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Research Article

In Vitro Investigated Study of the Relationship among Fluoride of Plaque and Caries

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Abstract

Many researchers have worked on the relation between individual caries occurrence and total fluoride of plaque including the diverse structures of fluoride, but none gave any real justification about this relation. Thus, we will extend the scope of the study to involve the links between individual caries experience, hydrogen ion content and ionized- and bound- fluoride concentrations in resting plaque. The participants were 75 children (10 - 11 yrs) living in two Syrian towns and received little/no dental care and their oral hygiene was poor. We took from them plaque samples which adhered to the teeth surfaces. Caries prevalence was assessed as decayed, missing and filled surfaces (DMFS). The *In vivo* and *in vitro* tests were referred to that no detectable amount of surface enamel was removed during the sampling. We found that the weights of the fermenting plaque were higher, but with no effect of sucrose rinsing, than the resting and plaque water was unvarying in all the samples. Fermenting plaque was more acidic and rich with fluoride more than resting plaque, but with meager quantity of bound fluoride. Moreover, the more acidic samples had significantly higher total fluoride amount. Noticeably, a positive correlation between DMFS and H+ concentration in fermenting plaque was observed which refers to the cariogenic potential of plaque. Consequently, we think that bound fluoride is metabolic which the responsible factor for caries protection is.

Keywords: *Fluoride; Caries; Teeth surfaces; Plaque Water; Cariogenic; Bacteria*

Introduction

Previous studies demonstrated inverse associations between individual caries experience and the total F content of resting plaque in industrialized and primitive communities [1,2]. These results support the concept that one of the mechanisms by which F protects against caries is mediated through its presence in plaque [3].

In-vitro findings indicate that plaque contains ionized, ionizable (relatively weakly bound) and strongly-bound F [4,5]. Available evidence favors the view that the bound fractions are associated with, or held within, bacteria [6], although Bullock., *et al*. [7] suggested that the greater proportion of F is attached to extracellular mineral components. The ionized-F concentration of resting plaque appears to be low; Bullock., *et al*. [7] found a mean value of 0.038 parts/10^6 in plaque fluid and reported less than 0.1 parts/106 in plaque following saline extraction using extractant:plaque ratios µl/mg wet wt) ranging in value from 2 to 10. Progressively more ionized F is extracted from plaque on stepwise treatment with acid buffers or complexing agents [8] or following the pH drop induced by sucrose [9].

Digestion with strong acid, well beyond physiological limits, is needed for releasing strongly-bound F [4]. These studies have made substantial contributions to our understanding of the states, and by inference, the potential effects of plaque F but contain no information concerning the relation between various forms of F and caries experience.

Our aim was to examine interrelations between individual caries experience, hydrogen-ion (H+) concentration and ionized- and bound-F levels, in resting plaque, and following fermentation induced in situ. The work was carried out during the first examination in a three-year longitudinal investigation of several potential determinants of past and incremental caries experience.

Materials and Methods

The subjects were 75 school-children (39 males, 36 females) aged 10 - 11 yr (mean 10.7 yr, SD ± 0.8), lifelong residents of Salamis and Kafat, two neighboring towns in the arid center of Syria (< 0.26 parts/10^6 F in the water supplies) [10]. The children received little or no dental care and their oral hygiene was poor. Clinical examination and questioning led to the conclusion that less than 10% of the children cleaned their teeth and then only occasionally; those who did were unaware of the existence of F-containing dentifrices. 20% of the children were of nomad descent.

We defined plaque here as soft deposits adhering to tooth surfaces, other than obvious food debris or similar extraneous material which were removed, together with excess moisture, with explorers and cotton pellets prior to plaque collection. The first individual plaque specimen was collected not less than 1.5 h after the last known meal or drink, using austenitic (soft) stainless steel chisels modeled from dental hand-instruments (Ash No. PFI 179, Amalgamated Dental Trade Distributors Ltd, London, England). All accessible surfaces of alternate teeth were sampled with gentle pressure to ensure that no outer enamel was removed and that the gingival crevice and visible supragingival calculus were avoided. *In vivo* and *in vitro* tests simulating the collection method resulted in Ca and F values within the blank range, indicating that no detectable amount of surface enamel was removed.

