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Changes in Lactate and Other Ions in Plaque and Saliva after a Fluoride Rinse and Subsequent Sucrose Administration

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

Abstract
The purpose of this study was to examine plaque and saliva composition after a fluoride rinse and subsequent sucrose application. Fifteen subjects accumulated plaque for 48 h, and then rinsed with a fluoride rinse based on 228 μg/g (ppm) Na_2SiF_6 and some received no rinse. After 60 min, upper and lower buccal molar plaque samples and 1-min saliva samples were collected. The subjects then rinsed with 10% g/g sucrose solution, and 7 and 15 min later, a second and a third set of samples were collected. Plaque fluid and clarified saliva were then recovered from these samples by centrifugation, and the remaining plaque acid extracted. The plaque fluid, centrifuged saliva, and plaque extract samples were then analyzed using micro techniques for pH, free calcium, phosphate, organic acids (plaque fluid and saliva only) and fluoride. Considering both the fluoride rinse and no-rinse groups, the most notable compositional changes in saliva 7 min after the sucrose rinse were pH –0.40 unit, free calcium 0.42 m*M*, lactate 5.2 m*M*, phosphate –1.3 m*M*, and fluoride 2.8 μ*M*; while in plaque fluid, the corresponding changes were pH –1.59 unit, free calcium 1.5 m*M*, lactate 35 m*M*, phosphate –1.6 m*M* and fluoride –26 μ*M*. After sucrose rinsing, undersaturation was found with respect to dicalcium phosphate dihydrate in saliva and plaque fluid and with respect to tooth enamel in some plaque fluid samples. Plaque fluid composition appeared to be strongly influenced by salivary clearance, diffusive loss of ions into the water phase of the rinse, and lower jaw pooling of the sucrose and fluoride components of the rinses. After the experimental rinse, the fluoride concentration in plaque fluid [86 ± 22 m*M* (upper molar site), 162 ± 150 m*M* (lower molar site)], saliva (26 ± 18 m*M*), and whole plaque [99 ± 97 μg/g (upper molar site), 197 ± 412 μg/g (lower molar site)] was comparable to the values in previous studies using this rinse. These very high plaque fluid fluoride concentrations, compared with the 'no-rinse' samples, induced an approximately 0.3-unit increase in the plaque fluid pH 7 min after the sucrose rinse, a small decrease (approximately 20%) in lactate production and a modest increase in enamel saturation. Although these changes were all statistically significant, no correlation was found between the decrease in lactate concentration and plaque fluid fluoride, pH or whole plaque fluoride.

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The demineralization of dental tissue at low pH caused by the production of lactic acid in dental plaque is generally acknowledged as the primary factor in the mechanism of caries [Nikiforuk, 1985]. However, while the effect of fluoride on remineralization of teeth appears to be well established, its effect on the demineralization process in the oral environment is not fully elucidated, although several mechanisms appear to be important. One mechanism, which postulates a fluoride-induced reduction in the production of lactic acid, especially at low pH, where cellular HF diffusion may acidify the cytoplasm, is central to the concept that the antimicrobial effect of fluoride has a role in caries prevention [Hamilton, 1990; Marquis, 1990]. However, experimental evidence for this concept is primarily based on in vitro studies [Van Loveren, 1990; Wahab et al., 1993], and most of the in vivo studies done on humans show only a modest effect on pH, unless the fluoride was administered nearly simultaneously with the sucrose rinse [Tatevossian, 1990]. The more meaningful [Hamilton, 1990] examination of lactate production following a sucrose rinse subsequent to a fluoride rinse has apparently been examined in only one in vivo study [Simone et al., 1992]. This study, which found no difference in lactate production following a fluoride dentifrice, employed pooled samples, which have been identified as a confounding factor in such measurements [Rankine et al., 1985; Vogel et al., 1990; Ekstrand et al., 1990]. A second mechanism by which fluoride may exert a cariostatic effect during demineralization involves a reduction in calcium phosphate solubility [Chow, 1990] and hence a reduction in the rate of dissolution of enamel mineral. Especially noteworthy in regard to this mechanism are studies suggesting that the observed very large effect of solution fluoride on enamel dissolution [Margolis and Moreno, 1990] may be attributed to the formation of fluorapatite (FAp) or partially fluoridated hydroxyapatite (OHAp) at the active sites of mineral destruction [Arends and Christoffersen, 1990].

