Dentinogenesis and the Calciotraumatic Response to the Injection of Lead or Fluoride Ions

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A number of ions can disturb the formation of dentine resulting in a calciotraumatic response. The calciotraumatic response following the injection of sodium fluoride was investigated using backscattered electron imaging in the scanning electron microscope and compared with the response to lead acetate. With fluoride, there was formation of a hypermineralized band succeeded by a relatively hypomineralized band, but with lead acetate, only a hypomineralized band was produced. However, there were some differences in the response between the labial and lingual dentine with both ions. In the labial dentine following injection of sodium fluoride, the onset of hypermineralization was less abrupt than in the lingual dentine. Furthermore, the transition from hypermineralization to relative hypomineralization was more abrupt in the labial dentine. Sometimes there was an increased thickness of labial dentine between the hypermineralized layers towards the apex of the tooth and this dentine was less homogeneously mineralized. Normal incremental lines were occasionally seen both labially and lingually. Lead acetate produced a more severe disruption of dentine formation labially than lingually. These differences in response may be related to the pattern of mineralization labially and lingually and to the systemic effects following the injection of sodium fluoride.

Key Words: Dentine, calciotraumatic response, fluoride ions, lead ions, calcium transport.

Dentine formation, in common with other hard tissues, takes place in two distinct phases, that is, the production of an organic matrix and its subsequent mineralization. The persistently erupting incisors of rodents are ideal to study this process since they grow continuously and dentine is deposited on their pulpal surfaces to compensate for their constant attrition at the incisal edge. Since dentine formation is rhythmic with periods of activity and quiescence, this results in the formation of structures which reflect this activity, that is, incremental lines. Additionally, if the process is interrupted or disturbed at any stage, it can result in exaggerated incremental lines being formed. This led Erdheim (1911) to suggest that the rat incisor might be used as a graphical indicator of mineral metabolism having earlier (Erdheim, 1906) seen changes in dentine formation following parathyroidectomy. More recently this tooth has been used as a model system for investigating the mechanisms of calcium transport during dentinogenesis (Appleton, 1988, 1991; Linde and Lundgren, 1990).

The structure of incremental lines can, therefore, be altered experimentally by agents which are known to affect mineral metabolism. The injection of sodium fluoride (Schour and Smith, 1934) and calciferol or parathyroid hormone (Schour and Ham, 1934) or parathyroid hormone after parathyroidectomy (Schour et al., 1937a, b) were said to produce a similar effect, that is, a hypomineralized band succeeded by a hypermineralized band. Following haematoxylin and eosin staining, the latter noted a haematoxylin stained line, the calciotraumatic line, at the junction with the eosinophilic hypomineralized layer. Such calciotraumatic lines which stain intensely with haematoxylin were used at an early stage as markers for measuring the rate of growth of dentine (Weinmann, 1942).

The earliest contact microradiographic studies on enamel mineralization (Applebaum, 1943) supported the relationship between hypomineralization and eosinophilia and hypermineralization and haematoxylin staining, although because of the large grain size, film resolution was poor. The later works of Irving (Irving, 1943; Irving and Weinmann, 1948; Irving et al., 1948a, b, c)
suggested that there was a consistent response in the dentine, both chronologically and morphologically, to a variety of ions including strontium and fluoride. Therefore, Irving and Weinmann (1948) suggested the use of the term "calciotraumatic response" to encompass and describe the sum total of the effects of exposure to these ions. Haematoxylin and eosin staining showed the calciotraumatic response to consist of a calciotraumatic line, a layer of hypomineralized dentine, and a layer of hypermineralized dentine (Fig. 1).

The advent of high resolution microradiography clearly showed, however, that the calciotraumatic response to sodium fluoride consisted of an external hypomineralized band and an internal hypermineralized band (Yaeger and Eisenmann, 1963; Osmanski and Yaeger, 1964). This response is consistent and forms at the time of injection (Yaeger and Eisenmann, 1963; Yaeger et al., 1964).

