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Structural changes in fluorosed dental enamel of red deer (*Cervus elaphus* L.) from a region with severe environmental pollution by fluorides

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ABSTRACT

A macroscopic, microradiographic and scanning electron microscope study was performed on the structure of fluorosed dental enamel in red deer from a fluoride polluted region (North Bohemia, Czech Republic). As was revealed by analysis of mandibular bone fluoride content, the rate of skeletal fluoride accumulation in the fluorotic deer was about 6 times that in controls taken from a region not exposed to excessive fluoride deposition. In all fluorosed mandibles, the 1st molar was consistently less fluorotic than the other permanent teeth. This was related to the fact that crown formation in the M_1 takes place prenatally and during the lactation period. Fluorosed teeth exhibited opaque and posteruptively stained enamel, reduction or loss of enamel ridges, moderately to grossly increased wear and, in more severe cases, also enamel surface lesions of partly posteruptive, partly developmental origin. Microradiographically, fluorosed enamel was characterised by subsurface hypomineralisation, interpreted as a result of fluoride interference with the process of enamel maturation. In addition, an accentuation of the incremental pattern due to the occurrence of alternating bands with highly varying mineral content was observed in severely fluorosed teeth, denoting fluoride disturbance during the secretory stage of amelogenesis. A corresponding enhancement of the incremental pattern was also seen in the dentine. The enamel along the more pronounced hypoplasias consisted of stacked, thin layers of crystals arranged in parallel, indicating that the ameloblasts in these locations had lost the distal (prism-forming) portions of their Tomes processes. The findings of the present study indicate that red deer are highly sensitive bioindicators of environmental pollution by fluorides.

Key words: Teeth; bioindication; enamel hypomineralisation; enamel hypoplasia; fluoride; fluorosis.

INTRODUCTION

During the past few decades there has been increasing concern about the effects of environmental pollution by fluorine compounds on wildlife (Shupe et al. 1984; Walton, 1988; Kierdorf & Kierdorf, 1990; Boulton & Cooke, 1994). Most of the studies in this field have been conducted on free-ranging ruminants, especially deer, proving that they are well suited as bioindicators of excessive fluoride deposition into their habitats (Karstad, 1967; Kay, 1975; Kay et al. 1975; Newman & Yu, 1976; Newman & Murphy, 1979; Shupe et al. 1984; Suttie et al. 1987; Kierdorf, 1988; Walton & Ackroyd, 1988; Machoy et al. 1991; Kierdorf et al. 1993). A particular advantage when working with deer is that their skulls and mandibles are collected and kept by hunters as trophies and for age estimation of the animals, respectively. Huge samples of this material are therefore readily available both for analysis of bone fluoride content and for the diagnosis of fluorotic alterations in teeth and bones.

In man, as well as in domestic and laboratory animals, the effects of increased fluoride exposure on forming dental hard tissues have been studied extensively and our current knowledge has recently been reviewed (Fejerskov et al. 1994). Most of the work has concentrated on dental enamel, the hardest tissue of the mammalian body. Enamel is especially suited for studying fluoride influences, as it does not undergo remodelling and therefore any significant disturbance

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during its formation leaves a permanent record in the tissue. However, despite significant progress during recent years in understanding the pathogenetic mechanisms leading to the fluorotic alterations observed in dental hard tissues, the ways in which varying levels of fluoride affect certain processes, e.g. the different phases of amelogenesis, are at present far from being clear.

The macroscopic changes present in fluorosed deer teeth have been reported for a number of species (Karstad, 1967; Kay et al. 1975; Newman & Yu, 1976; Newman & Murphy, 1979; Shupe et al. 1984; Suttie et al. 1985, 1987; Kierdorf, 1988). By contrast, information on the histological and ultrastructural characteristics of fluorosed dental hard tissues is presently available only for the roe deer (Kierdorf & Kierdorf, 1989; Kierdorf et al. 1993, 1994). These studies revealed among other things that increased fluoride exposure during tooth formation had marked effects on both the secretory and the maturation stage of amelogenesis.

In order to broaden our knowledge of the spectrum of dental lesions occurring in deer exposed to increased levels of fluoride in the environment and to further elucidate the pathogenetic mechanisms underlying the formation of these alterations, studies on the histology and ultrastructure of fluorosed dental hard tissues in other cervid species are clearly needed. The present paper reports our findings on the structure of fluorosed dental enamel of red deer from North Bohemia, Czech Republic, as revealed by macroscopic, microradiographic and scanning electron microscope analyses.

MATERIALS AND METHODS

Geographical area

The North Bohemian brown coal belt is one of the most polluted areas on earth. Major sources of this pollution are thermal power plants burning low quality brown coal with high contents of both sulphur and fluoride (Carter, 1993). Average yearly fluoride emissions from coal combustion in this area are about 12000 tons (Kotesovec, 1990). In 1992, fluoride fallout in the region of the town Chomutov, located in the coal basin, amounted to 366 kg per km^2 (Czerny, 1992).

Specimens

In total, 24 mandibles or hemimandibles of North Bohemian red deer stags aged between 2 and 12 y were at our disposal. Except for a single mandible belonging to a stag that was found dead, the specimens originated from animals that had been taken during normal hunting operations in the vicinity of the two towns Karlovy Vary and Chomutov between 1985 and 1993. On macroscopic inspection, the dentition of all stags exhibited different degrees of fluorotic alteration. Thirty-nine mandibles or hemimandibles taken from red deer of known age, living in a region of Germany (Harz mountains, State of Lower Saxony) with no increased fluoride deposition, served as controls.

Fluoride measurements

In order to obtain information on the fluoride exposure of the stags exhibiting dental fluorosis, bone fluoride concentration was measured in all of the 24 fluorotic jaws. For this, samples of cortical bone were obtained by drilling holes into the ventral side of the body of the dried and defatted mandibles and collecting the bone powder. Precisely weighed samples (between 3 and 12 mg) of this powder were dissolved in 1.5 ml of 0.5 M perchloric acid. The solution was then buffered with 6 ml of 0.5 M sodium citrate and analysed for fluoride using a fluoride ion-specific electrode (model 96-09, Orion, Cambridge, MA). For comparison, the previously determined mandibular bone fluoride concentrations (Kierdorf et al. 1995) for the 39 control red deer are also given. All fluoride values are expressed as μg F^- per g (= ppm) of dried bone.

Preparation and examination of teeth

Teeth assigned for scanning electron microscopy were extracted from the jaws that had previously been immersed in water for several hours. For inspection of enamel surfaces, the teeth were first treated with a 5% (v/v) aqueous solution of NaOCl for 80 min and afterwards ultrasonicated for 20 min, in order to remove the posteruptively acquired deposits typically present on ruminant teeth. The specimens were then rinsed with distilled water, air dried, mounted on aluminium stubs, sputter coated with gold and examined in a Hitachi S 520 scanning electron microscope (SEM) operated at 15 kV.

For SEM inspection of sections through cheek teeth, these were first cleaned as described above. They were then embedded in epoxy resin (Struers Co., Copenhagen, Denmark) and cut in a buccolingual direction. The cutting surface was polished with silicone carbide sandpaper and subsequently etched

with 34% (v/v) phosphoric acid for 15 s, followed by a thorough rinse in distilled water. The teeth were then further processed as described above.

For microradiography, buccolingual (premolars and molars) and labiolingual (incisors) ground sections of ~ 100 µm in thickness were prepared. Contact microradiographs of these sections were obtained with Ni-filtered copper radiation of 20 kV and 20 mA and a target distance of 8.6 cm in a Matchlett tube AEG 50, using Kodak spectroscopic plates (type 649-0). Exposure times varied between 1.5 and 3 min.

RESULTS

Mandibular bone fluoride levels in the red deer stags from North Bohemia (n = 24; range 948–4680 ppm) were much higher than in the controls (n = 39; range 208–1026 ppm) from the Harz mountains (Fig. 1). In both groups, bone fluoride content was positively correlated with age ($P < 0.001$). As can be deduced from comparing the regression equations given in Figure 1, the average rate of skeletal fluoride uptake in the fluorotic deer was about 6 times that in the controls.

Generally, teeth both of control and fluorotic deer were covered to a varying extent by dark mineralised deposits. The enamel of the control teeth was of a glossy, translucent, creamy-white appearance (Fig. 2). In premolars and molars in which the dentine had become exposed as a result of dental wear, the enamel, due to its greater hardness compared with dentine, had formed distinct ridges on the occlusal surfaces.

