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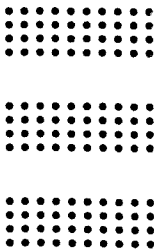
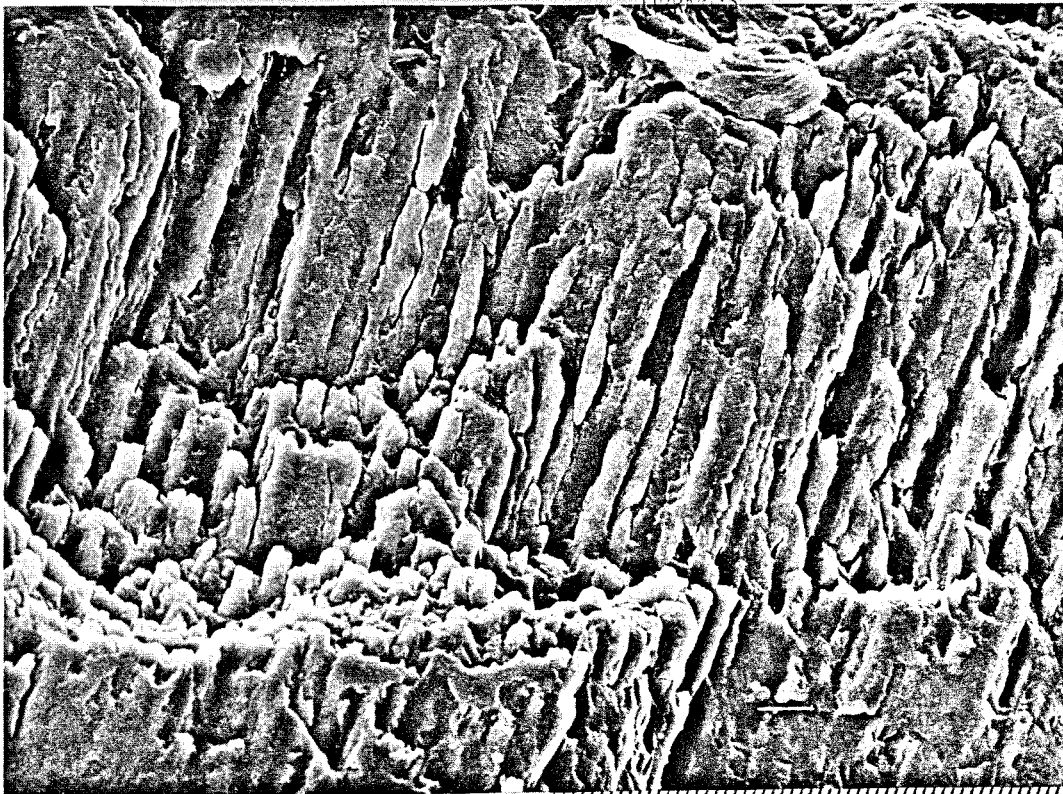
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Influence of Fluoride and pH on in vitro Remineralization of Bovine Enamel

Key Words

Bovine enamel
 Demineralization
 Fluoride
 pH
 Remineralization

Abstract

Subsurface lesions in bovine enamel slices were remineralized. The remineralization solutions contained either 0.03, 0.3, or 1.0 ppm fluoride at either pH 5.5 or 6.8. The amount of remineralization was determined after periods of up to 610 h, using quantitative microradiography. The results showed that after 126 h of remineralization in the presence of 0.03 ppm fluoride significantly ($p < 0.05$) more remineralization occurred at pH 6.8 than at pH 5.5. At 0.3 and 1.0 ppm fluoride no significant differences between pH 5.5 and pH 6.8 were observed. An interaction between fluoride and pH was observed. The observed differences in the rates of remineralization are explained by the formation and subsequent transformation of the precursors octacalcium phosphate (pH 6.8) and brushite (pH 5.5) into (fluor)apatites.