Transmission electron microscope (TEM) examination of rodent dentine following injection of sodium fluoride (Yaeger, 1963; Appleton, 1988) showed differences in electron density in the mineralized phase. Both studies demonstrated that there were more crystallites per unit volume in the more electron dense hypermineralized dentine, but Yaeger (1963) showed that in the less electron dense hypomineralized dentine, the crystallites were of greater width than in normal dentine. Although there were no apparent differences in the organic matrices of hypo and hypermineralized dentine, polarizing microscopy revealed randomly arranged collagen fibrils in the hypomineralized bands (Grady and Yaeger, 1965). Irregular mineralization and discrete clusters of crystallites were observed in fluorotic dentine by Eisenmann and Yaeger (1972) and Walton and Eisenmann (1975). It was not possible to identify hypermineralized dentine in transmission electron micrographs. With time, normal dentine formed within the fluoride response areas, so that, eventually only small regions of unmineralized dentine remained adjacent to the odontoblast process.

Fejerskov et al. (1979) analysed the effect of acute and chronic exposure of rats and humans to fluoride using high resolution contact microradiography. The effects of acute exposure and chronic administration are summarized in Figs. 2 and 3, respectively. This study confirmed the unique response of these tissues to acute fluoride administration, i.e., a hypermineralized band succeeded by a hypomineralized band while other ions are said to produce only hypomineralized zones.

An ultrastructural and microradiographic study of the effect of sub-cutaneously injected strontium showed that there were different effects on the labial and lingual dentine four days after injection (Ogawa et al., 1981). The labial wall frequently showed two hypomineralized layers separated by about 30 µm. In the lateral, medial and lingual walls, however, there was sometimes only a
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single hypomineralized layer. Ogawa et al. (1981) suggested that the internal hypomineralized layer corresponded to the base of the odontoblast process at the time of injection. Electron microscopy showed that the external layer was less dense than normal dentine, but there were no differences between their matrices. The internal hypomineralized layer, however, contained sparse collagen fibres and large numbers of ruthenium red positive granules indicating the presence of glycosaminoglycans or proteoglycans.

The injection of lead acetate is also known to effect hard tissue formation by the production of the so called 'lead line'. This is also accompanied by rapid, but temporary, induction of hypercalcemia and hyperphosphatemia (Kato et al., 1977; Appleton, 1991).

This paper presents new information on the structure of dentine in the persistently erupting rat incisor following exposure to fluoride, using backscattered electron (BSE) imaging, and relates this information to the existing body of knowledge while making a particular comparison with the effect of lead acetate on dentine structure. With BSE imaging in the electron microscope, the resulting contrast in the image is a measure of mean atomic number or, in effect, the density of the specimen. There is considerably improved resolution over contact microradiography, and detailed differences in specimen density are such that even minor variations in the degree of mineralization can be visualized (Boye and Jones, 1983; Boyde et al., 1990). However, contrast due to surface topography must be eliminated by examining specimens in which the surface has been polished as smooth as is practically possible using the techniques described below.

Materials and Methods

Lead. Ten black and white rats weighing between 150-175 gm were anaesthetised with Immobilon (C-Vet) and given, via the femoral vein, a single injection of lead acetate in aqueous solution (3 mg/100 gm body weight (b.w.)). Ten control rats were given sodium acetate in aqueous solution (1.65 mg/100 gm b.w.). All the animals were killed one week after injection.

Fluoride. Ten black and white rats weighing between 150-175 gm were given a single intra-peritoneal (I.P.) injection of sodium fluoride in aqueous solution (17 mg/100 gm b.w.) and ten control rats an equivalent volume of normal saline. A second injection was given 48 hours later and the animals killed after a further 48 hours and the incisor teeth removed.

All the teeth were fixed in Analar methanol for 24 hours and embedded in polymethyl methacrylate (PMMA). The methacrylate monomer was washed three times in 5% NaOH followed by three washes in distilled water and stored over molecular sieve 4A in a fridge at 4 °C. Styrene monomer was added to produce a more stable block material on polymerization. The specimens were then placed in methyl methacrylate containing Perkadox 16 (0.1 gm/10 ml) (Akzo Cheni UK Ltd.), for a further five days. Plastoid N (2.5 ml/10 ml methyl methacrylate) was added to the final mixture and allowed to polymerize at room temperature.