The enamel of fluorotic deer exhibited marked variation in appearance between the teeth of a single mandible (Figs 3–9). Thus, of the permanent cheek teeth, the 1st molar showed either no clinical signs of fluorosis (Figs 3, 4) or only slight to moderate alterations (Figs 5–9). By contrast, the other molars and the permanent premolars regularly exhibited more or less severe fluorosis. In the specimens with a complete set of incisors and incisiform canines present for inspection (n = 13), all front teeth showed fluorotic alterations.

Macroscopically, fluorosed enamel was characterised by opacity and (posteruptively acquired) brown staining of different intensity and extent (Figs 10–15). In only slightly affected teeth, these changes were most clearly seen in the cuspal regions (Figs 3, 4). In more severe cases, the enamel of the whole tooth crown was opaque and stained, with cloudy areas of increased opacity and discoloration often being present (Fig. 10). Other teeth exhibited a distinct horizontal banding of alternating darker and markedly opaque and more lightly stained and less opaque zones of surface enamel in the middle and coronal

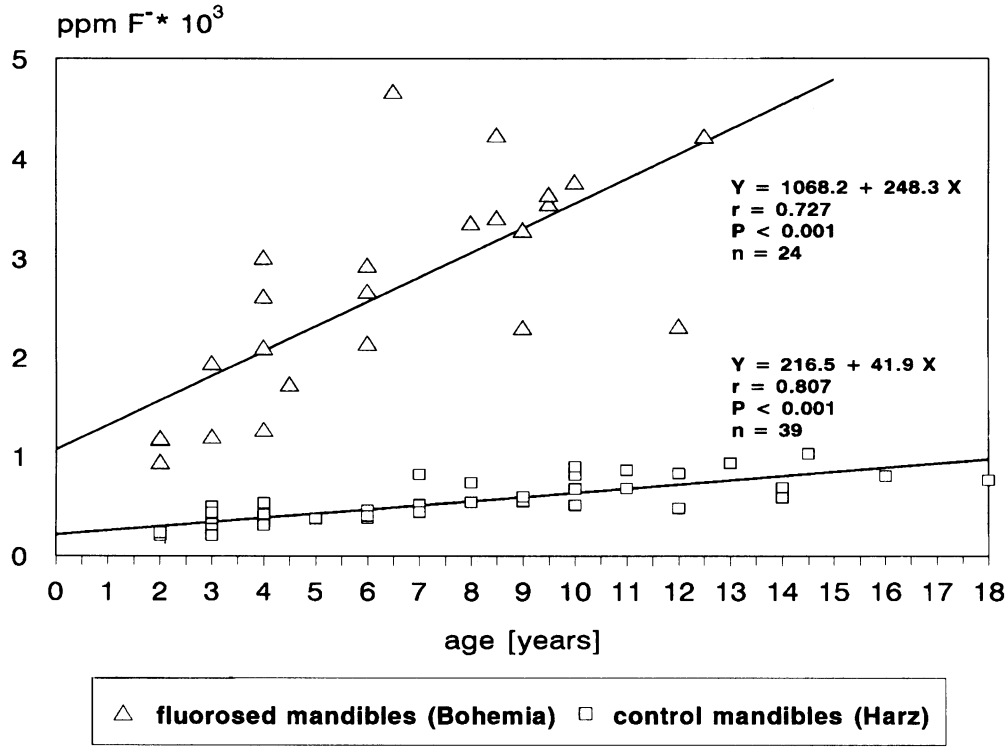
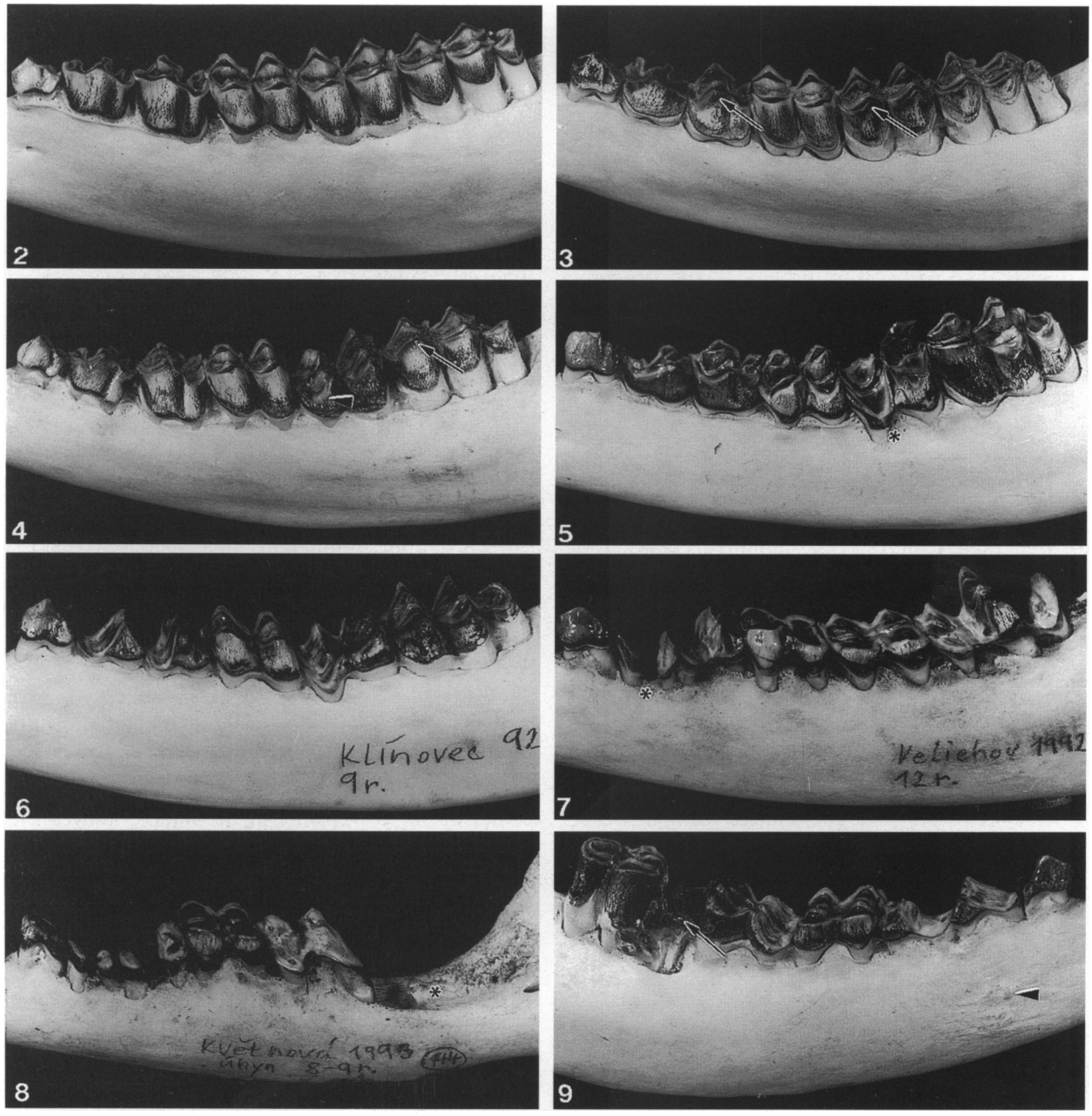


Fig. 1. Relationship between mandibular bone fluoride content (ppm dry weight) and age (y) for fluorosed red deer from North Bohemia and controls from the Harz mountains, Germany.



Figs 2–9. Rows of permanent mandibular cheek teeth from control (Fig. 2) and fluorosed red deer (Figs 3–9) in bucco-occlusal view. Fig. 2. Control, all teeth appear normal and show physiological wear. Fig. 3. Normal appearance of M_1 and slight fluorosis in the other teeth, discoloration of cusp tips (arrows) in P_3, P_4, M_2 and M_3. Fig. 4. Normal appearance of M_1 and slight to moderate fluorosis in the other teeth, increased wear (arrowhead) and intense staining of M_2 and discoloration of cusp tips in P_4 and M_3 (arrow). Fig. 5. All teeth affected to different extent by fluorotic alterations; increased wear of M_2 has led to local regression of alveolar process (asterisk). Fig. 6. Slight fluorosis in M_1 and severe alterations of the other teeth, gross disfigurement of P_3, P_4 and M_2 due to increased wear. Fig. 7. Severe disfigurement of P_3, P_4 and M_3, partial regression of alveolar process (asterisk). Fig. 8. Animal found dead; only M_1 has retained a functional shape, other teeth more or less destroyed or completely lost (M_3); partial regression of the alveolar process and complete loss in the area formerly occupied by M_3 (asterisk). Fig. 9. Severe wear on P_3, P_4 and M_2 and fracture of mesial lobe of M_3 (arrow), partial regression of alveolar process and localised bone apposition on mandibular body (arrowhead).

parts of their crowns (Fig. 11). Furthermore, a reduction in height or complete loss of the enamel ridges was observed on the occlusal surfaces of the fluorosed teeth (Figs 3–9, 14, 15), denoting diminished enamel hardness. Thus the transition zone between enamel and dentine on the occlusal surfaces of the fluorosed cheek teeth often appeared more or less flat (Fig. 15). Sometimes, the occlusal surface was even sloped from the dentinoenamel junction towards the anatomical surface of the enamel (Fig. 14).

