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MICRO-PIXE (PROTON-INDUCED X-RAY EMISSION) STUDY OF THE EFFECTS OF FLUORIDE ON MINERAL DISTRIBUTION PATTERNS IN ENAMEL AND DENTIN IN THE DEVELOPING HAMSTER TOOTH GERM

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Abstract

Micro-PIXE (proton-induced X-ray emission) analysis was performed on unfixed and anhydrously prepared sections from developing enamel and dentin from hamsters injected with a single dose of 20 mg NaF/kg body weight. Fluoride, apart from inducing the formation of the characteristic paired response in the enamel (i.e., a hyper- followed by a hypomineralized band in the secretory enamel), also induces the formation of sub-ameloblastic cystic lesions under the transitional and early secretory enamel accompanied by relatively intense hypermineralization of the underlying cystic enamel surface. These cystic lesions, however, were only found to be associated with certain isolated populations of these cells. In addition, these lesions were restricted to the smooth surfaces of the tooth germ only. Cystic lesions such as those seen under the transitional and early secretory ameloblasts were not observed under the fully secretory or maturation stage ameloblasts.

Why fluoride induces the formation of cystic lesions in some ameloblast populations while other cells in the same stage of development apparently remain unaffected, is a matter which needs further investigation.

Key words: Fluoride, calcium, phosphorus, enamel, dentin, mineralization, Proton-induced X-Ray Emission, amelogenesis, dentinogenesis, hamster.

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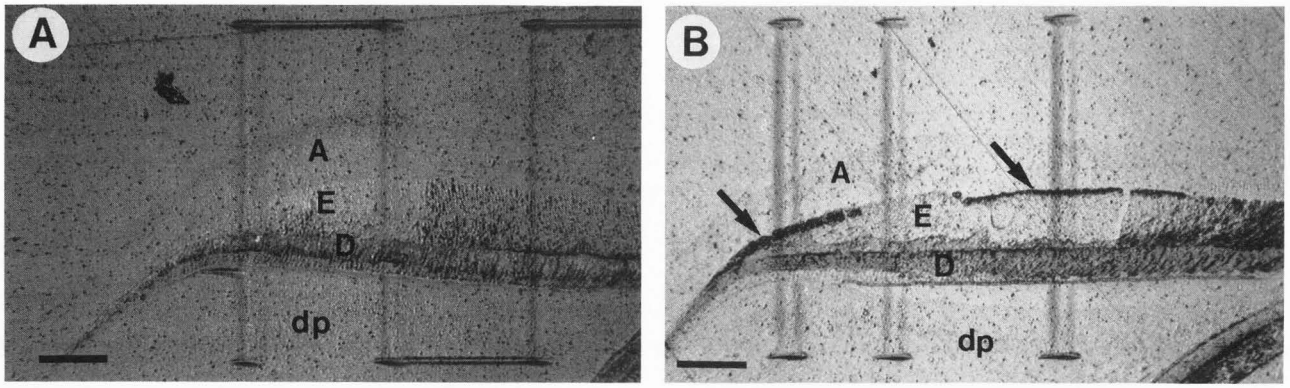
Introduction

Administration of fluoride to experimental animals during the secretory phase of amelogenesis induces a paired response in the developing enamel, i.e., the formation of a hyper- followed by a hypomineralized layer in the developing enamel (Weber and Yaeger, 1964; Fejerskov et al., 1979; Walton and Eisenmann, 1974; Mörnstad and Hammarström, 1978; Ishida et al., 1983). Apart from the aforementioned effect, fluoride may also induce the formation of the so-called sub-ameloblastic cysts in the developing enamel organ (Lange Nordlund et al., 1986; Simmelink and Lange 1986; Lyaruu et al., 1989a). Ultrastructural examination of these cystic lesions suggests that the underlying cystic enamel surface is hypermineralized (Simmelink and Lange, 1986). In addition, recent micro-PIGE (proton-induced gamma-ray emission) studies of these cystic lesions in developing hamster molar tooth germs have shown that the enamel surface under the cysts also contain the highest concentrations of fluoride measured in the developing enamel (Lenglet et al., 1988; Lyaruu et al., 1989a). The formation of these cystic lesions in the enamel seems to be restricted only to certain ameloblast populations (Lyaruu et al., 1989a). However, it is not clear why only certain ameloblast populations are affected by fluoride while others in the same stage of development apparently remain unaffected. The aim of this investigation, therefore, was to systematically find out with the aid of the micro-PIXE (proton-induced X-ray emission) technique, whether the administration of a 20 mg NaF/kg body weight fluoride dose to neonatal hamsters always leads to the formation of sub-ameloblastic cysts in the developing tooth germ, and whether the enamel surface under the cystic lesions is always hypermineralized.

Materials and Methods

Experimental animals and fluoride administration

Seventeen four-day old hamsters from three litters (nine experimental and eight controls) were used. All the animals were weighed before and after the experiments in order to find out whether fluoride affected body weight. The experimental animals were administered a single sub-cutaneous dose of 20 mg NaF/kg body weight (b.w.). The NaF was dissolved in distilled water and sterilized through a 0.2 micrometers filter before administration. The injected volume was 20 microliters/gram b.w. The controls were injected with an equimolar amount of Cl^- (as



Figures 1A and 1B. Micrographs taken after the measurements directly from the targets (specimen holders) using a CCD (charge coupled device video) camera mounted on a microscope. The dark lines across the micrographs show the position and the dimensions of the linescans. These lines were due to beam irradiation of the plastic embedding medium. The lines were used to determine the position of the histological structures in the element distribution patterns obtained after the analyses. Figure **1A** shows a control germ and Figure **1B**, a fluoride-treated germ containing a cystic lesion under both the transitional and early secretory ameloblasts (arrows). (A; ameloblasts, E; enamel, D; dentin, dp; dental pulp). Bars = 100 micrometers.