The embedded teeth were sectioned longitudinally and transversely using a diamond impregnated wafering blade with a low speed precision saw (Isomet, Buehler Ltd., USA) and the surface polished on a Minimet (Buehler Ltd., USA) polishing machine using diamond pastes down to a particle size of 0.25 µm. The polished surfaces were coated with carbon in a sputter coater, and examined in a JEOL 35C scanning electron microscope (SEM) operating at an accelerating voltage 35 kV. The SEM was equipped with a Robinson BSE detector (EPT Semra Pty Ltd., Australia).

Results

Using BSE imaging the lead line was seen to consist of a distinct but fine band of hypomineralized dentine (Figs. 4, 5) formed by continuous interglobular spaces (Fig. 6). After the formation of the initial hypomineralized band, dentine formation was more severely disturbed labially so that the dentine formed after injection had an irregular tubular structure, large unmineralized areas were present, and mineralization was heterogeneous (Figs. 5, 6).

The calciotraumatic response of the dentine to intra-peritoneal injection of sodium fluoride was clearly visible at low magnification as two fine white lines of hypermineralized dentine, concentric in transverse section (Fig. 7) and parallel in longitudinal section (Fig. 8), separated by between 132-140 µm of dentine. In transverse section, it is evident that there is a greater distance between the lines in the mesial and distal dentine but this is probably associated with an oblique plane of section.

Additional incremental lines can often be seen in relation to the dentine formed between calciotraumatic lines on both the lingual and labial surfaces of the dentine (Fig. 9). These are alternate bands of relative hyper and hypomineralization. Sometimes in longitudinal section, it is evident that there is a distinct difference in response between the lingual and labial dentine (Fig. 9). This is seen as an increasingly thicker layer of dentine, both after the first and second injection of fluoride, on the labial surface towards the apical half of the tooth. Moreover, the variation in the level of mineralization in the dentine between the two calciotraumatic lines is more marked than elsewhere (Fig. 9). However, the lines are usually parallel throughout the length of the tooth (Fig. 8).

Close examination shows that the hypermineralized component of the calciotraumatic response is the more distinctive. In the labial dentine, the hypermineralized line is about 10 µm in width and there is a gradual increase in the level of mineralization within the line on the pulpal facing surface (Fig. 10). There is an abrupt transition from the relatively high level of mineralization within the line to that found in the dentine formed between the hypermineralized lines. The level of mineralization within the dentine formed between the calciotraumatic lines is lower than in the pre-injection dentine that
is relatively hypomineralized. Lingually, the hypermineralized line is more irregular and its pulpal facing surface shows small isolated areas of hypermineralized dentine with small areas of interglobular dentine (Fig. 11).

Discussion

It has been known for some time that there are numerous ions which temporarily disturb the formation of dental hard tissues (Irving, 1944; Eisenmann and Yaeger, 1969). In these studies the effects of ions on hard tissue structure was assessed using histological and microradiographic techniques. It is only recently that these effects have been examined with the improved resolution of the electron microscope (Yaeger, 1963; Grady and Yaeger, 1965; Walton and Eisenmann, 1975; Appleton, 1988, 1991). Of particular significance is the use of BSE imaging as used in this and an earlier study (Appleton, 1991) since it shows with high resolution the variations in distribution and density of the mineral phase.

This study has confirmed that the calciotraumatic response of dentine to fluoride is unique amongst all the ions investigated (Eisenmann and Yaeger, 1969). There is a paired response in which the formation of a hypermineralized band of dentine is succeeded by a hypomineralized band. Furthermore, it has shown that there are distinct differences in response between the labial and lingual dentine. This is also true following exposure to strontium chloride (Ogawa et al., 1981; Appleton, unpublished data) and lead acetate (Appleton, 1991).

These differences in response may be related to differences in the pattern of mineralization, the rate of formation and the structural differences which have been shown to exist between labial and lingual dentine and the associated odontoblasts. The labial dentine mineralizes by means of large calcospherites, while in the lingual dentine, they are small and linear mineralization predominates. Furthermore, the crystallites and collagen fibres are randomly orientated in the lingual dentine and more regularly orientated in the labial dentine with crystallites and collagen fibres parallel (Mishima and Sakae, 1986; Mishima et al., 1988, 1991). The linear mineralization in the lingual dentine, therefore, probably results in the more distinct incremental lines in this region.
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Figure 6. The 'lead line' (arrowed) consists of continuous interglobular spaces. In the pre-injection (pij) dentine, there is a normal tubular structure but in the post-injection (poj) dentine, the tubular structure is disrupted and there are large unmineralized spaces in the dentine and small isolated calcospherites. Longitudinal section, BSE image.

