Dental plaque fluoride and pH in children exposed to different water fluoride levels

Fábio Correia Sampaio and Pål Arneberg

Department of Social and Clinical Dentistry, Federal University of Paraiba, João Pessoa, Brazil, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, University of Oslo, Oslo, Norway

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The aims of this study were to determine plaque fluoride concentrations in children not exposed to topical fluorides but to different fluoride levels in the drinking water (0.1 and 2.0 ppm), and to observe whether plaque fluoride was related to plaque pH. Twenty-five children (6 to 7 years old) were selected from two rural villages in Brazil. A sub-set of subjects was examined for resting and fermenting plaque pH before sampling. A maximum of 14 sites was studied in each subject (vestibular and interproximal of first molars and central incisors). Plaque fluoride was extracted and measured with an inverted fluoride electrode under oil. Amounts of plaque were determined by protein analysis. Mean values in the 0.1 ppm village were 1.3 ngF/mg of plaque wet weight (SD = 1.1) and in the 2.0 ppm village 2.5 ngF/mg (SD = 2.1) and were not statistically different (Kruskal-Wallis test, $P = 0.09$). Plaque fluoride varied considerably from site to site in the same mouth. Combining sites in all subjects, plaque fluoride concentrations were positively related to resting and fermenting pH (regression analysis, $P < 0.01$–0.001, adj$R^2 = 0.12$–0.31). On an individual basis the same trend was found for fermenting, but not for resting pH. In conclusion, our findings showed a moderate influence of water fluoride upon dental plaque fluoride concentrations and give some support to the theory that low fermenting pH may contribute to the release of bound plaque fluoride. □ *Caries; fermentation; fluoride analysis; water fluoridation*

Fabio C. Sampaio, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, University of Oslo, PO Box 1109, Blindern, N-0317, Oslo, Norway. Tel. +47 22 85 21 42, fax. +47 22 85 23 44, e-mail. fabios@student.odont.uio.no

Trace amounts of fluoride in the cariogenic environment favourably affect de- and re-mineralization processes (1, 2). The main part of fluoride in plaque is bound (3) and possibly not available as an active (free) fluoride in these processes. It has been suggested, however, that a release of free fluoride may take place at low pH when saturation is critical (4). Thus, a plaque rich in mineral-ion reservoirs (fluoride, calcium phosphate) might have a great capacity to cope with a sucrose challenge (5).

Studies linking total fluoride in dental plaque to corresponding levels in the drinking water show a remarkable variation among population and subject's means. In areas with low water levels ($\leq$0.1 ppm), population means for total plaque fluoride levels range from 0.2 to 10 ppm (ng F/mg plaque wet weight), and in areas with optimal levels (1 ppm) between 3 and 100 ppm (6–10). Apparently, in some of these studies, concentrations in the fluoride extracts may have been below the sensitivity limit of the fluoride electrode (10); in others, the exposure to topical fluoride may have obscured the effect of different water fluoride levels (11).

Active caries lesions are usually restricted to localized sites in a dentition. Improved micro-analytical techniques have opened up possibilities for studying fluoride turnover at localized cariogenic and non-cariogenic plaques in vivo (12). A recent study in orthodontic patients on a heavy topical fluoride programme showed lowest total fluoride levels at the sites with the lowest resting and sucrose challenged plaque pH (13). The observation was attributed to a depletion of fluoride in the low pH plaque environment.

One aim of the present study was to study plaque fluoride levels at different sites of the dentition of subjects exposed to high and low levels of fluoride in the drinking water, but not to topical fluoride. A second aim was to see whether these levels were related to plaque pH under sucrose fermenting and non-fermenting conditions at the same site. In an attempt to relate plaque pH and fluoride to demineralization processes, we decided to study dental plaque on newly erupted teeth.

