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FORMATION AND STRUCTURE OF DENTINE IN THE RAT INCISOR AFTER CHRONIC EXPOSURE TO SODIUM FLUORIDE

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Summary

Weanling rats were chronically exposed to sodium fluoride by feeding them for eight weeks with a standard powdered diet incorporating sodium fluoride at 0.025%, 0.05% and 0.1% by weight. After eight weeks blood was removed by cardiac puncture and the levels of plasma calcium and phosphate determined. The incisor teeth were removed and their structure examined in the scanning electron microscope (SEM) using backscattered electron imaging to determine the relative concentration and distribution of the mineral phase in the dentine. There was no significant alteration to the normal serum calcium and phosphate levels. Small scattered interglobular spaces were seen in the incisor dentine of rats consuming the 0.025% sodium fluoride diet. The principle feature with the 0.05% diet were marked striations in the circumpulpal dentine but with the 0.1% diet there was severe disruption of dentine structure with continuous bands of interglobular spaces. These spaces were larger labially than lingually reflecting differences in the size and shape of calcospherites. The distribution of interglobular dentine would suggest that its formation takes time to establish.

Key Words: Dentine, dentine formation, mineralisation, sodium fluoride, rat incisor.

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Introduction

The acute effects of single or multiple injections of sodium fluoride on dentine formation and mineralisation in rats is well established and results in the formation of discrete paired bands of hyper and hypomineralised dentine (Schour and Smith, 1934; Yaeger, 1963; Yaeger and Eisenmann, 1963; Weber and Yaeger, 1964; Yaeger *et al.*, 1964; Fejerskov *et al.*, 1977, 1979; Appleton, 1988, 1992). Less well known is the effect of chronic exposure to sodium fluoride on dentine with early histological studies (Schour and Smith, 1934) showing no effect except with high fluoride diets. Studies of rat dentine using contact microradiography, histochemistry, and polarising microscopy describe striations, hypomineralised interglobular spaces, hypoplastic defects and gross deformations of the external outline of the dentine (Yaeger, 1966; Fejerskov *et al.*, 1979). These abnormalities form part of a continuous spectrum of alterations to dentine structure and mineralisation which include the changes seen in human chronic endemic fluorosis. Using contact microradiography, detectable changes in human dentine mineralisation have so far only been seen in the most severe dental fluorosis (Baud and Almi, 1970) and, although striations may be present in enamel, they are not seen in the dentine of the same tooth (Gustafson, 1961).

The purpose of this investigation, therefore, was to examine the structure of dentine from the incisors of rats chronically exposed to a range of concentrations of dietary sodium fluoride over a period of eight weeks, during which, in normal circumstances, the whole tooth should have been replaced (Schour and Massler, 1949). The dentine was then examined using backscattered electron (BSE) imaging in the scanning electron microscope (SEM). This technique facilitate the production of images which are a measure of mean atomic number or, in effect, the density of the specimen. Therefore, these images show variation in the density of the mineral with greater resolution than can be achieved using contact microradiography (Boyde and Jones, 1983).

Additionally, at the conclusion of the experiment,

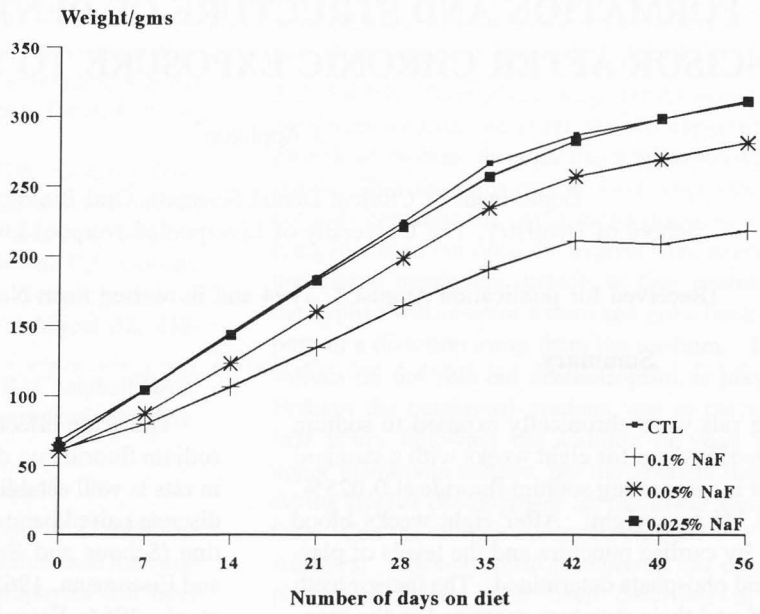


Figure 1. The graphs show that at the end of the experiment there was a statistically significant difference between the weights of the control and the 0.1% and 0.5% sodium fluoride diets.

Table 1. Serum Calcium and inorganic phosphate after eight weeks of dietary chronic exposure to sodium fluoride (pooled results from five animals in each group). SD = Standard deviation.

