

# Microradiography and electron-microprobe analysis of some caries-like lesions of enamel prepared in vitro in human teeth

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## MICRORADIOGRAPHY AND ELECTRON-MICROPROBE ANALYSIS OF SOME CARIES-LIKE LESIONS OF ENAMEL PREPARED *IN VITRO* IN HUMAN TEETH

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**Summary**—Six lesions were made on the buccal surfaces of premolars. The volume percentage of mineral was determined as a function of depth by microradiography. Using the electron microprobe, the signals for Ca, Na, Mg, P and Cl were recorded as a function of depth both through the lesions and through the adjacent sound enamel. In the demineralized parts, there was a preferential loss of Na and a preferential retention of chloride. In the surface layers, the Na:Ca and Cl:Ca ratios were almost the same as in the adjacent sound enamel, indicating that the surface layers were not formed by gross dissolution of the original mineral followed by gross precipitation of another, less-soluble calcium phosphate, but remained probably because their microcrystals were protected by a thin layer of precipitated fluorapatite or fluoridated hydroxyapatite. The same had been found for surface layers in natural caries.

### INTRODUCTION

The mineral in tooth enamel shows a typical behaviour in dental caries *in vivo*. Preferential dissolution of carbonate has been reported (Coolidge and Jacobs, 1957; Little, Cuetto and Rowley, 1962a; Johansen, 1965; Hallsworth, Weatherell and Robinson, 1973) of magnesium (Johansen, 1965; Suga, 1970; Hallsworth, Robinson and Weatherell, 1972) and of sodium (Little, Posen and Singer, 1962b). Using microradiography in combination with electron-microprobe analysis, Driessens *et al.* (1986) confirmed the preferential loss of sodium, but they also found a preferential retention of chloride in natural carious lesions.

The *in vivo* carious challenge is exerted intermittently during the course of the process: pH and degree of undersaturation of the plaque fluid vary continuously with time. This was probably not so during the formation of caries-like-lesions *in vitro*, at least not pH which tends to be constant at about 4 to 5.5. Some investigators (e.g. Theuns *et al.*, 1984; Theuns, Driessens and van Dijk, 1986) even keep the degree of undersaturation constant during demineralization *in vitro*. Our aim was to investigate whether preferential dissolution of sodium and magnesium and preferential retention of chloride occurred under continuous demineralization *in vitro*.

### MATERIALS AND METHODS

Two human premolars extracted for orthodontic reasons were covered with wax except for three small windows on the buccal surface. The window surfaces were brought in contact with buffers undersaturated with hydroxyapatite (pK 117.2) and supersaturated with fluorapatite (pK 121.2). Table 1 shows the times of demineralization and the characteristics of the buffers. The degree of undersaturation of the buffers with hydroxyapatite can be calculated from their

value for the negative logarithm of the ionic product for hydroxyapatite OHA defined as

$$pI_{\text{OHA}} = 10 p\text{Ca} + 6 p\text{PO}_4 + 2 p\text{OH}.$$

For this calculation, the activity coefficients and complexation constants were as given by Driessens (1982). Their fluoride content was also known so that the negative logarithm of the ionic product for fluorapatite FA could be calculated. The volume of buffer was 100 ml per tooth. During demineralization, the buffers were changed twice a week.

After demineralization, the teeth were sectioned longitudinally and a slice about 200  $\mu\text{m}$  thick was ground planoparallel to a thickness of about 80  $\mu\text{m}$ . Contact microradiograms were made from the thinned slices together with an aluminium stepwedge using  $\text{CuK}\alpha$  radiation (Groeneveld and Arends, 1975). The density of the microradiograms was measured with a Leitz densitometer. The volume percentage of mineral as a function of depth was calculated from these densities and that of the stepwedge

Table 1. Demineralization conditions for the six artificial lesions under investigation (pH of buffers was 4.5, their  $pI_{\text{OHA}}$  124 and their total acetate content 50 mmol  $\text{l}^{-1}$ ; the lesions contained no chloride and their  $\text{Na}^+$  content was brought to a level to make the pH 4.5)

Lesion	Demineralization time (days)	Buffers $pI_{\text{FA}}$
A	16	114.0
B	32	114.0
C	64	114.0
D	16	115.6
E	32	115.6
F	64	115.6

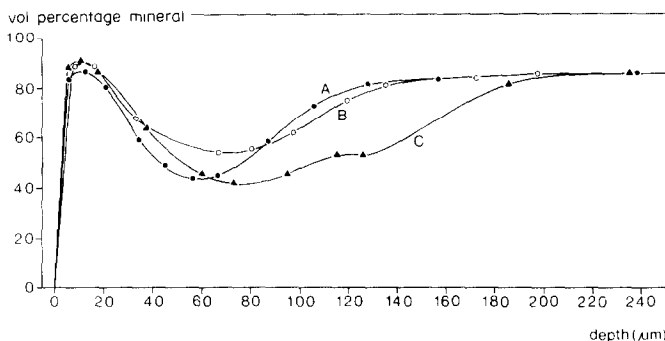


Fig. 1. Volume percentage of mineral as a function of depth for the lesions A, B and C.

using the mineral content for human tooth enamel as described by Angmar, Carlström and Gilas (1963).