Concomitant with the disappearance of the enamel ridges was a moderately to grossly increased wear of

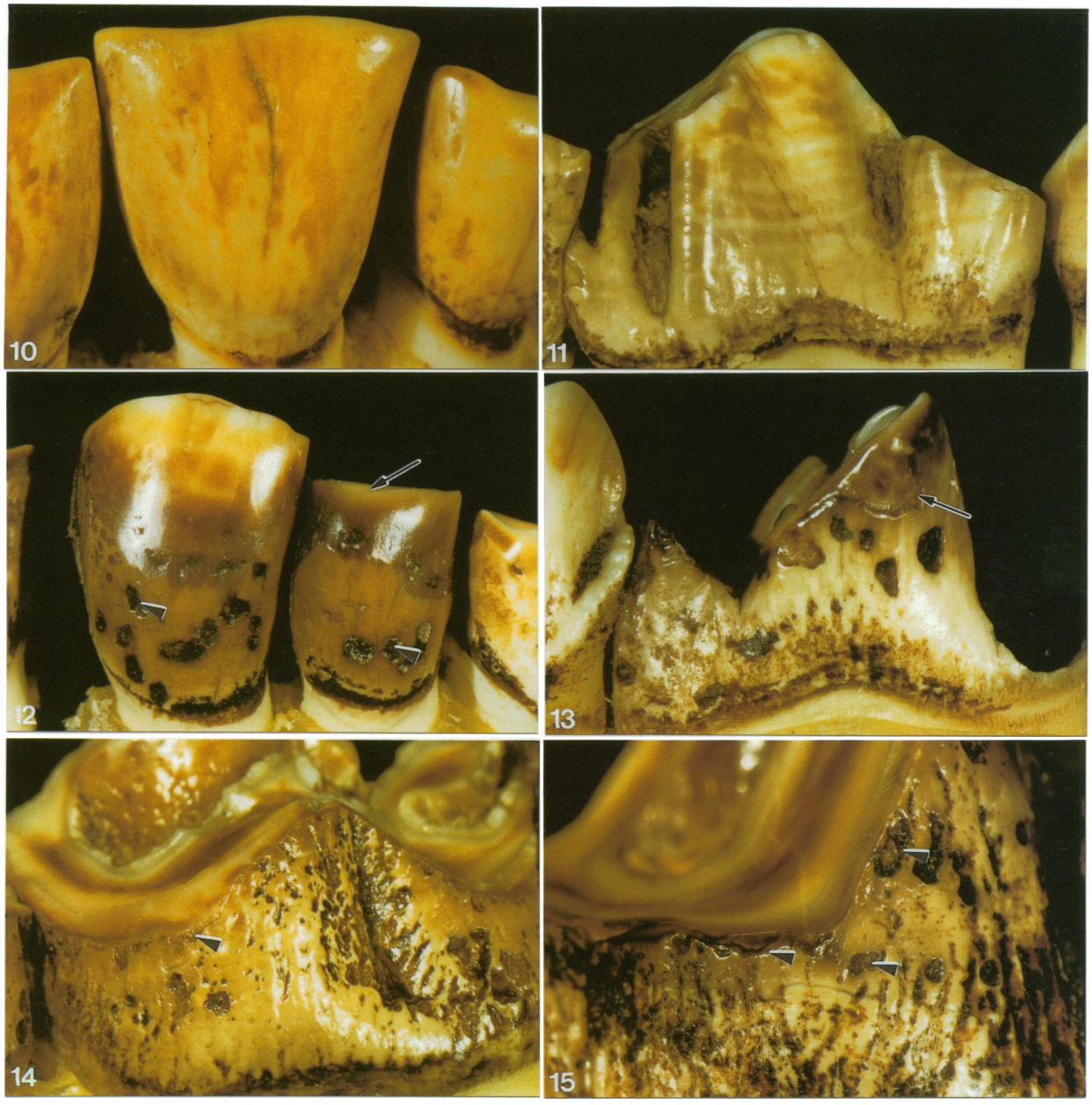


Fig. 10. Fluorosed red deer I_1, labial view. The enamel surface is opaque and stained, with cloudy areas of more intense discoloration.

Fig. 11. Fluorosed red deer P_3, lingual view. Enamel surface with alternating horizontal bands of dark and light staining.

Fig. 12. Severely fluorosed I_2 and I_3 of red deer, labial view. Opaque and heavily stained enamel. Note occurrence of enamel surface lesions (arrowheads) and abnormal wear of the incisors (arrow).

Fig. 13. Severely fluorosed red deer P_4, lingual view. The enamel exhibits opacity, discoloration and surface lesions. Extended areas of posteruptive surface enamel loss in cuspal region (arrow).

Fig. 14. Severely fluorosed red deer P_4, bucco-occlusal view. The tooth exhibits posteruptive enamel surface lesions (arrowhead) and loss of enamel ridges resulting in an abnormal slope of the occlusal surface.

Fig. 15. Severely fluorosed red deer P_4, buccal view. Complete reduction of enamel ridges on the occlusal surface and occurrence of posteruptive enamel surface lesions (arrowheads).

the cheek teeth (Figs 3–9). In older animals, this eventually led to severe dental disfigurement and loss of a functional tooth shape (Figs 6–8). Additionally, molars were fractured in 5 specimens (Fig. 9). Increased wear and disappearance of the enamel ridges were also observed in the incisors of the fluorotic deer (Fig. 12). In mandibles with cheek teeth worn down to the gum line, the alveolar process exhibited signs of regression (Figs 5–9). In the 2 most severe cases, periodontal breakdown was so extensive that individual teeth had been lost (Fig. 8).

The enamel surface in the slightly or moderately

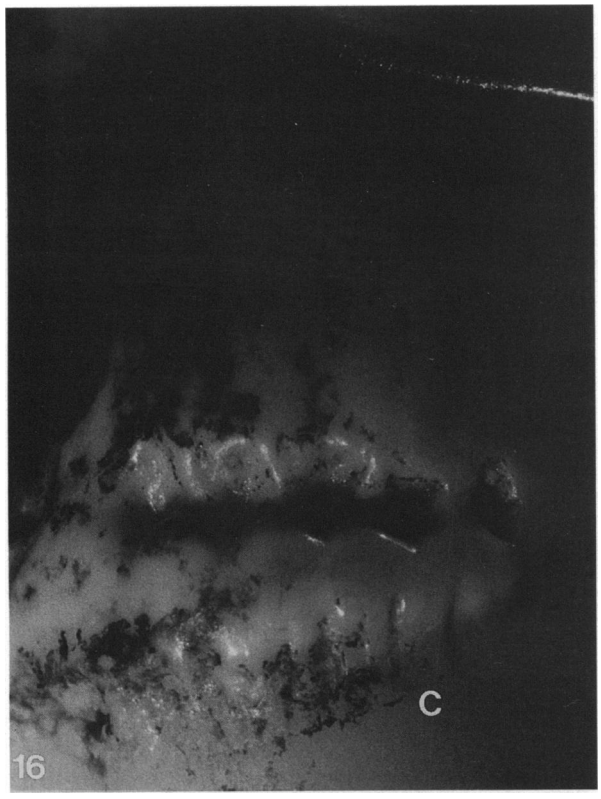
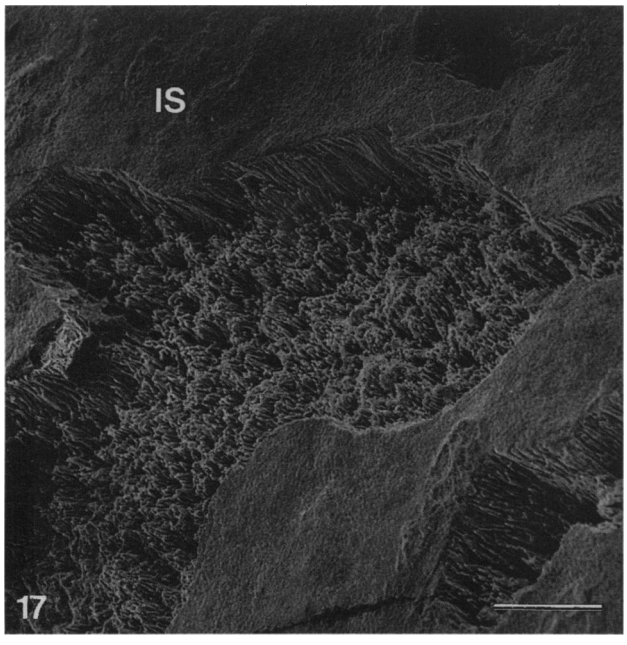