Remineralization of caries lesions in enamel is possible [ten Cate and Arends, 1977, 1980; ten Cate, 1979; Silverstone et al., 1981; Larsen and Fejerskov, 1989]. Clinically this process is very important, as it counteracts the process of demineralization [Backer Dirks, 1966; Pot and Groeneveld, 1976; Koulourides and Cameron, 1980]. In vivo remineralization occurs by saliva [Koulourides et al., 1965]. However, the ability of individuals to remineralize enamel differs, as saliva and plaque also differ in composition between and within individuals.

Of probable importance with respect to remineralization are fluoride concentration (0.01-1.0 ppm) and pH [McCann, 1968; Jenkins, 1978; Driessens, 1982]. A number of studies reported about the influence of fluoride on crystal growth and on remineralization of enamel [Meyer and Nancollas, 1972; Eanes and Meyer, 1978; Eanes, 1980; ten Cate and Arends, 1977; ten Cate and Duijsters, 1982; Lam-

mers et al., 1990a, b]. Both stimulating and inhibiting influences of fluoride on remineralization have been reported in the studies cited above. Most remineralization studies have been carried out at a pH of around 7. This is the pH of resting saliva and plaque [McCann, 1968; Jenkins, 1978; Driessens, 1982; Jensen and Schachtele, 1983]. However, during an acid attack, the pH of plaque decreases to an acid value and increases to the resting level, as the acid is removed or neutralized. Depending on the type of foods consumed [Jensen and Schachtele, 1983], the pH of the plaque may reach a value of about 5-6 for some time. The question is whether remineralization may occur at such pH values, although crystal growth studies showed that precipitation is possible [Barone et al., 1976; Barone and Nancollas, 1978a, b].

The aim of this study was to investigate the influence of fluoride and pH on remineralization.

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Materials and Methods

Preparation of Sections and Lesions

Thirty-six slices with a thickness of about 125 μm were cut from sound bovine incisors perpendicular to the labial surface. Sandwiches were prepared from these slices as described elsewhere [Lammers et al., 1990a]. The sandwiches were demineralized during 40–69 h at 25 °C under stirring in a solution containing 2.2 mM Ca, 2.2 mM P, 50 mM acetic acid, and 5 μM (0.1 ppm) fluoride (pH 5.0). This solution was undersaturated with respect to hydroxyapatite (OHA) [van Dijk et al., 1979]. The solution was refreshed every day. Of this solution 10 ml/sandwich was used.

Remineralization

After demineralization the sandwiches were washed in distilled water and divided into six groups. These groups were subjected for 610 h to remineralization solutions (25 °C) containing at pH 6.8 1.54 mM Ca, 1.54 mM P, and 20 mM acetic acid/ammonium acetate buffer. At pH 5.5, the solutions contained 5.5 mM Ca, 5.5 mM P, and 20 mM acetic acid/ammonium acetate buffer. At both pH values fluoride was added to a final concentration of either 0.03, 0.3, or 1.0 ppm. Although chemicals of analytical grade were used, the solutions always contained trace amounts of fluoride. The amount varied from about 0.019 to 0.026 ppm, depending on the composition of the buffer used. Therefore, even without addition of any fluoride to the solution, the fluoride concentration was not virtually 0 [see also Theuns et al., 1983]. In order to keep the fluoride concentration at a defined level, the amount of fluoride in the solutions was determined, and just enough fluoride was added to obtain the final fluoride concentration desired. To simulate saliva, all solutions were adjusted with KCl to obtain a ionic strength of about 44 mM [Shannon et al., 1974].

The solutions at pH 6.8 were refreshed twice a day in order to avoid the influences of precipitation reactions. The solutions at pH 5.5 were refreshed once a day. The solutions were stirred at 150 rpm. Per sandwich 34 ml solution was used. The solutions simulated the oral environment for fluoride (saliva and plaque) and pH (unstimulated and stimulated condition and a certain value during food consumption) [McCann, 1968; Jenkins, 1978; Driessens, 1982; Jensen and Schachtele, 1983].