		0.1%	0.05%	0.025%	control
Calcium	mean	2.62	2.59	2.59	2.67
mmol/l	SD	0.14	0.05	0.05	0.07
Phosphate	mean	2.31	2.09	2.01	2.04
mmol/l	SD	0.17	0.07	0.23	0.16

blood chemistry was examined to evaluate the status of calcium and phosphate in the test animals receiving the sodium fluoride diets.

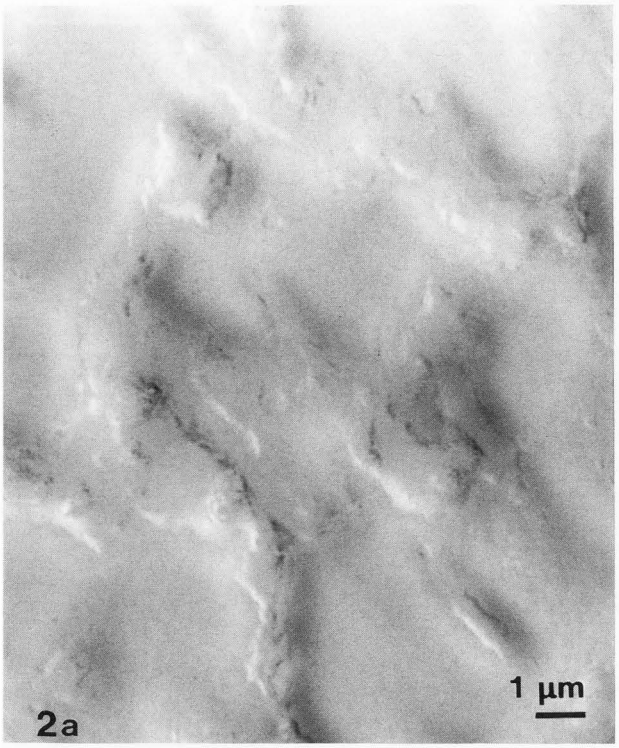
Materials and Methods

Black and white weanling rats of the Liverpool strain and weighing 60-70 gm ± 10 gm were divided into four groups of five. The control group was fed on a powdered rodent maintenance diet [RMI (E) (G) Special Diet Services, Essex, England] and the test groups on the same powdered diet to which had been mixed crystalline sodium fluoride at concentrations of either 0.025%, 0.05% or 0.1%. The rodent maintenance diet contained 0.71% calcium and 600 i.u. vitamin D per kg. The food intake was not measured but regular monitoring showed that the food was consumed by all the groups and required replenishment daily. The animals were given water *ad libitum* which was fluoride-free although the diet contained traces of fluorine (17 mg/kg). Each group was weighed at the beginning of the experiment and daily until the animals were killed eight weeks later and blood removed by cardiac puncture. Student's paired t-test was used to test for differences amongst the control and experimental group weight gains.

The blood was placed in heparinised tubes and centrifuged to separate the cells. The resultant plasma was then carefully decanted with a pipette and frozen prior to analysis using a Technicon sequential multi-analyser with computer (SMAC) system. Results were obtained from each sample of the concentration of calcium and phosphate. Student's paired t-test was used to determine if there were significant differences between the control and experimental groups.

The teeth were removed and fixed in Analar Methanol (BDH Poole, Dorset) for 48 hours after which they were routinely embedded in polymethyl methacrylate (Appleton, 1991, 1992, 1993) and then sectioned longitudinally or transversally using an Isomet (Buehler UK Limited, Coventry) equipped with a diamond impregnated wafer blade. The cut surface was polished to minimise surface topography using a Minimet (Buehler UK) and water soluble diamond pastes down to a particle size of 0.25 μm.

The specimens were examined in either a JEOL 35C SEM operating at an accelerating voltage of 15 kV equipped with a Robinson scintillator type backscattered electron detector or a JEOL 6310 SEM operating at an



