water indicates that plaque buffer capacity and the mineral stores of these ions in plaque are sufficient to overcome an approximately 30% volume fraction dilution. The increases observed in acetate and propionate anion concentration during the three-hour storage of plaque samples are in agreement with a recent review describing the effect of plaque storage (Margolis and Moreno, 1994) and with observations made during studies of plaque buffer capacity (Shellis and Dibdin, 1988).

Table 2. Plaque Fluid Composition, pH, and -log IAP Values (ion activity products) Before and After Addition of 0.5 mol/L HCl to Plaque (0.1 µL/mg) Obtained 1 hr after a 12 mmol/L (228 ppm) NaF, a "Controlled-release" (CR) Fluoride Rinse, or with No Rinse (baseline)[a]

Rinse	Type	pH	Fluoride µmol/L	Free Calcium mmol/L	Phosphate mmol/L	Acetate mmol/L	Propionate mmol/L	-log IAP DCPD[b]	-log IAP enamel-HAp[b]	-log IAP FAp[b]	-log IAP CaF_2[b]
					UPPER MOLAR SITES						
No-rinse	before	6.90 ± 0.29[c]	5.5 ± 2.1	0.76 ± 0.26	14.4 ± 4.4[f]	15.8 ± 6.5	4.1 ± 2.8	6.26 ± 0.16	49.7 ± 1.4	47.9 ± 1.1	14.45 ± 0.44
No-rinse	acid	5.15 ± 0.54	4.2 ± 2.0	2.6 ± 1.6	20.3 ± 5.2[f]	16.5 ± 6.0	5.4 ± 3.8	7.02 ± 0.71	57.9 ± 4.4	54.6 ± 3.9	14.22 ± 0.50
NaF	before	7.00 ± 0.27	54 ± 54	1.05 ± 0.50	14.7 ± 2.6[f]	16.9 ± 7.9	4.8 ± 3.1	6.10 ± 0.25	48.6 ± 1.5	46.1 ± 1.4	12.65 ± 0.88
NaF	acid	5.35 ± 0.45	94 ± 48	3.1 ± 1.5	19.3 ± 2.9[f]	14.9 ± 3.9	5.3 ± 2.3	6.76 ± 0.48	56.2 ± 3.2	51.7 ± 2.8	11.47 ± 0.65
CR	before	6.84 ± 0.24	124 ± 184[d]	0.90 ± 0.54	14.0 ± 3.8[f]	17.7 ± 5.7	4.3 ± 2.5	6.26 ± 0.19	49.9 ± 1.4	47.0 ± 0.9	12.3 ± 1.1
CR	acid	5.22 ± 0.52	254 ± 174[e]	3.6 ± 5.4[d]	16.9 ± 4.5[f]	17.3 ± 5.2	6.0 ± 3.8	7.03 ± 0.55	57.7 ± 3.5	52.7 ± 2.9	10.80 ± 0.83
					LOWER MOLAR SITES						
No-rinse	before	6.79 ± 0.45	5.9 ± 2.2	0.95 ± 0.39	12.2 ± 3.7	19.0 ± 11.3	6.7 ± 6.3	6.33 ± 0.47	50.2 ± 3.4	48.2 ± 3.0	14.29 ± 0.45
no-rinse	acid[g]	5.07 ± 0.71	4.3 ± 1.8	2.5 ± 1.7	15.0 ± 4.6	14.4 ± 4.2	6.0 ± 3.3	7.29 ± 0.9	59.2 ± 5.6	55.7 ± 4.9	14.22 ± 0.50
NaF	before	6.80 ± 0.27	75 ± 66	1.28 ± 0.40	11.7 ± 3.0	15.9 ± 6.4	6.1 ± 2.5	6.17 ± 0.22	49.3 ± 1.6	46.5 ± 1.3	12.25 ± 0.92
NaF	acid	5.00 ± 0.54	69 ± 32	2.8 ± 1.8	13.2 ± 4.3	14.6 ± 7.8	7.9 ± 5.9	7.33 ± 0.71	59.4 ± 4.4	54.7 ± 3.8	11.73 ± 0.36
CR	before	6.76 ± 0.22	97 ± 97	1.17 ± 0.79	11.4 ± 4.2	17.4 ± 9.5	5.7 ± 4.9	6.30 ± 0.21	50.1 ± 1.3	47.1 ± 1.0	12.14 ± 0.89
CR	acid	5.13 ± 0.39	273 ± 244[e]	2.2 ± 1.5	13.3 ± 4.8	16.4 ± 6.3	7.3 ± 4.5	7.31 ± 0.47	59.1 ± 2.9	54.0 ± 2.5	10.93 ± 0.95