NaCl) using the same volume and under identical experimental conditions. The injections were administered between 15:00 and 15:30 hr. The hamsters were marked and returned to their respective mothers after the injections.

Twenty four hours later, the hamsters were weighed again to check the effect of fluoride on body weight. Statistical analysis of the body weight data (Students' t-test) showed that fluoride had no significant effect on the body weights of the experimental animals (4.34 ± 0.30 g (n=9) vs. 4.28 ± 0.35 g (n=8), experimental vs. control, respectively, at the onset of the experiment, and 5.00 ± 0.30 g vs. 4.96 ± 0.39 g experimental vs. control, respectively, after the experiment). Thus, using this criterion, the fluoride dose used (20 mg NaF/kg b.w.) does not seem to induce systemic side effects in neonatal hamsters 24 hours after administration. In contrast to this, preliminary experiments had shown that a dose of 30 mg NaF/kg b.w. or higher significantly reduced the hamster body weight after the experiment, which is reminiscent of systemic side effects. Thus, using this criterion, a single dose of 20 mg NaF/kg b.w. administered sub-cutaneously is considered the maximum fluoride dose which does not induce systemic side-effects in the 4-5-day-old neonatal hamsters. Higher doses were, therefore, not used in this investigation.

Tissue processing

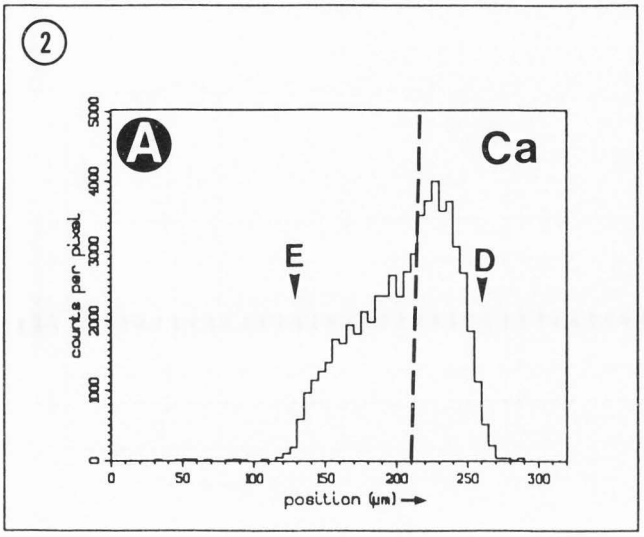
The hamsters were killed by decapitation 24 hours after the injections. The maxillary first molars (M^1) were then dissected out of the surrounding alveolar bone and quickly frozen in liquid nitrogen-cooled propane. The tissues were kept in liquid nitrogen until all the dissections were completed. The germs were then transferred to a liquid nitrogen-cooled metal block and freeze-dried for three days at -45°C. After freeze-drying, the tissues were slowly brought to room temperature under vacuum. The germs were then infiltrated with Spurrs' low viscosity resin, also under vacuum.

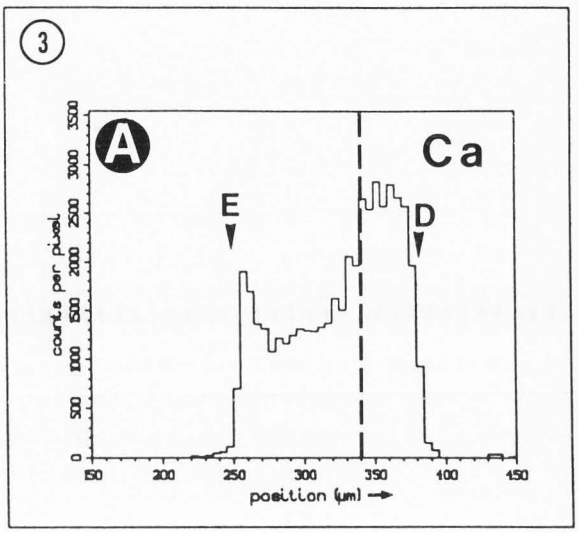
After polymerization, 1-2 micrometers thick sections were serially prepared at room temperature in a buccal-lingual plane using a diamond knife and a Reichert UltraCut microtome (Reichert-Jung, Austria). The sections were stained with dilute toluidine blue in sodium tetraborate solution and were examined immediately. Serial sectioning was continued until a fluoride-induced cystic lesion was encountered in the enamel organ. Then, from each experimental germ, 2 micrometer sections were cut and mounted on Histophane foil (4 micrometers thick, coated with a few nanometers of aluminum) for PIXE analysis. Due to the hardness of the material, sectioning artefacts were often encountered associated with the hypermineralized cystic enamel surfaces. For this reason, only those sections free of artefacts, as judged by phase contrast microscopy (250X), were used for the analyses. In addition, in order to reduce mineral loss, section contact with water during sectioning was kept to absolute minimum (approximately 30 seconds). All sections used (both experimental and control) originated from the anterior cusps. Sections of similar thickness from each control germ, were cut from the anterior pair of cusps and also analyzed.