The solutions at pH 6.8 were saturated, i.e., in equilibrium, with respect to octacalcium phosphate (OCP; pI_{OCP} 68.6) and brushite (DCPD; pI_{DCPD} 6.69) and supersaturated with respect to OHA (pI_{OHA} 100.3) and fluorapatite (FAP; pI_{FAP} 97.7, 95.7, and 94.7 for 0.03, 0.3, and 1.0 ppm fluoride, respectively). pI_{OCP} , pI_{DCPD} , pI_{OHA} , and pI_{FAP} represent the negative logarithms of the ionic products of OCP, DCPD, OHA, and FAP respectively. At pH 5.5 the solutions were saturated, i.e., in equilibrium, with respect to DCPD (pI_{DCPD} 6.69) and supersaturated with respect to OHA (pI_{OHA} 108.3) and FAP (pI_{FAP} 103.2, 101.2, and 100.2 for 0.03, 0.3, and 1.0 ppm fluoride, respectively) [Driessens, 1982].

The above pI values were obtained by calculations using the extended Debye-Hückel formula [Robinson and Stokes, 1970].

Microradiography/Microdensitometry

At 0, 22, 62, 126, 192, 329, and 610 h of remineralization, contact microradiographs of the sandwiches and an aluminum stepwedge were made, using $\text{CuK}\alpha$ radiation [Groeneveld, 1974; Theuns et al., 1984, 1985; Lammers et al., 1990a, b]. The density of the microradiographs was measured quantitatively and transformed in mineral contents (vol%) as a function of depth (μm) [Angmar et al., 1963; Lam-

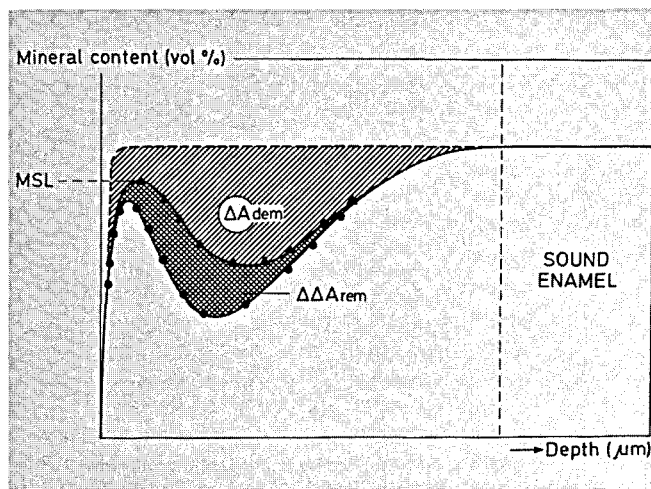


Fig. 1. Mineral content as a function of depth in enamel with a subsurface lesion. Measurements were performed after demineralization (●) and after de- and subsequent remineralization (▲). For further explanation see tables 1 and 2.

mers et al., 1990a, b]. It should be noted that some drying of the sections is unavoidable during this procedure, as the specimens are out of solution for some time for evaluation. In principle, this drying may have had an influence on the remineralization. But as every section was treated in an identical way, it was assumed that this influence was the same in all experimental groups.

Data Handling

The lesion parameters obtained from the densitometric profiles were averaged. The averaged values represent the results for one sandwich at one time. These values for the sandwiches in one group were averaged to obtain values for that group.

Data were subjected to two-way analysis of variance, combined with multiple comparisons (Tukey) in order to detect significant differences between the groups, as sometimes an interaction between fluoride and pH was observed. Residuals were checked for normality. (As no serious deviations from normality could be found, these statistical methods were applied.)

Results

After demineralization, the enamel slices showed subsurface lesions. A schematic presentation of the mineral content as a function of depth is given in figure 1. The mean subsurface lesion depth of a group ($n=6$) ranged from 110 ± 9.8 to 133 ± 25.0 μm .

The remineralization solutions, differing in pH and fluoride concentrations, are all able to induce remineralization (table 1; see footnotes for further explanations). For most preparations the remineralization occurred mainly

Table 1. ΔA_{dem} and $\Delta\Delta A_{\text{rem}}$ after 22, 62, 126, 192, 329, and 610 h of remineralization at either pH 5.5 or 6.8 and either 0.03, 0.3, or 1.0 ppm fluoride (n = 6; mean \pm SD)