Materials and methods

Test subjects

Pupils (6 to 7 years old) from two small rural village schools in Paraiba state, northeast Brazil were included. Both areas have similar low socio-economic levels (14). One village is located in an Indian reservation about 1–2 km from the Atlantic coast, the other in a semi-desert area about 500 km from the coast. Each village has a single water source with stable fluoride levels of 0.1 ppm and 2.0 ppm, respectively (15). The selection of the children ($n = 25$) was based on the presence of newly erupted teeth with visible dental plaque (permanent central incisors and

Table 1. Fluoride in plaque (mean and SD), plaque amounts, DMFS, dmfs and OHI-S in children from the areas with 0.11 and 2.0 ppm of fluoride in the drinking water

Water F levels	N (subjects)	N (sites)	DMFS	dmfs	OHI-S	Protein (μg) mean (SD)	Fluoride (ng) mean (SD)	Plaque fluoride (ng F/50 μg protein) mean (SD)*
0.1 mg / mL	11	135	5.5 (4.3)	21.2 (16.4)	1.7 (0.6)	32.3 (5.4)	0.7 (0.8)	1.2 (1.1)
2.0 mg /mL	14	144	0.9 (1.1)	3.4 (5.6)	1.3 (0.4)	29.6 (7.2)	1.3 (1.7)	2.4 (2.1)
P values†	—	—	0.001	0.001	0.20‡	0.66	0.15	0.09

* Values correspond to ppm.
† Kruskal-Wallis test from individual mean values ($n = 25$).
‡ Statistics by Mann-Whitney U test ($Z = -1.2$).

first molars). Toothpaste and topical fluoride were not used by any of the children. Forty percent had a toothbrush. A 24-h dietary recall was obtained from the children with the assistance of the teacher or a health agent. The study protocol was approved by the local ethics committee.

Plaque pH-recordings

Five children from the coastal (0.1 ppm) and three children in the semi-desert village school (2.0 ppm) were selected. Touch pH-microelectrodes (Beetrode MEPH1 or MEPH3) were connected with a BEE-CAL cable (WPI Inc., Sarasota, FL, USA) to a meter (Orion 720A) and to the domestic electrical supply via a voltage stabilizer (CP 500, 220 V). Resting plaque pH was determined at least 2 h after the last meal (16). Then, fermenting pH was measured after a 1 min 10% sucrose rinse as described (13). The rinse was prepared with non-fluoridated boiled water. The selected sites were vestibular surfaces of 1st molars and central incisors (maximum 8 sites) as well as the mesial surfaces of these teeth when in approximal contact (maximum 6 sites). Duplicate measurements in resting plaque showed a mean difference of <0.1 pH units.

Plaque fluoride analysis

Plaque samples were collected from each of the 14 sites specified above in all 1st grade children in the two villages. Sampling was carried out more than 2 h after meal and when the plaque pH measurements had been completed. Vestibular samples were harvested as described previously (13). A fluoride-free dental floss (Butler, Chicago, IL) was used at the approximal sites. Plaque samples were

Table 2. Dental plaque parameters (protein, fluoride and pH) for each subject according to water fluoride in the drinking water

		Dental plaque				
				Plaque pH (Mean SD)		
					Fermenting pH	
Subjects (*n*)	Sites (*n*)	Protein (μg) Mean (SD)	ppm F* Mean (SD)	Resting pH	Minimum	Mean†
Low fluoride area (0.1 mg/L)						
A	10	29.2 (18.4)	0.6 (0.6)	6.8 (0.7)¶	5.4 (0.4)	6.2 (0.3)
B	9	27.9 (19.2)	0.6 (0.3)	6.7 (0.3)	5.6 (0.9)¶	6.3 (0.7)
C	8	54.4 (12.7)	1.1 (2.1)	7.2 (0.2)	4.4 (0.2)	5.4 (0.2)
D	8	33.0 (10.1)	1.4 (0.8)	7.8 (0.2)	6.3 (0.9)¶	7.2 (0.4)
E	10	33.6 (16.5)	0.6 (0.3)	6.7 (0.4)	5.3 (0.6)	6.2 (0.5)
Mean	9	35.1 (17.9)	0.9 (1.0)	7.0 (0.6)**	5.4 (0.8)**	6.3 (0.7)**
High fluoride area (2.0 mg/L)						
F	6	20.6 (08.0)	3.9 (5.0)	6.8 (0.6)	6.1 (0.6)	6.6 (0.4)
G	13	25.5 (12.3)	0.9 (0.7)	6.6 (0.4)¶	5.0 (0.5)	5.9 (0.2)
H	6	20.3 (09.1)	0.6 (0.1)	6.6 (0.4)	—	—
Mean	8.3	23.1 (10.6)	1.5 (2.7)	6.7 (0.5)	5.4 (0.7)‡***	6.1 (0.4)‡***
All	70	30.8 (16.7)	1.1 (1.8)	6.9 (0.5)**	5.4 (0.8)‡***	6.3 (0.6)‡***
p value§	—	0.001	0.01	<0.0001	<0.0001	<0.0001