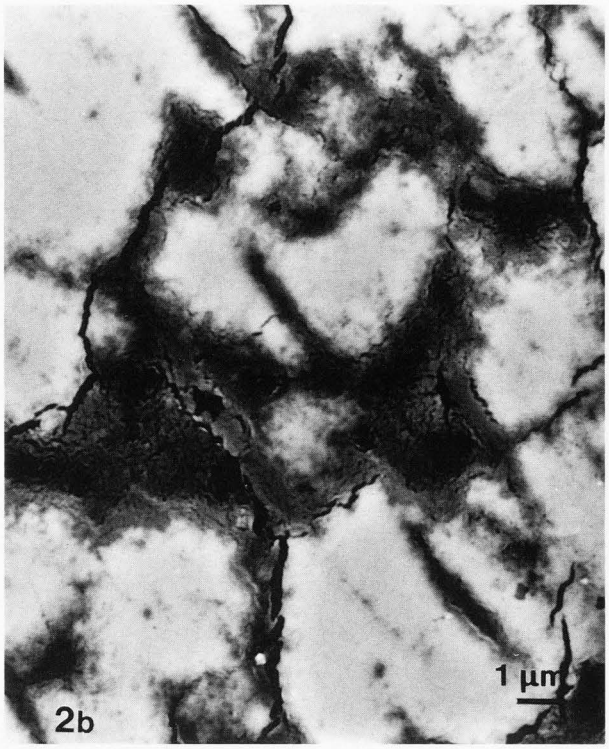


Figure 2. The topographical image of a typical tooth surface (**a**) shows that topography was not sufficient to contribute to the compositional image in which cracks can be seen resulting from the embedding process (**b**).

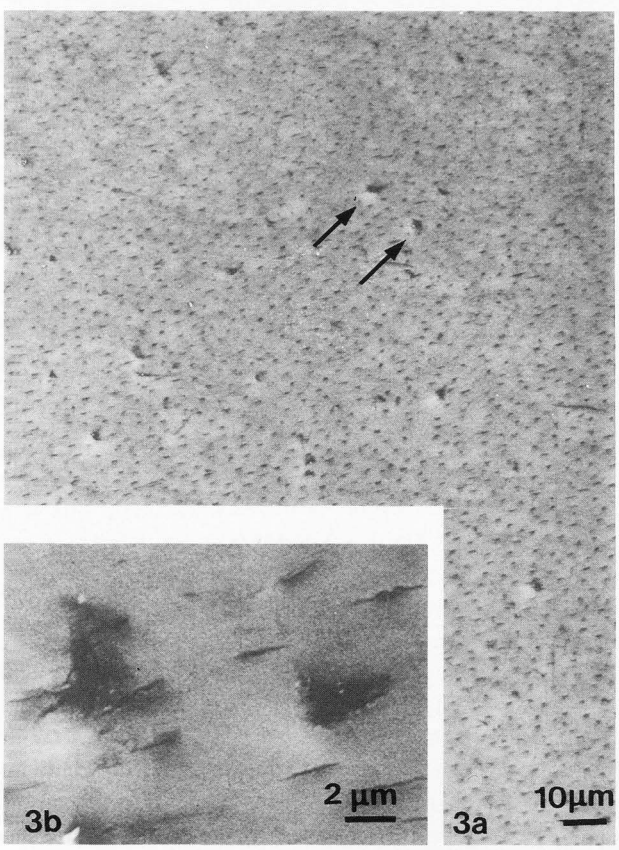


Figure 3. **a)** With 0.025% sodium fluoride there were small scattered interglobular spaces bounded by calcospherites (arrowed). **b)** Higher magnification of an interglobular space.

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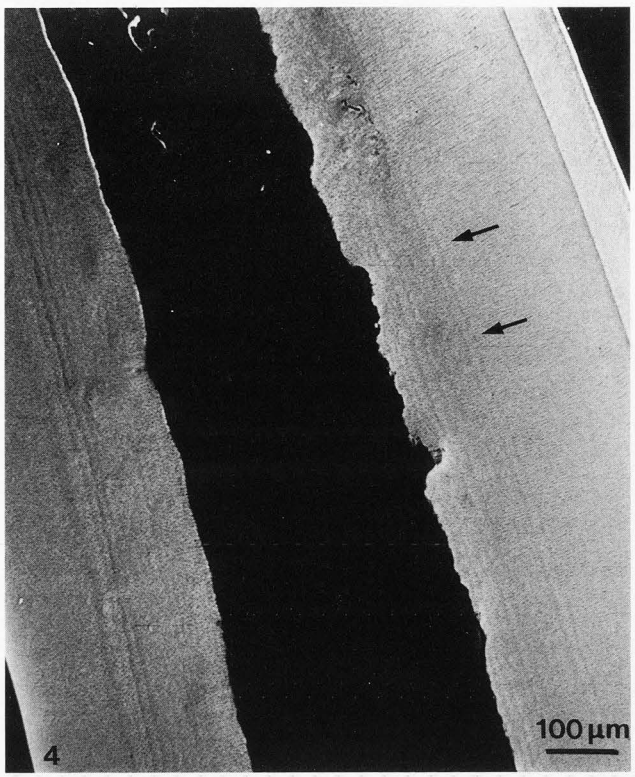
accelerating voltage of 15 kV equipped with a JEOL two-segment solid state backscattered electron detector. With the solid state detector, both compositional and topographical images of the specimen surface were obtained.