[a] n = 11 subjects unless noted. The pH values (and hence the IAP values) depend on the ratio of acid added to plaque weight, which is only approximate.

[b] Samples with -log IAP values > -log Ksp are undersaturated. The -log Ksp values for the relevant phases: dicalcium phosphate dihydrate (DCPD) = 6.6, hydroxyapaptite (HAp) = 58.5, tooth enamel (calculated as HAp; see text) = about 54.3, fluorapatite (FAp) = 60.5, and calcium fluoride (CaF_2) = 10.4. Average—log IAP calculated as the average of the individual IAP values.

[c] ± = standard deviation. pH calculated as the average of the individual pH values rather than the negative log of the average hydrogen ion concentration (Margolis *et al.*, 1988).

[d] One very high sample was in each of these upper molar CR rinse groups ("before" group fluoride = 638 µmol/L, "acid' group free calcium = 19.6 mmol/L). These were statistical outliers according to the criteria of Grubbs and Beck (1972). With these numbers omitted, the average values that were substantially changed are: ["before" group] fluoride = 72 ± 74; $-\log(IAP)_{CaF_2}$ = 12.02 ± 0.78; ["acid" group] free calcium = 1.97 ± 0.72. Exclusion of these values does not affect the conclusions.

[e] CR rinse statistically greater than NaF at $p < 0.05$.

[f] Upper site > lower site at $p < 0.05$.

[g] n = 10.

The no-rinse plaque fluid fluoride values (Table 2) are somewhat lower than previously obtained: 12.7 ± 2.8 µmol/L and 14.7 ± 4.9 µmol/L (Vogel *et al.*, 1992a) and 8.3 ± 4.1 µmol/L (Vogel *et al.*, 1997). However, the no-rinse free calcium and phosphate concentrations are similar both to our previous values (Carey *et al.*, 1986; Vogel *et al.*, 1998) and to the values obtained by others (Margolis and Moreno, 1994). Since there were no statistically significant differences in composition in the pre-acidified samples, except for fluoride, neither the fluoride in the rinses nor the calcium and acetate contained in the CR rinse altered the plaque fluid composition.

Although the differences are not statistically significant, the data (Table 2 with the outlier omitted) are in agreement with previous observations that fluoride concentrations in the plaque fluid are higher at lower molar sites compared with upper molar sites (Weatherell *et al.*, 1988; Vogel *et al.*, 1992b; Ekstrand, 1997). The opposite distribution for plaque fluid phosphate agrees with the distribution of whole plaque phosphate noted by others (Ashley, 1975) and with recent studies in this laboratory (Vogel *et al.*, unpublished data). Previous studies with these rinses (before acid values) found a significantly lower concentration of plaque fluid fluoride one hour after rinsing with NaF compared with the CR rinse (Vogel *et al.*, 1992a). However, the relative difference between the rinses in the current study was lessened, and the standard deviations were higher. Thus, no statistically significant difference was found in plaque fluid fluoride following the two rinses.