* ppmF = ng of fluoride / 50 μg of protein.
† Arithmetic mean of fermenting pH (0–60 min).
‡ Values do not include subject H (total $n = 64$).
§ Inter-subject differences calculated by ANOVA.
Direct related to plaque fluoride levels : ¶ ($P < 0.05$), ** ($P < 0.01$).

Table 3. Regression analysis of fluoride concentrations (log10 transformed) in relation to resting and fermenting pH in dental plaque from high and low fluoride areas

Areas	*N* (sites)	Plaque pH	Adjusted R2	B (SE)	Beta	*P* value
	45	Resting pH	0.17	0.32 (0.10)	0.44	0.002
0.1 mg/ mL		Fermenting pH				
	45	Minimum	0.32	0.28 (0.06)	0.57	<0.001
	45	Mean	0.24	0.31 (0.08)	0.50	<0.001
	25	Resting pH	0.06	0.20 (0.14)	0.28	0.165
2.0 mg/mL		Fermenting pH				
	19	Minimum	0.31	0.30 (0.09)	0.59	0.007
	19	Mean	0.36	0.54 (0.16)	0.63	0.004
All	70	Resting pH	0.11	0.25 (0.07)	0.35	<0.01
		Fermenting pH				
	64	Minimum	0.31	0.29 (0.05)	0.57	<0.0001
	64	Mean	0.24	0.33 (0.07)	0.50	<0.0001

transferred to 0.2 ml Eppendorf test tubes by sliding the floss through a slit cut in its lid. Measurable fluoride contamination from the dental floss as well as from dust in the air was ruled out by controls. The tubes were centrifuged within 5 min at 2000 × *g* and stored frozen until the fluoride was extracted in 0.25 N perchloric acid and neutralized as described (13). Four out of 309 fluoride extracts were not within the required pH range (5–5.5) and excluded. Quantitative fluoride analysis was performed in duplicate with <1 µl samples on an inverted fluoride electrode membrane (Orion 9409) under oil, and by operating a KCl micro-reference electrode with a micro-manipulator under the microscope (12). The mean difference in the reading of duplicate samples from the