The second specimen was collected from the remaining teeth in an identical manner, beginning 60s after two supervised rinses with 15% aqueous sucrose solution, over 45s each, separated by a rest for 45s.

Immediately following collection, the samples were weighed in a moist atmosphere to the nearest 0.01 mg, dispersed using a vortex in a small volume of glass-distilled, deionized water (GDDW; pH 6.1 - 6.4), centrifuged for 9 minutes at 2000g and the supernatant and sediment separated. The volume of GDDW used for dispersion was varied according to the wet weight of the samples, aiming for a GDDW:plaque ratio of 2 (µl/mg wet *wt*), but slightly increasing the amount of GDDW for small samples, to yield at least 10 µl recoverable supernatant. The low GDDW:plaque ratio (mean 2.2 µl/mg wet *wt*, SD ± 0.8) was used because a point of interest was to approximate the concentration of free ionized F present in plaque, as opposed to the amount extractable using high extractant:plaque ratios. The interval between the commencement of plaque collection and the separation of the supernatant varied between 17.5 and 19 minutes, introducing corresponding variation into the fermentation period.

The pH of the supernatant was determined immediately after separation, on 3 µl aliquots diluted 1:1 with GDDW, using a custom-made combination microelectrode (Ionode Pty. Ltd, Brisbane, Australia), and the values were used to calculate the H^+ concentration in relation to the wet weight of plaque (parts/10^9). The results were expressed in this form to avoid the assumption that the buffering capacity of plaque would always fully compensate for individual variations introduced by the contribution of plaque fluid to the total volume of supernatant, by deviations of GDDW:plaque ratios from the mean and by possible differences in the buffering capacities of plaque samples, especially at different pH levels. Moreover, while it is valid to average H+ concentrations, averaging pH values is conceptually incorrect, because of the logarithmic nature of the pH scale. The F- ion activity in the supernatant was determined on 5 µl aliquots added to 5 µl buffer (TISAB III, Orion Research Inc., Cambridge, Mass., U.S.A.) using a combination F electrode (Model 96-09, Orion). The values were expressed as the weight of F- per unit weight of plaque collected (parts/10^6). The sediment was homogenized and dried at 80°C for 8h, after which a representative portion of about 1 mg was weighed to the nearest 0.001 mg, and used for F determination by a technique based on the principles described by Bullock., *et al* [7]. The sample was placed in an inner container custom-made from polypropylene tubing (PP280, Portex, Boots Pure Drug Co., Kent, England) digested in 23 µl of a 1:1 mixture of 9.2 M $HClO_4$ (Aristar, B.D.H. Chemicals

Ltd, Dorset, England) and 15.8 M HNO_3 (Aristar, B.D.H.) at 60°C for 2h, cooled to 4°C and mixed with 100 µl of TISAB III containing 2.5 M NaOH (Pro analysi, E. Merck, Darmstadt, West Germany) and 0.24 M trisodium citrate (Univar. Ajax Chemicals, Sydney, Australia), held within an outer airtight polypropylene container (No. MCP 5505C, Camelec Agencies, Adelaide, Australia). When necessary, the final pH was adjusted to 5.3 with the same acid and alkali solutions before determining the F- activity on a combination F electrode. Although this part of the sample must have contained a small amount of plaque fluid, and therefore some F- from the original sample, the contribution of the latter to the F found in the sediment would have been negligible. Thus, the F found in the sediment was regarded as representing the fraction of F which was not ionized in situ, and subsequently will be termed "bound F" (parts/10^6). The total F (TF) content of plaque was derived by summing the ionized- and bound-F values (parts/10^6).