Previous studies where a decrease in plaque fluid pH was produced by the *in vivo* administration of sucrose (Rankine *et al.*, 1985, 1989; Margolis and Moreno, 1992; Margolis et al., 1993) exhibited an increase in total calcium, which is consistent with the free calcium values measured in the current experiments (Table 2). However, the increase in total phosphate observed here has been found in some studies (Rankine *et al.*, 1985; Margolis and Moreno, 1992) but not in others (Rankine *et al.*, 1989; Margolis *et al.*, 1993). Although phosphate utilization of cells has been postulated to explain the lack of increase in these *in vivo* studies (Margolis and Moreno, 1992), this mechanism would not be expected to be of significance in the fasted samples used in the current experiment, where the pH drop was induced by HCl addition rather than by sucrose. Diffusion of phosphate into the aqueous phase of a sucrose rinse may also be a factor, since it has been found (Vogel *et al.*, unpublished data) that simply rinsing with water produced a decrease in plaque fluid phosphate that was similar in magnitude to the increases in phosphate seen here. Recent studies (Vogel *et al.*, 1998) have also found a larger decrease in plaque fluid phosphate when salivary flow, and hence diffusion into saliva, is increased during the chewing of sugarless gum. The post-acidification release of either calcium or phosphate (Table 2) was also not affected by the fluoride in the NaF rinse or by the presence of both calcium and fluoride in the CR rinse administered 1 hr before the acid addition. The integral buffer capacities (Fig. 3) are generally lower than those

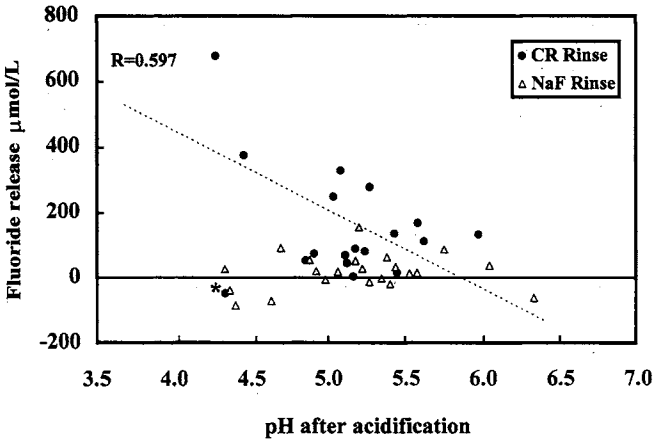

Figure 2. Fluoride release in plaque fluid after acidification *vs.* pH, *i.e.*, the acidified plaque fluoride minus the pre-acidified fluoride value from the same sample. These values may be somewhat lower than the actual increase in fluoride upon acidification, because they do not consider a small increase in fluid volume when the acid is added. The linear correlation shown in this Fig. refers to the CR rinse data with the value corresponding to the single outlier point noted in the footnote to Table 2 removed (point noted by * in the Fig.).

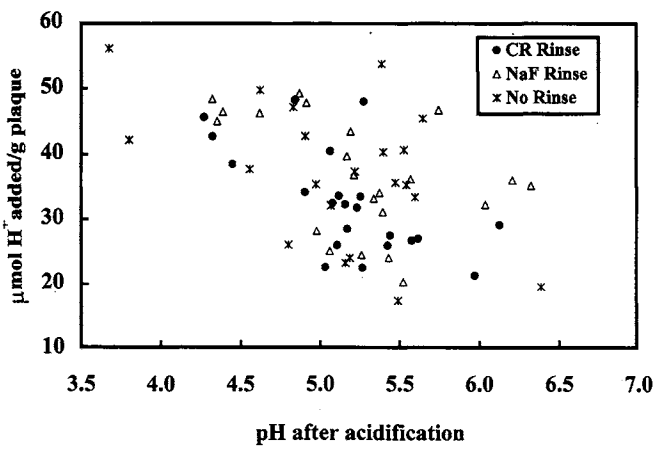

Figure 3. Buffer capacity of plaque *vs.* pH.

