Mechanism and Timing of Fluoride Effects on Developing Enamel

Pamela K. Den Besten, DDS, MS

Abstract

Fluoride appears to specifically interact with mineralizing tissues, causing an alteration of the mineralization process. In enamel, fluorosis results in a subsurface hypomineralization. This hypomineralized enamel appears to be directly related to a delay in the removal of amelogenins at the early-maturation stage of enamel formation. The specific cause for this delay is not known, although existing evidence points to reduced proteolytic activity of proteinases that hydrolyze amelogenin. This delay in hydrolysis of amelogenins could be due to a direct effect of fluoride on proteinase secretion or proteolytic activity, or to a reduced effectiveness of the proteinase due to other changes in the protein or mineral of the fluorosed enamel matrix. The formation of dental fluorosis is highly dependent on the dose, duration, and timing of fluoride exposure. The early-maturation stage of enamel formation appears to be particularly sensitive to the effects of fluoride on enamel formation. Although the risk of enamel fluorosis is minimal with exposure only during the secretory stage, this risk is greatest when exposure occurs in both secretory and maturation stages of enamel formation. The risk of fluorosis appears to be best related to the total cumulative fluoride exposure to the developing dentition. [J Public Health Dent 1999;59(4):247-51]

Key Words: fluorosis, enamel, mechanisms.

Exposure of dental enamel to above-optimal levels of fluoride during enamel development can cause a mineralization defect of the enamel, referred to as enamel fluorosis. The histopathology of fluorotic enamel shows a subsurface porosity deep into a well-mineralized surface zone (1-4). With increasingly severe fluorosis, the porous enamel is extended toward the dentinal-enamel junction and posteruptive breakdown of the enamel surface can occur, resulting in pitting of the enamel (5).

Studies to determine the mechanisms by which fluoride affects developing enamel are relatively few. These studies include those using animal models, as well as epidemiologic studies that have been used to draw conclusions relative to timing of the fluoride effects. Animal studies generally use high levels of fluoride either in food or drinking water to produce disturbances in the enamel. Although these levels of fluoride exposure result in serum levels within the physiologic range of those found in humans, they are higher than those found in humans ingesting optimally fluoridated drinking water. However, these studies have been useful in defining how fluoride interacts with the developing enamel matrix and cells.

Mechanisms that have been proposed for the formation of dental fluorosis include a systemic effect of fluoride on calcium homeostasis, altered protein secretion, impaired matrix biosynthesis, direct effects on extracellular proteins and proteinases, and specific effects on cell metabolism and function. The first of these proposed mechanisms, an alteration of calcium homeostasis, appears to be relevant only to humans consuming water with fluoride levels high enough to result in skeletal fluorosis. Thus, this possible effect of fluoride will not be discussed further. The other proposed mechanisms involve an effect of fluoride on cell function either directly through interactions with the developing ameloblasts or more indirectly through interactions with the extracellular matrix.

The morphologic features of the ameloblasts and/or the appearance of the extracellular matrix that they produce have been used to define the stages of enamel development (6). Although a number of different classifications for stages of enamel formation exist, most generally identify the stages of amelogenesis as the presecretory, the secretory, and the maturation stages. During the presecretory stage, the differentiating ameloblasts acquire their phenotype, and prepare to secrete the organic matrix of enamel. At the secretory stage, ameloblasts secrete amelogenin proteins to form a protein matrix over the full thickness of the enamel, and mineralization of the matrix begins shortly after protein secretion. The maturation stage begins with a rapid loss of amelogenin protein from the enamel matrix. Mineralization occurs more rapidly as protein loss continues throughout maturation to yield a fully mineralized tissue with less than 1 percent protein by weight remaining. The mechanisms of fluoride interaction may affect more than one stage of enamel formation. To better understand the effects of fluoride on enamel development, the following discussion will identify the changes that are caused by fluoride at each stage as well as their relative importance. The discussion is summarized in Table 1.