Surprisingly, with regard to the site of caries attack in the plaque, no in vivo studies have been performed in which fluoride, lactic acid production, and changes in enamel solubility were examined after fluoride administration and a subsequent sucrose challenge. Unfortunately, such studies are confounded by the potential synergistic or antagonistic interactions of the various fluoride anticaries mechanisms. For example, a fluoride-induced reduction in the rate of dissolution of tooth mineral or calcium phosphate minerals in plaque, would reduce the release of phosphate buffers, and hence would tend to decrease plaque pH after a sucrose rinse, especially adjacent to the enamel surface. Similarly, a fluoride-induced decrease in lactic acid production would increase the pH, but this might decrease the amount of fluoride released from acid-labile fluoride sources in plaque. Other complicating factors include the potential increase in oral clearance of fluoride and other cariostatic ions by diffusion: (1) into the water component of the sucrose rinse, (2) into saliva, due to a sucrose-mediated increase in salivary clearance and, in the case of fluoride, (3) into the cells at low pH. Recently, in vitro acidification [Vogel et al., 2000] and water-rinsing experiments [Vogel et al., 2001] were done to separately address some of these factors. The purpose of the current study was to examine the changes in fluoride, pH, lactate and mineral ions in plaque fluid and whole plaque (fluoride only) after a sucrose rinse given 1 h after a fluoride rinse, and to use the results of these previous studies (which are briefly described in the Methods section) to interpret our observations. The concentrations of ions in saliva were also examined to study the effect of fluoride and sucrose on this phase.

Materials and Methods

Choice of Experimental Conditions and Rinse Formulation

The sucrose rinse was administered 1 h after a fluoride rinse because this was considered to be the shortest postfluoride rinse time that could be anticipated before a subsequent cariogenic attack, and because previous studies suggested that, for a short period of time, plaque ions may be depleted by the water component of the fluoride rinse [Vogel et al., 2001]. The controlled-release experimental fluoride rinse, which uses the controlled release of fluoride from the hydrolysis of Na_2SiF_6 in the presence of $CaCl_2$ to enhance the deposition of fluoride in oral tissue [Vogel et al., 2000, 2001; Chow et al., 2000], was chosen for these experiments for the following reasons: (1) in agreement with the studies described in the introduction, a preliminary experiment using similar experimental conditions found no effect on lactic acid production with an NaF rinse [Vogel, unpubl. data], and (2) compared with an NaF rinse, the experimental rinse produced a much higher concentration of plaque and plaque fluid fluoride and a greater release of fluoride when plaque was acidified [Vogel et al., 1992, 2000, 2001], suggesting that a positive effect might be observed. More importantly, the plaque fluid concentration of ions other than fluoride has been shown to be very similar after either an NaF rinse or the experimental controlled-release rinse so that the results found here should be similar to those obtained with a conventional NaF rinse [Vogel et al., 2000, 2001]. Finally, the fluoride concentration of the rinse, 12 m*M* (i.e., 228 ppm, here m*M* is used for the SI unit mmol/l), was chosen because it approximates current over-the-counter formulations and because it was used in these previous experiments.

Subjects, Fluoride Administration and Sample Sites

After a protocol review and approval by the institutional review boards, 13 male and 2 female volunteers (aged 25–56 years) were

Table 1. Salivary mass, saliva concentrations and IAP 60 min (time 0) after a fluoride rinse, or no rinse ('control') and 7 or 15 min after a subsequent sucrose rinse (n = 15)

Rinse	Time min	Mass g	pH	Free Ca m*M*	Fluoride μ*M*	Acetate m*M*	Lactate m*M*	Phosphate m*M*	Propionate m*M*	–log (IAP_{DCPD})[1]	–log (IAP_{HAp})	–log (IAP_{FAp})
No rinse	0	1.43 ± 0.53[2]	6.68 ± 0.26[3]	1.04 ± 0.28	1.91 ± 0.59	5.3 ± 2.6	0.31 ± 0.27	6.7 ± 2.7	0.85 ± 0.51	6.43 ± 0.18	50.5 ± 1.5	49.0 ± 1.2
F rinse[4]	0	1.54 ± 0.59	6.69 ± 0.23	0.95 ± 0.30	26 ± 18	4.2 ± 2.0	0.18 ± 0.25	6.3 ± 1.7	0.75 ± 0.48	6.50 ± 0.20	50.9 ± 1.3	48.3 ± 1.1
No rinse	7	1.86 ± 0.48	6.21 ± 0.49	1.56 ± 0.31	1.83 ± 0.34	3.1 ± 1.7	6.0 ± 2.4	5.6 ± 2.4	1.10 ± 0.90	6.69 ± 0.44	52.8 ± 3.2	50.8 ± 2.8
F rinse	7	2.03 ± 0.79	6.36 ± 0.44	1.27 ± 0.37	32 ± 20	2.6 ± 1.0	4.9 ± 2.1	4.8 ± 1.6	1.00 ± 0.36	6.73 ± 0.37	52.6 ± 2.8	49.6 ± 2.4
No rinse	15	1.76 ± 0.51	6.32 ± 0.42	1.37 ± 0.25	1.86 ± 0.48	3.6 ± 1.6	3.2 ± 1.8	5.4 ± 2.0	1.12 ± 0.74	6.66 ± 0.31	52.4 ± 2.6	50.5 ± 2.2
F rinse	15	1.92 ± 0.72	6.45 ± 0.34	1.16 ± 0.33	24 ± 14	4.0 ± 1.6	3.0 ± 2.2	5.4 ± 2.6	1.40 ± 0.51	6.67 ± 0.37	52.3 ± 2.3	49.4 ± 2.0

[1] Samples with –log IAP values > –log Ksp are undersaturated. The –log(Ksp) values for the relevant phases at 25 °C: DCPD = 6.58, HAp = 58.5, tooth enamel (calculated as HAp) see text, FAp = 60.5. The sources for these values are listed in Vogel et al. 2000. Average -log(IAP) calculated as the average of the individual IAP values.