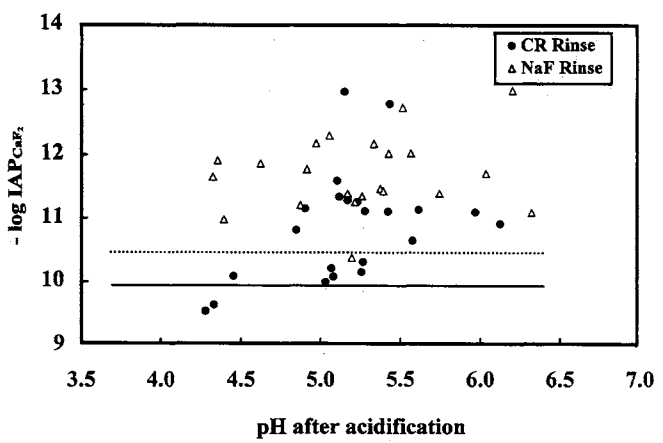

Figure 4. Ion activity products of CaF_2 *vs.* pH after acidification (no-rinse points not shown). The lines show the solubility product of CaF_2 (dotted line) and the ion activity products of this mineral when formed in a phosphate-containing solution (solid line). Points above the respective lines are undersaturated; those under the lines are supersaturated.

obtained by Shellis and Dibdin (1988) in a step-wise titration, but they are similar to those obtained by Strålfors (1948). The increase in whole plaque buffer capacity with decreasing pH is in agreement with these previous studies. The similarity of the pH response in this Fig. demonstrates that the various samples, in spite of their great differences in fluoride concentration, have a similar buffer capacity. This suggests that the influence of fluoride on buffer capacity must be small.

Although the release of calcium and phosphate ions at low pH (Table 2) would be consistent with mineral stores in plaque (Vogel *et al.*, 1990b: Margolis and Moreno, 1992), it is probable that there are several processes involved. Specifically, more phosphate was released than calcium, a process inconsistent with the stoichiometry of calcium phosphate minerals likely to form in plaque at resting pH. Given the increase in calcium and phosphate in almost all the individual samples after acid addition, the lack of a pH correlation for these ions is certainly a reflection of the various kinds of calcium and phosphate stores and the different amounts of these stores in the samples. For example, pH-sensitive calcium binding has been demonstrated in dental plaque (Rose *et al.*, 1994), and a very large standard deviation is often found in studies of the calcium and phosphate content of plaque (Ashley, 1975).

Nearly all of the samples became undersaturated with respect to enamel after acidification. But only the two no-rinse samples (with pH < 4 and fluoride concentrations of less than 3.2 µmol/L) became undersaturated with respect to FAp. This result is difficult to reconcile with studies demonstrating that shark enamel, which is a mass fraction of about 75% fluorapatite, can demineralize in an *in vivo* model (Øgaard *et al.*, 1988). Nearly all the samples became undersaturated with respect to the nominal solubility of enamel -log $(IAp)_{HAp} = 54.3$, indicating that sufficient acid was added for demineralization of tooth mineral. Although previously it has been suggested that plaque is in equilibrium with DCPD (Vogel *et al.*, 1990b), most of the samples became undersaturated with respect to this mineral after acidification, which suggests that the amount of this mineral in two-day-old plaque is small.

Acidification of the no-rinse samples produced a small decrease in plaque fluid fluoride consistent with the dilution of the samples by the added acid. These results are in agreement with recent *in vivo* studies after sucrose administration (Tanaka and Margolis, 1999) and suggest that labile stores of fluoride in plaque samples must be low in samples collected 48 hrs following the use of a fluoride dentifrice. Although the CR rinse was devised to enhance deposition of CaF_2 in the oral environment, the fate of deposited CaF_2 after either rinse is the subject of some controversy, because the relatively high solubility of CaF_2 suggests that it would rapidly be lost by dissolution into saliva (McCann, 1968). One explanation for this observed persistence of CaF_2 is the presence of phosphate-containing calcium fluoride deposits (referred to as "calcium-fluoride-like"). The formation of these deposits has been found to diminish the rate of dissolution of pure CaF_2 dramatically when this mineral is exposed to phosphate-containing solutions (Christoffersen *et al.*, 1988, 1995; Lagerlöf *et al.*, 1988; Rølla and Saxegaard, 1990). Such deposits, which are very likely to form in saliva and more particularly in plaque fluid, given its very high phosphate content (Table 2), may explain how the NaF and CR rinse plaque samples could maintain a relatively high CaF_2 undersaturation in plaque fluid before acidification.