Results

Only in the group with 0.1% sodium fluoride in the diet was there a marked effect on the overall growth and development of the animals. The group receiving 0.05% sodium fluoride showed a smaller weight loss. After eight weeks animals in the 0.1% test group weighed about 100 gm less than the control group (Fig. 1). The statistical tests showed that at the end of the experiment there was a significant difference between the weights of the controls and the 0.1% and 0.05% test groups but no significant difference with the 0.025% group. The results from the calcium and phosphorus analyses showed that the levels of calcium and phosphate



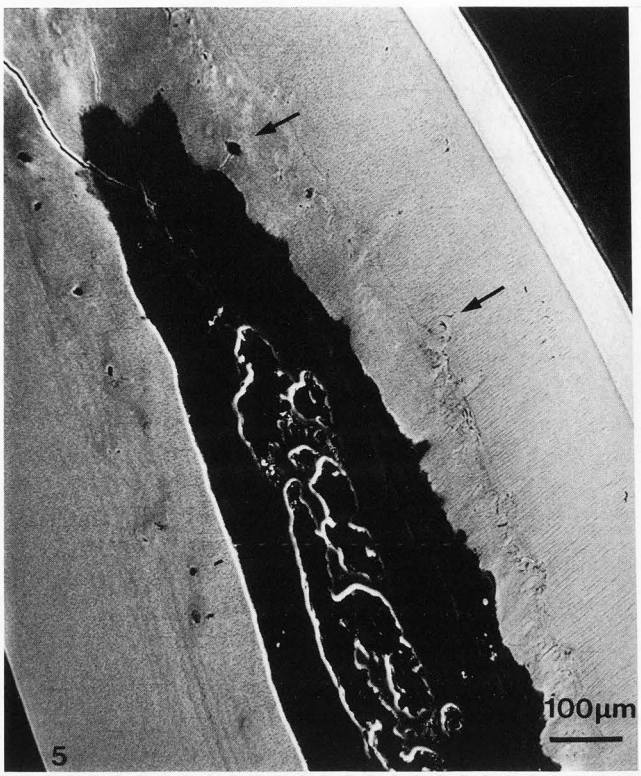


Figure 4. Longitudinal section showing striations are present in the circumpulpal dentine (arrowed) towards the apex of the tooth (0.05% sodium fluoride in the diet).

Figure 5. Longitudinal section showing that towards the incisal edge there is a marked disruption of dentine structure (arrowed) (0.05% sodium fluoride in the diet).

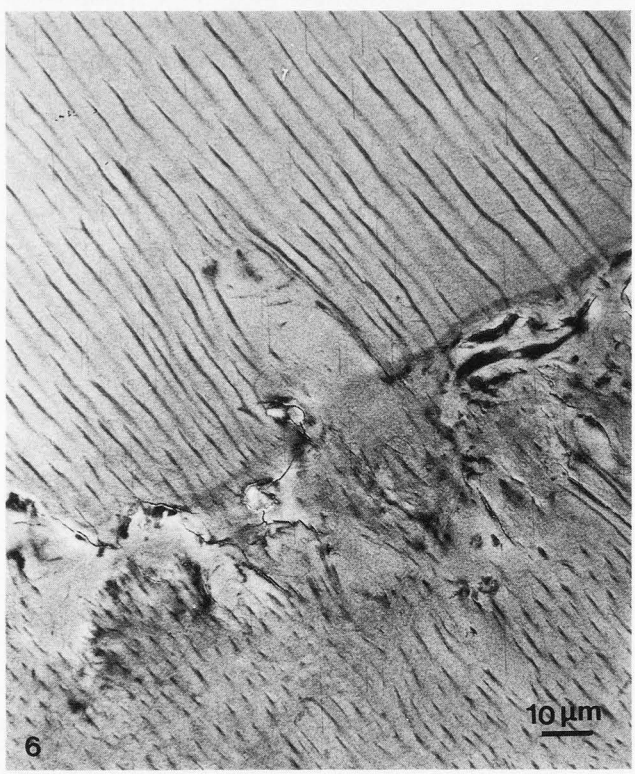


Figure 6. Severe disruption of the dentinal tubules and large unmineralised spaces are present in the circumpulpal dentine (0.05% sodium fluoride in the diet).