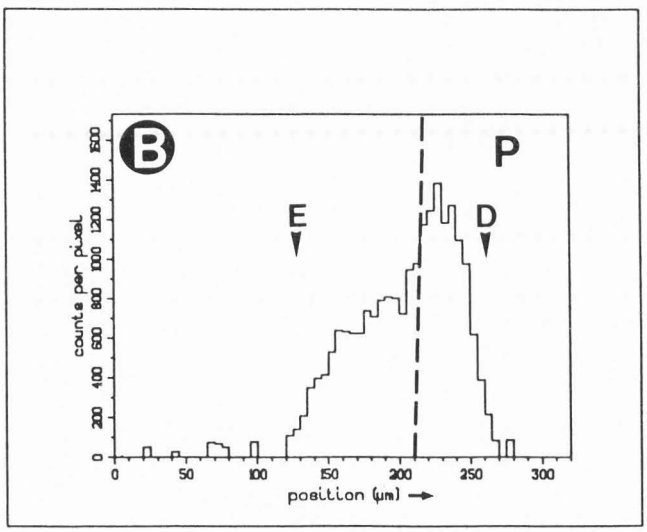
Micro-PIXE analysis

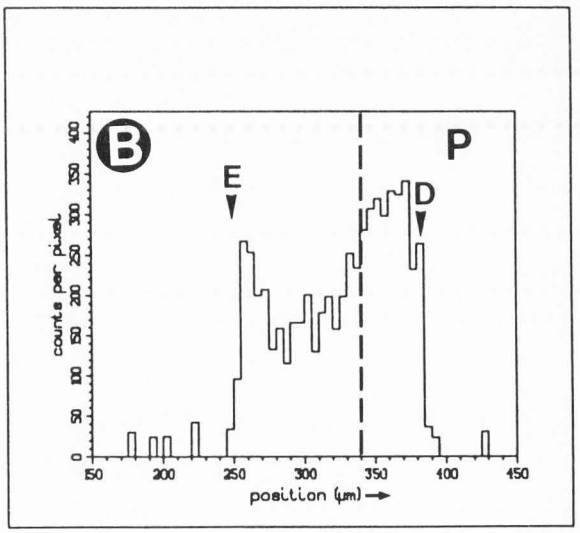
The micro-beam set-up at the Vrije Universiteit (Amsterdam) was used to produce a 3 MeV pulsed proton beam. The time interval between two successive proton bursts is approximately 166 ns. In this investigation, the proton beam was scanned over a track length of about 400-600 micrometers across the area of interest, i.e., over the ameloblasts, enamel, dentin, predentin, odontoblasts and pulp (Figures 1A and 1B) using a beam spot of 10x60 micrometers^2 and a typical average beam current of approximately 1 nA. The smallest beam spot dimension was always tuned parallel to the scan direction which was perpendicular to the enamel surface. The average measurement time for each linescan was about twenty minutes. Detailed description of the analysis parameters and techniques used in this investigation has been reported elsewhere (Tros et al., 1990).

A minimum of three linescans was performed for each experimental and control tooth germ: one linescan across the late secretory-to-transitional ameloblasts; a second scan across the mature secretory ameloblasts, (i.e., those secretory ameloblasts