Fig. 16. Severely fluorosed red deer P_4. Horizontal row of deep surface hypoplasias in cervical enamel. C, root cementum.

fluorosed teeth exhibited no morphological irregularities (Fig. 11). By contrast, enamel surface lesions of greatly varying shape and extent were observed in the more severely fluorosed specimens (Figs 12–15). Morphologically, two types of lesions could be distinguished. The first type consisted of infoldings of the enamel surface, sometimes aligned in horizontal rows (Fig. 16). These lesions had smooth rounded walls and were regarded as developmental in origin, i.e. as true enamel surface hypoplasias.

Lesions of the second type were sharply demarcated against the surrounding enamel. The transition between these defects, that were considered posteruptive in origin, and the surrounding enamel of normal thickness, was either sharp (Fig. 17) or, in cuspal or incisal areas that had been exposed to abrasion for some time, partly smoothed (Figs 13, 15). In the scanning electron microscope, these lesions appeared as 'punched out' areas, where the outer enamel layer had been lost, thus exposing the underlying subsurface enamel (Fig. 17). The lesion walls were steep (Fig. 17) and individual enamel prisms running perpendicular to the anatomical surface of the teeth could be identified (Fig. 18). The bottom of the 'punched out' lesions exhibited a typical structure, consisting of numerous small depressions surrounded by interprismatic enamel (Figs 17, 18).

Microradiographically, the enamel of control teeth showed a uniform high radiodensity, indicative of normal mineral content (Fig. 19). By contrast, the enamel of fluorosed teeth always exhibited different degrees of subsurface hypomineralisation, the zone of



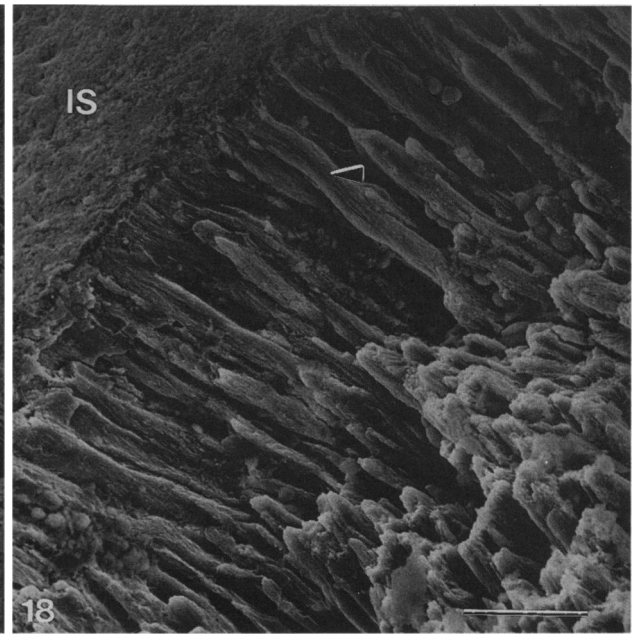


Fig. 17. Scanning electron micrograph of a severely fluorosed red deer P_3. Posteruptive 'punched out' lesions of the enamel surface. IS, intact surface enamel. Bar, 100 μm.

Fig. 18. Higher magnification of the wall of the large posteruptive enamel surface lesion shown in Figure 17. Individual enamel prisms (arrowhead) running to the anatomical surface can be identified. IS, intact surface enamel. Bar, 20 μm.

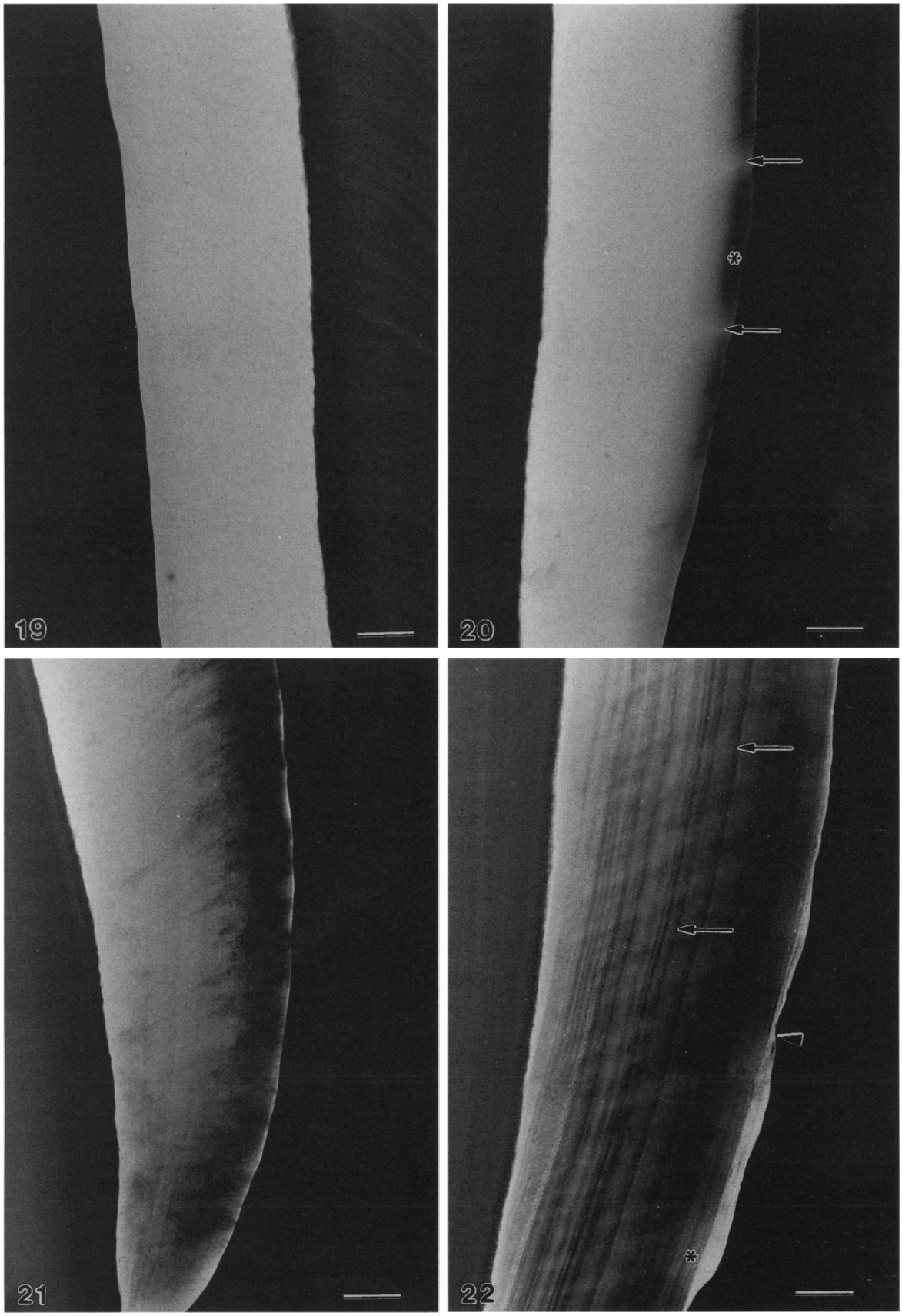


Fig. 19. Microradiograph of section through the enamel of an M_1 from a control red deer. Enamel exhibits uniform high radiodensity indicative of normal mineralisation. Bar, 250 μm.

increased radiotranslucency typically located deep to a thin outermost enamel layer with higher mineral content (Figs 20–23). The extension of the hypomineralised subsurface zone towards the dentino-enamel junction varied considerably both between different fluorosed teeth as well as along the vertical axis of individual teeth (Figs 20–23). In only slightly fluorosed specimens, the zone of increased radiotranslucency was confined to the outer enamel. Sometimes, markedly hypomineralised, rather shallow subsurface lesions were separated by narrow areas of significantly higher mineral content (Fig. 20). More severely fluorosed enamel often exhibited a continuous band of subsurface hypomineralisation along the entire vertical axis of the tooth crown (Fig. 21). Moreover, the zone of lower mineral content extended much deeper into the enamel. Often, cervically only a narrow juxtadentinal rim of higher radiodensity was left (Fig. 21).