[2] ± = Standard deviation.

[3] 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].

[4] All saliva fluoride rinse values from one subject were statistical outliers using the criteria previously described [Vogel et al., 1997] and were omitted. The values were at times 0, 7, 15 min, 325, 187, and 132 μ*M*, respectively.

recruited to participate in the study. Volunteers were excluded from the study if the oral examination revealed active caries lesions, unfilled cavities or denture prostheses. Fluoride administration and the collection of samples were performed after receiving informed consent from the subjects. The subjects lived in an area with fluoridated water and used fluoride-containing dentifrices. During the experimental periods, they were frequently reminded to be meticulous in tooth brushing and flossing. Before each part of the experiment, the subjects accumulated plaque for 48 h by not brushing, and fasted overnight before sample collection. In the morning, some subjects rinsed for 1 min with 20 ml of the fluoride rinse, and some received no rinse (control). Plaque and saliva samples were then collected 1 h later. Immediately following the collection of these samples, the subjects rinsed with 2 g of sucrose in 18 g of water. Saliva and plaque samples were then collected at 7 and at 15 min after the sucrose rinse.

Collection of Samples

Separate pools of upper- and lower-arch plaque were recovered from the easily accessible supragingival buccal-interproximal surfaces of the first and second molar and premolar teeth. The plaque collection and sample preparation have been extensively described [Vogel et al., 1990, 1997] and are only summarized here: (1) collection of about 1 mg of plaque with plastic strips, (2) determination of the plaque mass under mineral oil to prevent evaporation and loss of CO_2, (3) centrifugation to separate plaque fluid and whole plaque residue, (4) recovery of plaque fluid (approximately 0.1 μl) with mineral oil-filled micropipettes, and (5) extraction of the remaining plaque mass with 1 *M* perchloric acid. A weighed 1-min saliva sample was collected immediately following the plaque samplings by expectoration, without prior swallowing, as previously described [Vogel et al., 1998]. This sample was also clarified by centrifugation.

Analysis Procedure

The analysis procedures are very similar to those previously employed and are only summarized [Vogel et al., 1990, 1997, 2000, 2001]. Plaque fluid or centrifuged saliva samples were analyzed for free calcium and pH using microelectrodes. All samples were analyzed for fluoride using an inverted fluoride electrode apparatus after dilution 9:1 with TISAB III (Orion, Cambridge, Mass., USA). However, with the plaque acid extract, the TISAB solution also contained NaOH to neutralize the samples. The plaque fluid or saliva samples were analyzed for acetate, lactate, phosphate and propionate by capillary electrophoresis. The estimated standard uncertainty of these measurements is less than 0.02 pH unit and less than 7% w/w (w/w refers to mass fraction) in the free calcium [Vogel et al., 1990]. Because smaller diluted aliquots were used for fluoride and acetate or phosphate measurements, these analyses were performed in triplicate to increase the precision. The precision of these replicates ranged from 3 to 10% w/w depending on the type and concentration of the ion.

Calculation of Ion Activity Products

The calculation of the ion activity products (IAP) for dicalcium phosphate dihydrate (DCPD), HAp, and FAp from these analytical data was performed as previously described [Vogel et al., 1998, 2000; Carey and Vogel, 2000]. Samples with a –log(IAP) greater than or less than –log(Ksp) are undersaturated and supersaturated, with respect to these phases, respectively, where Ksp is the solubility product for the mineral in question. The solubility products at 25 °C are given in the footnotes to table 1. The calculated solubility product for tooth enamel, assuming that its stoichiometry is similar to HAp, is quite variable [Patel and Brown, 1975]. Although, the value $-\log(Ksp)_{enamel}$ = 54.3 has been frequently cited [Margolis et al., 1993; Margolis and Moreno, 1992], several studies suggest that the value may be between 56 and 58 [Vogel et al., 1988; Shellis et al., 1993].