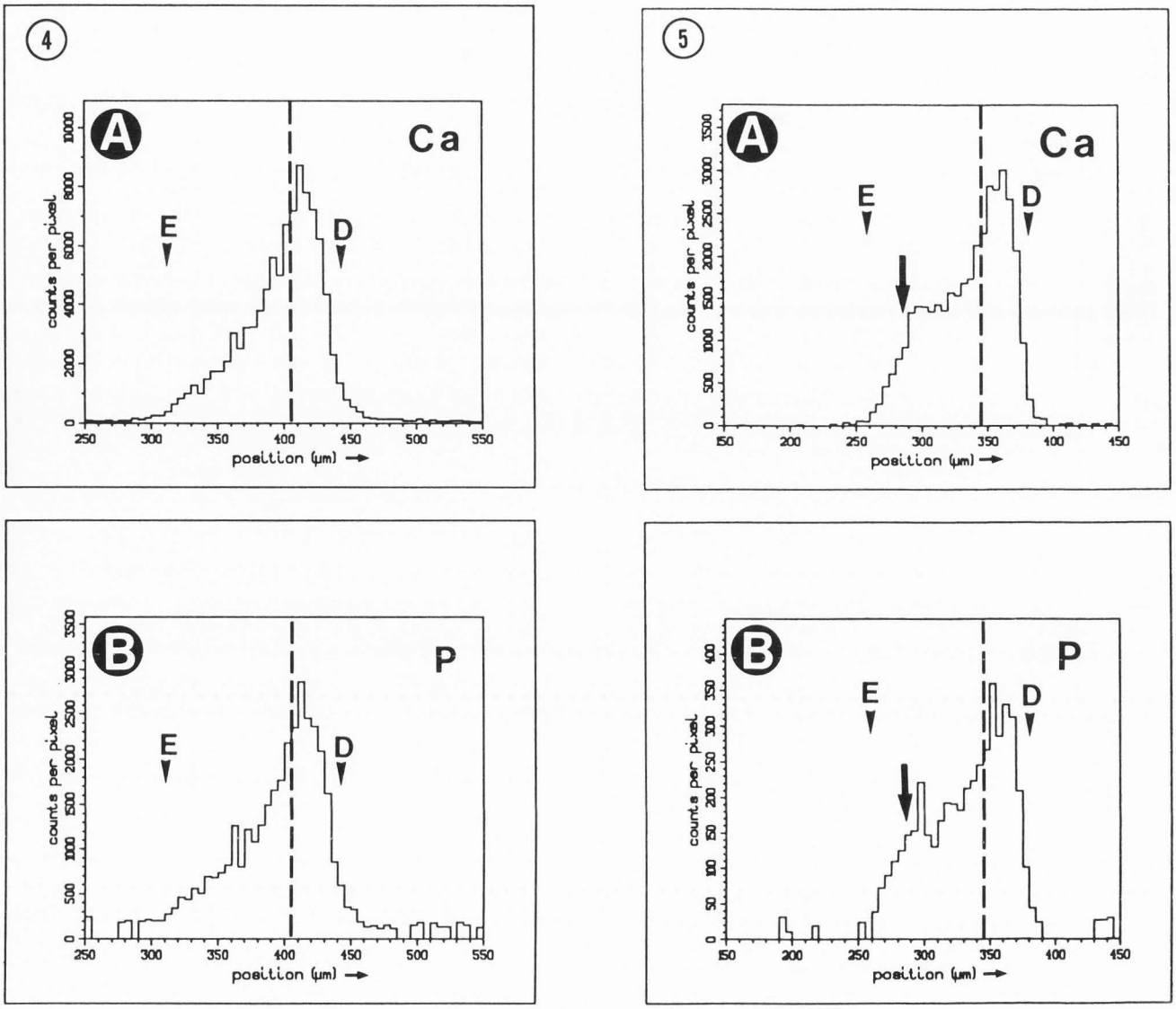




Figures 2A and 2B. The Ca and P element distribution pattern recorded across the transitional ameloblasts from a control tooth germ are shown in Figures 2A and 2B, respectively. The letters E and D, indicate the positions of the enamel surface and dentine-predentin junction respectively, (see also Figures 1A and 1B). The broken line shows the position of the dentin-enamel junction (DEJ). The lowest counts for both Ca and P were recorded on the enamel surface and gradually increased in the direction of the dentin-enamel junction. The highest number of counts for both elements were recorded in the dentin near the dentin-enamel junction. Figures 3A and 3B. Figures 3A and 3B show, respectively, the Ca and P distribution across the transitional enamel of a fluoride-treated tooth germ containing a sub-ameloblastic cyst. The letters E and D, indicate the positions of the enamel surface and dentin-predentin junction, respectively, and the broken line the position of the DEJ (see also Figures 1A and 1B). The cystic enamel surface is clearly hypermineralized (cf. Figures 2A and 2B).

which had attained their maximum height); and the third scan across the early secretory ameloblasts (Figures 1A and 1B). It was not always possible to tune the smallest spot dimension perpendicular to the enamel surface in those scans performed across the early secretory enamel in the cervical loop region because of the natural curvature of the tooth germ surface. This geometrical aberration was not corrected for when the positions of the different histological structures were being determined from the micrographs. Thus, the enamel thickness determined from the analyses in some of the linescans in this region may be greater than the actual enamel thickness in the tooth germs.

The peak contents of the elements of interest in the analyses, (i.e., $Ca_{K(\alpha + \beta)}$ and $P_{K(\alpha + \beta)}$ peaks) were determined using a spectrum fitting programme called "Specfit" (Blok et al., 1975). No attempt was made to estimate the absolute concentrations of these elements in the spectra, only their



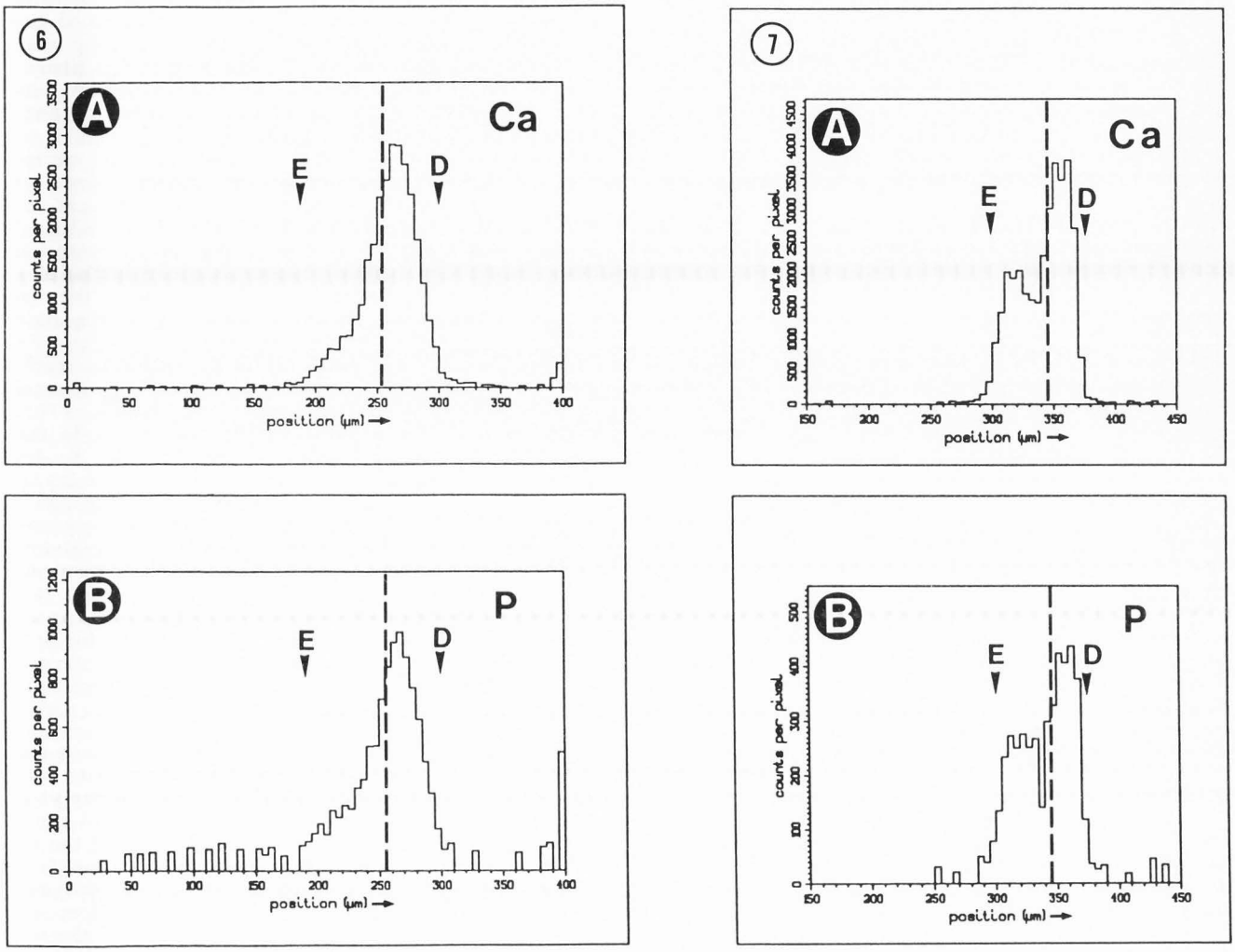
Figures 4A and 4B. These figures show, respectively, the distribution of Ca (Figure 4A) and P (Figure 4B) recorded across the secretory enamel of a control tooth germ. This mineral distribution pattern shows the gradual increase of mineralization with depth characteristic of developing enamel. The letters E and D, indicate the positions of the enamel surface and dentin-predentin junction, respectively, and the broken line, the position of the DEJ (see also Figures 1A and 1B).

