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X-RAY MICROANALYSIS OF DENTIN: A REVIEW

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Abstract

The aim of this review was to present a condensed summary of the literature on X-ray microanalysis of dentin, including both energy-dispersive (EDS) and wavelength-dispersive (WDS) analysis. Estimations of concentrations by XMA of dentin should be regarded as semiquantitative values. The Ca level in rat odontoblasts was elevated in the secreting end of the cell body. In predentin Ca accumulated at a concentration of 2% that of mineralized dentin. In coronal dentin the peritubular areas were hypermineralized (Ca, P, Mg). Primary caries lesions showed a decrease of Ca, P, Mg and Cl, and usually an increase of S and Zn. The mineralized surface often present contained especially high concentrations of F and K. Considerable uptake of various ions in cavity walls exposed to filling materials was assessed: from silver amalgam, Zn and Sn, from silicate cement and glassionomer cement F, Al and Zn, and from zinc oxide-eugenol cement, Zn. The highest F concentrations following topical application were found with solutions of TiF₄ and with the varnishes Duraphat[®] and FluorProtector[®]. Dentin wall lesions adjacent to amalgam fillings exhibited considerably reduced Ca and P values, but concomitantly considerable amounts of Zn and Sn, that explained the increased radiopacity seen in some microradiographs.

KEY WORDS: Dentin, human, animal, normal, anomalous; caries lesions; uptake of ions.

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Introduction

The literature contains many reports on X-ray microanalysis (XMA) of dentin. The variants of the method, energy-dispersive analysis (EDS) as well as wavelength-dispersive analysis (WDS), have been adopted by the authors. Correlation with the micromorphology of the tissue has been obtained with a variety of methods, as ordinary and polarized light microscopy, microradiography and scanning and transmission electron microscopy (SEM, TEM). In some studies X-ray and electron diffraction have been used as well.

This review gives a condensed presentation of the various studies. Further the methodological aspect and technical problems associated with X-ray microanalysis of dentin are discussed, as well as the importance of the studies for our understanding of the biology of the dentin.

Development of dentin

In odontology, X-ray microanalysis (XMA) is best known as a method primarily applicable to hard dental tissue and metals. The method has, however, been applied to soft tissue as well. In a qualitative study by Boyde and Reith (1977) rapidly frozen, growing rat incisors were freeze fractured and freeze dried in preparation for energy-dispersion X-ray microanalysis (EDS) in a scanning electron microscope. It was shown that Ca levels were elevated in the distal, i.e., secreting cell body of odontoblasts, where the peaks were seven times as high as in the basal part of the cell. The P peaks followed a somewhat different pattern, the peaks being high throughout the entire cell, probably reflecting in part the presence of nucleic acids. However, in the distal part of the cell where the Ca peaks were highest, the P peaks also increased to their highest levels. In an odontoblastic process singled out for analysis, the Ca and P levels were high approximately equivalent

to the levels in the distal cytoplasm of the odontoblast. The results indicated that in the odontoblasts, there appears to be some mechanism for concentrating Ca within the cells, and presumably this Ca will find its way into the dentin. Such a mechanism does not rule out the likelihood that simple diffusion is also involved in the calcification of dentin.

Høhling et al. (1967), Ashton et al. (1973) and Nicholson et al. (1977) have studied mineralization of the pre-dentin in rats. The specimens were prepared as alcohol fixed embedded ultrathin sections, unfixed vacuum embedded dry cut ultrathin sections and as thin cryostat sections. The measurements of Ca, P and S distribution in the pre-dentin could be correlated to the shape of the developing mineral front. The results indicated for one thing that Ca is tightly bound to the matrix, whereas P can be easily washed out by water and alcohol during preparation of sections. The Ca/P ratio gradually increased when proceeding from the unmineralized into the mineralized region. The concentrations related to dry tissue mass for Ca, P and S were in the ranges 0.2-0.4%, 1-4% and 0.5-0.7%, respectively.

Engel (1981) and Engel and Hilding (1984) have studied calcifying matrices and formative cells in developing, 5-14 days old, mouse molars by EDS. Their method for preparation of sections avoided or minimized loss of diffusible substances and translocation or degradation of cellular and matrix components. In the odontoblasts the Ca content was low and contrary to the ameloblasts showed only a small increase with maturation. P and K constituted the most prominent intracellular elements. The P counts were essentially the same among all age groups whereas the K counts were lowered by about 50%. The S content in the odontoblasts was suggested to be a component of both the cytoplasmic and the glycosaminoglycan (GAG) or proteoglycan precursors of the matrix. These authors stressed the high variability in the analysis of pre-dentin, but observed some striking features which differentiated pre-dentin from dentin: 1) Ca and P counts were much lower than in dentin. 2) Pre-dentin contained a significant concentration of S; this element occurred at very low levels in dentin. 3) The Ca/P ratio in old pre-dentin was 1.15 i.e. considerably lower than that characteristic of apatite, suggesting the presence of either amorphous forms of calcium phosphate or protein-bound calcium. 4) Pre-dentin contained some K, probably reflecting the inclusion of odontoblastic processes.

In dentin the Ca and P content was found to stabilize at 8-14 days post partum at a level about 50% higher than

in 5 days old animals. Values for Ca/P ratio lay in the range from 1.8-2.3 (mean = 2.1). A gradient of mineralization was observed from the pulp to the dentino-enamel junction (DEJ), the latter zone being the most highly mineralized. Also Takuma et al. (1972) and Nagai and Takuma (1973) found the S content in pre-dentin variable and in many cases slightly higher than in the mineralizing dentin.