Blanks were prepared in the field daily and analyzed for F together with the samples; the mean F concentration was 7 x 10^{-7} M (SD ± 2 x 10^{-7}, n = 40). For standard recovery tests, known amounts of F were mixed with pooled aliquots of supernatant and sediment, aiming to match the F content of the matrices. Duplicate determinations before and after additions were used to calculate F recoveries in the supernatant (98%, SD ± 4.7, *n* = 6) and sediment (92%, SD ± 8.0, *n* = 11).

Caries prevalence was assessed as decayed, missing and filled surfaces (DMFS) following plaque collection, according to the clinical criteria of the World Health Organization. The surface score assigned to a missing tooth was determined using the mean decayed surfaces:decayed teeth ratio as a multiplier [11].

Associations between variables were examined using bivariate linear regression analysis. Where data plots indicated a curvilinear relation between two variables, the transformation offering the best fit was applied prior to regression. Differences between means were tested using Student's t-test. Paired t-tests were used to isolate and examine differences attributed to sucrose rinsing.

Results

Data summaries according to residence and sex disclosed no significant differences between respective mean values for any of the variables examined. Therefore, all analyses were carried out for the combined sample.

Individual DMFS scores ranged from 0 to 21 surfaces (mean 6.7, SD ± 5.0, *n* = 75). The low level of dental care was reflected by the small proportion of the F (filled) component of the index (19.6%).

Table 1 shows the means and standard deviations of wet and dry weights of plaque collected before and after the sucrose rinse. The mean weights of the fermenting plaque samples were 8-10% higher than those of the resting samples, but the differences were not significant. However, paired t-tests indicated that individuals had significantly higher weights of plaque after, rather than before, fermentation (wet weight: t = 2.24. p < 0.05, degrees of freedom (d.f.) = 74; dry weight: t = 2.82, p < 0.01, d.f. = 74).

Weight or ratio	RP		FP		t	p <
	Mean	SD	Mean	SD		
Wet wt[(1)]	18.1	12.7	19.6	12.7	0.71	ns
Dry wt[(2)]	3.0	2.2	3.3	2.4	0.91	ns
Wet wt: Dry wt	6.3	0.9	6.2	1.2	0.43	ns

Table 1: *Wet and dry weights (mg) and wet:dry weight ratios of resting plaque (RP) and fermenting plaque (FP) samples. Means and standard deviations; n = 75.*

[(1)]: Before extraction

[(2)]: After extraction

As the wet weights were determined immediately after collection, whereas the dry weights were based on the residues recovered after centrifugation, the wet:dry weight ratios (Table 1) have been slightly overestimated.

However, taking as a guide the amount of solid components found in plaque fluid as by Vogel., *et al*. [1] the error caused by omitting from the calculation of the ratio the weight of material dissolved in the supernatant is unlikely to exceed 4% in fermenting plaques (FP) and 2% in resting plaques (RP). In contrast to the high standard deviations of the mean plaque weights (coefficient of variation from 65 to 73%), the wet:dry weight ratios varied within much narrower limits (coefficient of variation from 14 to 19%), indicating considerable uniformity of plaque water content regardless of variations in absolute quantity.

The H^+, F^- and bound-F concentrations in plaque were calculated on the basis of both wet and dry weights. To determine whether the data should be processed using either or both sets of values, the relation between wet and dry weights was examined by regression analysis. The close correlation ($r = 0.98$, $p < 0.0001$, $n = 75$) indicated that the results would be very similar, and it was decided to limit further analyses to data based on wet weight.

Table 2 shows the means and standard deviations of H+, F- and bound-F concentrations, and the proportion of total F ionized in RP and FP. Despite very high individual variations with respect to all parameters included in the table, FP contained significantly greater concentrations of H^+ and absolute and proportionate amounts of F- than did RP. In contrast, less bound F was found in FP than in RP, but the means were not significantly different. Almost identical results were obtained when the corresponding variables were examined using paired r-tests.