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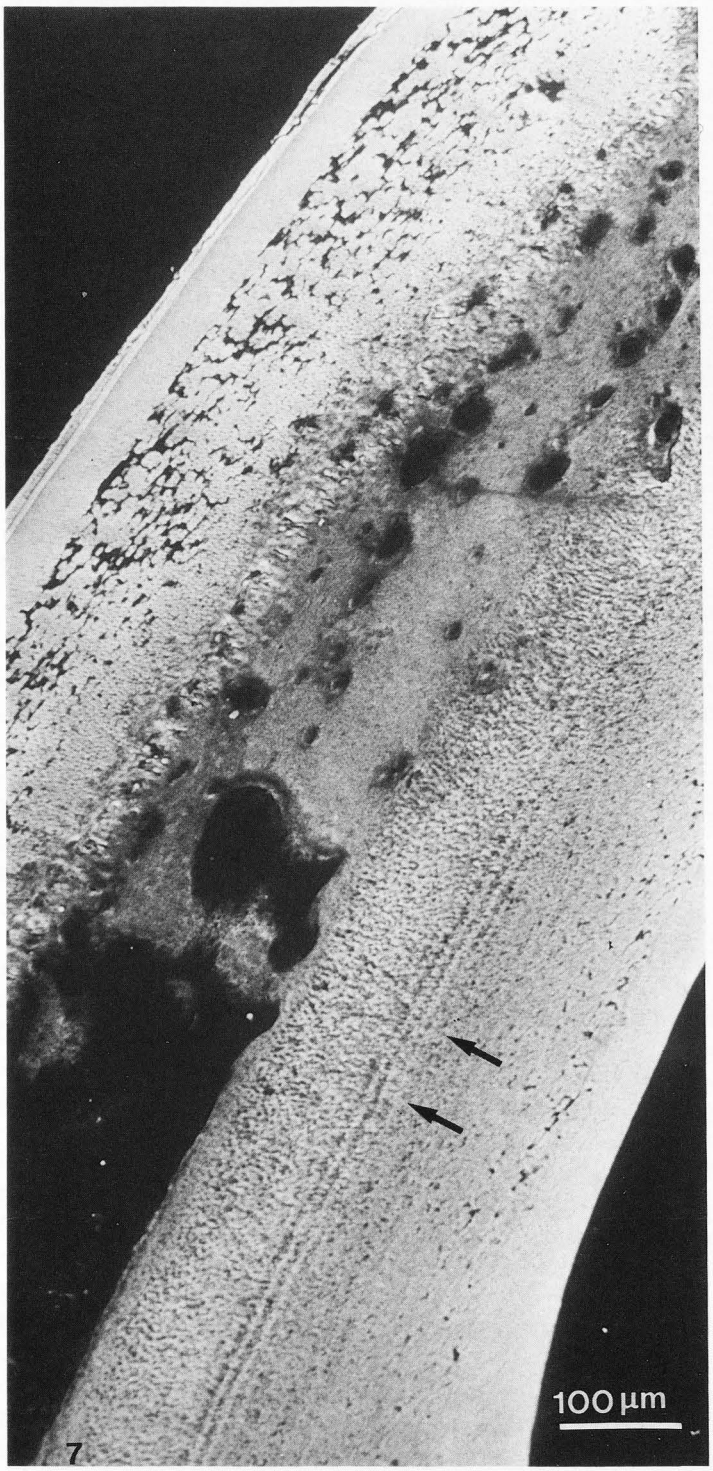
at the end of the experiment were not significantly different when compared with the controls (Table 1).

The efficacy of the polishing regime was judged by the examination of the topographical images of the cut and polished tooth surfaces. This showed that there was some surface topography but that it was not sufficient to contribute to the contrast in the compositional image as demonstrated in Figures 2a and 2b.

In the animals receiving 0.025% sodium fluoride in the diet there were distinctive small scattered areas of interglobular dentine up to 10 μm in diameter both labially and lingually (Figs. 3a and 3b) which were not present in the animals receiving the control diet. No other detectable effects were observed.

The animals receiving 0.05% sodium fluoride in the diet showed distinct disturbances to dentine formation. There were striations of markedly consistent widths and densities in both the labial and lingual dentine which were most evident in the apical two-thirds of the tooth (Fig. 4). Incisally there were more distinctive changes.