In more severely fluorosed enamel, the pattern of incremental lines was enhanced due to the occurrence of alternating bands of enamel with highly varying mineral content (Figs 21–23). The width of these striae or bands varied considerably between different teeth.

Some of the fluorosed teeth exhibited shallow surface defects of the type shown in Figure 22. The Retzius lines did not follow the outlines of these lesions. Instead, the cervical margins of the defects corresponded to the openings of the incremental lines on the anatomical surface of the teeth. As can be seen on Figure 22, a number of cloudy areas with somewhat higher mineral content were present in the outer enamel of this specimen. Other enamel surface lesions seen in the microradiographs appeared as infoldings of varying depths and extent that were characterised by rounded walls (Fig. 23). In these (hypoplastic) lesions the Retzius lines exhibited a distinct bending according to the outlines of the defects.

In Figure 23 all types of developmental disturbances are present in the fluorosed enamel of a single tooth specimen. The changes include increased subsurface radiotranslucency, an accentuation of the incremental pattern and occurrence of hypoplastic enamel surface lesions. A marked enhancement of the incremental pattern was also seen in the dentine of this tooth, as was the case in other teeth revealing a distinct accentuation of the Retzius lines.

Scanning electron microscopy of the etched tooth sections revealed that the bulk of the enamel consisted of prisms arranged in longitudinal rows, separated by interrow sheets of interprismatic enamel (Fig. 26). Sections corresponding to the deep hypoplastic enamel surface lesions (Fig. 24) showed that the enamel located external to a grossly accentuated, hypomineralised incremental line had lost this characteristic structure. Instead, the enamel along the hypoplasias consisted of numerous stacked, thin layers of crystals all arranged in parallel (Fig. 25). The individual sheet-like layers of this 'aprismatic enamel' were of varying thickness and clearly separated from each other. Figure 26 shows a more coronal aspect of the grossly enhanced incremental band seen at the base of the structurally altered enamel in Figure 24. In contrast to both the internally as well as the externally adjacent enamel, no distinct prism/interprism pattern was discernible in the enamel forming this band, which apparently was rich in organic material.

DISCUSSION

The results of the bone fluoride analyses in the red deer from North Bohemia clearly demonstrate that these animals had been exposed to much higher levels of fluoride in their environment than the controls. The higher correlation coefficient for the relationship of bone fluoride concentration to age in the latter sample is most probably mainly attributable to the fact that the age in years was known in these animals whereas it had to be estimated in the fluorosed deer. Moreover, the degree of fluoride pollution within the North Bohemian study area and consequently fluoride uptake by the deer can also be expected to vary between different locations.

The variation in the degree of fluorotic alteration observed between the 1st molar and the other permanent mandibular cheek teeth can be related to the developmental sequence of the dentition in the red deer. According to Brown & Chapman (1991) mineralisation of the M_1 starts in utero and crown formation is complete at 4 months postpartum.

Fig. 20. Microradiograph of section through the enamel of a fluorosed red deer M_1. Hypomineralised subsurface lesions (asterisk) beneath a thin surface layer of higher radiodensity. Arrows, small areas of higher mineral content. Bar, 250 μm.

Fig. 21. Microradiograph of section through the enamel of a fluorosed red deer P_3. A zone of severe subsurface hypomineralisation extends deep into the enamel. Note thin surface layer of higher mineral content and moderate enhancement of incremental pattern. Bar, 250 μm.

Fig. 22. Microradiograph of section through the enamel of a fluorosed red deer P_3. The enamel deep to a thin radiodense surface layer exhibits overall marked hypomineralisation and a pronounced enhancement of the incremental pattern with individual grossly hypomineralised Retzius lines (arrows). Note occurrence of shallow surface lesions, the cervical margins of which correspond to the opening of individual Retzius lines (arrowhead) and of cloudy subsurface areas of somewhat increased mineral content (asterisk). Bar, 250 μm.

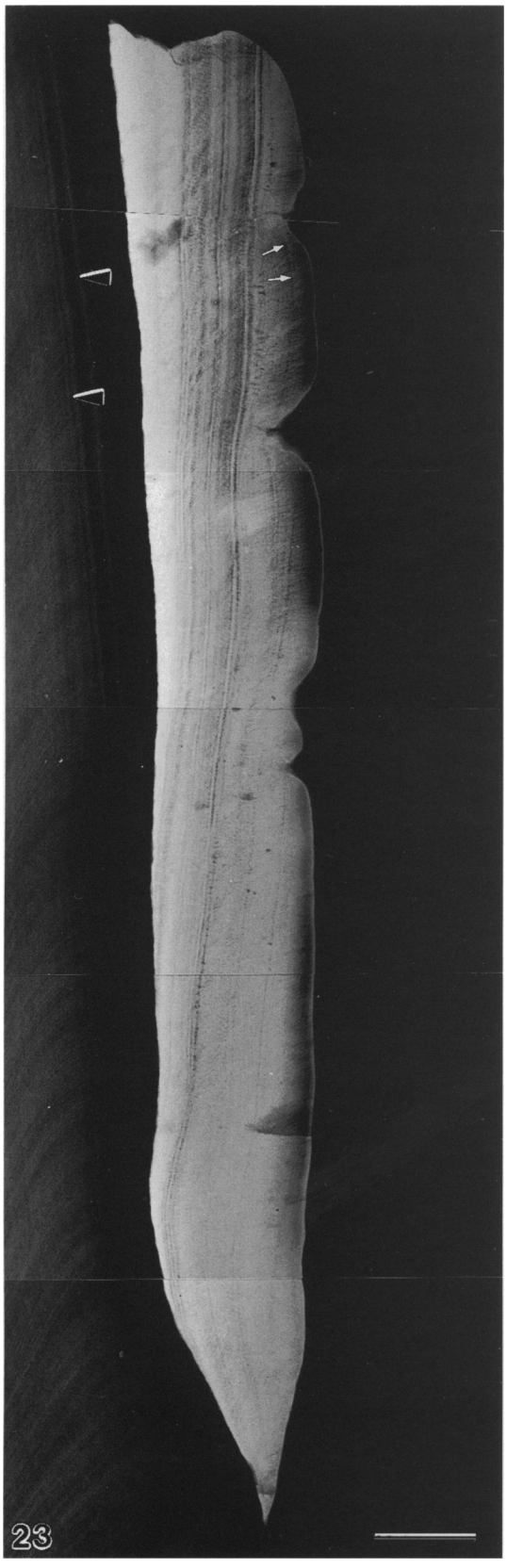
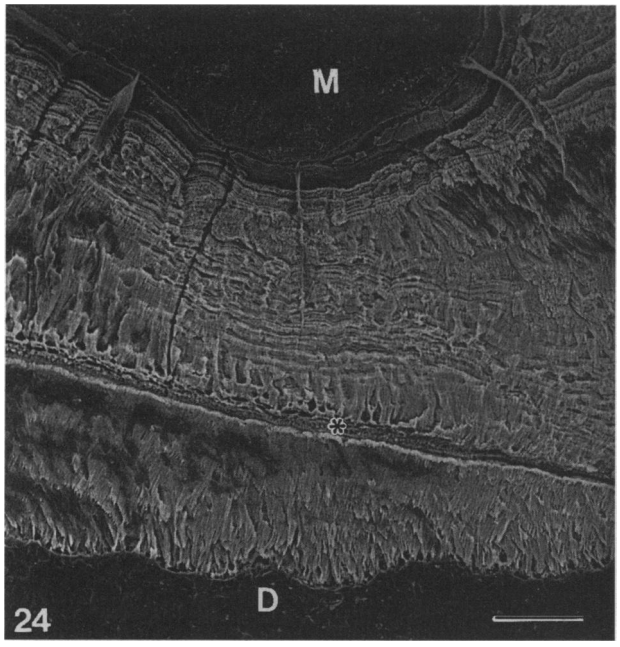


Fig. 23. Microradiograph of a section through enamel and dentine of a fluorosed red deer M_2. The enamel exhibits subsurface hypomineralisation of different extent along the coronocervical axis, a marked enhancement of the incremental pattern with individual severely hypomineralised Retzius lines and 4 hypoplastic surface lesions. Note bending of the incremental lines according to the hypoplasias (arrows). An enhancement of the incremental pattern (arrowheads) is also visible in the dentine of this specimen. Bar, 500 μm.