One half of each premolar was used for electron-microprobe analysis. Each half was embedded in copper-containing polymethyl methacrylate, polished with a diamond paste in oil and covered with a thin layer of carbon. Tracings for Ca, Na, Mg, P and Cl were made with the electron microprobe Jeol Superprobe 733 starting at the tooth surface after the locations of the lesions were established with an electron back-scattering image. The excitation voltage was kept at 10 kV and the current at 15 nA in order to suppress the evaporation of Na from the excited volume. The tracings were taken by steps of 5  $\mu\text{m}$ ; counting was done during 10 s at each step. According to the degree of reproducibility of the signals and to the height of the background, the standard deviations in the signals for Ca, Na, Mg, P and Cl were about 1, 3, 6, 1 and 3 per cent respectively. Therefore, variations larger than twice those percentages were significant at the 0.05 level.

Two tracings were made through each lesion. In addition, one tracing was made through the adjacent sound enamel located on the cervical side of the lesion and one through the adjacent sound enamel located on the occlusal side of the lesion. The last two tracings were averaged to obtain what was called the tracing for sound enamel through the lesion. The results were expressed on a semi-quantitative scale by taking the ratios of the signals Na:Ca, Mg:Ca, P:Ca and Cl:Ca before comparing these with the results of microradiography.

## RESULTS

Figure 1 gives the volume percentage of mineral as a function of depth for the lesions A, B and C. The microprobe signals for Ca and P did not vary with depth nor did they differ between lesions A, B and C or sound enamel. The ratio of the signals P:Ca and Mg:Ca did not differ significantly for sound enamel and artificial lesions. Therefore, the preferential loss of magnesium reported by other workers for natural lesions could not be detected on our artificial lesions due to the low intensity of the  $\text{MgK}\alpha$  signal.

A comparison was made (Fig. 2) between the microradiogram and the Na:Ca Cl:Ca ratio of lesion A. The corresponding results for lesions B and C were similar. Sodium was preferentially lost and chloride

was preferentially retained in the body of the lesion, whereas their concentration in the surface layer were nearly the same as in the surrounding sound enamel. As Fig. 1 shows, volume percentage mineral in the body of lesions A, B and C did not differ much. Accordingly, the amounts of preferential loss of sodium and preferential retention of chloride in lesions B and C were nearly the same as those shown for lesion A (Fig. 2) as would be expected.

As Fig. 3 shows, the volume percentage of mineral in the body of lesions D, E and F differed considerably. So did their ratio for the signals Na:Ca (Fig. 4); the more mineral lost, the higher was the preferential loss of sodium. The main reason why base lines for the Na:Ca ratio of sound enamel were different for lesions D, E and F is probably that the lesions

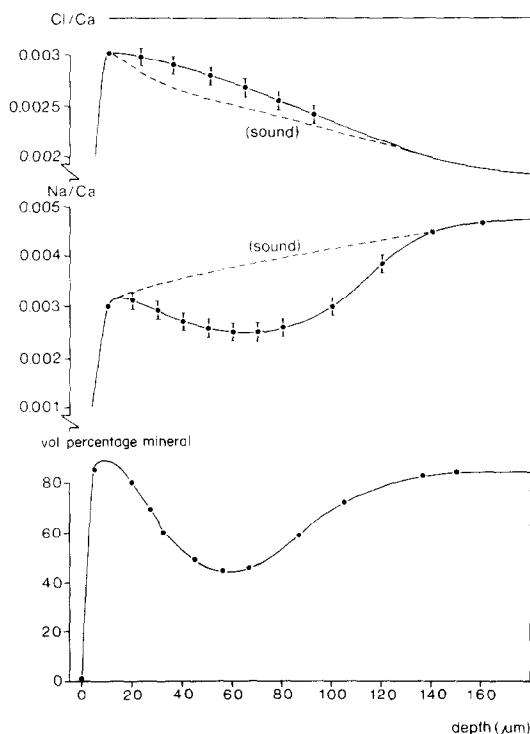


Fig. 2. Volume percentage of mineral and signal ratios Na:Ca and Cl:Ca for lesion A. The signal ratios for the lesion are compared with values for adjacent sound enamel.