Description of Relevant Previous Studies

Two previous experiments have been reported that are relevant to the current study: an in vitro acidification study and a water rinse study. In the acidification study [Vogel et al., 2000], the release of fluoride and other ions was examined in plaque that was acidified, in vitro, to a 'cariogenic' pH (approximately 5.2), i.e., a pH at which enamel mineral should dissolve in plaque fluid. In the water rinse study [Vogel et al., 2001], the effect of an in vivo water rinse on fluoride and other ion concentrations in plaque fluid and saliva was also examined. Both of these experiments were performed using identical

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

Fixed variable	Mass g	pH	Free Ca m*M*	Fluoride μ*M*	Acetate m*M*	Lactate m*M*	Phosphate m*M*	Propionate m*M*	–log (IAP_{DCPD})[1]	–log (IAP_{HAp})	–log (IAP_{FAp})
Rinse											
F rinse – no rinse	0.13	0.09	–0.20*	26*	–0.36	–0.48	–0.40	0.05	0.04	0.00	–1.11*
Time											
7 min – 0 min	0.46*	–0.40*	0.42*	2.8	–1.88*	5.18*	–1.25	0.22	0.25*	2.02*	1.57*
15 min – 0 min	0.37*	–0.30*	0.27*	–1.2	–0.91	2.87*	–1.04	0.43*	0.20*	1.56*	1.30*
15 min – 7 min	–0.10	0.11	–0.15	–4.0	0.97*	–2.31*	0.22	0.22	–0.05	–0.46	–0.28

Statistical comparisons were done using the Newman-Keuls multiple comparisons test. The numbers 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 (*).
[1] See footnote for table 1.

techniques as used here, on a panel composed mostly of the same subjects, with samples being taken at the same 1 h after the rinse time point as employed in the current experiment. However, in addition to the experimental rinse employed here, these experiments were also done on subjects who received a conventional NaF rinse.

Statistical Methods

Statistical analyses were done with a commercial statistical package (Texasoft, Cedar Hill, Tex., USA) using a 2- or 3-way ANOVA with rinse treatment (CR, no rinse), time (0, 7, 15 with 0 referring to presucrose rinse and 7 or 15 min referring to the time elapsed after the sucrose rinse) and site (upper molar site, lower molar site, plaque and plaque fluid samples only) as factors. The Newman-Keuls multiple-comparisons test was used to determine which experimental groups belonged to the same population. A significance level of $p < 0.05$ was used in all statistical tests. As in similar studies, a very large range of fluoride values was found, especially in the fluoride plaque data after the experimental rinse, which, as in previous studies, was usually due to one or two outliers in the data set [Duckworth, 1994; Vogel et al., 1997, 2000, 2001]. Three 'extreme' outliers from one individual were removed from the salivary data set using the criteria previously described [Vogel et al., 1997]. Additionally, some data from one subject were omitted from the fluoride rinse group because of very low plaque pHs in the presucrose samples. All omitted data are given in the footnotes to the tables. The ± 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 average salivary and plaque fluid compositions are given in tables 1 and 3. Except for plaque fluid fluoride, no significant interaction between the factors (rinse, time and site) was found, permitting the independent examination of the effects of individual variables, as shown in tables 2 and 4. The interaction between rinse and site for plaque fluid fluoride is examined in table 5.

Saliva

Except where noted, the data below refer to table 2. The average mass of saliva recovered in this study was 1.76 ± 0.63 g.

Rinse Effects. The fluoride rinse produced a significantly higher salivary fluoride concentration and a decrease in $-\log(IAP_{FAp})$, indicating increased saturation with respect to this mineral. No other changes were seen except a decrease in free calcium.

Time (Sucrose) Effect. Sucrose administration increased the flow of saliva at 7 and 15 min, induced large and significant increases in the concentrations of lactate and free calcium, and a small increase in propionate. Significant decreases in pH and acetate (7 min only), and a nonsignificant decrease in phosphate also occurred. The significant decline in salivary pH after sucrose administration significantly increased the –log(IAP) values with respect to all mineral phases, indicating a decreased degree of saturation.

Plaque

Except where noted, the data below refer to table 4. The average plaque mass was 1.47 ± 0.47 mg. The plaque masses are not reported in the data tables because the amount collected does not represent the total amount of plaque available from these sites and is, therefore, without biological significance.

Rinse Effects. The free calcium and propionate concentrations remained nearly unchanged as a result of the fluoride rinse. As expected, the fluoride rinse produced a very large increase in whole plaque fluoride, plaque fluid fluoride and FAp saturation (decreased $-\log(IAP_{FAp})$. But there was a significant site and rinse interaction in the case of plaque fluid fluoride so that the increase was great-

Table 3. Whole plaque fluoride concentration and plaque fluid composition and IAP of samples from upper and lower molar buccal sites 60 min (time 0) after a fluoride rinse, or no rinse ('control'), and 7 or 15 min after a sucrose water rinse (n = 15)