Figures 5A and 5B. These figures show the element distribution patterns for Ca (Figure 5A) and P (Figure 5B) for the linescans performed across the secretory enamel of a fluoride treated tooth germ. The letters E and D, indicate the positions of the enamel surface and the dentin-predentin junction, respectively. The solid arrow shows the approximate position of the fluoride-induced hypermineralized band in the secretory enamel and the broken line, the position of the DEJ. The mineralization pattern in the surface layers of the enamel up to the fluoride-induced hypermineralized band resembles that seen on the surface enamel layers of the control tooth germs (cf. Figures 4A and 4B).

relative concentrations within the scans. The distribution of the mineral elements (Ca and P, see Figs. 2 to 7) along the scans was correlated with the position of the respective histological structures using micrographs of the tooth germs (final magnification: 100X) taken after the measurements were performed (Figures 1A and 1B).

Results

Serial sectioning of the experimental tooth germs confirmed the presence of fluoride-induced sub-ameloblastic cystic lesions under the late secretory-to-transitional ameloblasts as well as under those ameloblasts which had just become secretory at the onset of fluoride treatment in all experimental tooth germs examined. These lesions were, however,



Figures 6A and 6B. The Ca (Figure 6A) and P (Figure 6B) distribution patterns obtained for the early secretory enamel from a control tooth germ. The positions of the enamel surface and dentin-predentin junction are indicated by the letters E and D, respectively, and that of the DEJ by the broken line.

Figures 7A and 7B. The general element distribution patterns for Ca (Figure 7A) and P (Figure 7B) recorded for the early secretory enamel containing a sub-ameloblastic cystic lesion were comparable to those recorded across the transitional ameloblasts also containing a cystic lesion (cf. Figures 3A and 3B). Note that in the case of the early secretory enamel lesion, the relative difference in mineral content between that recorded on the hypermineralized enamel surface and that recorded in the body of the lesion is greater than that recorded for the cystic lesions under the transitional ameloblasts (see also Figures 3A and 3B). The position of the DEJ is indicated by the broken line and that of the enamel surface and dentin-predentin junction by the letters E and D, respectively.

not found under all the transitional or the early secretory ameloblasts; they were found to be associated with only certain populations of these cells. In addition, these lesions were restricted to the smooth surfaces of the developing tooth germ only; they were not observed in the fissures.

Light microscope examination of the cystic lesions indicated that the enamel surface was highly hypermineralized as could be judged by the lack of toluidine blue staining and the presence of numerous sectioning artefacts often observed in these affected regions of enamel. Cystic lesions, such as those seen under the early secretory or transitional ameloblasts, were not observed under the "mature" secretory ameloblasts, i.e., those ameloblasts which had attained their maximum dimensions at the time of fluoride administration. However, a faint staining line separating the pre-exposure enamel from the enamel formed after exposure to fluoride was always observed in the enamel in this region.

Typical Ca and P element distribution patterns for the control tooth germs recorded for the analyses performed in the enamel organ across the transitional, secretory and early secretory ameloblasts are shown in Figures 2A and 2B, 4A and 4B, and 6A and 6B, respectively. This mineral distribution pattern was in general identical for all control germs analyzed. The highest concentration of both elements in the mineralized tissues was found in dentin near the dentin-enamel junction (DEJ). In the developing enamel, the Ca and P content (i.e., mineral content) was lowest on the enamel surface and increased in the direction of the DEJ. However, in the enamel under the transitional ameloblasts, the mineral content seemed to increase much faster with depth than that recorded for the younger enamel regions.