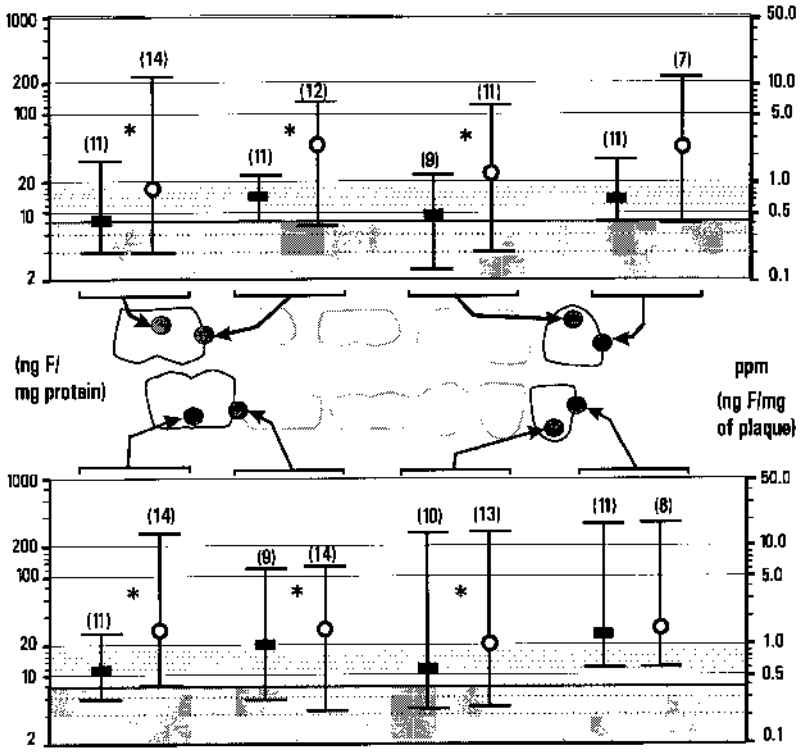

Fig. 1. Medians and ranges of total fluoride concentrations (ngF/mg protein and ngF/mg in plaque) at indicated tooth-sites in 6–7 year olds. The numbers in brackets correspond to the number of subjects at 0.1 ppm (■) and at 2.0 ppm water F area (○), respectively. Left and right corresponding sites were averaged when possible and plaque amounts correspond to 5% protein in plaque. Most values (17 samples) in the shaded area are semi-quantitative data. Note logarithmic scale for fluoride. (*) Significant differences by Mann-Whitney U test ($P < 0.05$).

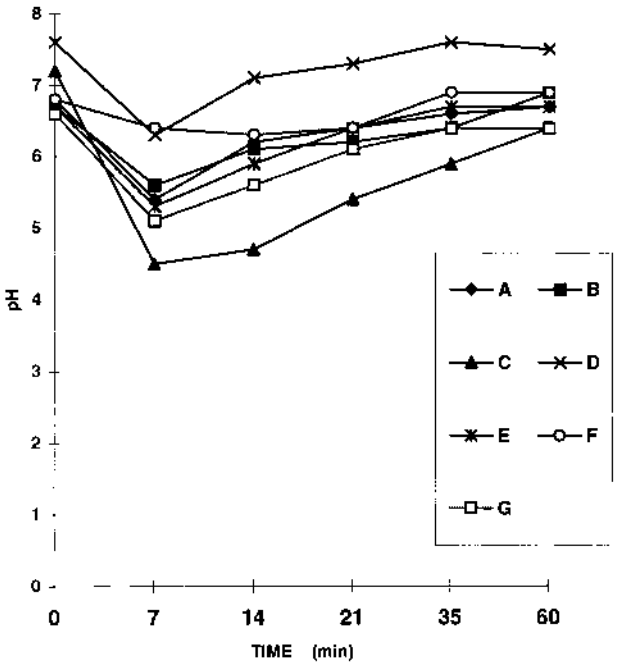

Fig. 2. Plaque pH before and after sucrose rinse. Mean values for sites of 7 subjects. Number of sites is shown in Table 1. Subjects F and G are from a high water fluoride area.

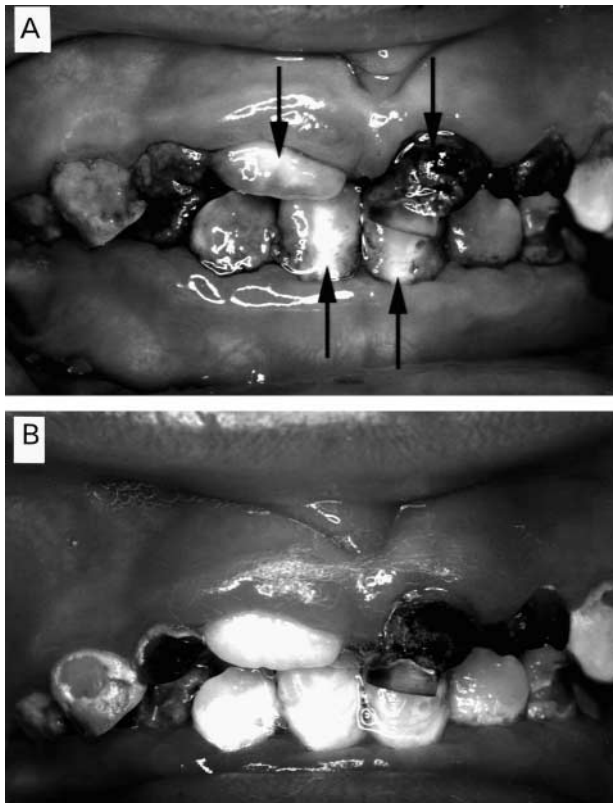

Fig. 3. Clinical pictures of subject C. (A) Arrows indicate vestibular test sites for pH and plaque sampling. (B) Sampling sites after toothcleaning. Note white spots at 31 and 41 and close to the test site at 11. Cavitation at 61.