Rinse	Time min	Plaque fluid										Whole plaque
		pH	free Ca m*M*	fluoride μ*M*	acetate m*M*	lactate m*M*	phosphate m*M*	propionate m*M*	–log (IAP_{DCPD})[1]	–log (IAP_{HAp})	–log (IAP_{Fap})	fluoride μg/g
Upper molars												
No rinse	0	6.94 ± 0.29[2, 3]	0.86 ± 0.33	15 ± 22	25 ± 11	–[4]	15.1 ± 4.7	8.5 ± 5.3	6.2 ± 0.18	49.3 ± 1.5	47.3 ± 1.3	2.7 ± 1.8
F rinse	0	7.08 ± 0.17[5]	0.76 ± 0.27	86 ± 80	19.6 ± 7.4	–	13.3 ± 3.4	7.0 ± 4.8	6.2 ± 0.23	49.0 ± 1.2	46.3 ± 1.1	99 ± 97
No rinse	7	5.28 ± 0.30	2.7 ± 1.4	4.5 ± 1.6	14.2 ± 4.0	37 ± 16	14.9 ± 4.9	5.6 ± 2.9	7.0 ± 0.43	57.2 ± 2.4	53.9 ± 2.2	3.0 ± 2.4
F rinse	7	5.53 ± 0.39	2.6 ± 2.4	54 ± 47	15.0 ± 4.2	31 ± 15	12.6 ± 4.7	6.6 ± 2.8	6.9 ± 0.43	56.2 ± 2.7	52.2 ± 2.1	83 ± 71
No rinse	15	5.53 ± 0.50	1.72 ± 0.53	5.6 ± 3.0	20.7 ± 8.4	26 ± 17	13.3 ± 5.0	8.5 ± 8.1	7.0 ± 0.465	56.6 ± 3.4	53.5 ± 2.9	2.4 ± 1.8
F rinse	15	5.65 ± 0.35	2.1 ± 2.3	78 ± 74	18.4 ± 7.2	22 ± 14	11.3 ± 3.4	7.1 ± 3.6	7.0 ± 0.41	56.1 ± 2.5	52.1 ± 2.3	109 ± 154
Lower molars												
No rinse	0	6.82 ± 0.26	0.98 ± 0.24	10.5 ± 8.8	23.4 ± 8.9	–[4]	13.9 ± 4.0	9.2 ± 4.8	6.2 ± 0.21	49.6 ± 1.2	47.6 ± 1.0	1.9 ± 1.4
F rinse	0	6.82 ± 0.22	1.06 ± 0.56	162 ± 150	21.3 ± 8.1	–	11.9 ± 2.5	7.5 ± 5.2	6.3 ± 0.23	50.1 ± 2.1	47.0 ± 1.6	197 ± 412[6]
No rinse	7	5.07 ± 0.24	2.2 ± 1.2	4.7 ± 1.7	14.2 ± 4.7	38 ± 12	11.9 ± 3.6	6.0 ± 3.2	7.4 ± 0.43	59.7 ± 2.4	56.1 ± 2.1	1.8 ± 1.7
F rinse	7	5.40 ± 0.35	1.99 ± 0.93	108 ± 76	13.9 ± 6.8	31 ± 12	8.8 ± 2.4	6.1 ± 3.3	7.2 ± 0.40	57.7 ± 2.6	53.3 ± 2.1	122 ± 279[6]
no rinse	15	5.41 ± 0.32	1.84 ± 0.60	5.0 ± 1.4	17.3 ± 5.7	28 ± 16	10.4 ± 2.0	7.4 ± 3.6	7.2 ± 0.41	57.7 ± 2.7	54.5 ± 2.3	1.9 ± 1.9
F rinse	15	5.42 ± 0.29	1.70 ± 0.66	108 ± 77	16.7 ± 6.5	28 ± 13	9.3 ± 2.4	7.5 ± 3.9	7.2 ± 0.29	57.7 ± 2.0	53.3 ± 1.7	81 ± 113.4[6]

[1–3] These footnotes are the same as in table 1.

[4] Before sucrose, lactate in plaque fluid was very low and not determined.

[5] Both upper and lower time zero (before sucrose) pH and calcium values from one individual was omitted from the fluoride rinse group because the pHs were very low (6.39, 5.37, respectively) and, as a consequence, the free Ca were very high (2.61 m*M*, 3.09 m*M*, respectively).

[6] One subject had very high whole plaque lower site values after the fluoride rinse. These values were not eliminated from the data set. With this subject eliminated, the whole plaque values are 92 μg/g ± 58 μg/g, 51 μg/g ± 39 μg/g, 53 μg/g ± 32.3 μg/g at time 0, 7 and 15 min, respectively.

Table 4. Multiple comparisons of marginal means for whole plaque and plaque fluid data (table 3) using rinse, time and site as fixed variables

Fixed variable	Plaque fluid										Whole plaque
	pH	free Ca m*M*	fluoride μ*M*	acetate m*M*	lactate m*M*	phosphate m*M*	propionate m*M*	–log (IAP_{DCPD})[1]	–log (IAP_{HAp})	–log (IAP_{Fap})	fluoride μg/g
Rinse											
F rinse – no rinse	0.12*	0.00	IA[2]	–1.7	–2.9[3]	–2.1*	–0.6	–0.02	–0.4[3]	–1.3*	113.1*
Time											
7 min – 0 min	–1.59*	1.48*	–25.9	–7.9*	34.5*	–1.6*	–2.0*	0.91*	8.2*	6.8*	–26.6
15 min – 0 min	–1.41*	0.94*	–19.6	–4.0*	25.6*	–2.6*	–0.5	0.87*	7.6*	6.3*	–22.7
15 min – 7 min	0.18*	–0.54*	6.3	4.0	–8.8*	–1.0	1.5	–0.04	–0.7	–0.5	3.9
Site											
upper – lower	0.18*	0.17	IA[2]	1.0	–1.1	2.4*	0.1	–0.24*	–1.5*	–1.2*	–17.7

Statistical comparisons were done using the Newman-Keuls multiple comparisons test, except where noted. Factors showing a difference at the 0.05 significance level by this test are marked with an asterisk (*).