Mineralisation of the other molars and the permanent premolars commences postnatally (between 3 months (M_2) and 13 months (premolars) of age) and crown formation is complete much later in ontogeny (at 9, ~ 18 and 26 months in M_2, the permanent premolars and the M_3, respectively). Replacement of the front teeth occurs between 15 (I_1) and 19–22 (C inf.) months of age (Habermehl, 1985). Since in red deer calves the period of intense milk feeding extends to about 3 months postpartum (Wagenknecht, 1988), we conclude that the M_1, even in animals living in a highly fluoride polluted environment, is not exposed to grossly increased levels of fluoride during development and therefore exhibits either no or only slight to moderate fluorotic alterations. By contrast, crown formation in the other permanent mandibular teeth occurs largely or completely postweaning and partly during a period of reduced body and skeletal growth (winter), when the ability of the skeleton to act as a 'sink' for fluoride is clearly reduced. We assume that the teeth mineralising later in ontogeny are therefore exposed to higher plasma fluoride levels and in consequence exhibit more severe degrees of fluorosis.

For a free ranging ruminant living in a fluoride polluted region an intermittent rather than a uniform fluoride exposure has to be regarded as the typical situation (Kierdorf et al. 1993). In a study on dairy cattle, Suttie et al. (1972) showed that exposure of young animals to alternating high and low dietary fluoride intakes caused more severe pathological changes in their molars than exposure to a constant dose resulting in the same average yearly intake. We suppose that the sometimes grossly enhanced wear as well as the disfigurement observed in the cheek teeth of the red deer can likewise be explained as a result of intermittent fluoride action, leading to large differences in the degree of mineralisation both between different teeth of the same dentition as well as within individual teeth.

The variety of structural changes observed in the enamel of the red deer from North Bohemia we interpret as a result of fluoride interference with the different processes involved in amelogenesis. A band of subsurface hypomineralisation located deep to an outermost layer with a higher mineral content has previously been observed in fluorosed teeth of man (Fejerskov et al. 1975, 1977; Thylstrup & Fejerskov, 1978), rats (Shinoda, 1983), sheep (Suckling & Purdell-



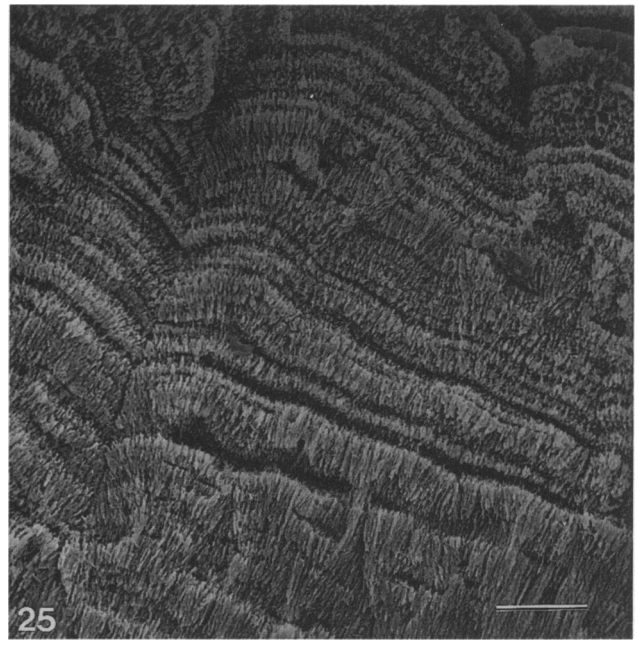


Fig. 24. Scanning electron micrograph of etched section through the base of a deep enamel surface hypoplasia of a fluorosed red deer P_4. External to a severely hypomineralised 'calciotraumatic' band (asterisk), the enamel has lost its normal structure. D, dentine; M, embedding medium. Bar, 50 μm.

Fig. 25. Higher magnification of the 'aprismatic enamel' shown in Figure 24. The enamel has a layered appearance and consists of thin stacked sheets of crystals arranged in parallel. Bar, 10 μm.

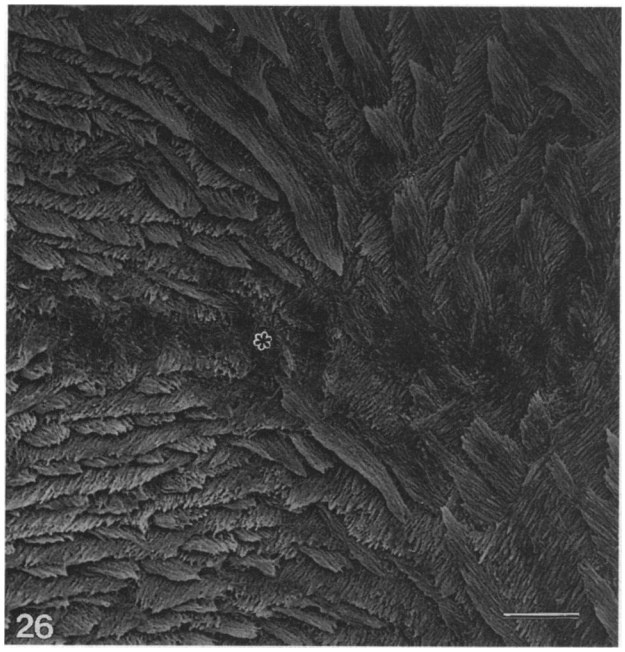


Fig. 26. Coronal aspect of (full thickness) enamel of the specimen depicted in Figure 24. An incremental line (asterisk) exhibiting loss of the prism/interprism structure and apparently rich in organic matter forms the coronal extension of the 'calciotraumatic band' seen in Figure 24. Bar, 10 μm.

Lewis, 1982), cattle (Shearer et al. 1978) and roe deer (Kierdorf et al. 1993). This hypomineralisation can thus be regarded as typical of enamel fluorosis in mammals. In summarising the available data, Fejerskov et al. (1994) recently hypothesised that this subsurface hypomineralisation results from fluoride action on the maturation phase of amelogenesis. Conclusive experimental evidence for this hypothesis derives from controlled feeding experiments on pigs (Richards et al. 1986) and sheep (Suckling et al. 1988; Milhaud et al. 1992).

A high degree of variation in the extension of the hypomineralised subsurface layer towards the dentino-enamel junction as well as along the coronocervical axis both within and between teeth was previously also observed in fluorosed enamel of roe deer (Kierdorf et al. 1993). These variations most probably reflect differences in fluoride intake between animals and fluctuations of fluoride exposure during formation of single teeth, respectively.

The increased subsurface porosity of the fluorosed enamel leads to its opaque appearance and post-eruptive uptake of stain. The conspicuous horizontal enamel banding observed in some of the teeth (Fig. 11) was also recorded for fluorosed roe deer enamel (Kierdorf, 1988). According to Suckling et al. (1988), alternating transverse bands of opaque and translucent surface enamel are present in sheep incisors during the late maturation phase of amelogenesis. With further development, the whole enamel surface then attains a translucent appearance. Based on these

findings, it can be hypothesised that the alternating horizontal bands of different opacity (and staining) seen in the fluorosed enamel of the deer teeth result from an impairment of the later stages of enamel maturation.

The reduction or loss of the enamel ridges in the fluorosed deer teeth as well as their increased wear likewise result from enamel hypomineralisation (Kierdorf, 1988; Kierdorf et al. 1993). In a free-ranging ruminant like the red deer, loss of a functional tooth shape as was demonstrated in our material will inevitably lead to a more or less intense fitness reduction. A further consequence of this hypomineralisation is the posteruptive loss of surface enamel that has also been reported for fluorosed teeth of humans (Thylstrup & Fejerskov, 1979; Thylstrup, 1983), sheep (Deutsch et al. 1979), and roe deer (Kierdorf & Kierdorf, 1989). It occurs as a result of masticatory stress acting on the hypomineralised tissue and leads to exposure of the underlying porous subsurface enamel which in man has been shown to be able to take up considerable amounts of mineral from the oral environment (Thylstrup & Fejerskov, 1979; Fejerskov et al. 1991).

Shallow enamel surface defects corresponding to those depicted in Figure 22 were also observed in microradiographs of fluorosed roe deer cheek teeth (Kierdorf et al. 1993). Since the present study revealed that the cervical margins of these lesions correspond to the openings of Retzius lines, we regard them as posteruptive in origin. We further assume that the areas of higher mineral content occurring in the outer enamel of these specimens denote posteruptive mineral uptake.