same extract was 3.0 (SD = 4.2) mV, corresponding to 11% difference in fluoride concentrations. A deviation of the log-linear standard curve was usually not apparent until about 0.03–0.02 ppm F. Readings of 0.01 and 0.005 ppm standards differed by 8–12 mV, suggesting a low level of fluoride contamination during analysis (17). As a reference for plaque mass, quantitative protein analysis of the plaque sediments was performed in duplicate with the sensitive (1 μg) BCA method upon digestion in strong base (13). The mean difference between measurements of the duplicate protein samples was 5%. Twenty-six samples contained <10 μg of protein (about 0.2 mg of plaque) and were excluded, giving a final number of 279 samples.

Dental examinations

The IHO-S index (18) was recorded using a disclosing agent. The examiner assisted the children in removing the remaining plaque with a toothbrush. DMFS and dmfs (19) were then determined under indirect daylight and a headlamp as light source when needed. Active and inactive white spot caries lesions at the pH-measurement sites were recorded according to criteria described by Ismail et al. (20).

Data analysis

The mV readings of the fluoride extracts on the fluoride electrode were converted to fluoride amounts and related to protein amounts in the samples. Results are presented in ppm (ngF/mg of plaque wet weight, or ngF/ per 50 μg protein) in order to facilitate comparisons (13). Further, the lowest fermenting pH for each site was recorded; and its resting and mean fermenting pH values were calculated (13). Parametric (Student's *t* test, paired *t* test, ANOVA) and non-parametric tests (Kruskal-Wallis and Mann-Whitney-U) were used when appropriate. Due to the skewed distribution of fluoride concentrations, log10 transformed values were related to plaque pH parameters by linear regression as described (13).

Results

Dietary recall

The recall showed a mean of 2.7 daily meals for the coastal (0.1 ppm) village and 2.2 for the semi-desert (2.0 ppm) village (ANOVA; $P > 0.1$). Mean daily sweet intakes between meals were 2.0 and 0.7 for these two areas ($P < 0.01$). A low caloric and protein intake was found, particularly in the semi-desert (2.0 ppm) area. Five

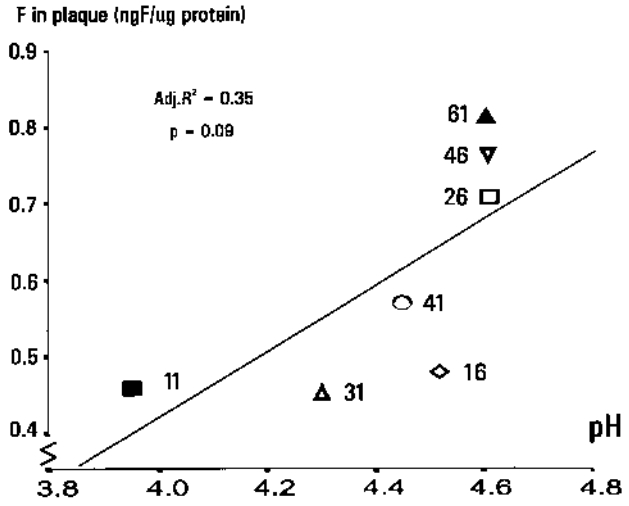

Fig. 4. Total fluoride plotted against minimum fermenting pH for vestibular sites in subject C. Open symbols refers to white spot lesions (teeth, 46 ▽, 26 □, 16 ◇, 41 ○, and 31 △), black symbols to normal enamel (tooth 11 ●) and cavity (61 ▲).

children from the coastal village reported intake of sea food (fish) during the last 24 h.

Clinical findings

Table 1 gives the mean values for DMFS and dmfs, OHI scores, number of plaque sampling sites, fluoride amounts and concentrations for the children from the coastal (0.1 ppm) and from the semi-desert (2.0 ppm) village. Caries prevalences were significantly higher among the coastal children and also the OHI-S score tended to be higher.