[1] See footnote for table 1.

[2] An interaction (IA) was found between the variables (see table 5 for these data).

[3] Using a paired difference test to examine the 7 min post sucrose data, a decreased production of lactate (average decrease – 6.1 m*M* ± 12.2 m*M*, $p < 0.01$, n = 30) and a decrease in –log(IAP_{HAp}) (–1.5 ± 2.5, $p < 0.001$, n = 30) was found. The corresponding pH increase is 0.29 ± 0.35 unit ($p < 0.001$).

est at the lower molar sites (table 5). A significant increase was also seen in plaque pH in the fluoride-rinsed samples. Phosphate significantly decreased after the fluoride rinse. A decrease was also seen in the case of acetate, lactate and –log(IAP_{HAp}), as a result of fluoride rinse administration, but these decreases fell just short of significance in the ANOVA analysis (table 4). When a paired difference test (fluoride rinse – no rinse) was used to examine 7-min post-sucrose lactate and –log(IAP_{HAp}) values, a procedure which minimizes the influence of intersubject and inter-

Table 5. Difference of marginal means for plaque fluid fluoride (μM) showing the interaction between the rinse and site (see table 4)

Rinse	Upper molar site	Lower molar site
F rinse – no rinse	64.3*	120*
Site	No rinse	F Rinse
upper – lower	1.6	–54*

Factors showing a difference at the 0.05 significance level are marked with an asterisk (*).

site variations, the fluoride rinse group had a significant decrease in lactate and –log (IAP_{HAp}) ($p < 0.01$ and 0.001, respectively, n = 30).

Time (Sucrose) Effect. Sucrose produced a significant decrease in plaque fluid pH and a large increase in plaque fluid lactate at both time points. A significant increase in plaque fluid free calcium with time after sucrose rinsing was also apparent (table 3), but the concentrations of the acetate, propionate, and phosphate all significantly decreased. A nonsignificant decrease can also be seen in the whole plaque and plaque fluid fluoride (tables 3, 4). Due primarily to the large decrease in pH, the saturation of plaque fluid with respect to all mineral phases significantly decreased (increased –log(IAP) values).

Site Effects. Although plaque fluid fluoride concentrations were significantly higher at the lower molar sites after the fluoride rinse (table 5), pH and phosphate were significantly lower in this region (table 4). This produced a significant increase in the –log(IAP_{DCPD}), –log(IAP_{HAp}) and –log(IAP_{FAp}) values (decreased saturation) relative to the upper molar site. Lactate concentrations were similar at the upper and lower sites.

Discussion

The time zero salivary concentrations and saliva mass values (table 1) are similar to previous studies using similar collection methods [Vogel et al., 1992, 1998, 1997, 2001]. The fluoride rinse produced a significantly higher salivary fluoride concentration (tables 1, 2), resulting in a large decrease in –log(IAP_{FAp}) (an approximately 500% increase in saturation in non-log units). The reason for the decrease in salivary free calcium 1 h after the fluoride rinse (table 2) is unclear.

As expected, sucrose stimulated salivary flow (tables 1, 2) and the significant decrease in acetate in the 7-min postsucrose samples may be related to salivary dilution. Salivary stimulation is nominally associated with an increase in pH [Dawes and Macpherson, 1992; Chow et al., 1994]. However, a decrease in postsucrose salivary pH is observed here, which may be associated with the large increase in salivary lactic acid (lactate in tables 1, 2). This lactate may originate from bacterial sucrose metabolism in both plaque and salivary sediments [Singer et al., 1983; Ryan and Kleinberg, 1995]. The postsucrose increase in salivary free calcium and the decrease in phosphate (table 2) have also been seen in studies when saliva flow was stimulated [Vogel et al., 1998; Chow et al., 1994; Lagerlöf, 1983]. However, in the current experiment, the pH-mediated release of ions from plaque or salivary sediments may also have influenced the concentrations of these ions. The increase in salivary fluoride in the 7-min postsucrose fluoride rinse group samples (table 1) appears to be the result of sucrose metabolism, since salivary clearance should decrease the fluoride concentration. The saliva samples were highly saturated (table 1) with respect to HAp before and after the sucrose rinse. However, these samples were only slightly supersaturated with DCPD before sucrose, but became saturated or slightly undersaturated afterwards.