Rosenberg and Simmons (1980a) identified in rabbit incisors a variety of biological rhythms involved in the apposition and mineralization of dentin. Fourier analysis was applied to the structural rhythms, observed on stained decalcified sections, as well as to data obtained by XMA (the compositional rhythms). The Fourier analysis is a mathematical method for spectrum analysis. It makes it possible to identify components of a number of "pure" waveforms (harmonics) which are defined by their periodicity, amplitude, and phase. Based on such analyses the authors suggested that Ca and S deposition, representing mineral and GAGs, respectively, were not simply inversely proportional, and that hematoxylin-stained structural increments did not solely reflect differences in the distribution of the mineral components in dentin. Rosenberg and Simmons (1980b) have also shown that differences in Ca rhythms reflect that the labial side of the rabbit incisors grows slightly faster than the lingual side.

Mineralized dentin

In scans across human dentin from the DEJ or the cementodentinal junction (CDJ) to the pulp cavity, the Ca and P profiles were largely horizontal (Besic et al., 1969, Inoue et al., 1971). The Mg profiles were described as somewhat variable, though generally ascending towards the pulp cavity. However, Tøtdal and Hals (1985) and Hals and Tøtdal (1985a) found that in the pulp-near dentin of human and red deer roots and crowns the Ca, P and Mg profiles sloped downward. Marked elevation of the Zn profiles and moderate elevations of the F and S profiles in this region were also seen (Figs. 1,2). Neiders et al. (1972) analyzed peripheral root dentin. The mineral content of the granular layer of Tomes was significantly lower than the underlying tubular dentin and a narrow hypermineralized layer in the region of the CDJ.

In the four following studies the peritubular zones (PTD), which according to Høhling et al. (1972) are almost collagen free and rich in proteoglycans, have been compared with the collagen-rich intertubular dentin (ITD) (Fig. 3). Miller et al. (1971) found that coronal

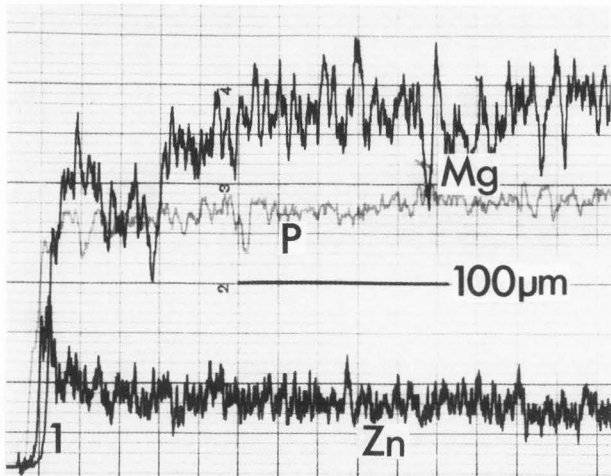


Fig. 1. Downward slopes of P and Mg, elevation of Zn profiles toward pulp cavity (left). WDS linear scans across red deer coronal dentin. From Hals and Tøtdal (1985a). Scand J Dent Res. Courtesy Munksgaard International Publishers Ltd., Copenhagen.

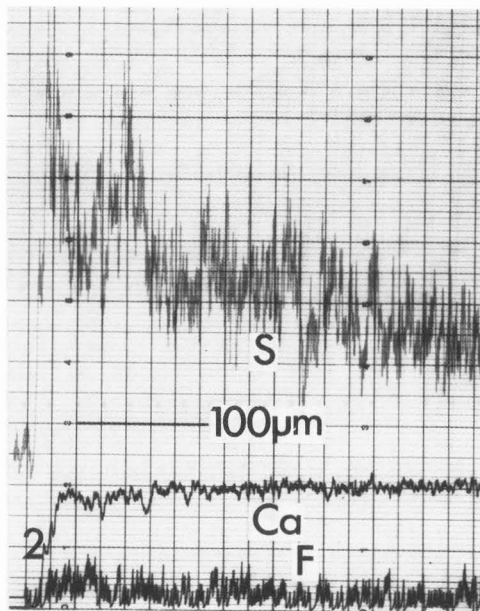


Fig. 2. Downward slope of Ca, elevation of F and S profiles toward pulp cavity (left). WDS linear scans across red deer coronal dentin. From Hals and Tøtdal (1985a). Scand J Dent Res. Courtesy Munksgaard International Publishers Ltd., Copenhagen.

Fig. 3. Diagrammatic representation of a dentin area including an interglobular area (IGD). ITD, intertubular dentin. DT, dentinal tubule. PTD, peritubular dentin.

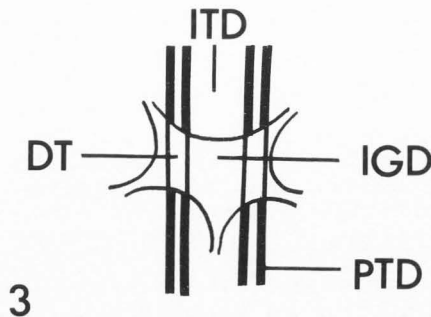
PTD in young human teeth was usually hyper-mineralized (total Ca, P and Mg) by 2-4%, but in some cases up to 9%. Boyde et al. (1961) found an increase of Ca of approximately 10%. On the other hand Høhling et al. (1972) reported an increase of Ca of 40%. In horse teeth the PTD zone is more prominent than in other mammals. Takuma et al. (1966) found the increase of Ca and P concentration in PTD in these teeth to be high, in one case even twice as high as in the ITD.