Figure 7. The calciotraumatic response to two sodium fluoride injections is seen as two bright lines in an oblique transverse section of a rat incisor. BSE image.

The processing of pre-dentine into dentine is also more rapid lingually where the odontoblast, in contrast to those lining the labial surface, had secretory granules which were not loaded with electron dense particles 30 nm in diameter (Beersten and Niehof, 1986). This may reflect differences in the non-collagenous components of dentine matrix such as phosphoprotein. Steinfort et al. (1989) have shown, for example, that the content of higher phosphorylated phosphoproteins was four times greater in the labial dentine than in the lingual dentine but the reverse was true for the lower phosphorylated phosphoproteins.

Following the acute exposure of developing dentine to fluoride, the physiological mechanisms by which the characteristic response is generated are still uncertain. If Ca\(^{45}\) and F\(^{18}\) are injected simultaneously in mice, then both ions target on the mineralizing tissues (Ericsson and Hammarström, 1964) which would suggest that fluorapatite formation is promoted leading to the removal of calcium and phosphorus from the plasma (Larsen et al., 1981). Furthermore, this and other studies (Larsen et al., 1977; Larsen and Thorsen, 1984; Monsour et al., 1985, 1987) have shown that following either intra-venous (I.V.) or I.P. injection of sodium fluoride, there is a rapid increase in serum fluoride concentration, and this fluoride is rapidly cleared from the system. Moreover, these studies demonstrated that injection of sodium fluoride produced significant, but temporary, hypocalcemia and hyperphosphatemia.

The degree of response to fluoride in dental tissues is related to the amount of injected fluoride (Eisenmann and Yaeger, 1969), and the period during which the hypermineralized layer forms is related to the high plasma fluoride levels. Additionally, it has been shown that the hypermineralized dentine following fluoride injection, contains considerably more fluoride than adjacent areas (Suga, 1972). It has been suggested, therefore, that the formation of fluorapatite in the hypermineralized zone is related to the associated fall in plasma calcium (Larsen et al., 1978; Larsen and Thorsen, 1984). However, apart from a slight fall initially, serum phosphorus rises (Baker, 1974; Monsour et al., 1985, 1987). Since the rise in plasma phosphate is associated with increases in plasma urea and creatinine, this would suggest impaired kidney function and that fluoride given in this experiment was harmful to the kidney. In addition to the systemic effect, it is possible that fluoride also directly effects the odontoblasts, although...
there are no apparent ultrastructural alterations to these cells (Appleton, 1988). However, one hour after injection of sodium fluoride, there is a marked alteration in the intracellular and extracellular distribution of ionic calcium as indicated by increased pyroantimonate deposits within the odontoblasts and predentine (Appleton, 1988). This suggests that one effect of fluoride is on membrane enzyme systems which maintain calcium concentration gradients between the dental pulp, odontoblasts and predentine (Appleton, 1988; Linde and Lundgren, 1990). Extracellular concentration of Ca$^{++}$ is in the millimolar range, and intracellular, in the micromolar range (Linde and Lundgren, 1990). If fluoride temporarily inhibits the enzyme systems which maintain these concentration gradients, then calcium will pass into the pre-dentine until equilibrium is reached (Appleton, 1988). This, in turn, may promote the formation of additional mineral nuclei to produce relative hypomineralization. During recovery and establishment of equilibrium, there may be a transient phase in which mineralizing front is relatively deficient in calcium ions, and thus, there will be relative hypomineralization.

In contrast to fluoride following the injection of lead acetate, there is a rapid rise in serum calcium and phosphorous (Kato et al., 1977; Appleton, 1991). It has been suggested that this is due to the direct effect of the lead ion on the mineral phase with the rapid replacement of calcium and phosphorus in the crystal lattice (Kato et al., 1977). However, this and earlier work (Appleton, 1991) has shown that the lead line in dentine represents continuous interglobular spaces followed by disruption of tubular structure. Furthermore, attempts to identify localized concentration of lead ions using energy-dispersive X-ray microanalysis (EDX) were not successful, but it is possible that it was undetectable by this technique. The displacement of lead ions during processing is unlikely since lead apatites have a very low solubility particularly in Analar methanol (Neuman and Neuman, 1958). It is possible, therefore, that as with fluoride, lead ions act on the odontoblasts effecting their ability to maintain calcium concentrations gradients between the odontoblasts and predentine, and resulting in the loss of intracellular calcium.