The 'no-rinse' and postfluoride rinse (time 0) plaque pH, phosphate, free calcium (table 3) are similar to previous values [Carey et al., 1986; Margolis and Moreno, 1992, 1994; Margolis et al., 1993; Vogel et al., 1992, 1997, 1998, 2001]. The values were also similar to those obtained in the in vitro acidification and water rinse studies described in Materials and Methods [Vogel et al., 2000, 2001], in which an NaF rinse was also examined, so that the results obtained here can be compared with these previous results. The mass of the plaque samples recovered here was also very similar to these earlier studies.

The pH and organic acid concentrations after sucrose rinsing (table 3) are also similar to the reported values using similar methods [Margolis and Moreno 1992; Margolis et al., 1993]. In studies using different methodologies, these concentrations were somewhat greater [Pearce et al., 1999; Oliveby et al., 1990] or less [Higham and Edgar, 1989] than reported here but, in all cases (tables 3, 4), a large increase in lactate and a decrease in the concentration of other organic acid anions, particularly acetate, were observed after sucrose administration. With respect to acetate, the decrease has been ascribed to the uptake of this ion by enamel at low pH [Margolis and

Moreno, 1994; Oliveby et al., 1990]. However, the previous water rinse experiment [Vogel et al., 2001] found a decrease in acetate, phosphate and fluoride. This suggests that the acetate decrease in the current experiment may be due to a loss by diffusion either into the water component of the sucrose rinse or, given the low salivary acetate concentration, by diffusion into the increased amount of saliva subsequent to the sucrose rinse [Higham and Edgar, 1989]. A decrease in whole plaque and plaque fluid fluoride, especially in the fluoride rinse group (table 3) and phosphate (table 4), is also seen in the current study after the sucrose rinse. With regard to fluoride, the decrease in this ion is too rapid to be accounted for by salivary clearance in the absence of sucrose [Vogel et al., 1992; Zero et al., 1992]. Since an increase in the plaque fluid concentration of both of these ions was observed in the in vitro acidification study [Vogel et al., 2000], it appears that the sucrose-mediated increases in salivary clearance or the diffusion into the water phase of the sucrose rinse are more dominant factors in determining plaque fluid fluoride and phosphate concentration than is acid-induced release of these ions. However, bacterial phosphate utilization during sucrose metabolism [Margolis and Moreno, 1994] as well as increased intercellular diffusion of fluoride at low pH may be lesser factors [Hamilton, 1990]. The approximately 2-fold increase in free calcium (tables 3, 4) after a sucrose rinse is in agreement with previous studies [Margolis and Moreno, 1994], and with the in vitro acidification study, showing a pH-mediated release of this ion. The large increase in plaque fluid free calcium with sucrose administration is, however, not sufficient to offset the overall decrease in phosphate, fluoride and especially in pH. Thus, the saturation of all the mineral phases decreased (increased –log(IAP) values), particularly at the lower molar site where the pH decrease was greatest (tables 3, 4). Although the plaque fluid samples remained highly saturated with respect to FAp, suggesting that this mineral would not dissolve in plaque fluid 7 min after sucrose administration, they became undersaturated with respect to DCPD. More importantly, the HAp saturation of plaque fluid samples in the current study decreased to a level that is just saturated or even undersaturated (table 3) with respect to the putative solubility of tooth enamel (Material and Methods). Given the homogenized nature of these samples, it is probable that enamel undersaturation is obtained in many of the samples.

After the fluoride rinse, higher concentrations of fluoride were found at the lower sites (tables 3, 5) which were attributed in previous studies to lower jaw pooling of the fluoride ion during rinse application [Vogel et al., 2000, 2001]. As noted above, clearance by the water component of the sucrose rinse and/or the increase in salivary flow appear to be responsible for a decrease in phosphate, acetate, and propionate following sucrose. These effects also seem to be enhanced at the lower molar sites (table 4), and it is noteworthy that the water rinse study found a greater loss of fluoride, phosphate and acid anions in this region that was attributed to water pooling in this region. Thus, both increased exposure to the rinse components and enhanced clearance, from both saliva and the water component of the rinse, appear to be occurring at the lower molar site. However, no lactate site differences were seen (table 4) perhaps due, by the same mechanism, to an increased lower jaw sucrose exposure (i.e., increased lactate production after the sucrose rinse) compensating for the higher clearance at this site. Surprisingly, the pH at the lower molar sites were lower than the upper molar sites; however, once the lower site pHs were decreased by a higher sucrose exposure, they would remain depressed (table 4) due to the high buffer capacity of whole plaque [Shellis and Dibdin, 1988]. This mechanism, which suggests that lactate loss from plaque is more rapid than pH changes, is supported by the fact that in the current experiment, where clearance was a factor, about 50% less acid (as lactic acid) was found in the 7-min postsucrose samples than the amount of acid required to reach the same pH values in the in vitro acidification experiment [Vogel et al., 2000], where clearance factors were absent. The higher concentration of free calcium at the lower jaw sites (table 4) appears to be due primarily to lower pH at this location, but the enhanced lower jaw clearance of calcium-binding anions may also be a factor.