Figures 3, 6 and 7, show the distribution of Ca and P recorded across the three different regions of the enamel organ of a typical fluoride-treated germ. This was the general distribution pattern for the experimental tooth germs analyzed. Figures 3A and 3B show the distribution pattern for Ca and P from a linescan across the transitional ameloblasts of a fluoride-treated tooth germ containing a cystic lesion, and Figures 7A and 7B show the distribution of the elements in a linescan across the early secretory ameloblasts, also containing a cystic lesion. Figures 5A and 5B show the distribution patterns for Ca and P obtained across the secretory ameloblasts. Cystic lesions were not observed under the mature secretory ameloblasts. It should be mentioned here that cystic lesions in the enamel organ were in most cases not simultaneously present in both locations (i.e., under the transitional and early secretory enamel) on the same section analyzed. Normally, only one of the two lesions could be analyzed from one single section.

The Ca and P content (i.e., mineral content) in the fluoride-treated enamel was highest on or near the cystic enamel surface irrespective of the position of the lesion in the enamel organ, i.e., either under the transitional or early secretory ameloblasts. Hypermineralized cystic lesions were observed in all experimental tooth germs. The mineral content in the cystic lesions in the enamel under the ameloblasts, after an initial increase near the surface, decreased in the direction of the body of the lesion and increased again in the direction of the DEJ.

In the secretory enamel of the fluoride-treated tooth germ, the mineral content in the enamel layer formed after fluoride administration gradually increased with depth and in the same way as that seen in the control secretory enamel up to the junction between the exposure and pre-exposure enamel (cf. Figures 4A and 4B and Figures 7A and B). On or just under the junction, the mineral content increased at a faster rate than that seen in the controls. The mineral content then levelled off and then increased again in the direction of the DEJ as in the controls.

Typical element distribution patterns for Ca and P recorded across a fluoride-induced lesion under the early secretory ameloblasts are shown in Figures 7A and 7B. A comparison of the mineral content recorded on the surface of the lesion with that recorded in the dentin shows that the surface of the lesion was also highly hypermineralized, in some cases even approaching that recorded for dentin.

Discussion

The pattern of the distribution of calcium and phosphorus counts observed in the linescans of the control tooth germs indicate that the developing enamel analyzed was not yet in the maturation phase. On the other hand, a progressive mineralization pattern was evident when the element distribution patterns from the different regions were compared; i.e., the mineral content in that enamel situated under the transitional ameloblasts was higher than that recorded for the secretory regions of the enamel organ. In all element distributions, the highest mineral content was always recorded in the dentin near the DEJ. When the absolute counts obtained in the element distributions were used as a measure for the mineral composition in the enamel organ, the data indicated that the mineral in dentin near the DEJ had the highest Ca/P ratio, while the mineral associated with the surface layers of developing enamel had the lowest Ca/P ratio (data not shown). This suggests that the mineral in the enamel up to the transitional stage of amelogenesis is still in its formative stages and probably contains a mineral with a low Ca/P ratio such as octacalcium phosphate and/or its transition product intermediate to octacalcium phosphate and hydroxyapatite. Octacalcium phosphate is currently thought to be the precursor mineral in developing enamel (Brown et al., 1987).

Administration of a single fluoride dose, apart from inducing the formation of the characteristic paired response in the enamel (i.e., a hyper- followed by a hypomineralized band in the secretory enamel) (Weber and Yaeger, 1964; Fejerskov et al., 1979; Walton and Eisenmann, 1974; Mörnstad and Hammarström, 1978; Ishida et al., 1983), also induced the formation of cystic lesions in the ameloblasts accompanied by hypermineralization of the underlying enamel surface under both the transitional and early secretory ameloblasts. A rough comparison of the Ca counts recorded across the cystic enamel surface (approximately 20 micrometers) with those recorded in the dentin near the DEJ (also about 20 micrometers thick; highest counts in the DEJ=100%) indicated that the hypermineralized cystic enamel surface layer contained approximately 55-70% of the calcium counts recorded in dentin near the DEJ, indicating that fluoride induces a high degree of hypermineralization on the cystic enamel surface in comparison with that recorded for the control enamel surface (control calcium content on enamel surface is less than 20% DEJ). Regarding the hypermineralization of the cystic enamel lesions, the data presented in this investigation agree with the data reported for cystic enamel lesions in the rat enamel organ (Simmelink and Lange, 1986; Lange Nordlund et al., 1986; Lange Nordlund and Lindskog, 1986). However, it should be mentioned here that with respect to the mineral content on the hypermineralized cystic enamel surface, it is likely that the mineral content recorded in this region was lower than the actual mineral content present in situ due to possible mineral loss in these regions during the preparation of the sections. Although all necessary care was taken to prevent sectioning artefacts, even in those sections which were judged to be of optimal quality, in the hypermineralized cystic enamel surface regions, the sectioning knife appeared to chop rather than section the tissue, a factor which could invariably lead to mineral

loss. Also, mineral loss due to the brief contact with water during sectioning, although kept to an absolute minimum, cannot be ruled out completely.