As was recently concluded (Fejerskov et al. 1994), our knowledge of how fluoride affects the secretory stage of amelogenesis is scarce. The enhancement of the pattern of Retzius lines seen in the present study clearly indicates that the stage of enamel secretion was affected by mechanisms at present still unknown. Similar findings were made on fluorosed teeth of humans (Fejerskov et al. 1977, 1979, 1991), rats (Fejerskov et al. 1979; Shinoda, 1983), and roe deer (Kierdorf et al. 1993) under a variety of chronic exposure conditions. We assume that the most pronounced lines of hypomineralisation present in our material reflect periods of especially intense fluoride induced disturbances of enamel secretion. The fact that the dentine of the fluorosed red deer teeth also exhibited a pronounced accentuation of its incremental pattern likewise matches earlier observations in man, rats, and roe deer (Fejerskov et al. 1979; Kierdorf et al. 1993). Since both enamel and dentine apparently react in a very similar way to fluoride exposure it may be assumed that the pathogenetic mechanisms leading to this type of lesion are of a generalised nature.

The observation of true enamel surface hypoplasias in the fluorosed red deer teeth parallels earlier findings in roe deer (Kierdorf & Kierdorf, 1989; Kierdorf et al. 1993) and denotes a severe toxic fluoride induced effect on the secretory ameloblasts, leading to a marked reduction in enamel matrix production. Hypoplastic enamel surface lesions have been produced experimentally in sheep incisors by daily oral application of 2, 4 or 6 mg fluoride per kg body weight (Suckling & Purdell-Lewis, 1982; Suckling & Thurley, 1984; Suckling et al. 1988) and in cows fed a varying amount of fluoride (yearly average of $\sim$ 40 ppm) in their forage (Shearer et al. 1978).

The arrangement of prisms and interprismatic material seen in the bulk of the red deer enamel corresponds to pattern 2 of the classification by Boyde (1971), as was also observed in other deer species (Kierdorf et al. 1991). In both red deer (this study) and roe deer (Kierdorf et al. 1993), stacked layers of 'aprismatic enamel' were found corresponding to the deep hypoplasias. We hypothesise that the loss of the characteristic arrangement of the enamel crystals into prisms and interprismatic material was due to the fact that the secretory ameloblasts lost the distal (prism forming) portions of their Tomes processes (Warshawsky et al. 1981). As a result of this, the subsequently formed crystals were all laid down with their long axes perpendicular to the now flattened cell surfaces at the distal poles of the ameloblasts. This type of crystal orientation corresponds to that seen in the initially formed innermost enamel layer secreted by the ameloblasts prior to the establishment of their prism growth regions (Boyde, 1967; Warshawsky et al. 1981; Kierdorf et al. 1991). As becomes particularly evident from Figure 25, the (reduced) matrix production by the ameloblasts clearly continued in a rhythmic fashion, giving rise to the layered appearance of the enamel in these locations.

Formation of severely hypomineralised incremental bands concomitant with disturbance of normal crystal growth, corresponding to that shown in Figures 24 (cervical aspect) and 26 (more coronal aspect) has repeatedly been observed in rats after parenteral administration of high fluoride doses (Kruger, 1969; Walton & Eisenmann, 1974; Takuma et al. 1983; Monsour et al. 1989). These 'calciotraumatic bands' were shown to contain remnants of the Tomes processes that, contrary to normal development, remained within the enamel and were not replaced by

crystals. Since after a certain recovery period, the enamel laid down external to these bands (reported to be largely or completely devoid of crystals) was found to be of normal structure, the fluoride effect on the process of enamel secretion was interpreted as a transient one with no permanent damage to the ameloblasts (Walton & Eisenmann, 1974).

Our observations, however, indicate that groups of ameloblasts apparently reacted in different ways to the same insult, depending on their location along the coronocervical axis of the tooth and, therefore, their stage of secretory activity. Thus, coronally, the enamel external to the severely hypomineralised incremental band exhibited a typical (prism/interprism) structure (Fig. 26) as well as normal thickness, which is seen as indicative of a resumption of normal enamel formation. By contrast, cervically the ameloblasts, being in an earlier stage of their secretory activity, were permanently damaged, leading to the formation of 'aprismatic enamel' of reduced thickness external to the 'calciotraumatic band'. At present, the reasons for these differences in susceptibility between groups of ameloblasts being in different stages of their secretory activity are not clear. The same applies to the variation in reaction of cells being in the same stage of matrix formation, i.e. having the same position along the coronocervical axis of a forming enamel layer.

In developing molars of rats, occurrence of subameloblastic cysts, i.e. of cysts located between the ameloblast layer and the surface of the already formed enamel was reported after single injections of 15, 30 or 60 mg NaF per kg body weight (Lange Nordlund & Lindskog, 1986; Lange Nordlund et al. 1986; Simmelink & Lange, 1986). As a result of this cyst formation, seen especially during the late secretory stage of amelogenesis, enamel surface hypoplasias were reported. More recently, Lyaruu et al. (1990) likewise induced formation of subameloblastic cysts in developing molars of hamsters by injection of 20 mg NaF per kg body weight. In their study, cystic lesions were only found under ameloblasts at an early stage of matrix formation, whereas no such changes were seen under fully secretory or maturation stage ameloblasts.

In comparing the findings of the present study and those of our previous work on fluorosed roe deer enamel (Kierdorf et al. 1993, 1994) with those of the above-mentioned experimental studies in rodents, it must be stressed that we did not observe anything indicating detachment of the ameloblast layer from the forming enamel surface in our material. When using the term hypoplasia for fluoride induced enamel surface lesions of developmental origin it should therefore be noted that these defects are very probably not always formed in the same way.

As can be concluded from our results, red deer are highly sensitive bioindicators of environmental pollution by fluorides. Moreover it has been demonstrated that by analysing the structural changes of dental enamel in the animals it is possible to draw a number of conclusions about the effects of fluoride on the different stages of amelogenesis and the causation of the changes observed in fluorosed enamel. However, very little is still known about how fluoride interferes with the basic mechanisms involved in biomineralisation and further studies in this field are therefore needed.

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REFERENCES

BOULTON IC, COOKE JA (1994) Fluoride accumulation and toxicity in wild small mammals. *Environmental Pollution* **85**, 161–167.

BOYDE A (1967) The development of enamel structure. *Proceedings of the Royal Society of Medicine* **60**, 923–928.

BOYDE A (1971) Comparative histology of mammalian teeth. In *Dental Morphology and Evolution* (ed. A. A. Dahlberg), pp. 81–94. Chicago: University of Chicago Press.

BROWN WAB, CHAPMAN NG (1991) Age assessment of red deer (*Cervus elaphus*): from a scoring scheme based on radiographs of developing permanent molariform teeth. *Journal of Zoology (London)* **225**, 85–97.

CARTER FW (1993) Czechoslovakia. In *Environmental Problems in Eastern Europe* (ed. W. F. Carter & D. Turnock), pp. 63–88. London: Routledge.

CZERNY J (1992) Internal report. Cesky Geologicky Ustav. Prague, Czechoslovakia (in Czech).

DEUTSCH D, WEATHERELL JA, ROBINSON C (1979) Pre-eruptive and post-eruptive dental fluorosis. *Caries Research* **13**, 113–114.

FEJERSKOV O, SILVERSTONE LM, MELSEN B, MØLLER IJ (1975) Histological features of fluorosed human dental enamel. *Caries Research* **9**, 190–210.

FEJERSKOV O, THYLSTRUP A, LARSEN MJ (1977) Clinical and structural features and possible pathogenic mechanisms of dental fluorosis. *Scandinavian Journal of Dental Research* **85**, 510–534.

FEJERSKOV O, YAEGER JA, THYLSTRUP A (1979) Microradiography of the effect of acute and chronic administration of fluoride on human and rat dentine and enamel. *Archives of Oral Biology* **24**, 123–130.

FEJERSKOV O, YANAGISAWA T, TOHDA H, LARSEN MJ, JOSEPHSEN K, MOSHA HJ (1991) Posteruptive changes in human dental fluorosis—a histological and ultrastructural study. *Proceedings of the Finnish Dental Society* **84**, 607–619.

FEJERSKOV O, LARSEN MJ, RICHARDS A, BAELUM V (1994) Dental tissue effects of fluoride. *Advances in Dental Research* **8**, 15–31.

HABERMEHL KH (1985) *Altersbestimmung bei Wild- und Pelztieren*, 2nd edn. Hamburg and Berlin: Paul Parey.