Presecretory Stage

Cell Proliferation. A few reports in the literature deal with the effects of fluoride on the proliferation of epithelial cells of the enamel organ—in particular, cells of the inner enamel epithelium, which are precursors of the ameloblasts. Bronckers and Wolt-

Send correspondence to Dr. Den Besten, Department of Pediatric Dentistry, University of California San Francisco, PO Box 0438, 707 Parnassus Avenue, D1014, San Francisco, CA 94143-0512. E-mail: pkdb@itsa.ucsf.edu. Paper as presented at the Dietary Fluoride Supplement Workshop, Chicago, IL, January 31–February 1, 1994.

gens (7) showed that fluoride concentrations of up to 1.13 µmol/L had no effect on DNA synthesis and therefore, presumably, no effect on proliferation in hamster tooth organ culture. Using the same system, Lyaruu et al. (8) also reported no changes in the frequency of mitotic figures at up to 1.06 µmol/L fluoride in the culture medium. Proliferation can, however, be affected at much higher concentrations, i.e., above 1 mmol/L. Mammalian cell cultures show growth inhibition and some cell death (9) at fluoride levels of 6.36 mmol/L. However, this cytotoxic effect of fluoride occurs at fluoride levels that are much greater than those that would be found in human serum.

Cell Differentiation. The effects of fluoride on cell differentiation have been studied in vivo by injecting rats twice daily with 7.8 mg NaF/kg body weight, with no observable effects on the differentiation pattern of rat incisor ameloblasts (10). Bronckers and coworkers (11) studied the effects of fluoride on hamster molar enamel organ cells in vitro and found no detectable effects on differentiating inner enamel epithelium histology below 265 µmol/L of fluoride in the culture medium after a 16-hour exposure. Although at higher fluoride levels (millimolar) the earliest differentiating ameloblasts were found to be affected (12), these effects are not relevant to the physiologic levels of fluoride found in human serum. Therefore, low fluoride levels do not appear to affect ameloblasts at the presecretory stage of enamel formation.

Secretory Stage

Histologic Changes. Following a single injection of sodium fluoride, histologic changes that occur include the formation of subameloblastic cysts below the secretory ameloblasts, with an increase in lysosome-like structures. The maturation ameloblasts remain relatively unaffected in this animal model (13-15). Bands of altered enamel seen beneath the cysts include a hypocalcified and a hypercalcified band of enamel. Similar changes have been found in the enamel of rats chronically ingesting high levels of fluoride in the drinking water (Nanci A. Personal communication, 1993), although in these animals the maturation-stage of enamel formation also is affected.

Protein Synthesis and Secretion. The effect of fluoride on the uptake of amino acids for protein synthesis by rat ameloblasts in vivo was first reported by Kruger (16,17), who showed that uptake of radioactively labeled serine and proline was inhibited by fluoride. Cell culture studies using fibroblasts (9) have indicated that fluoride has an effect on amino-acid transport, perhaps via coupled ATPase activity. Such an inhibition of amino-acid uptake could be responsible for the reported inhibition of protein synthesis (18). In vitro studies by Bronckers and Woltgens (7) have shown that fluoride levels up to 0.5 µmol/L in the culture medium had little effect on ^{3}H proline incorporation into enamel proteins. Above this level, however, a reduction was observed. The total amount of secretory matrix also has been shown to be reduced in rats ingesting chronic high levels of fluoride in drinking water (19,20). Therefore, it appears that at high levels of fluoride the amount of protein secreted into the enamel matrix is reduced.

Although the relative amount of enamel matrix proteins that are secreted can be altered at high fluoride concentrations, the amino acid composition of proteins in fluorosed enamel do not appear to be altered. A change in amino acid composition of the enamel matrix relative to fluoride levels has been reported (21); however, the changes that included an increase in polar residues were minimal. Aoba and co-worker (22) studied the secretory enamel proteins of rats ingesting either 0, 25, 50, or 100 ppm fluoride in drinking water, and found no differences in the amino acid composition of enamel from the various fluoride exposed groups.

Effects of fluoride on posttranslational modifications of enamel proteins have not been identified. Phosphorylation of proteins, studied by incorporation of ^{32}P in the hamster tooth organ culture system, was not altered in the presence of up to 1.325 µml fluoride/L in the culture medium (23). Comparisons of the two-dimensional electrophoretic patterns of matrix proteins from fluorosed secretory rat enamel as compared to control secretory rat enamel showed no differences between the two groups (24), further suggesting that fluoride does not cause posttranslational changes in the developing enamel matrix.