It has been suggested that the phosphate-containing "calcium-fluoride-like" material formed *in vivo* after these rinses loses phosphate and releases fluoride under acidic conditions (Rølla and Saxegaard, 1990). Consistent with this model, with the proposed mechanism of CaF_2 deposition after the use of a CR rinse, and with the very large *in vivo* and *in vitro* deposition of fluoride previously seen when this rinse is used (Chow and Takagi, 1991; Chow *et al.*, 1992; Vogel *et al.*, 1992a, 1997), acidification of the CR rinse plaque samples releases nearly 9 times more fluoride than acidification of the NaF samples (Fig. 2). The release of fluoride from these samples is negatively correlated with pH (correlation coefficient = 0.587, $p < 0.05$) if the point corresponding to the one fluoride outlier noted in Table 2 is removed from the dataset. No relationship between pH and fluoride release is seen in the NaF samples.

Recent studies found that, 1 hr after a NaF or CR rinse, whole plaque samples similar to those used here contain approximately 3.9 μg/g (NaF rinse) and 49 μg/g (CR rinse) extractable fluoride, measured by a procedure that extracts only loosely bound fluoride (Vogel *et al.*, unpublished data). However, the difference in plaque fluid fluoride between these rinses (Table 2 "before" values) is much more modest, suggesting that the effect of phosphate "stabilization" of these deposits is difficult to predict. Specifically, phosphate, besides decreasing the rate of release of fluoride from pure CaF_2, can, as described above, increase the ion activity product (and thus makes this material more soluble) and can also act as a crystal growth poison (Christoffersen *et al.*, 1988, 1995). The ion activity products with respect to CaF_2 (Fig. 4) after acidification of the CR rinse plaque samples are consistent with the former mechanism, since many of the samples became supersaturated with respect to pure CaF_2, but only two of these samples became slightly supersaturated with respect to the ion activity product of CaF_2 formed in the presence of phosphate. These latter two values may be explained by the variability of the ion activity products of "calcium-fluoride-like" deposits in the presence of very high plaque phosphate levels. The complex nature of the phosphate effect may explain why, in previous studies, where phosphate was present in the applied rinse, the CR rinse produced a relatively greater plaque fluid fluoride concentration, compared with the NaF rinse, than in the current study. Finally, it is important to note that, although the behavior of these samples, especially the CR samples, is consistent with the formation of "calcium-fluoride-like" deposits, it is also consistent with a recent bacterial fluoride binding model that suggests that fluoride is held by pH-sensitive calcium bridges (Rose *et al.*, 1996).

Since, as noted above, the CR fluoride rinse deposits a much higher percentage of water-extractable fluoride in plaque, and since several studies have found that this rinse deposits up to 6 times more total fluoride than the NaF rinse (Vogel *et al.*, 1992a, 1997), the moderate difference in plaque fluid fluoride concentration following these rinses suggests that a dramatic reduction in the rate of fluoride release must have been induced after the CR rinse. This high deposition and slow release at resting pH may be responsible for the maintenance of the high plaque fluid fluoride concentration found overnight after application of the CR rinse (Vogel *et al.*, 1997). More significant, given the apparent importance of fluoride release during the caries process (Arends and Christoffersen, 1990; Margolis and Moreno, 1990; White *et al.*, 1994), is the very large release of fluoride from plaque following use of a CR rinse at a low pH. These results suggest that a "controlled release" fluoride rinse may produce a superior cariostatic effect without increasing the amount of fluoride administered.

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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 ADA Health Foundation or that the material or the equipment identified is necessarily the best available for the purpose.

REFERENCES

Ashley FP (1975). Calcium and phosphorus levels in human dental plaque—variations according to site of collection. *Arch Oral Biol* 20:167-170.