Variable	RP		FP		t	p <
	Mean	SD	Mean	SD		
H^+ (parts/10^9 wet wt)	11.7[3]	20.3	113.5[3]	47.6	15.5	0.001
F^- (parts/10^6 wet wt)	0.07	0.07	0.16	0.14	4.84	0.001
Bound F (parts/10^6 wet wt)	4.96	7.62	3.69	4.50	1.24	ns
F^- (% total F)	2.82	2.54	8.44	7.72	6.00	0.001

Table 2: *Hydrogen-ion (H^+) and fluoride (F^-) concentrations and the proportion of total F ionized in resting plaque (RP) and fermenting plaque (FP) samples. Means and standard deviations; n = 75.*

[3]: Corresponding to RP: 5.9 pH units, FP: 4.5 pH units.

The relation between increasing H^+ concentration owing to fermentation and the associated release of F- was also examined using regression analysis. The variables considered were H^+, F^- and bound-F concentrations and the proportion of total F ionized; all were expressed as the difference between the respective values in RP and FP (net increase or decrease). As the data plots suggested straight-line relationships, the variables were submitted to the analysis without transformation, using the F-concentration values as dependent variables.

The results in table 3 show that the net increase in H^+ concentration was positively correlated with both the net increases in F^- concentration and the proportion of total F ionized. No association could be demonstrated between the net decrease in bound-F concentration and the increase in H^+ concentration.

Variables	r	S	I	p <
F^- FP-F^- (RP) vs. H^+ FP- H^+ (RP)	0.28	0.001	1.01	0.010
F^- % (FP)-F^- (RP) vs. H^+ (FP)- H^+ (RP)	0.44	0.065	6.94	0.001

Table 3: *Net increases in F^- concentration (parts/10^6) and in the proportion of total F ionized (%) in plaque, regressed on the net increase in H^+ concentration (parts/10^9 wet wt) due to fermentation; n = 75.*

r: Correlation Coefficient; S: Regression Slope; I: Intercept of Regression Line.

Associations between individual cumulative caries experience (DMFS, dependent variable) and the following plaque parameters were tested by regression analysis:total plaque quantity (wet *wt*, RP + wet *wt*, FP), H^+, F^- and bound-F concentrations and the proportion of total F ionized in RP and FP, and the net differences between the corresponding RP and FP values. The data plots suggested curvilinear associations for DMFS *vs.* total plaque quantity, H^+ concentration (in FP), bound-F concentration (in RP and FP) and the proportion of total F ionized (in RP and FP). In each case, the best fit was offered by the exponential curve, suggesting $\log_{10}$, transformation of the DMFS values. To avoid conceptually unacceptable weighting of subjects with zero DMFS (since $\log_{10} 0 = -\infty$) but maintain the form and strength of associations unchanged, one DMFS unit was added to each subject's score prior to transformation. The plot for DMFS *vs*. the increase in H^+ concentration due to fermentation (H^+, FP minus H^+, RP) indicated a straight-line relationship. For the remaining variables, no relationship was perceived from the data plots, but the lack of association was confirmed by regressing non-transformed values. The results are summarized in table 4, which includes the pairs of variables between which significant correlations were found. An additional noteworthy result was the positive trend between DMFS and total plaque weight, which failed to reach the 5% significance level by a small margin.

$\log_{10}$ (DMFS + 1)[4] *vs*.		***r***	***S***	***I***	***p* <**
H^+ (parts/10^9 wet *wt*)	FP	0.28	0.002	0.55	0.010
H^+ (parts/10^9 wet *wt*)	FP-RP	0.30	0.002	0.58	0.010
F^- (parts/10^6 wet *wt*)	FP	-0.23	-0.57	0.87	0.050
F^- (% of total F)	RP	0.40	0.05	0.63	0.001
	FP	0.28	0.01	0.67	0.010
Bound F (parts/10^6 wet *wt*)	RP	-0.57	-0.03	0.90	0.001
	FP	-0.50	-0.04	0.92	0.001

Table 4: *Summary of regressions of DMFS on variables related to resting plaque (RP) and fermenting plaque (FP); n = 75.*

[4]: Dependent variable in all regressions except vs. H^+ in FP - RP where no transformation was indicated.

r: Correlation Coefficient; S: Regression Slope; I: Intercept of Regression Line.