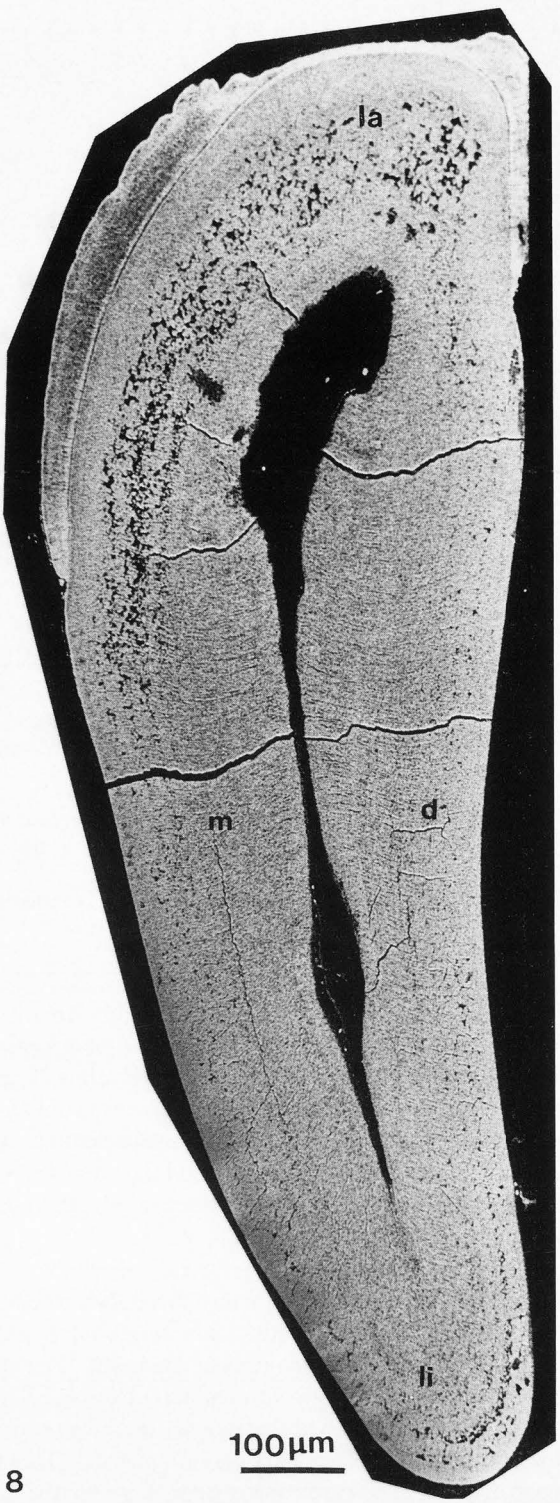


Figure 7. Longitudinal sections in which a continuous band of interglobular dentine (arrowed) can be seen labially (la) and striations are present lingually (li, arrowed) (0.1% sodium fluoride in the diet).

Figure 8. Transverse section of a tooth showing the continuous bands of interglobular dentine both labially (la) and lingually (li). Some striations can be seen mesially (m) and distally (d) (0.1% sodium fluoride in the diet).

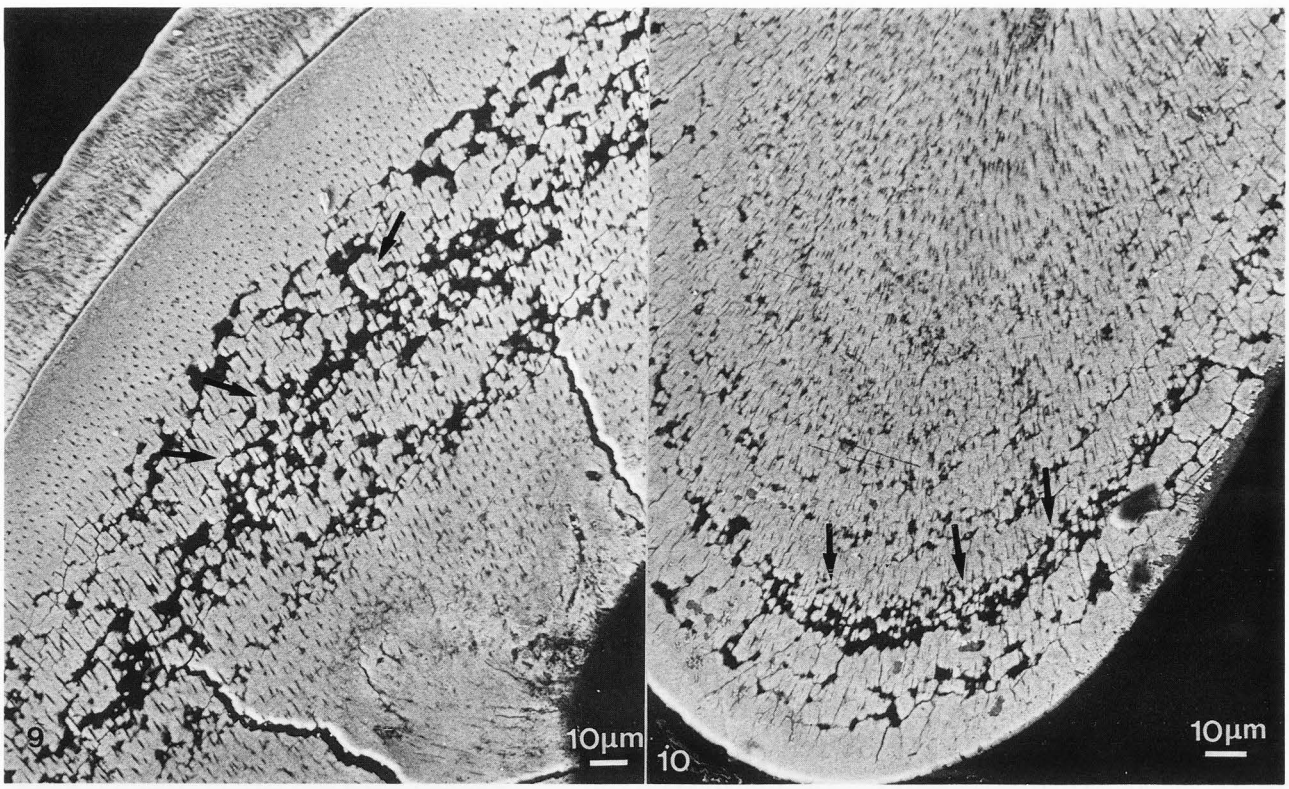


Figure 9. Large calcospherites (arrowed) are present in the labial dentine surrounding large continuous unmineralised spaces in the dentine (0.1% sodium fluoride in the diet).