As was the case in an earlier study (Lyaruu et al., 1989a), the cystic lesions observed under both types of ameloblasts in this study were restricted to the transitional and early secretory ameloblasts. These observations indicate that these cells are more sensitive to fluoride than other cells of the enamel organ. In addition, the fact that only certain populations of these cells were affected also implies that the sensitivity of ameloblasts to fluoride is not only dependent upon the stage of development, but that there is also a difference in sensitivity to fluoride between cells of the same developmental stage within the same tooth germ specimen. One possible explanation for the high sensitivity of the transitional ameloblasts to fluoride could be that during this stage of development (in which the cells are being remodelled for another totally different function, i.e., the transition from a secretory into a resorptive function), they become temporarily vulnerable to external influences. This explanation could also be applicable in the case of the early secretory ameloblasts because these cells are in fact also in a transitional stage, i.e., these cells are still in the process of transformation into fully highly active secretory cells. This explanation, however, does not answer one important question posed by the data presented in this study namely: Why are only certain isolated populations affected while other cells of the same developmental stage remain unaffected?

Micro-PIGE studies specifically designed for the detection of fluorine in developing hamster tooth germs have demonstrated that the hypermineralization of the cystic enamel lesion is also accompanied by increased fluorine content in the enamel mineral (Lyaruu et al., 1989a). The fluorine detected in the fluorotic enamel mineral in the above mentioned study was most probably incorporated into the enamel crystals rather than present in ionic form because the experimental conditions used were purposely not permissive for the retention of ions in the tissues. Also, it is unlikely that the fluoride was bound to or associated with the organic matrix because the fluorine concentration in the developing enamel was lowest on the enamel surface; a region where one expects the highest content of enamel matrix proteins. Also, in vitro culture experiments using hamster tooth germs explanted during the secretory phase of amelogenesis have shown that unmineralized enamel matrix secreted during fluoride exposure retains its capacity to support mineralization when the ion is removed from the culture environment and that the hypermineralization induced by the fluoride treatment is irreversible (Lyaruu et al., 1986; 1987; 1989b). In addition, in vitro experiments have shown that fluoride increases the rate of precipitation of calcium ions from solutions (Larsen and Thorsen, 1984). Thus, in this context, the increased mineral deposition observed in the affected hypermineralized cystic enamel areas is due to increased fluoride influx into the enamel mineralization front. Current data suggests that the precursor for the mineral in developing enamel is octacalcium phosphate, and that its subsequent transformation (maturation) into hydroxyapatite is accelerated by fluoride (Brown et al., 1987). Thus, presence of elevated fluoride levels in the cystic lumen will result in accelerated formation of (fluoridated) hydroxyapatite and may thus be the explanation for the hypermineralization of cystic enamel surface.

Acknowledgements

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References

Blok HP, de Lange JC, Scotman JW. (1975). A new peak search method for automatic spectrum analysis program. Nucl. Instr. Methods. 128, 545-556.

Brown WE, Eidelman N, Tomazic B. (1987). Octacalcium phosphate as a precursor in biomineral formation. Adv. Dent. Res. 1, 306-313.

Fejerskov O, Yaeger JA, Thylstrup A. (1979). Microradiography of the effect of acute and chronic administration of fluoride on human and rat dentine and enamel. Archs. Oral Biol. 24, 123-130.

Ishida T, Ogawa Y, Yagi T. (1983). Effects of fluoride injection on the enamel formation in rats. In: Mechanisms of Tooth Enamel Formation, Suga, S. (Ed.), Quintessence Publ. Co., Tokyo, pp. 285-303.

Lange Nordlund A, Lindskog S, Hammarström L. (1986). Fluoride-induced cystic changes in the enamel organ of the rat molar. J. Oral Pathol. 15, 87-92.

Lange Nordlund A, Lindskog S. (1986). Immunoglobulin G in cystic lesions of rat enamel organ following fluoride intoxication. Scan. J. Dent. Res. 94, 15-18.

Larsen MJ, Thorsen A. (1984). A comparison of some effects of fluoride on apatite formation in vitro and in vivo. Calcif. Tissue Int. 36, 690-696.

Lenglet WJM, Stoof CJJ, Lyaruu DM, Vis RD, Verheul H. (1988). Fluorine determination in hamster tooth germs. Nucl. Instr. Methods B30, 429-433.

Lyaruu DM, Lenglet WJM, Wöltgens JHM, Bronckers ALJJ. (1989a). Micro-PIGE determination of fluorine distribution in developing hamster tooth germs. J. Histochem. Cytochem. 37, 581-589.

Lyaruu DM, Blijleven N, Hoeben-Schornagel K, Bronckers ALJJ, Wöltgens JHM. (1989b). X-ray microanalysis of the mineralization patterns in developing enamel in hamster tooth germs exposed to fluoride in vitro during the secretory phase of amelogenesis. Adv. Dent. Res. 3, 211-218.

Lyaruu DM, de Jong M., Bronckers ALJJ, Wöltgens JHM. (1987). Ultrastructure of in vitro recovery of mineralization capacity of fluorotic enamel matrix in hamster tooth germs pre-exposed to fluoride in organ culture during the secretory phase of amelogenesis. Archs. Oral Biol. 32, 107-115.

Lyaruu DM, de Jong M, Bronckers ALJJ, Wöltgens JHM. (1986). Ultrastructural study of fluoride-induced in vitro hypermineralization of enamel in hamster tooth germs explanted during the secretory phase of amelogenesis. Archs. Oral Biol. 31, 109-117.

Mörnstad H, Hammarström L. (1978). Morphologic changes in the rat enamel organ following a single intraperitoneal injection of sodium fluoride. Scand. J. Dent. Res. 86, 211-220.