References


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Figure 8. The calcio-traumatic response to two sodium fluoride injections is seen as two bright parallel lines running the whole length of the tooth. BSE image.

Figure 9. The amount of dentine formed between the first and second injection of fluoride is greater in the labial dentine (la) towards the apex of the tooth and it is unevenly mineralized. Incremental lines are distinct in the lingual dentine (arrowed). BSE image.

Figure 10. The hypermineralized lines in labial dentine following the injection of sodium fluoride at intervals of 48 hours. Dentine between the lines is hypomineralized with respect to the pre-injection dentine. BSE image.

Figure 11. The hypermineralized lines in lingual dentine in an area where the tubules are sectioned transversely. These lines are more irregular than in the labial dentine: there are small calcospherites and interglobular spaces on the pulpal facing surface (arrowed). BSE image.


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Discussion with Reviewers

D.R. Eisenmann: Is it possible that the apparent differences in thickness of dentine on the labial side, towards the apical half of the tooth, as illustrated in Figure 9 might be attributed to an oblique plane of section? The pulp chamber appears to narrow towards the apical, which also would not be the case in a true longitudinal section.

H. Mishima: The increased thickness of labial dentine was seen between the hypermineralized layers towards the apex of the tooth. Do you have any ideas on what causes the increased thickness of labial dentine?

Author: It is possible that an apparent increase in the thickness of dentine could arise from the plane of section. In the case of Figure 9 it is possible that the longitudinal section passed through the periphery of the pulp chamber so that the distance between the hypermineralized lines would appear greater than it is in both labial and lingual dentine. However, this is not the case, since the only apparent increase is in the width of the dentine between the hypermineralized lines in the labial dentine. We have also had a similar response with strontium chloride where 48 hours after the initial calcio-traumatic line, there was a thicker layer of labial dentine towards the apex of the tooth. So clearly there is a difference in susceptibility between labial and lingual which may be related to known differences in rate of growth or to histological environment.

D.R. Eisenmann: It is noted that additional incremental
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lines are seen in dentine formed between injection induced calcio-traumatic lines. In addition, the overall level of mineralization between the calcio-traumatic lines is lower than in pre-injection dentine. Do you have any explanation for these observations?

Author: This has been observed before in the pulpal dentine of human teeth where there is an enhancement of the lines of von Ebner after chronic exposure to fluoride (Fejerskov et al., 1979). So these additional increment-al lines may result from an enhancement of the normal cyclical deposition of dentine throughout the development of the tooth. It is tempting to speculate that fluoride is effecting calcium homeostasis, but no studies have ever produced fluorotic-like lesions by interfering with calcium regulating mechanisms. On exposure to fluoride, crystal growth may be enhanced and facilitate rapid influx of calcium from the pulpal blood supply by damaging enzyme systems which maintain normal extra- and intra-cellular calcium concentration gradients. At the same time, fluoride may prevent crystal nucleation in predentine resulting in the distinctive hyper- and hypo-mineralized layers.

M.B. Engel: Fluoride is a highly reactive electro-nega-tive anion. Is it possible that in addition to its effect on apatite crystal, it affects the protein matrix and alters its calcifiability?

Author: All the evidence suggests that the fluoride in the body is present as inorganic fluoride, usually as the ion F⁻, and is not bound to the protein matrix.

M.B. Engel: Lead is incorporated into mineralizing tissues. Could the binding of this cation displace calcium from the matrix?

Author: After the injection of lead ions, there is a rapid but transient rise in serum calcium. About 2% of the injected lead is incorporated in the mineralized tissues and since the formation of lead apatite by the displacement of calcium is a slow process, the very rapid rise in serum calcium cannot be explained by this process alone. Therefore, it is reasonable to suggest that some calcium may be displaced from the organic phase by the lead ion to contribute to this rise in serum calcium.