Remineralization solution		ΔA_{dem}	$\Delta\Delta A_{\text{rem}}$					
pH	F, ppm		22 h	62 h	126 h	192 h	329 h	610 h
5.5	0.03	3,288 \pm 470	27 \pm 88	233 \pm 147	269 \pm 252	434 \pm 181	484 \pm 173	694 \pm 216
5.5	0.3	3,542 \pm 937	196 \pm 212	346 \pm 199	396 \pm 203	604 \pm 255	887 \pm 142	1,152 \pm 261
5.5	1.0	3,380 \pm 764	225 \pm 92	222 \pm 195	422 \pm 219	485 \pm 258 ^b	768 \pm 344	825 \pm 436 ^b
6.8	0.03	3,097 \pm 511	268 \pm 220	494 \pm 146	748 \pm 239 ^a	958 \pm 223 ^a	1,194 \pm 333 ^a	1,663 \pm 416 ^a
6.8	0.3	2,721 \pm 310	139 \pm 99	337 \pm 207	468 \pm 202	697 \pm 211	898 \pm 200	1,205 \pm 259
6.8	1.0	3,005 \pm 724	344 \pm 168 ^a	329 \pm 150	399 \pm 277	602 \pm 364	730 \pm 418	860 \pm 535 ^b
Remarks ^c		NS	NS	NS	pH, I	pH	pH, I	pH, I, F

ΔA_{dem} = Area (vol% \times μm) above the profile of treated enamel; ΔA after demineralization is represented by the hatched area in figure 1. $\Delta\Delta A_{\text{rem}}$ = Area (vol% \times μm) above the profile after demineralization minus the area (vol% \times μm) above the profile after de- and subsequent remineralization; $\Delta\Delta A$ after some remineralization is represented by the cross-hatched area in figure 1.

^a Significantly (p < 0.05) different from pH 5.5, 0.03 ppm fluoride.

^b Significantly (p < 0.05) different from pH 6.8, 0.03 ppm fluoride.

^c Analysis of variance (p < 0.05) resulted in: pH = effect of pH; I = interaction between pH and F; F = effect of fluoride; NS = no significant difference.

Table 2. MSL_{dem} and ΔMSL_{rem} after 22, 62, 126, 192, 329, and 610 h of remineralization at either pH 5.5 or 6.8 and either 0.03, 0.3, or 1.0 ppm fluoride (n = 6; mean \pm SD)

Remineralization solution		MSL_{dem}	ΔMSL_{rem}					
pH	F, ppm		22 h	62 h	126 h	192 h	329 h	610 h
5.5	0.03	70.2 \pm 3.3	1.8 \pm 1.5	4.0 \pm 1.3	4.3 \pm 1.5	4.7 \pm 1.8	5.2 \pm 1.7	6.7 \pm 1.9
5.5	0.3	69.1 \pm 6.4	8.1 \pm 5.2 ^a	9.3 \pm 4.6	11.9 \pm 5.8 ^a	12.5 \pm 5.4 ^a	13.6 \pm 6.5 ^a	13.5 \pm 6.4
5.5	1.0	69.8 \pm 6.0	8.8 \pm 3.3 ^a	9.1 \pm 2.9	10.9 \pm 2.6	11.3 \pm 3.3 ^a	10.2 \pm 4.7	8.9 \pm 4.6 ^b
6.8	0.03	71.2 \pm 2.6	4.6 \pm 4.1	6.0 \pm 3.2 ^b	9.4 \pm 3.4	9.3 \pm 2.8	11.2 \pm 2.1	12.1 \pm 4.3
6.8	0.3	63.9 \pm 4.5	8.8 \pm 2.2 ^a	12.7 \pm 3.7 ^a	12.6 \pm 4.6 ^a	15.8 \pm 4.4 ^a	17.2 \pm 3.4 ^a	16.9 \pm 4.7 ^a
6.8	1.0	69.5 \pm 4.5	8.7 \pm 1.7 ^a	8.4 \pm 1.6	8.3 \pm 3.0	9.8 \pm 3.7	11.6 \pm 5.0	10.8 \pm 4.5
Remarks ^c		NS	F	F	F	F	pH, F	pH, F

MSL_{dem} = Mineral content (vol%) of the surface layer after demineralization before remineralization. ΔMSL_{rem} = Mineral content (vol%) of the surface layer after de- and subsequent remineralization minus the mineral content (vol%) of the surface layer after demineralization.