According to Miller et al. (1971), the radicular PTD zones in young teeth were either hypo- or isomineralized when compared with the ITD. In most teeth the coronal dentin was more highly mineralized than the root dentin in both PTD and ITD areas.

Miake et al. (1973) compared the content of various elements in interglobular areas (Fig. 3) with that of the adjacent dentin. Teeth in young and aged people were compared. In young teeth the interglobular areas showed a marked lowering of the Ca, P, Mg, Zn and Na profiles, whereas in aged teeth the profiles of these elements showed less depression. On the other hand, in young teeth the S and Cl profiles revealed peaks, whereas in aged teeth they showed marked lowering. These observations substantiate the generally held opinion that the interglobular areas seem to undergo a secondary mineralization with age.

Even human dentinal fluid has been submitted to X-ray microanalysis. The preliminary analytical results of Coffey et al. (1970) indicated that the Na/K ratio in this fluid was approximate to that of interstitial fluid.

In their XMA study of coronal dentin in red deer Hals and Tøtdal (1985a) also analyzed the so called giant tubules, which may be up to 40 µm wide. They occur frequently in human teeth (Miller, 1981; Hals, 1983a) and are regularly present in red deer (Hals 1983b) and bovine teeth (Dyngeland and Fosse 1986). WDS profiles crossing giant tubules revealed deep clefts (Fig.4). In some instances the walls of these tubules caused slight to moderate elevations of the Ca, P, Mg and



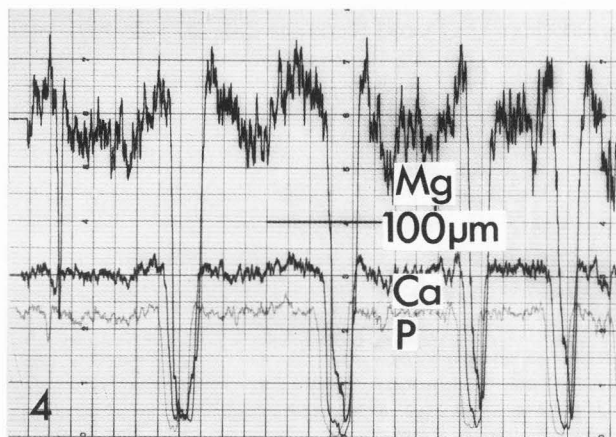


Fig. 4. Axiomesiodistal WDS linear scans traversing five giant tubules in transversal section of red deer coronal dentin. Elevation of Ca, P and Mg profiles in wall area of these tubules, in nos. 1-3, 5 from left on both sides of lumen, with the possible exception for Ca in no. 5. In no. 4 elevation on one side only. From Hals and Tøtdal (1985a). Scand J Dent Res. Courtesy Munksgaard International Publishers Ltd., Copenhagen.

Zn profiles. These appeared on both sides of the lumen or on one side only.

Giant tubules may be partly or completely filled with a fairly radiopaque material. Two transversal sections from the exposed incisal dentin of a human tooth showing giant tubules with such inner material were subjected to XMA. The material revealed concentrations of Ca and P that in the various tubules were either higher or lower than in the surrounding dentin, whereas the Mg concentrations in all instances were remarkably increased. It was suggested that the mineralization to a large extent must be of extrinsic origin (Hals and Tøtdal, 1985b).

Inoue et al. (1971) observed that in teeth with abrasion and secondary dentin on the wall of the pulp chamber, the analytical values for Ca and P decreased slightly, in some cases considerably, from the surface of the abraded dentin to the wall of the pulp chamber. On the other hand, there was an increase in the values of Mg in the same direction, that was greater than that in the control teeth. The distribution of Pb in the dentin of human deciduous teeth was studied by Carroll et al. (1972). In almost all cases the lead was deposited either in, or at the periphery of, hypomineralized areas. The same pattern of deposition was also noted in root dentin, especially in areas adjacent to the pulp. Takuma et al. (1977) have shown that in rat incisors the concentration of S, and

therefore of GAGs, was higher in the osteodentin matrix than in the dentin matrix.

The amorphous area in the dentin in teeth with regional odontodysplasia has been analyzed by Gardner and Sapp (1977). This area, appearing grey in sections stained with hematoxylin and eosin, revealed higher concentrations of Ca and P than either normal dentin or the relatively normal dentin adjacent to the amorphous areas in regional odontodysplasia. In an apparently unusual case of this anomaly, Kerebel and Kerebel (1982) registered the opposite situation. Knychalska-Karwan et al. (1982) produced experimental rickets in rats. Disturbances in the general mineral balance were more evident in the dentin than in the enamel. A higher Ca/P ratio was the result of a decrease in P values.

Johnson and Singer (1967) have studied the incorporation of Sr into the incisors of rats which were given a diet high in Sr content. It was shown that Sr produced decreased mineralization of enamel and dentin, and that an inverse relationship existed between Sr and Ca contents.