Figure 10. Small calcospherites (arrowed) are present in the lingual dentine mid-way between the incisal edge and apex of the tooth (0.1% sodium fluoride in the diet).

The pre-dentine dentine interface was highly irregular in outline, the tubular pattern was disrupted and large unmineralised spaces were present in the dentine (Figs. 5 and 6). The full thickness of the dentine was not affected so that the area adjacent to the amelo-dentinal junction appeared normal in structure and the striations were confined to the circumpulpal dentine for a depth of about 400 μm.

The diet containing 0.1% fluoride produced severe disruption of the dentine structure throughout the teeth. There were large and continuous interglobular spaces, particularly in the labial dentine of the tooth. For about 100 μm beneath the amelo-dentinal junction, dentine structure was undisturbed, but deeper, there were continuous bands of unmineralised dentine matrix. This was most distinctive towards the incisal edge of the tooth (Figs. 7 and 8). The outline of the calcospherites bounding the interglobular spaces demonstrated that the calcospherites of the labial dentine were large spheres of mineral up to 100 μm in diameter (Fig. 9). In the lingual dentine the interglobular spaces were smaller and more diffuse and clearly demonstrated that the calcospherites were smaller than the labial calcospherites (Fig. 10). These smaller inter-globular spaces formed a continuous band or bands about 100 μm beneath the cementum dentine junction and in transverse section, were seen to be continuous with the labial inter-globular spaces (Fig. 7). A consistent feature of the labial dentine was the presence of distinct striations particularly in the circumpulpal dentine similar to those seen in the 0.05% diet. The mineralising front at the pre-dentine dentine junction was irregular and deeply scalloped (Fig. 8).

Discussion

It is evident from this study that chronic exposure to sodium fluoride incorporated into a standard rodent maintenance diet retards the overall growth and development of the animals as evidenced by the lower body weights at the end of the experiment. With the exception of the animals on the 0.025% diet they all gained weight at a slower rate which is in contrast to the experiment of Yaeger (1966) where the animals lost weight between weeks two to four but gained weight rapidly

thereafter. In this study, there was also overall impairment of dental development so that teeth were smaller in the test animals. All the animals appeared to consume the same amount of food daily. Since the diet contained all the essential ingredients for normal development and growth, it is probable that in the test animals, the presence of fluoride affected the normal functions of the gastric mucosa (Pashley *et al.*, 1984). Therefore, it is reasonable to assume that fluoride is responsible for the lesions seen in the dentine and that they are not a function of malnutrition. However, this study confirms earlier studies which show that in the presence of an adequate dietary calcium and vitamin D, the obvious dental fluorosis in these animals is not related to changes in calcium and phosphate metabolism (Fejerskov *et al.*, 1983). Furthermore no studies have so far been able to produce lesions which resemble those of dental fluorosis simply by interfering with calcium homeostasis.

Following ingestion, sodium fluoride is absorbed into the blood stream via the gastric and intestinal mucosa so that after a few minutes there is a detectable rise in plasma fluoride concentration (Ekstrand *et al.*, 1977; Whitford and Pashley, 1984). However, the rate of absorption is greatly affected by diet and gastric pH. For example, in the presence of ions such as calcium and magnesium, which can complex with fluoride, the rate of absorption is retarded (Ekstrand and Ehrnebo, 1979). This would probably apply in this experiment where the maintenance diet has ions present which will complex with fluoride. In rats, the influence of gastric pH on gastric absorption has been studied in detail and shows that there is an inverse relationship between gastric acidity and the rate of absorption. There is also evidence to show that a fluoride concentration of only 0.005 mol/l (95 ppm) affected the normal secretory and absorption functions of the gastric mucosa (Pashley *et al.*, 1984).

Clearance rates of fluoride from plasma are inversely related to age (Ekstrand and Whitford, 1984) and this rate is higher in rats than other species such as the dog (Whitford, 1989). This experiment began with weanling rats in which, therefore, once the fluoride was in the plasma, it would probably be cleared rapidly. Also, there is no homeostatic regulation of plasma fluoride concentrations; they rise and fall according to the pattern of fluoride intake (Ekstrand *et al.*, 1977). There is, however, a circadian rhythm in plasma fluoride concentrations which is independent from fluoride intake. This rhythm is probably caused by circadian variations in the kinetics and renal dynamics of fluoride (Whitford *et al.*, 1983).