Plaque fluoride levels

Mean values were about twice as high in the semi-desert (2.0 ppm) village as in the coastal (0.1 ppm) village, although this difference was not statistically significant because of a pronounced inter-subject variation in both groups (Table 1). Pronounced variations from site to site in the same dentition were also apparent (mean coefficient of variation 83%, SD = 41%). Fig. 1 gives the group medians and shows the wide ranges for plaque fluoride values at each vestibular and approximal sampling site in children from each village area on a Log10 scale. On the right-hand scale the fluoride-protein values have also been converted to ppm/wet weight values assuming 5% protein in plaque (13). Medians were always higher in the 2.0 ppm area and this difference was statistically significant by non-parametric test at most sites (Fig. 1); although (Student's *t* test) this was seldom the case due to a pronounced variance. Approximal plaque contained higher mean fluoride levels than vestibular plaque in all subjects (paired *t* test; $n = 25$, $P < 0.001$). Mean individual plaque fluoride concentrations were unrelated to mean plaque protein amount, OHI-S, DMFS, and dmfs (Pearson's r; $P > 0.3$).

Relationship of plaque pH to plaque fluoride

Because of small amounts of plaque on some surfaces and a few missing approximal contacts, no child gave complete data from all 14 sites, the numbers ranging from 13 to 6 (Table 2). On sucrose challenge, approximal

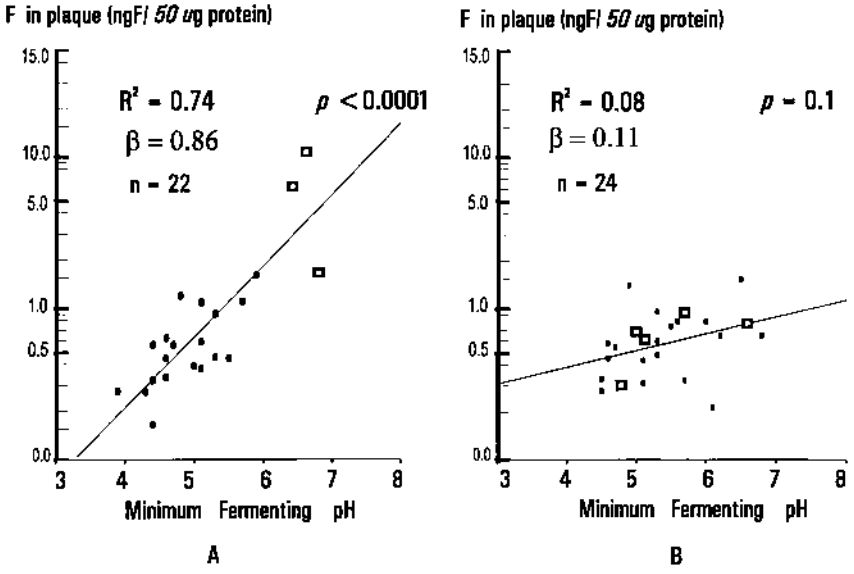

Fig. 5. Scatter diagram of total plaque fluoride concentrations versus minimum fermenting pH at vestibular surfaces of incisors (A) and molar teeth (B). Plaque samples are from low (●) and high fluoride area (□).

plaque gave smaller pH declines than vestibular plaque on the same tooth. This difference was significant in the upper arch (paired *t* test; $n = 12$; $P = 0.004$), but not in the lower ($n = 10$; $P = 0.1$). Fig. 2 shows the mean plaque pH (Stephan curves) for each of the examined subjects. In subject H, who had fewest sites, plaque levels were too low to give stable plaque pH recordings during fermentation (Table 2). Subject D showed the highest resting and fermenting pH values, subject F gave the weakest pH response and subject C the strongest. This latter subject had the lowest fermenting pH values (Fig. 2), the highest plaque amounts, OHI-S scores (Fig. 3A), dmfs, and number of sugar intakes. Moreover, he was the only one with active white spot lesions at the lower incisor sites (Fig. 3B). For this patient, a non-significant decline in plaque fluoride levels by declining fermenting pH is illustrated (Fig. 4). Such a trend was seen in 6 of the 7 subjects tested (mean $AdjR^2 = 0.13$). However, with the low number of individual sites, these relationships were statistically significant in 2 subjects only (Table 2). For resting pH values, no such trend was apparent.