In dietary experiments with rats Wesenberg (1982) showed by atomic absorption spectrophotometry that absorption of Cd altered the normal Ca, Mg and P levels in the molars. However, increased dietary Cd had no effect on the Ca/P ratios in either enamel or dentin, as revealed by XMA (EDS).

Kantola (1972) analyzed dentin that had been irradiated with a CO₂ laser, which caused formation of a crater in the wall of which were two radiopaque layers. The Ca and P contents of these layers were clearly higher than those of normal dentin. It was stated that the layers were produced from dentin under laser effect, and that the enamel had not contributed to the formation of these radiopaque deposits.

Caries lesions in dentin

Takuma et al. (1969) registered a decrease in the concentration of Ca, P, Mg and Cl, and usually an increase of S and Zn in these lesions. Gradients of decrease or increase of these elements were coincident with the decalcification patterns suggested by microradiography. Takuma et al. (1975) showed that the remineralized surface layer often present in caries lesions in dentin, contained especially high concentrations of F and K. There were many cases in which Zn, S, and Fe concentrations were higher in the lesions than in the intact area; this was especially the case in the surface area. In many cases the decrease in Mg concentration seemed to start from an area deeper than where the decrease in

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Ca and P concentrations started. This has been shown by Suga et al. (1967), Inoue et al. (1971) and Hals and Selvig (1977) as well.

The analyses in dog teeth by Kato and Fusayama (1970) revealed a partial *in vivo*-remineralization of artificially demineralized cavity floor dentin. Superficial remineralization occurred when the cavities were exposed to saliva or Ca(OH)₂ cement, even in teeth without pulps. The cement increased only the Ca, not P in underlying dentin.

Corrosion products in dentin

Arvidson and Wroblewski (1978) showed that screwposts (60% Cu and 40% Zn) may corrode under *in vivo* conditions although no signs of corrosion products were seen in the radiographs. Cu and Zn were demonstrated in the dentin.

Posts made of nonprecious metals have been commonly used for the retention of artificial crowns and large dental restorations. It has been claimed that corrosion of such posts *in situ* in the root canals may cause fracturing of the roots (Angmar-Månsson et al., 1969). The purpose of the investigation of Silness et al. (1979) was to study corrosion mechanisms which might be responsible for root fracturing of teeth restored by means of stainless steel posts. EDS, microradiography and electron microscopy were used. It was shown that penetration of corrosion products containing Ca, P, Fe, Cr, Ni, Zn, Sn and other elements may continue until available dentinal tubules have been filled up. According to a hypothesis proposed by these authors such blocked tubules can no longer absorb the pressure exerted by the growing corrosion products. If the forces exerting this pressure exceed the strength of the root, fracturing will take place. However, in cases described in the literature displaying both a root fracture and corrosion of a post or a pin, the possibility that corrosion is secondary to an existing root fracture should not be overlooked (Simonsen et al., 1973).

Uptake of ions into dentin

(Fluorine analysis will be reviewed in the next section).

In several studies, uptake of ions from filling materials and penetration into dentin have already been assessed. Silver amalgam is still one of the most widely used filling materials in restorative dentistry, and dentin exposed to this material for some time has been analyzed for Sn, Zn, Hg, and Ag (Wei and Ingram, 1968; Kurosaki and Fusayama, 1973; Hals and Halse, 1975; Halse, 1975; Grossman et al., 1986). High contents of Zn and Sn have been found in discolored

and carious parts of the dentin cavity walls, while "healthy" dentin does not seem to take up such elements to any significant degree. Regularly, Ag and Hg from amalgam were not present in the dentin cavity walls in detectable concentrations.

Up to 4% Zn and 6% Al were found in the outer 10-60 μm of the cavity walls in teeth which had been exposed to silicate cement for 6 months. Further, it was shown that a cavity varnish, Copalite[®], could not prevent penetration of these ions into the dentin (Tveit and Hals, 1977). When cavities had been filled with the glassionomer cement Aspa[®] for periods of 1-3 months, Al concentrations in the dentin up to 1.5% and scattered accumulations of Zn were found (Wesenberg and Hals, 1980).

XMA has been used to clarify whether increased radiopacity of dentin observed beneath silicate fillings on radiographs was caused by an elevation of Ca content or by elements originating from the filling material (Tveit and Halse, 1979). The analysis revealed that both Ca and Zn may contribute to the increased radiopacity.

In a basic study to obtain some information for development of a new method for pulp capping, Fujimasa et al. (1979) used WDS to assess the distribution of metallic constituents in dentin subjected to iontophoresis. By this method the metallic constituents were driven into the dentinal tubules by an electric force based on potential difference. The electrolyte solutions used were a 3% water solution of silver diamine hydroxide and an 8% water solution of zinc chloride. The teeth were exposed to a current 1-2 mA for 5-10 minutes. The material consisted of freshly extracted human third molars in which Class I cavities had been prepared. It appeared that the distribution of a metallic constituent in the dentin decreased gradually with an increase in the distance from the cavity bottom. Peaks of Ag were assessed up to 600 μm below the cavity bottom, whereas infiltration of Zn was 20% deeper than that of Ag.

Camargo and Ribeiro (1976) used EDS to determine the penetration of sodium hypochlorite into the dentinal tubules by registration of the uptake of Na and Cl ions. In one of the two teeth investigated the scores showed a marked increase of Cl in the dentinal tubules, compared to a control. However, no information of the depth of penetration was given. No increase of Na was observed.