Several investigators have suggested that the secretory enamel matrix proteins bind fluoride (25-27). The binding of fluoride by the secretory matrix may either alter enamel formation in the secretory stage, or as proposed by Crenshaw and Bawden (26), may act as a reservoir for fluoride that is later released when proteins are hydrolyzed at the maturation stage. Later studies by the same investigators showed that with extensive dialysis, fluoride binding to the secretory matrix was eliminated (28) drawing into question the hypothesis that secretory enamel matrix can act as a fluoride reservoir. However, more recent studies of fluoride binding to rat secretory and maturation enamel following chronic ingestion of fluoride suggest

TABLE 1
Fluoride Effects Relative to Stage of Enamel Formation

Stage of Formation	Effect of Fluoride Exposure
Presecretory (cell proliferation and differentiation)	No effect at physiologically relevant fluoride doses
Secretory (protein synthesis and secretion, early mineralization)	No effect on protein synthesis Inhibited protein secretion at high F levels Increased fluoride in matrix
Maturation	Fewer bands of modulating ameloblasts Altered rate of ameloblast modulation Decreased height of enamel organ Slower removal of amelogenin proteins Increased fluoride and magnesium in enamel

that fluoride is bound to enamel protein extracts, possibly related to calcium-binding proteins (29).

Maturation Stage

Although fluoride can affect secretory enamel formation at high levels, the maturation stage appears to be affected most directly by fluoride exposure during development. The modulating ameloblasts and associated papillary layer cells show abnormalities in morphometric parameters as well as the extracellular matrix.

Histologic Effects. Histologic studies showing the effects of fluoride at the maturation stage of enamel formation have been done following chronic fluoride exposure in several animal models. In these animal models, the levels of chronic fluoride ingestion is much higher than that ingested by humans. However, the relatively low serum fluoride levels found in the animals following fluoride ingestion indicate that they are appropriate models to study fluoride mechanisms. For example, a number of studies have been done with rats chronically ingesting 100 ppm fluoride in drinking water, which results in a serum fluoride level of approximately 0.16 ppm fluoride (20,24). This serum fluoride level is similar to that of humans ingesting water containing 5 to 10 ppm fluoride (30-32).

Morphometric studies of the ameloblasts from rats ingesting 100 ppm fluoride in drinking water show significantly fewer bands of smooth-ended and ruffle-ended ameloblasts in the exposed rats as compared to controls. In addition, fluoride alters both the rate and pattern of ameloblast modulation as ameloblasts modulate from smooth-ended cells to ruffle-ended cells and back again (20,33) in what appears to be a dose-dependent manner. Smith et al. (20) showed that the rate of ameloblast modulation in fluoride-exposed rats was initially slower, followed by a possible increase in the modulation rate as maturation progressed. Other morphometric parameters shown to be altered following fluoride ingestion by rats included an overall decrease in the height of the enamel organ, particularly in the papillary layer.

Matrix Removal. A number of studies using histochemical staining (34,35), scanning electron microscopy (36-39), and quantitative measurement of nitrogen (40) and carbon (41,42) have shown an increase in the amount of organic material in fluorosed enamel compared to normal enamel. Studies of fluoride effects on developing rat enamel show that amelogenin proteins are retained longer in the maturation stage of fluorosed enamel (19,24,43). This prolonged retention of amelogenin as maturation progresses may slow the growth of the enamel crystals, resulting in the less mineralized enamel that occurs in enamel fluorosis.

The mechanism responsible for the delay in hydrolysis and removal of amelogenin has not yet been determined. However, fluoride may alter the quantity or activity of extracellular proteinases (19,44,45) needed to degrade enamel proteins during the maturation stage of amelogenesis. While proteinases are present in the enamel in both the secretory and maturation stages of enamel formation, the proteinases most active in hydrolysis of amelogenin are the serine proteinases present at the maturation stage (46-49). Some evidence suggests that the proteolytic activity of these serine protein-ases is reduced in fluorosed as compared to control enamel (45).