When using site as a unit of analysis, strong and highly significant relationships of fluoride concentrations to resting and fermenting pH values were observed in both fluoride areas (Table 3). This fluoride–pH relationship was stronger in vestibular incisor plaque than in corresponding molar plaque (Fig. 5A and B).

Discussion

Under the field conditions of the study, plaque mass was assessed exclusively by quantitative protein analysis. This may have caused an overestimation of the fluoride concentration in caries active sucrose plaques, since these contain high proportions of carbohydrate (21). The few permanent tooth surfaces with sufficient amounts of plaque for pH-recordings were a problem, in particular for subjects from the village with the 2 ppm fluoride in the water source. The sensitivity of the LaF_3 electrode is another limiting factor in analysis of small plaque samples (10), which is related to the progressive deviation towards the lower end of the log-linear standard curve. This deviation can be reduced when fluoride contamination is controlled, however (17). The under oil technique of Vogel et al. (12) seemed to function well in this respect. By considering readings below the 0.01 standard as semi-quantitative the sensitivity limit will be 0.2 ng fluoride in our 20 µl extracts. Out of 135 samples from the coastal (0.1 ppm) village 10 were below this level, and of 144 from the semi-desert (2.0 ppm) village 7 were below (Fig. 1). Although all lower ranges in Fig. 1 are not strictly quantitative, small samples were excluded on a protein, and not on a fluoride basis to avoid systematic bias.

The caries prevalence (DMFS) between water fluoride groups suggests a difference approximately 6 times higher (Table 1). This is higher than in other comparisons between high and low water fluoride areas (22), and it is probably related to the dietary differences observed between the study groups. Assuming a 5% protein content in plaque, the mean fluoride level was about 2.5 ppm in the village with 2 ppm in the water, and about one half of this in the coastal village with 0.1 ppm water (Table 1). The latter mean value is almost 5 times higher than observed in Brazilian children from a city where a water fluoridation programme had been stopped for several months (8), but similar to pooled plaque levels in subjects not using topical fluoride from areas with 0.1 ppm (23), 0.6 ppm (24) or 1.0 ppm (25) in the drinking water. In addition to some differences in methodology (10), other dietary factors or sources of fluoride (sea food) might have contributed to our different results. Plaque fluoride levels in the 2.0 ppm village are similar to those observed when using fluoride toothpaste (26) or rinsing solutions (23); but only about one quarter of those observed in Norwegian orthodontic patients 2 days after a combined fluoride programme (rinsing and toothpaste) had been stopped (13). Since toothpaste and the rinsing solution concentrations are more than 500 and 100 times higher, precipitation and build-up of calcium fluoride in plaque is more conceivable than with 2.0 or 0.1 ppm in the drinking water (27).

Considerable variations in plaque fluoride levels were noted between subjects using the same water source as well as between tooth surfaces in the same mouth. Approximal plaque tended to have higher fluoride concentrations than vestibular plaque (Fig. 1), which is in accordance with the observations of Gaugler & Bruton (28), but in contrast to those of Wilson & Ashley (29). These authors used pooled plaque samples, whereas we collected plaque material at specific sites, which seems to be more relevant considering the localized cariogenic environment.

The relationship between total fluoride and pH parameters in plaque was highly significant for pooled sites (Table 3) and comparable to that observed among Norwegian orthodontic patients (13). Using site as a unit of measurement (as in Table 3) may be questioned due to subject influence. However, the intra-subject relationships in the present model were fairly consistent with fermenting, although not to resting, pH values. In the orthodontic patient model, factors like plaque fluoride levels, number of sites per subject and also subject age were considerably higher than in the present study. In addition, the slower and more variable oral sugar clearance pattern in our younger children may have contributed (30). Despite the limitations of this in vivo model, our data support low pH possibly having an effect on bound plaque fluoride release (4, 5). Based on in situ experiments in an urban fluoridated area of Brazil, Cury et al. (31) suggested that plaque fluoride from a (0.7 ppm) water source may not give sufficient plaque levels to control a demineralization process during repeated sucrose exposures. This might also be the case for the rural areas of Paraiba, where subjects are not exposed to topicals. Since in our study the fluoride in the drinking water (2.0 ppm) gave only moderate build up of plaque fluoride, the caries preventive

effect of water fluoride might be related more to high frequency of exposure than to plaque fluoride build-up.

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