Plaque fluid acetate and phosphate decreased with fluoride rinsing (tables 3, 4) for reasons that are unclear. However, a decrease in these ions has been observed at some sites after a fluoride rinse in the previous studies [Vogel et al., 2000, 2001]. With respect to lactate, figure 1 is a plot of the 7-min postsucrose plaque fluid lactate differences (fluoride rinse lactate – 'no-rinse' lactate) matched for each subject and site, as a function of presucrose (time = 0) fluoride in the fluoride-rinsed samples. In accordance with the paired difference test, this plot shows that there is a net decrease in lactate after administration of fluoride (average decrease about 20%). Similarly, and in agreement with table 4, approximately 2/3 of the subject-site-matched samples had a higher pH (average increase = 0.3 pH) and an increase in HAp saturation (average decrease in $-\log(IAP_{HAp})$ = 1.45 unit) in the 7-min postsucrose fluoride rinse group (footnote table 4). The

average pH increase is similar to the value observed in the subjects who chewed an NaF-fortified gum before sucrose administration [Ekstrand et al., 1985]. Surprisingly, figure 1 shows no correlation between fluoride exposure and the lactate decrease. Similarly, no correlation was found when these paired values (fluoride rinse lactate – 'no-rinse' lactate) were plotted against the presucrose pH, HF activity, or whole plaque fluoride of the fluoride rinse group and, more importantly, no correlation was obtained when the post-sucrose value of these quantities were plotted. Since the inhibitory effect of fluoride on bacterial glycolysis is usually ascribed to an accumulation of fluoride in the cells, especially at low pH where enhanced intercellular diffusion of HF can occur [Hamiltion, 1990; Marquis, 1990; Van Loveren, 1990], the lack of a correlation is surprising. Furthermore, no correlation was found when the site-subject-matched pH or $-\log(IAP_{HAp})$ was similarly plotted vs. the above presucrose or postsucrose values. These results suggest that confounding factors may be present that hinder the observation of correlations that would be anticipated based on the proposed anticaries mechanisms of fluoride noted in the introduction. For example, the lack of correlation between pH and fluoride might suggest a fluoride-induced decrease in plaque buffering that might compensate for a fluoride-mediated decrease in acid production. However, the acidification study demonstrated that plaque buffer capacity was not affected by either an NaF rinse or the experimental rinse used here. Based on the data presented here, a more important factor appears to be patterns of fluoride and sucrose rinse delivery and clearance that appear to maintain a higher concentration of both sucrose (which leads to an increase in lactate) and fluoride at the same oral sites [Oliveby et al., 1990].

As described above, the oral level of fluoride and other ions after the experimental rinse used here were comparable with previous studies in which this rinse produced large increases in oral fluoride compared with an NaF rinse of the same total fluoride concentration. Specifically, plaque fluid and saliva fluoride increased about 2 times, whole plaque fluoride about 4 times, water-extractable fluoride about 11 times, and fluoride release after in vitro acidification approximately 9 times. Given the profound effect of low levels of fluoride on the de- and remineralization process [ten Cate and Duijsters, 1983; Margolis and Moreno, 1990], the rather small effects of this high fluoride level on the lactate production found here suggest a rather modest biological influence of this ion (except shortly after fluoride administration). However, this study also suggests that any quantitative examination of these mechanisms is confounded by the oral distribution and clearance of rinse components (fluoride, sucrose and water) and plaque fluid ions.

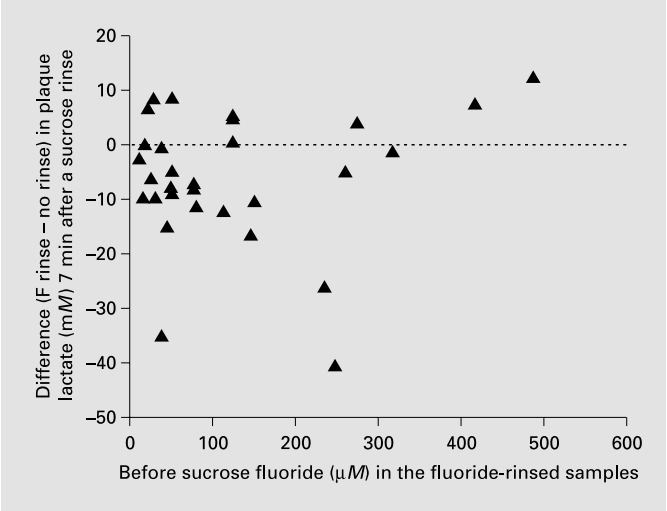

Fig. 1. Difference (fluoride rinse – no rinse) in plaque lactate for each subject and site, 7 min after a sucrose rinse, plotted against the presucrose (time = 0) fluoride concentration.

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