^a Significantly (p < 0.05) different from pH 5.5, 0.03 ppm fluoride.

^b Significantly (p < 0.05) different from pH 6.8, 0.3 ppm fluoride.

^c Analysis of variance (p < 0.05) resulted in: F = effect of fluoride; pH = effect of pH; NS = no significant difference.

in the outer half of the lesion. With 0.03 ppm fluoride significantly more remineralization occurred after 126 h of remineralization at pH 6.8 than at pH 5.5. Using 0.3 and 1.0 ppm fluoride, remineralization was found to be the same for both pH values. At pH 5.5 the presence of 0.3 ppm fluoride tended to cause a maximum in the remineralization rate. At pH 6.8 the presence of 0.3 and 1.0 ppm fluoride

caused an inhibition of the remineralization, tending to increase with increasing fluoride concentrations (table 1).

In table 2 (see footnotes for further explanations) the mean ΔMSL_{rem} is shown at several time points for both pH values and the three fluoride levels. At both pH values the ΔMSL_{rem} at 0.3 ppm fluoride showed the largest value as compared with the ΔMSL_{rem} at 0.03 and 1.0 ppm fluoride.

The remineralization ($\Delta\Delta A_{rem}$; vol% \times μm) after 610 h at both pH values and at the three fluoride levels is shown in figure 2. In this figure the maximum remineralization rate in the presence of 0.3 ppm fluoride at pH 5.5 and the inhibiting influence of fluoride on remineralization at pH 6.8 are visualized.

Discussion

In the presence of 0.03 ppm fluoride more remineralization occurred at pH 6.8 than at pH 5.5 (table 1, fig. 2). This observation may be explained by events observed in other studies during crystal growth experiments. At 0.03 ppm fluoride and pH 6.8, the solutions are saturated, i.e., in equilibrium, with respect to OCP and DCPD and supersaturated with respect to OHA and FAP [Driessens, 1982]. Remineralization may occur by initial formation of OCP followed by transformation into a fluoride-containing OHA, (F)OHA. The results of the studies of Eanes [1980], Eanes and Meyer [1978], and Driessens et al. [1978] support this view. At 0.03 ppm fluoride and pH 5.5, the solutions are saturated, i.e., in equilibrium, with respect to DCPD but not OCP and supersaturated with respect to OHA and FAP [Driessens, 1982]. Remineralization may occur by formation of DCPD, even though the solutions are supersaturated with respect to OHA and FAP. This is in accordance with the findings reported by Barone et al. [1976] and Barone and Nancollas [1978a, b]. Barone et al. [1976] reported DCPD formation and no OHA formation at pH 5.0 within 24 h, indicating that even though the solutions at acid pH were supersaturated with respect to OHA, no direct OHA formation was observed.

If these interpretations hold, it appears that at 0.03 ppm fluoride the OCP pathway, occurring at pH 6.8, is quicker than the DCPD pathway at pH 5.5. The fact that at pH 6.8 the amount of supersaturation with respect to OHA is greater than at pH 5.5 may contribute to the observed difference too. However, the main precipitation of mineral seems not to be directly mediated by OHA, but by precursors: DCPD at pH 5.5 and OCP at pH 6.8. This is supported by the results of the studies cited above.

At 0.3 and 1.0 ppm fluoride, no significant differences between both pH values were observed. Apparently fluoride influenced the crystal growth pathways at both pH values in such a way that the amounts of remineralization approached each other at both fluoride levels. The mechanism by which fluoride influences the rate of remineralization at both pH values may be explained in the following way.