Arends J, Christoffersen J (1990). Nature and role of loosely bound fluoride in dental caries. *J Dent Res* 69(Spec Iss):601-605.

Bruun C, Givskov H, Thylstrup A (1984). Whole saliva fluoride after toothbrushing with NaF and MFP dentifrices with different F concentrations. *Caries Res* 18:282-288.

Carey CM, Gregory TM, Rupp NW, Tatevossian A, Vogel GL (1986). The driving forces in human dental plaque fluid for demineralisation and remineralisation of enamel mineral. In: Factors relating to demineralisation and remineralisation of the teeth. Leach SA, editor. Oxford: IRL Press Ltd., pp. 163-173.

Chow LC, Takagi S (1991). Deposition of fluoride on tooth surfaces by a two-solution mouthrinse in vitro. *Caries Res* 25:397-401.

Chow LC, Takagi S, Shih S (1992). Effect of a two-solution fluoride mouthrinse on remineralization of enamel lesions *in vitro*. *J Dent Res* 77:443-447.

Christoffersen J, Christoffersen MR, Kibalczyc W, Perdok WG (1988). Kinetics of dissolution and growth of calcium fluoride and effects of phosphate. *Acta Odontol Scand* 46:325-336.

Christoffersen J, Christoffersen MR, Arends J, Leonardsen ES (1995). Formation of phosphate-containing calcium fluoride at the expense of enamel, hydroxyapaptite, and fluorapatite. *Caries Res* 29:223-230.

Ekstrand J (1997). Fluoride in plaque fluid and saliva after NaF or MFP rinses. *Eur J Oral Sci* 105:478-484.

Forward GC (1994). Non-fluoride anticaries agents. *Adv Dent Res* 8:208-214.

Gregory TM, Moreno EC, Brown WE (1970). Solubility of $CaHPO_4 \cdot 2H_2O$ in the system $Ca(OH)_2$-H_3PO_4-H_2O at 5, 15, 25, and 37.5°C. *J Res Natl Bur Stds (Phys and Chem)* 74(A):461-475.

Grubbs FE, Beck G (1972). Extension of sample sizes and percentage points for significance test of outlying observations.

Technometrics 14:847-854.

Lagerlöf F, Ekstrand J, Rølla G (1988). Effect of fluoride addition on ionized calcium in salivary sediment and in saliva. *Scand J Dent Res* 96:399-404.

Margolis HC, Moreno EC (1990). Physicochemical perspectives on the cariostatic mechanisms of systemic topical fluorides. *J Dent Res* 69(Spec Iss):606-613.

Margolis HC, Moreno EC (1992). Composition of pooled plaque fluid from caries-free and caries-positive individuals following sucrose exposure. *J Dent Res* 71:1776-1784.

Margolis HC, Moreno EC (1994). Composition and cariogenic potential of dental plaque fluid. *Crit Rev Oral Biol Med* 5:1-25.

Margolis HC, Duckworth JH, Moreno EC (1988). Composition of pooled resting plaque fluid from caries-free and caries-susceptible individuals. *J Dent Res* 67:1468-1475.

Margolis HC, Zhang YP, van Houte J, Moreno EC (1993). Effect of sucrose concentration on the cariogenic potential of pooled plaque fluid from caries-free and caries-positive individuals. *Caries Res* 27:467-473.

McCann HG (1968). The solubility of fluorapatite and its relationship to that of calcium fluoride. *Arch Oral Biol* 13:987-1001.

McDowell H, Gregory TM, Brown WE (1977). Solubility of $Ca_5(PO_4)_3OH$ in the system $Ca(OH)_2$-H_3PO_4-H_2O at 5, 15, 25, and 37.5°C. *J Res Natl Bur Stds (Phys Chem)* 81(A):273-281.

Moreno EC, Kresak M, Zahradnik RT (1977). Physicochemical aspects of fluoride-apatite systems relevant to the study of dental caries. *Caries Res* 11(Suppl):142-171.