Discussion

Plaque weight

The differences between RP and FP weights (Table 1) may have occurred by chance, but for individuals there was a significant increase following rinsing with sucrose. The outcome of the two types of t-tests also indicated that a substantial component of the standard deviation of plaque weights could be attributed to variations between individuals, which should be disregarded in testing the hypothesis that sucrose rinsing had no effect on plaque weight. We believe that the higher weight of FP reflected the additional amount of polysaccharide formed following exposure to sucrose. This would be in accord with our clinical observation that FP was more coherent than RP, and with the finding that the polysaccharide content of plaque rises rapidly and peaks at about 10min after rinsing with sucrose [12].

The mean wet:dry weight ratios were closely similar in RP and FP (Table 1) and corresponded to a mean water content of 84%.

Ionized and bound F in resting and fermenting plaque

The mean F- concentration found in resting plaque (Table 2) was about twice that reported by Lindfors and Lagerlöf [12] for undiluted plaque fluid. The difference may be attributed to the use of extractant and consequent dilution of plaque fluid in this study, which is known to contribute to the ionization of F [13]. As expected, the F- concentration increased with the ten-fold increase in H+ concentration following the sucrose rinse. However, whereas the absolute amount of F- recovered in FP was slightly more than double that found in RP, the calculated proportion of the total F that was ionized increased three-fold and the mean concentration of bound F was reduced by 25% in FP (Table 2).

The most plausible explanation for these differences is that part of the ionized F was lost to the oral environment or to enamel [14] during the 45 minutes elapsing between the first sucrose rinse and the completion of plaque collection. We conjecture that the amount of F lost corresponded to the difference between the mean TF concentrations found in RP and FP (5.03 - 3.85 = 1.18 parts/10^6 F), about 25% of the RP value. Such a difference is substantial in the context that, if the loss continued at a similar rate (i.e. 23-25% per 4 - 5 minutes), the plaques would be severely depleted of F during the low pH phase. If this were the case, plaques exhibiting the greatest pH drop, and presumably taking longest to return to resting pH, might be expected to contain relatively low F levels; the opposite would be true for plaques with a moderate pH drop.

Fermenting capacity and F content of plaque

Our data suggest that, given continued challenge, the loss of ionized F may enhance the development of a perpetually low plaque F level. This conclusion was reached by comparing the mean plaque F values of the 10 subjects with the highest H^+ concentration in FP with those of the 10 subjects with the lowest H^+ concentration (Table 5). The findings appear to support our assumption, in that subjects with low H^+ concentrations had significantly higher mean TF concentrations in both RP and FP than those whose H^+ concentration was high. The finding relating to the RP of these groups suggests that the difference in TF concentration is not of passing nature; it is probably maintained by the significantly greater rate of ionization of F in the FP of the high H^+ producers, also apparent from table 5. Although the primary determinants of plaque pH drop are the nature of the flora and the substrate supply, *in-vitro* evidence indicates that F reduces the utilization of carbohydrates by plaque bacteria [14,15]. Conversely, depletion of plaque F may facilitate fermentation, pH drop and continued reduction of F level. It is tempting to conclude that these findings also represent support *in vivo* for a cause-and-effect relationship between high plaque F and reduced acid production. However, as Higham and Edgar [16] demonstrated a significant direct association between the weight of plaque and its acid-producing capacity, the required evidence could only be obtained by comparing plaques of closely similar weights.