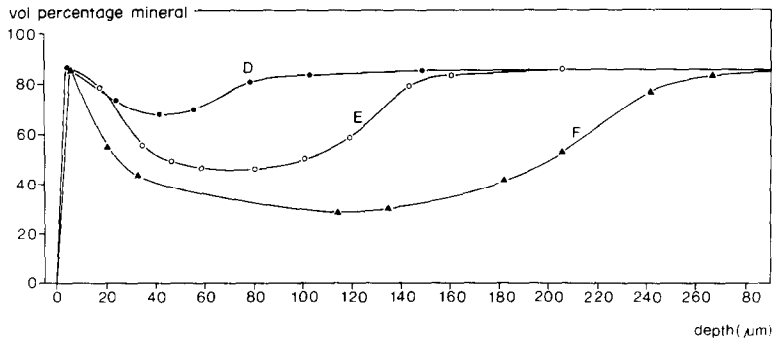


Fig. 3. Volume percentage of mineral as a function of depth for the lesions D, E and F.

were located at slightly different positions on the buccal surface. Lesion D was located in the middle, lesion E in cervical direction and lesion F in occlusal direction on the buccal surface of the same tooth. In these lesions there was a preferential retention of chloride too, but it was so slight that it was not clear whether the increase in the Cl:Ca ratio increased with increasing loss of mineral.

#### DISCUSSION

Comparison of the present findings with those obtained on natural carious lesions (Little *et al.*, 1962b; Driessens *et al.*, 1986) shows that the dissolution behaviour of the mineral of human tooth enamel is the same under continuous demineralization as under intermittent demineralization. In both, there is a preferential loss of sodium and a preferential retention of chloride. It is possible that re-precipitation of (fluoridated) hydroxyapatite occurs simultaneously with the dissolution of tooth enamel mineral, but it is unlikely that the increased Cl:Ca ratio is due to incorporation of chloride into the precipitate (Driessens, 1982).

Our findings indicate that Na and Cl are incorpo-

rated in different parts of the mineral of tooth enamel, whereby the Na-containing part is more soluble than the chloride-containing part, as has been presumed on theoretical grounds (Driessens, 1982; Driessens and Verbeeck, 1982; Verbeeck, 1986).

As in natural lesions (Driessens *et al.*, 1986), the surface layers of the artificial lesions contained nearly as much Na and Cl as the surrounding sound enamel. This probably means that these surface layers are not formed by gross dissolution of the original mineral followed by gross re-precipitation of a less-soluble mineral, but that they are more or less maintained during the period of continuous demineralization because, as proposed previously (Driessens *et al.*, 1986), the mineral particles in the surface layers are protected to a large extent against further dissolution by a thin re-precipitated layer of fluorapatite or fluoridated hydroxyapatite. Unfortunately, the microprobe used was not sensitive enough to make semi-quantitative tracings of the F:Ca signal ratio (Theuns *et al.*, 1986).

As observed from the Na:Ca ratios for sound enamel the two teeth differed about the same amount as this ratio, as a function of both depth and site. Theuns *et al.* (1984, 1986) also obtained good reproducibility on the buccal surfaces of premolars.

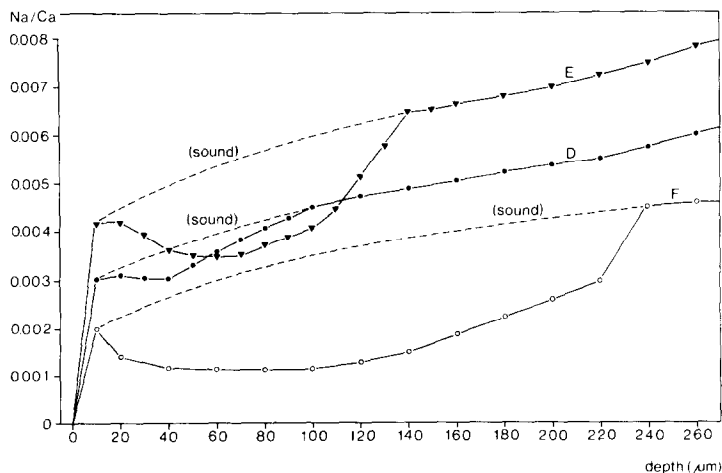


Fig. 4. Signal ratio Na:Ca as a function of depth through lesions D, E and F in comparison with values for adjacent sound enamel.

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