Simmelink JW, Lange A. (1986). Ultrastructure of altered rat enamel beneath fluoride-induced cysts. J. Oral Pathol. 15, 155-161.

Tros GHJ, Lyaruu DM, Vis RD (1990) The effect of fluoride on mineralization during tooth germ development in neonatal hamsters. Nucl. Instr. Meth. B49, 476-480.

Walton RE, Eisenmann DR. (1974). Ultrastructural examination of various stages of amelogenesis in the rat following parenteral fluoride administration. Archs. Oral Biol. 19, 171-183.

Weber DF, Yaeger JA. (1964). A microradiographic interpretation of abnormal enamel formation induced by subcutaneous sodium fluoride. J. Dent. Res. 43, 50-56.

Discussion with Reviewers

D.R. Eisenmann: The hypermineralization illustrated in Figures 7A and 7B is said to approach the mineral density of dentin. This is not evident in the figures. Could the authors please comment? Also, it has been shown in vitro that enamel organ stripped of its overlaying ameloblasts permit increased calcium uptake into the enamel. Could this phenomenon be a significant factor in the hypermineralization of the enamel surface under the cystic lesions?

Authors: The picture presented for the mineral content recorded for the cystic enamel surface layer shown in Figures 7A and 7B is slightly distorted. This is because the mineral content measured on and just under the cystic enamel surface in this particular area of the specimen is subject to geometrical aberration of the enamel surface due to the curvature of the tooth germ at the cervical loop region; i.e., in this region, the proton beam approaches the enamel surface at an angle instead of parallel to the surface as was the case with the other areas analyzed. This is because of the fact that in this experimental set-up, the specimen holder cannot be manipulated in order to correct for the above mentioned geometrical aberration. As stated in the "Materials and Methods", this aberration when present, was not corrected for in the element distribution patterns. In practice, this aberration was restricted only to some of the linescans performed across the early secretory enamel near the cervical loop and not in those scans performed across either the mature secretory or transitional enamel. Taking into consideration the above mentioned geometrical aberration, the absolute mineral content on the hypermineralized cystic enamel surface shown in Figures 7A and 7B is considered to approach that recorded for dentin near the DEJ.

Enamel stripped of its overlaying ameloblasts has been shown in vitro by Bawden and colleagues (e.g., see Bawden JW, Wennberg A, J. Dent. Res. 56, 313-319, 1977) to take up more radiolabelled Ca^{2+} than that from viable tooth germs containing an intact ameloblast layer. Thus using this model, it is pertinent to assume that the damaged ameloblasts overlaying the cystic lesions may have been affected to such a degree that they could no longer control the calcium influx into the enamel mineralization front. Consequently, calcium influx of calcium will take place from the extracellular space into the cystic enamel lesion until an equilibrium is established between the two compartments. Preliminary in vitro experiments from our laboratory, however, indicate that when experimentally damaged tooth germs containing a discontinuous layer of secretory ameloblasts are treated with fluoride (1-25 ppm F^-) for 24 hours, this does not necessarily result in hypermineralization of the cell-free enamel surface compared to that observed in the fluorotic enamel overlaid by intact cells either in vitro or in vivo (unpublished results). In retrospect, although the above mentioned phenomenon, postulated by the reviewer, may be involved in the hypermineralization of the cystic enamel surface, its participation in this process is probably only limited to the initial stages of lesion formation before an equilibrium is attained between the Ca^{2+} concentration at the enamel mineralization front and that of the extracellular fluid. Thus, it seems as if another mechanism is involved (probably induced by fluoride) which is responsible for the increased Ca^{2+} influx into the cystic lumen during the later phases of lesion formation resulting in hypermineralization of the enamel surface.

G.M. Roomans: What was the rationale of using the micro-PIXE technique instead of the electron microprobe which has higher resolution as well as the possibility of quantitative measurements? Also, the Ca/P count rate ratio was higher in the fluoride-treated tooth germs than in the controls. Was this increase significant?

Authors: The primary aim of this investigation was to study the relative changes in mineral distribution patterns in enamel and dentine induced by fluoride administration rather than absolute quantification of the mineral content in the specimens. The choice of this instrument, apart from having access to it, was mainly based on the fact that relatively large areas could be continuously scanned. In addition, if necessary, the experimental micro-PIXE set-up can be optimized for quantitative measurement of trace elements in biological tissues. However, the electron microprobe is the instrument of choice for elemental quantification if high resolution is a prerequisite.

Examination of the data indicated that, when compared to the controls, fluoride treatment increased the calcium/phosphorous count rate ratio ($Ca_{K\alpha}$ / $P_{K(\alpha+\beta)}$), in particular on the hypermineralized cystic enamel surface. Whether or not the observed fluoride-induced increase in Ca/P ratio is significant requires further investigation.

K. Malmquist: How were the scans performed? If a still beam was used, was radiation-induced damage observed in the specimens?

Authors: The scans were performed by moving the target continuously to and fro along the predetermined track. The stage is driven by a local autonomous computer. In order be sure of the position of the target when a particular count was registered, the x, y and z co-ordinates were continuously monitored by electronic gauges.

The proton beam was kept stationary during the measurements. After the analyses, some blackening of the section was evident along the linescans. Reanalysis of the same scan tracks as well as comparison of their mineral content with adjacent tracks indicated that there was no significant loss of either calcium or phosphorus from the specimen as a consequence of the analysis. Thus, the observed blackening of the scan tracks was judged to be due interaction of the proton beam with the plastic embedding medium rather than with the biological tissue being analyzed.