KARSTAD L (1967) Fluorosis in deer (*Odocoileus virginianus*). *Bulletin of the Wildlife Disease Association* **3**, 42–46.

KAY CE (1975) Fluoride distribution in different segments of the femur, metacarpus and mandible of mule deer. *Fluoride* **8**, 92–97.

KAY CE, TOURANGEAU PC, GORDON CC (1975) Industrial fluorosis in wild mule and whitetail deer from western Montana. *Fluoride* **8**, 182–191.

KIERDORF H, KIERDORF U, HOMMELSHEIM S (1991) Scanning electron microscopic observations on the development and structure of tooth enamel in Cervidae (*Mammalia: Ruminantia*). *Anatomia, Histologia, Embryologia* **20**, 237–252.

KIERDORF U (1988) Untersuchungen zum Nachweis immissionsbedingter chronischer Fluoridintoxikation beim Reh (*Capreolus capreolus* L.). *Zeitschrift für Jagdwissenschaft* **34**, 192–204.

KIERDORF U, KIERDORF H (1989) A scanning electron microscopic study on surface lesions in fluorosed enamel of roe deer (*Capreolus capreolus* L.). *Veterinary Pathology* **26**, 209–215.

KIERDORF U, KIERDORF H (1990) Chronic fluoride toxicosis in game animals. *Transactions of the 19th IUGB Congress*, Trondheim, pp. 266–270.

KIERDORF U, KIERDORF H, FEJERSKOV O (1993) Fluoride induced developmental changes in enamel and dentine of European roe deer (*Capreolus capreolus* L.) as a result of environmental pollution. *Archives of Oral Biology* **38**, 1071–1081.

KIERDORF U, SCHULTZ M, KIERDORF H (1994) Licht- und rasterelektronenmikroskopische Untersuchungen an Schmelzhypoplasien fluorotischer Rehzähne. *Zeitschrift für Jagdwissenschaft* **40**, 175–184.

KIERDORF U, KIERDORF H, ERDELEN M, MACHOY Z (1995) Mandibular bone fluoride accumulation in wild red deer (*Cervus elaphus* L.) of known age. *Comparative Biochemistry and Physiology* **110A**, 299–302.

KOTESOVEC F (1990) *Report on the state of the environment and its influence on the population of the Teplice district*. Ekoforum, Teplice, Czechoslovakia (in Czech).

KRUGER BJ (1969) Electron microscopy of enamel formed in the presence of fluoride and molybdenum. *Journal of Dental Research* **48**, 1303–1307.

LANGE NORDLUND A, LINDSKOG S (1986) Scanning electron microscopy of fluoride-induced disturbances on the enamel surface of rat molars. *Scandinavian Journal of Dental Research* **94**, 185–192.

LANGE NORDLUND A, LINDSKOG S, EKSTRAND J, HAMMARSTRÖM L (1986) Fluoride induced cystic changes in the enamel organ of the rat molar. *Journal of Oral Pathology* **15**, 87–92.

LYARUU DM, TROS GHJ, BRONCKERS ALJJ, WÖLTGENS JHM (1990) Micro-PIXE (proton-induced X-ray emission) study of the effects of fluoride on mineral distribution patterns in enamel and dentine in the developing hamster tooth germ. *Scanning Microscopy* **4**, 315–322.

MACHOY Z, DABKOWSKA E, NOWICKA W (1991) Increased fluoride content in mandibular bones of deer living in industrialized regions of Poland. *Environmental Geochemistry and Health* **13**, 161–163.

MILHAUD GE, CHARLES E, LOUBIÈRE ML, KOLF-CLAUW M, JOUBER C (1992) Effects of fluoride on secretory and postsecretory phases of enamel formation in sheep molars. *American Journal of Veterinary Research* **53**, 1241–1247.

MONSOUR PA, HARBROW DJ, WARSHAWSKY H (1989) Effects of acute doses of sodium fluoride on the morphology and detectable calcium associated with secretory ameloblasts in rat incisors. *Journal of Histochemistry and Cytochemistry* **37**, 463–471.

NEWMAN JR, YU MH (1976) Fluorosis in black-tailed deer. *Journal of Wildlife Diseases* **12**, 39–41.

NEWMAN JR, MURPHY JJ (1979) Effects of fluoride on black-tailed deer (preliminary report). *Fluoride* **12**, 129–135.

RICHARDS A, KRAGSTRUP J, JOSEPHSEN K, FEJERSKOV O (1986) Dental fluorosis developed in post-secretory enamel. *Journal of Dental Research* **65**, 1406–1409.

SHEARER TR, KOLSTAD DL, SUTTIE JW (1978) Bovine dental fluorosis: histologic and physical characteristics. *American Journal of Veterinary Research* **39**, 597–602.

SHINODA H (1983) Effects of long-term administration of fluoride on the enamel formation in rats. In *Mechanisms of Tooth Enamel Formation* (ed. S. Suga), pp. 273–284. Tokyo: Quintessence Publishing Co.

SHUPE JL, OLSON AE, PETERSON HB, LOW JB (1984) Fluoride toxicosis in wild ungulates. *Journal of the American Veterinary Medical Association* **185**, 1295–1300.

SIMMELINK JW, LANGE A (1986) Ultrastructure of altered rat enamel beneath fluoride-induced cysts. *Journal of Oral Pathology* **15**, 155–161.

SUCKLING GW, PURDELL-LEWIS D (1982) Macroscopic appearance, microhardness and microradiographic characteristics of experimentally produced fluorotic lesions in sheep enamel. *Caries Research* **16**, 227–234.

SUCKLING G, THURLEY DC (1984) Histological, macroscopic and microhardness observations of fluoride-induced changes in the enamel organ and enamel of sheep incisor teeth. *Archives of Oral Biology* **29**, 165–177.

SUCKLING G, THURLEY DC, NELSON DGA (1988) The macroscopic and scanning electron-microscopic appearance and microhardness of the enamel, and the related histological changes in the enamel organ of erupting sheep incisors resulting from a prolonged low daily dose of fluoride. *Archives of Oral Biology* **33**, 361–373.

SUTTIE JS, DICKIE R, CLAY AB, NIELSEN P, MAHAN WE, BAUMANN DP et al. (1987) Effects of fluoride emissions from a modern primary aluminum smelter on a local population of white-tailed deer (*Odocoileus virginianus*). *Journal of Wildlife Diseases* **23**, 135–143.

SUTTIE JW, CARLSON JR, FALTIN EC (1972) Effects of alternating periods of high- and low-fluoride ingestion on dairy cattle. *Journal of Dairy Science* **55**, 790–804.

SUTTIE JW, HAMILTON RJ, CLAY AC, TOBIN ML, MOORE WG (1985) Effects of fluoride ingestion on white-tailed deer (*Odocoileus virginianus*). *Journal of Wildlife Diseases* **21**, 283–288.

TAKUMA S, FURUI A, TOMODA F, OGIWARA H, KUMAMOTO Y, YANAGISAWA T (1983) Ultrastructural studies of disturbances in amelogenesis induced in rat incisors by fluoride and strontium administration. In *Mechanisms of Tooth Enamel Formation* (ed. S. Suga), pp. 259–272. Tokyo: Quintessence Publishing Co.

THYLSTRUP A (1983) Posteruptive development of isolated and confluent pits in fluorosed enamel in a 6-year-old girl. *Scandinavian Journal of Dental Research* **91**, 243–246.

THYLSTRUP A, FEJERSKOV O (1978) Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. *Community Dentistry and Oral Epidemiology* **6**, 315–328.

THYLSTRUP A, FEJERSKOV O (1979) A scanning electron microscopic and microradiographic study of pits in fluorosed human enamel. *Scandinavian Journal of Dental Research* **87**, 105–114.

WAGENKNECHT E (1988) *Rotwild*, 3rd edn. Berlin: VEB Deutscher Landwirtschaftsverlag.

WALTON KC (1988) Environmental fluoride and fluorosis in mammals. *Mammal Review* **18**, 77–90.

WALTON KC, ACKROYD S (1988) Fluoride in mandibles and antlers of roe and red deer from different areas of England and Scotland. *Environmental Pollution* **54**, 17–27.

WALTON RE, EISENMANN DR (1974) Ultrastructural examination of various stages of amelogenesis in the rat following parenteral fluoride administration. *Archives of Oral Biology* **19**, 171–182.

WARSHAWSKY H, JOSEPHSEN K, THYLSTRUP A, FEJERSKOV O (1981) The development of enamel structure in rat incisors as compared to the teeth of monkey and man. *Anatomical Record* **200**, 371–399.