Ashrafi et al. (1983) applied EDS to determine the relative concentrations of Zn in dentin following diffusion from a zinc oxide-eugenol dressing. Their conclusion was in accordance with previous observations, that this method is reli-

able to detect trace concentrations of Zn and that accordingly the diffusion behaviour in dentin and bone of a variety of metallic ions relevant to dentistry can be monitored.

Fluorine uptake by dentin from fluoride-containing solutions, varnishes, cements and filling materials.

Topical fluoride application on dental enamel has a caries-preventive effect. A corresponding effect might be expected from fluoride-application to exposed root surfaces, which in a clinical situation most often consist of dentin rather than cementum. Much emphasis has been put on studying the ability of different fluoride agents to deposit F into enamel and dentin. Using the XMA technique F uptake in root surfaces from different solutions and varnishes has been studied both in vitro (Tveit, 1980a; Hals et al., 1981; Tveit and Halse, 1982; Tveit et al., 1983), and in vivo (Tveit et al., 1985).

Solutions with varying pH values of NaF, SnF₂, APF (acidulated phosphate fluoride) and TiF₄ as well as Duraphat and Fluor Protector[®] varnishes were tested. The highest F concentrations were found when the root surfaces had been treated with TiF₄ (Fig. 5) or one of the

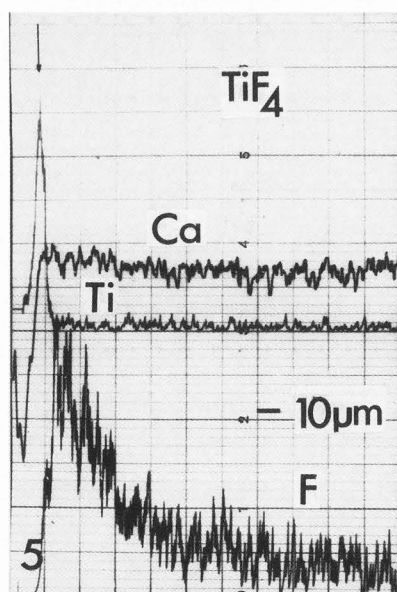


Fig. 5. Typical WDS scans showing the concentration profiles of Ca, F and Ti in a dentin surface 3 weeks after topical application of TiF₄. Position of specimen surface indicated by an arrow. Note the elevated F content in an 80- μ m wide surface layer and the presence of a Ti-rich coating external to the dentin. From Tveit et al. (1985). Courtesy S. Karger AG, Basel.

two varnishes. Application of SnF₂ and APF resulted in F depositions of the same order, but caused a marked demineralization of the dentin tissue. Compared to the NaF application, TiF₄ and the two varnishes caused a deep penetration of F into the tissue, i.e., increased concentration of fluorine could be observed up to 200 μ m from the surface (Tveit et al., 1985).

In some studies, the ability of cavity walls to take up F from different sources has been investigated. Treating the cavity walls with a 2% NaF solution before inserting the filling resulted in a small increase of the F concentrations in the dentin walls, while no F increase could be found when a fluoride-containing liner was used (Tveit et al., 1987). Application of the varnish Duraphat on the tooth surface after inserting the amalgam has resulted in uptake of F in the dentin cavity walls as well (Tveit, 1980b). Adding fluoride to the filling material is another means of F supply which will affect the development of secondary caries adjacent to the restorations. Fluorine uptake from silicate cement (Tveit and Hals, 1977), glass-ionomer cement, (Wesenberg and Hals, 1980) and fluoride-containing amalgam (Le Quang et al., 1976; Tveit and Tøtdal, 1981; Tveit et al., 1987), has been demonstrated by the XMA technique. In an animal study, F concentrations up to 0.9% were found in cavity walls which had been exposed to fluoride-containing amalgam for five months (Tveit et al., 1987). As far as the amalgam fillings are concerned, the results indicate that using fluoride-containing filling material is the most effective way of securing a F depot in cavity walls.

Secondary caries

The literature contains a few XMA studies of the so-called cavity wall lesion (Fig. 6) adjacent to amalgam fillings, with respect to content of minerals and penetration of elements from the fillings (Kurosaki and Fusayama, 1973; van der Linden and van Aken, 1973; Hals and Halse, 1975; Hals, 1976).

Dentin wall lesions where micro-radiographs had shown increased radiopacity relative to intact tissue, exhibited considerably reduced Ca and P values (Hals and Halse, 1975). The outer portions of the radiopaque areas contained 5-8% Zn and Sn, decreasing to 0.1% at a varying distance up to 130 μ m from the cavity wall (Fig. 7). There can be no doubt that the increased radiopacity of this zone is due to concentrations of Sn and/or Zn released from the fillings, overcompensating the radiolucency produced by the decalcification, as previously shown by van der Linden and