Øgaard B, Rølla G, Helgeland K (1983). Uptake and retention of alkali-soluble and alkali-insoluble fluoride in sound enamel in vivo after mouthrinses with 0.05% or 0.2% NaF. *Caries Res* 17:520-524.

Øgaard B, Rølla G, Ruben J, Dijkman T, Arends J (1988). Microradiographic study of demineralization of shark enamel in a human caries model. *Scand J Dent Res* 96:209-211.

Patel PR, Brown WE (1975). Thermodynamic solubility product of human tooth enamel: powdered sample. *J Dent Res* 54:728-736.

Rankine CA, Moreno EC, Vogel GL, Margolis HC (1985). Micro-analytical determination of pH, calcium, and phosphate in plaque fluid. *J Dent Res* 64:1275-1280.

Rankine CA, Prihoda TJ, Etzel KR, Labadie D (1989). Plaque fluid pH, calcium and phosphorus responses to calcium food additives in a chewable candy. *Arch Oral Biol* 34:821-824.

Rølla G, Saxegaard E (1990). Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition. *J Dent Res* 69(Spec Iss):780-785.

Rose RK, Hogg SD, Shellis RP (1994). A quantitative study of calcium binding by isolated streptococcal cell walls and lipoteichoic acid: comparison with whole cells. *J Dent Res* 73:1742-1747.

Rose RK, Shellis RP, Lee AR (1996). The role of cation bridging in microbial fluoride binding. *Caries Res* 30:458-464.

Shellis RP, Dibdin GH (1988). Analysis of the buffering system in dental plaque. *J Dent Res* 67:438-446.

Strålfors A (1948). Studies on the microbiology of caries. III. The buffer capacity of the dental plaques. *J Dent Res* 27:587-592.

Tanaka M, Margolis HC (1999). Release of mineral ions in dental plaque following acid production. *Arch Oral Biol* 44:253-258.

Tatevossian A, Gould CT (1976). The composition of the aqueous phase in human dental plaque. *Arch Oral Biol* 21:319-323.

ten Cate JM, Duijsters PP (1983). Influence of fluoride in solution on tooth demineralization. I. Chemical data. *Caries Res* 17:193-199.

Vogel GL, Carey CM, Chow LC, Brown WE (1987). Microelectrode techniques for the analysis of oral fluids. *J Dent Res* 66:1691-1697.

Vogel GL, Carey CM, Chow LC, Ekstrand J (1990a). Fluoride analysis in nanoliter- and microliter-size fluid samples. *J Dent Res* 69(Spec Iss):522-528.

Vogel GL, Carey CM, Chow LC, Tatevossian A (1990b). Micro-analysis of plaque fluid from single-site fasted plaque. *J Dent Res* 69:1316-1323.

Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S (1992a). *In vivo* fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. *J Dent Res* 71:448-452.

Vogel GL, Carey CM, Ekstrand J (1992b). Distribution of fluoride in saliva and plaque fluid after a 0.048 mol/L NaF rinse. *J Dent Res* 71:1553-1557.

Vogel GL, Mao Y, Carey CM, Chow LC (1997). Increased overnight fluoride concentrations in saliva, plaque, and plaque fluid after a novel two-solution rinse. *J Dent Res* 76:761-767.

Vogel GL, Zhang Z, Carey CM, Ly A, Chow LC, Proskin HM (1998). Composition of plaque and saliva following a sucrose challenge and use of an α-tricalcium-phosphate-containing chewing gum. *J Dent Res* 77:518-524.

Weatherell JA, Strong M, Ralph JP, Robinson C (1988). Availability of fluoride at different sites in the buccal sulcus. *Caries Res* 22:129-133.

Wefel JS, Harless JD (1984). Topical fluoride application and lesion progression *in vitro*. *J Dent Res* 63.1276-1278.

Welch BL (1951). On the comparison of several mean values: an alternative approach. *Biometrika* 38:330-336.

White DJ, Nelson DG, Faller RV (1994). Mode of action of fluoride: application of new techniques and test methods to the examination of the mechanism of action of topical fluoride. *Adv Dent Res* 8:166-174.