No measurements were made of plasma fluoride during this experiment, however, it is clear that although gastric and intestinal absorption may be reduced by nature of the diet and tissue damage, clearance rates of fluoride from the plasma would be high by virtue of the species involved and the age of the animals utilised. The plasma fluoride concentration in these rats can be estimated by converting the food concentrations (0.025, 0.05, 0.1%) of sodium fluoride to ppm which yields values of 113, 226 and 455 ppm. Each ppm in the diet produces a plasma fluoride level of about 0.1 μ mol/l. Therefore, the peak plasma fluoride levels in these animals would be 11, 23 and 45 μ mol/L (Whitford *et al.*, 1983) which is relatively high.

In human teeth, it has only been possible to record changes in dentine structure and mineralisation in the most severe cases of dental fluorosis, i.e., T F score 8 using the Thylstrup and Fejerskov (1978) classification of fluorosis. Using high-resolution contact microradiography, interglobular spaces and the enhancement of Von Ebner lines by hypomineralisation is evident (Baud and Almi, 1970; Fejerskov *et al.*, 1979). In the present experiment, using backscattered electron imaging, the earliest indications of dentine fluorosis was the appearance of small scattered interglobular spaces, but with no enhancement of the incremental lines in the animals receiving 0.025% sodium fluoride in their diet. Such small spaces would not be resolvable using high-resolution contact microradiography. These backscattered images could be interpreted with some confidence since the examination of surface topography clearly demonstrated that it was not a significant contributory factor to the contrast seen in the compositional image.

With 0.05% sodium fluoride in the diet, there was some enhancement of the incremental lines, particularly labially, similar to that seen in the acute response to fluoride. Following a single acute exposure a paired response has been reported in which a distinct hypermineralised band is succeeded by a relatively hypomineralised band (Appleton, 1988, 1992) and which was accompanied by transient but marked change in serum calcium and phosphorus metabolism. In chronic exposure, therefore, the enhancement of incremental lines may be related to the fact that there is no plasma fluoride homeostasis and that levels of plasma fluoride may oscillate with the eating cycle of the animals accompanied by naturally occurring circadian rhythms.

The acute and chronic responses to sodium fluoride are, therefore, markedly different with the chronic response always resulting in relative degrees of hypomineralisation and areas of complete failure of matrix mineralisation when calcospherites do not develop. The presence of interglobular spaces reveals the size and shape of the calcospherites which form their boundaries. The clear difference in the size of calcospherites, between the labial and lingual dentine, confirms the earlier works of Mishima *et al.* (1988, 1991). The extent of

the failure of matrix mineralisation is clearly related to the concentration of sodium fluoride in the diet but the mechanism of this response is uncertain. It is possible that matrix synthesis and secretion is modified, thereby, preventing apatite nucleation and the growth of calcospherites. For example, it has been shown that dentine proteoglycan synthesis is modified by fluoride (Embery and Smalley, 1980) and proteoglycans are thought to play an important part in the nucleation of mineral (Goldberg and Takagi, 1993; Goldberg *et al.*, 1993). Furthermore, our own work has shown that acute exposure to sodium fluoride in rats disturbs the normal calcium distribution in odontoblasts during dentinogenesis as demonstrated by potassium pyroantimonate staining (Appleton, 1988).

The overall development of the teeth in the animals on the highest fluoride diet is retarded, suggesting that the growth and replacement of the dentine tissues is slower than in the control animals, i.e., considerably less than 2 mm week (Yaeger, 1966). The fact that dentine appears normal beneath the enamel dentine junction and the cementum-dentine junction suggest, therefore, that this dentine probably formed at the beginning of the experiment and was not immediately effected by the sodium fluoride in the diet. The first effects are seen as bands of interglobular dentine continuous throughout the circumference of the tooth in the pattern as incremental lines. Internal to this, striations are seen particularly lingually and are probably enhanced because the calcospherites are small and in a more linear pattern in this area (Mishima *et al.*, 1988, 1991). It is possible, therefore, that the dentine becomes more resistant to the effects of the fluoride as evidenced by the striations. Indeed, with the lower dose of fluoride (0.05%), striations are the predominant feature and are only present in the circumpulpal dentine suggesting that the fluoride diet took some time to produce this effect. It seems unlikely that there is any substantive reparative process similar to that seen after acute exposure to fluoride or strontium (Yaeger, 1966).

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Discussion with Reviewers

D.H. Pashley: What is the effects of high levels of plasma fluoride on calcium transport in odontoblasts of the developing rat incisor?

Reviewer V: Please relate the appearance of striations to the influence of fluoride upon the secretory function of the odontoblast.

Author: Following acute exposure to sodium fluoride by injection we investigated the distribution of ionic calcium as indicated by the potassium pyroantimonate method (Appleton, 1988). There was a markedly altered and diffuse distribution of Ca-pyroantimonate precipitate when compared with the controls, suggesting that fluoride affects the membrane enzyme systems which maintain concentration gradients between the odontoblast and the extracellular matrix. This effect will be cyclical since there is no homeostatic regulation of plasma fluoride, and this may be a factor in the production of striations.