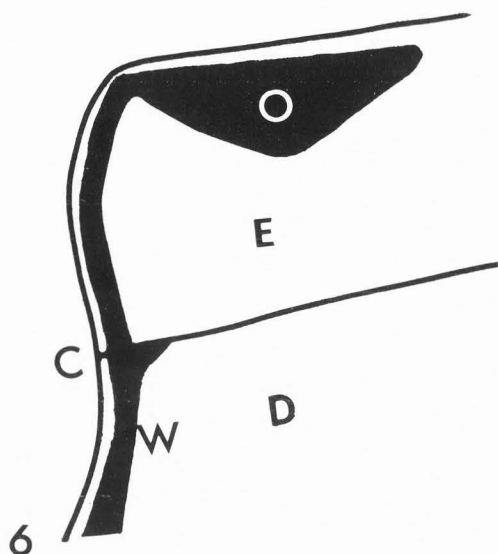


Fig. 6. Drawing of secondary caries lesion, modified from microradiograph. O, outer lesion. W, cavity wall lesion. C, cavity. D, dentin. E, enamel. From Hals and Halse (1975). Acta Odontologica Scandinavica Foundation, Oslo.

van Aken (1973). Dentin wall lesions adjacent to silicate fillings, where microradiographs had shown increased radiopacity relative to intact tissue, exhibited increased Ca and P values. Elements derived from the silicate fillings were regularly found in enamel and dentin. The concentrations of F and Zn amounted to 2-8% by weight close to the cavity wall and decreased gradually to 0.5% at depths of 600 μm and 400 μm , respectively, from the cavity. Aluminum most often occurred in a 20-40 μm -wide zone, showing a maximum concentration of 2-3% near the cavity. Sulfur was often present in the dentinal cavity walls of natural secondary caries, but not in the *in vitro* specimens. The findings indicate that F released from the silicate filling significantly modifies the progress of a caries lesion in the adjacent enamel and dentin. Through its tendency to form complexes with F, Al may possibly enhance the cariostatic effect of F (Hals 1976, Hals and Hals, 1976).

Discussion

The XMA has its methodological advantages and shortcomings, both of them felt when dentin is analyzed. Thus, simultaneous analysis of several elements under equal conditions in a few μm^3 of dentin, would be impossible with conventional chemical methods. Areas of

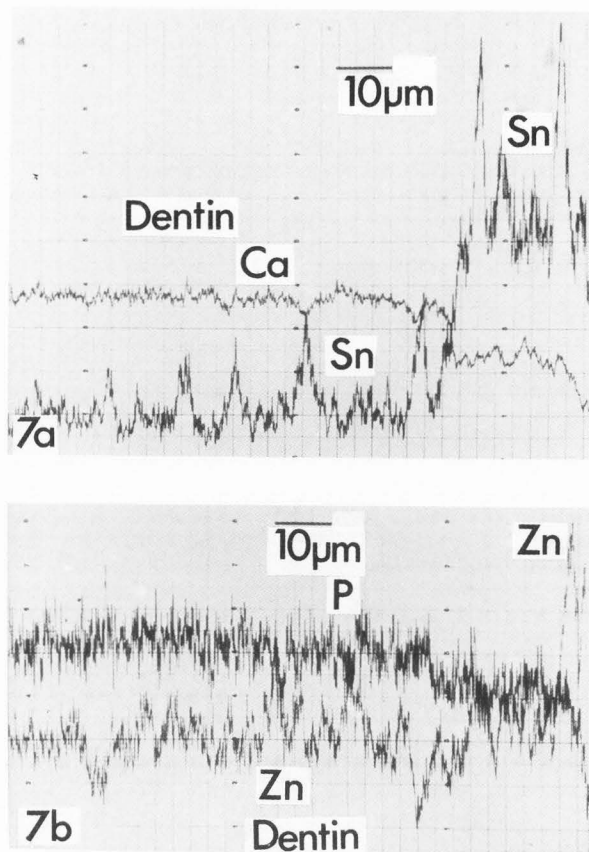


Fig. 7. Natural secondary caries lesion. Corresponding to the cavity wall lesion (right) the Ca (7a) and P (7b) profiles form uniformly lowered steps. In this demineralized area an accumulation of Sn (7a) and Zn (7b). In the corresponding microradiograph this area displayed increased radiopacity. From Hals and Halse (1975). Acta Odontologica Foundation, Oslo.

dentin confronting the chemists with special difficulties would be for example the predentin, the growth lines, the interglobular areas and the walls of prepared cavities. In this context XMA is also advantageous by being more or less non-destructive. Whereas a chemical concentration curve is based on measurements at a varying number of spots, the XMA (WDS) provides continuous concentration profiles.

The high content of organic matter in dentin (20%, dry weight) represents a main problem. In combination with the low thermal conductivity of the dentin this leads to irradiation effects on the organic material by the electron beam (Edie and Glick, 1976, 1978, 1979; Edie, 1977). The effect is noticeable for electron doses $\geq 10^{-10}$ C/ μm^2 .

With sample currents of ~ 50 nA and beam diameters of ≤ 1 μm the effect will occur within a small fraction of a second even for relatively swift scans. Reducing the sample current to 1 nA would prolong the "dynamic response time" to approximately 1 s for a beam diameter of 1 μm , but the count rates would be inconveniently small, at least for WD systems.

Small count rates would of course be even more problematic if the elements in question were minor constituents of the X-ray excitation volume. The small count rates connected to small sample currents can be somewhat improved by using ED instead of WD spectrometers. This will, however, in turn introduce inconveniently high background levels compared to the peak levels.

Another method to reduce the mass loss is to analyze the specimen at low temperatures. This will, however, introduce problems due to condensation of vapors upon the specimen, although this condensation may be reduced by introducing a cold trap close to the specimen.