Concentration or proportion Mean		High H^+ (>150, n = 10)		Low H^+ (<70, n = 10)		*t*	*p* <
		SD	Mean	SD			
H^+	FP	176	24	60	8.00	14.4	0.001
TF	RP	1.50	0.90	9.70	9.20	2.80	0.020
F^- (%TF)	RP	3.00	1.70	2.00	1.60	1.30	ns
TF	FP	1.10	0.60	6.00	3.60	4.10	0.001
F^- (%TF)	FP	15.6	5.60	2.70	2.40	6.70	0.001

Table 5: *Total fluoride concentration (TF, parts/10^6 wet wt) and proportion of total fluoride ionized (F^-, % TF) in resting plaque (RP) and fermenting plaque (FP) of subjects with high and low hydrogen ion (H^+) concentration (parts/10^9 wet wt) in FP.*

The findings discussed above raise the question of F uptake by plaque, which would also require F in ionized form, although not necessarily as a simple ion. As our findings show that low pH and associated ionization favor overall loss of plaque F, long-term uptake probably occurs at near-neutral pH levels under resting conditions.

Fermenting capacity of plaque and ionization of F

The positive association between the increase in H^+ concentration following sucrose rinse and the increases in absolute and proportionate amounts of F concentration (Table 3) were significant, but much weaker than the correlation reported by Turtola [17] between plaque pH and F- activity (r = -0.96) for *in-vitro* fermenting samples. Although the very high r value they obtained over a wide pH range is to an extent the consequence of including two distinct groups of data points in the same regression analysis, part of the discrepancy can be attributed to differences in experimental design and to specific constraints imposed in our *in-vivo* study.

The plaque samples used by Murata., *et al*. [6] were constituted portions of the mixed pools of plaque collected from several individuals. This procedure tends to minimize the adverse effect of the very substantial variation in F content between individual samples (Table 2) on correlations. Plaques with low original F concentration cannot produce the same absolute amount of F- for a given increase in H+ concentration as those with high F concentration, despite the greater proportion of total F ionized (Table 5). Thus a high correlation between increase in H+ and F- concentrations is precluded in populations where individual variation in plaque-F concentration is characteristic. Moreover, the implicit assumption we have made, that the initial F concentrations of the two plaque samples collected from each subject were identical, though justified for a group, is clearly untrue for each individual. This point is particularly important with respect to bound F, which represented over 97% of total F in RP, because the decrease due to ionization would not be apparent for an individual whose second plaque sample contained, fortuitously, slightly more F initially than the first sample. We believe that this is the reason why no association could be demonstrated between the increase in H^+ concentration and decrease in bound-F concentration on an individual basis.

Caries experience and the fermenting capacity and F content of plaque

The positive correlations demonstrated between DMFS and the H^+ concentration in FP, and the increase in H^+ concentration due to fermentation (Table 4), are in accord with the generally accepted view that the cariogenic potential of plaque is directly associated with its acid-producing capacity.

We discount the aetiological significance of the inverse association between DMFS and the F- concentration (Table 4) for several reasons: (1) the statistical significance level was marginal in FP analysis and no association or supporting trend was found in RP; (2) the association may be the consequence of the strong inverse correlation between DMFS and bound-F concentration, reflecting the higher absolute amount of F- released from plaques with high bound-F content; (3) at 0.07 and 0.16 parts/10^6 mean values in RP and FP, the F- concentrations were too low to envisage a short-term effect on bacterial metabolism, and negligible in comparison with the F content of enamel; (4) the evidence we obtained indicates no substantial build-up of F- concentration in fermenting plaque.

The proportion of total F ionized was directly correlated with DMFS in RP and FP. However, the latter association is explained by the inverse relation between DMFS and bound F because the bound-F fraction represents 92 and 98% of total F in FP and RP, respectively, and consequently the proportion of total F ionized equals approximately the reciprocal of bound F.

Conclusions

In contrast to the indefinite relation between DMFS and F- concentration, we reached to a conclusion that the highly significant association between DMFS and the bound-F content of RP and FP (Table 4) refers to the protective role of plaque F that is associated with the fractions which were not released under the conditions of this study. However, our data do not indicate the form of bound F, the work of Marquis [15] which suggests that about two-thirds of the F retained may have been associated with bacteria.

The significantly elevated mean F content of plaques with the lowest H+ concentration (Table 5) exactly supports the view that the bound-F fraction remains metabolically active and protects from caries through interfering with the carbohydrate metabolism of plaque bacteria.

Conflict of Interest

The authors have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

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