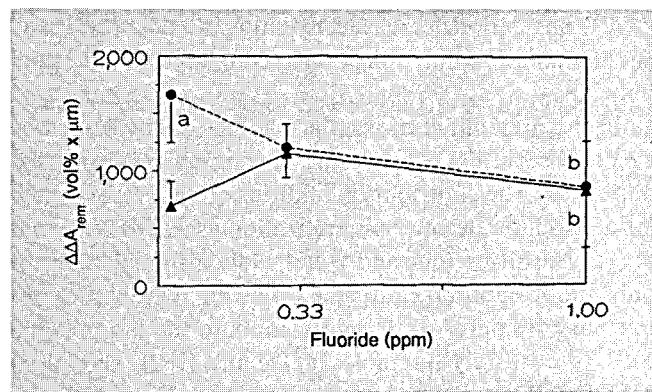


Fig. 2. $\Delta\Delta A_{rem}$ after 610 h of remineralization at either 0.03, 0.3, or 1.0 ppm fluoride and at either pH 5.5 (\blacktriangle) or 6.8 (\bullet). Bars indicate the SD ($n = 6$). ^a Significantly ($p < 0.05$) different from 0.03 ppm fluoride at pH 5.5; ^b significantly ($p < 0.05$) different from 0.03 ppm fluoride at pH 6.8.

At pH 6.8, in the presence of 1.0 ppm fluoride and possibly in the presence of 0.3 ppm fluoride too, very locally in the outer part of the lesion the fluoride concentration can become so high that the OCP is bypassed in favor of direct precipitation of FAPs [Driessens, 1986]. As a consequence, the fluoride concentration in the penetrating solution decreases in the deeper pores. The local conditions in these pores change at the expense of direct FAP precipitation. Now mineral can only precipitate via OCP formation, followed by transformation into (F)OHA. In addition, the local fluoride concentrations may be high enough to retard OCP formation, resulting in a lower net remineralization in the presence of 1.0 ppm fluoride, and possibly in the presence of 0.3 ppm fluoride too, as compared with 0.03 ppm (table 1, fig. 2). These proposed pathways are in agreement with findings in the literature [Eanes and Meyer, 1978; Eanes, 1980].

At pH 5.5 precipitated DCPD is able to react with fluoride in order to form (F)OHA [Chow and Brown, 1974]. With increasing amounts of fluoride in the solutions, a decrease in the amount of DCPD as fraction of the precipitated phase was observed, while a more basic calcium phosphate phase precipitated [Barone and Nancollas, 1978b]. Accordingly, in our experiments, at 0.3 ppm fluoride the net amount of remineralization increased, as the formation of DCPD and (F)OHA from DCPD was stimulated. At 1.0 ppm fluoride only the formation of (F)OHA was stimulated. The formation of DCPD during the precipitation phase decreased, and the formation of a more basic calcium phosphate phase increased. For this reason, the ability of the surface to act as a template for secondary

DCPD nucleation decreased, resulting in a decrease in the net amount of remineralization as compared with 0.3 ppm fluoride.

In the previous sections, the findings are explained by events observed in other studies during crystal growth experiments. The contribution of diffusion processes, which influence the rate of remineralization as well [Brudevold et al., 1982; Silverstone and Wefel, 1981], is thought to be of only minor importance for the following reasons. An important parameter for the diffusion into the lesion is the MSL (see Results and table 2) [Theuns, 1987]. The initial MSL for all groups was found to be not significantly different ($p > 0.05$) from each other. So the initial diffusion is expected to be the same for all groups. During remineralization the MSL changed (table 2). If the diffusion through the surface layer would be the rate-determining factor for remineralization, one would expect that after a given time of remineralization a low Δ MSL value corresponds with a high $\Delta\Delta A$ value and vice versa. As can be

seen from tables 1 (last column: 0.03 ppm fluoride at pH 5.5; 0.3 ppm fluoride at pH 5.5 and 6.8) and 2 (0.03 ppm fluoride at pH 5.5; 0.3 ppm fluoride at pH 5.5 and 6.8) this was not the case.

From this study it can be concluded that the pH causes only a significant difference in the rate of remineralization of tooth enamel at a low fluoride concentration (0.03 ppm). There is an interaction between fluoride and pH: the course of the influence of fluoride on remineralization depends on the pH of the remineralization solution.

With microradiographical analyses only changes in the amount of mineral at various locations in the lesion and in the total lesion can be determined. Therefore, although the amounts of remineralization are known, it is not known whether the precipitation mechanisms during the remineralization, as described here, apply. To obtain more precise information, other techniques such as microprobe analyses of the enamel or secondary ion mass spectrometry would be required.

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