Further, the tissue is intrinsically uneven in density. This interferes with the possibility to obtain an even and smooth surface when preparing sections which is of great importance in quantitative analysis with the XMA method. By itself the density of the specimen may affect decidedly the intensity of the emitted X-radiation. Frank et al. (1966) therefore suggested a method for bringing the sample to uniform density. Apparently, this idea does not seem to have been followed up in later studies. Neiders et al. (1972) explained that because the difference in density in the peripheral layers of dentin investigated by them "was not great", the method suggested by Frank et al. was not used. It is assumed that the methodological problems represented by the organic tissue would be less with the use of ultrathin sections. Approaches to quantitation with the XMA technique has been briefly reviewed by Levin (1986), but doubts have been expressed about how appropriate ZAF correction procedures for thick specimens are for low density materials (Yakowitz, 1975). The importance of specifying the complete organic matrix in biological XMA has been emphasized (Boekestein et al., 1983).

The analysis problems due to rough surface topography can be more or less overcome by the use of a ZAF procedure based on peak-to-background ratios (Small et al., 1978, 1979; Statham and Pawley, 1978; Statham, 1979a,b). The basic assumption of the method is that peak and background of the same wavelength are generated with approximately the same depth distribution, and are therefore absorbed similarly. The peak-to-background ratio should therefore be more

or less independent of the sample geometry. This method has also been used successfully for thick biological samples (Roomans, 1981; Boekestein et al., 1980, 1983).

In comparison, the element concentration values given for dentin up to now should be regarded as semiquantitative. As often pointed out, however, values on a relative scale should not be underestimated. According to Andersen (1967) one can take into account that these values will be approximately 15% higher than those provided by chemical analysis. In textbooks of cariology the data on element concentration in dentin usually concern whole dentin, and the range of concentration is great. On the other hand, the XMA technique allows the analysis of minute areas of highly different structure. As a result, also the range of concentration values within the individual specimen will be great.

If an electron beam is traversing a hole in a specimen, the corresponding X-ray signal is elevated on the rim of the hole that turns away from the spectrometer and is lowered on the opposite side, even if the sample is homogeneous. This phenomenon is due to the dependence of absorption on geometric conditions. In some of the profiles of Ca and P across the giant tubules (Fig. 4) there are, however, small but observable elevations on *both* sides of the lumen. This may therefore indicate a small but real increase of mineralization, an interpretation supported by the fact that microradiographs frequently revealed increased radiopacity in these areas (Hals, 1983a,b).

However, a zonation in the dentin as sometimes observed in microradiographs cannot in all cases be explained by the findings with XMA. The reason for this apparent lack of correspondence is probably that the microradiograph offers an image based on the whole thickness of the section, usually 60-150 μm - whereas with XMA only the superficial 1-2 μm of the specimen will be analyzed.

There can be no doubt that XMA studies have provided valuable new information on the mineralization of dentin and the content of and uptake of mineral ions into dentin. This also applies to dentinal anomalies and pathological conditions. Further, XMA studies have enlarged our knowledge of the distribution of various ions in the dentin. It is of importance that the distribution of the lesser constituents of dentin is not uniform. Fluorine, Pb and Zn reveal higher concentrations in inner dentin than in deeper layers. Ions which readily become attached to the apatite crystals tend to build up in those parts of the tooth which are exposed for the largest time to the body fluids. Inner dentin

tends to concentrate elements in the same way as outer enamel and cementum. On the other hand, Mg and Na are readily dissolved out from the calcified tissues by body fluids (Jenkins, 1978). Magnesium plays a specific role during evolution of the mineral phase and subsequently as a constituent of the latter. The mixed calcium-magnesium-phosphate salts have been expected to be less resistant to abrasive action than those of calcium apatite (Johnson, 1972). In this context the increased Mg in the dentin in teeth with abrasion (Inoue et al., 1971) is interesting and warrants further investigation. Lead and Zn are known to increase the resistance of the enamel to dissolution in acids (Jenkins, 1978). Probably these ions act in the same way in dentin. They do not seem, however, to have played any role in caries prophylaxis. In the caries lesions of dentin analyzed by Takuma et al. (1969, 1975) the increased S content reflects uptake of organic material as protein and microorganisms from the oral cavity. Presumably, the increased content of Zn, K, and Fe have been derived from food particles. Zn may also come from amalgam fillings (Jenkins, 1978).

The advancing front of large caries lesions are often seen to be restrained by pulp-near dentin, a phenomenon possibly related to the high fluoride content of pulp-near dentin (Levine 1974). This is directly supported by in vitro evidence that fluoride-pretreated dentin is more resistant to acid attack than untreated dentin (Selvig 1968).

It is well documented that fluorides promote remineralization in caries lesions of enamel. Presumably, fluorides act in the same way in caries lesions of dentin. Thus, in a recent textbook of cariology, topical application of fluoride on root surface caries lesions is advocated to affect the remineralization process (Thylstrup and Fejerskov 1986).

It has been shown by XMA that in most young teeth coronal dentin was more highly mineralized (total Ca, P and Mg) than root dentin (Miller et al. 1971). Chemical analysis of dentin from teeth of different ages shows a small but statistically significant increase in the mineral concentration, but in the apical region only (Jenkins 1978). To the authors' knowledge, no comparison between coronal dentin and root dentin with reference to caries susceptibility has been undertaken.

Summary

The studies reviewed reflect a variety of projects on X-ray microanalysis of dentin. These studies, con-

cerned with the concentration and distribution of elements in human and animal dentin, deal with odontoblasts, predentin, mineralized normal and anomalous dentin, primary and secondary caries lesions, corrosion products in dentin, and uptake of ions from filling materials, fluoride-containing solutions and varnishes. Ca and P, the main constituents of dentin, are most often focussed on, but some studies also deal with the lesser constituents Mg, Zn, S, F, Cl, Na, K, Zr and Cu. Caries lesions show loss of Ca, P and Mg, but increase of Zn, S, F, Fe and K. Various caries-prophylactic and therapeutic measures cause uptake of F, Al, Zn and Sn in dentin. Corrosion products contain Fe, Cr, Ni, Zn and Sn, in addition to Ca and P from the dentin.

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Discussion with Reviewers

M.B. Engel: What is the resolution of the method which the authors employ?

Authors: The volume of characteristic X-Ray production (the resolution) is dependent upon the acceleration voltage, the density of the specimen and the critical excitation energy of the X-ray line in question. The lateral spread is somewhat larger than the depth range, and can be assessed approximately by a nomogram (Reed, 1966). For F this gives a resolution of approximately 1 µm at E = 10 keV, and 2 µm at 15 keV. The nomogram is, however, based on parameters typical for metals, and should be used with care for dentin. In addition, one should be aware that the focusing of the electron beam on the specimen surface is in practice dependent upon the accelerating voltage and the beam current. High beam currents will generally increase the electron beam diameter, and consequently the region of X-ray production. A more conservative estimate would therefore be to claim a resolution of - 5 µm. This is also in agreement with the estimates of Engel and Hilding (1984).

M.B. Engel: How are the tissues prepared and what steps are taken to preserve diffusible elements?

Authors: Sections, 100-150 µm thick, of teeth were polished, dried for 3-20 hours at varying temperatures (25-60 degrees C) and glued to specimen blocks before analysis. Different degrees of polishing were performed in the various studies. It was observed, however, that the surface irregularities of the dentin could not be smoothed out whatever polishing technique applied, due to the inhomogeneous structure of the dentin tissue compared to enamel. Our investigations deal with mineralized tissue only, we have not examined cell tissue. Because our samples were kept away from contact with water as much as possible during the preparation procedures, we assumed that loss of substances should be minimal. Anyway, possible diffusion could be expected to act evenly all over the surface of the specimen. On this background we have assumed that the distribution pattern of the elements, which was our main interest, have not been affected.

M.B. Engel: How reliable are the results for such low Z elements as F?

Authors: Concentrations of the heavier elements in dentin seem to be some 15% higher than those generally found by chemical methods, even if ZAF corrections are being used. This is primarily due to the electron bombardment that changes the specimen constitution through removal of the light element constituents. Thereby the effective concentration of the other elements increases, and this will apply to F-results as well.

If count rate variations as a result of mass loss can be taken into account, it may be possible to achieve a precision of about 2 - 3%, at least for Ca and P, with proper use of weighted ZAF corrections (Edie and Glick, 1979). In heterogeneous specimens the volatility may vary considerably from point to point, thereby introducing major ambiguities. As for the measurement of F, it should also be mentioned that for acceleration voltages near 10 kV the F count rate versus electron dose shows a maximum even for fluorapatite (Edie, 1977).

We have not tried to correct for mass loss in our results, and have reported our dentin results as semi-quantitative only. The F values are probably elevated in a way similar to Ca and P. When ZAF corrections are applied to dentin (with a fluorapatite standard), the correction factors are so close to 1.0 that the uncertainty of the ZAF procedure is probably less important than the statistical uncertainty introduced by low count rates. A rough estimate of the accuracy of the F results might therefore be 10% "on top of" the elevation due to mass loss. For counting statistical reasons we have also ignored F concentrations below 0.15%.

J. Glawson: Your remarks regarding the validity of ZAF corrections for light elements are well noted, as is the statement to the effect that semiquantitative results are not without value. Do you think the usefulness of EDS for fluorine analysis is sufficiently improved by windowless and thin-window detectors to overcome ZAF limitations?

Authors: The limitations of the ZAF procedure for light elements are probably mainly due to uncertainties in physical parameters, and to some extent to the X-ray distribution function (Love, 1983). We therefore fail to see how these limitations can be influenced by the use of windowless detectors.

J. Glawson: Using WDS in conjunction with digital X-ray intensity mapping offers great potential in analyzing elements having a small signal due to low concentration. Do you feel this has usefulness for dentin studies of light elements?

Authors: Digital X-ray intensity mapping where separate intensities can be attributed to each picture element (pixel), is obviously very useful. With modern image analysis equipment one may record an X-ray intensity map of a background position of the spectrum, and then subtract this image, pixel by pixel, from the original X-ray intensity map of a characteristic X-ray line. Thereby the image sensitivity to element concentration variations is increased, and one gets semiquantitative information directly from the image. Unfortunately we do not have such equipment at our disposal.

K. Arvidson: Are there any differences in Ca and P dentin profiles of human vital teeth and endodontically treated teeth, and in very old teeth?

Authors: We found no XMA study in the literature that has compared the Ca and P profiles in vital and endodontically treated teeth. Miake et al. (1973), cited in our review, found that Ca and P profiles in interglobular dentin areas showed less depression in old teeth than in young teeth. As far as total dentin area is concerned, no XMA study comparing the profiles of these ions has been reported.

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