It appears that removal of protein during maturation is a critical step for final enamel mineralization. Both amelogenins and nonamelogenins have been shown to inhibit crystal growth in vitro (50-52). Therefore, a delayed withdrawal of amelogenin could be an important mechanism in the formation of enamel fluorosis by delaying the growth of enamel crystals; thus, when the tooth erupts, the enamel remains incompletely mineralized.

Mineral Deposition. The beginning of enamel maturation is defined by a secondary influx of mineral ions and is characterized by the white opaque zone described in rat incisors by Hiller et al. (53) and later shown to be present in most species (54,55). The white opaque zone and the preceding transitional enamel are characterized by a selective uptake of fluoride (56), possibly due to the high degree of hydration at this stage (57,58), allowing free access of fluoride to the enamel matrix (59). An increase in the influx of fluoride at the early-maturation stage may be partially responsible for the susceptibility of the early-maturation enamel to the effects of fluoride. The high concentrations of fluoride in the enamel matrix at the transition/early-maturation stage of enamel formation could reduce the available ionic calcium, resulting in reduced proteolytic activity at this critical stage (26,29).

Studies by Bronckers et al. (24) of the effects of fluoride on mineralization of hamster molar tooth organ culture showed that fluoride in the media irreversibly affected the existing mineralizing matrix, producing a more rapid deposition and disruption of crystal growth. Secondly, fluoride in the media interfered with the deposition of crystal in the new matrix. However, when fluoride was removed from the media, the matrix recovered and mineralized normally. These studies suggest that fluoride may interfere with initial nucleating sites in the matrix, perhaps by labile binding of the fluoride to the nucleating site in the matrix (26).

The nature of the mineral component is altered following exposure to fluoride. Fluorosed enamel can have an increased magnesium concentration (54); in bone mineral, fluoride can result in increased manganese (59) and decreased carbonate, citrate (61), and zinc (60). These changes in the mineral chemistry could affect mineral/matrix interactions and enzyme activity. For example, it has been suggested that enamel proteins produced in the presence of fluoride may be more tightly bound to fluorapatite, thereby making them less accessible to degradation by enamel proteinases (43,62).

Timing of Fluoride Exposure

A frequently asked question is which stage of enamel formation is most sensitive to the effects of fluoride, with the presumption that reducing fluoride exposure during this stage will decrease the incidence of fluorosis. Although available data is limited, it suggests that the early-maturation stage, sometimes referred to as the transition stage (between secretory and maturation) is most sensitive to the effects of fluoride exposure (29,63,64). Fluoride has been shown to affect the maturation stage of enamel formation without prior exposure of secretory enamel to fluoride in both animals (65,66) and humans (67).

The importance of timing in the formation of enamel fluorosis is shown in

a study by Ishii and Suckling (68). In that study they recorded the degree of fluorosis in children who were initially drinking 7.8 ppm fluoride in drinking water, but subsequently changed to 0.2 ppm fluoride. They found moderate-to-severe fluorosis of the upper left central incisor in children aged 35 through 42 months at the changeover, when the tooth would have been in the maturation stage of formation. In contrast, children aged 11 to 33 months prior to the change in fluoride levels who would have been mostly in the secretory stage of enamel formation had either very mild, questionable, or no fluorosis. Similarly, a study by Pendrys and Katz (69) using the Fluorosis Risk Index (FRI) determined that supplements used only during the first year of life (secretory stage) held little risk for fluorosis. In animal studies, as well, fluoride exposure during the secretory stage alone does not have the effect on maturation-stage ameloblast modulation when fluoride exposure is discontinued prior to the formation of the maturation-stage enamel (33).

Although these studies suggest that the maturation-stage is most sensitive to the effects of fluoride, the duration of exposure to fluoride prior to the early maturation stage does affect the severity of enamel fluorosis (29,63,68). Holm and Anderson (70) found that the prevalence of enamel fluorosis among children who had commenced fluoride tablet supplementation at the age of 6 months was 81 percent, compared with prevalences of 59, 38, and 33 percent for children who commenced taking supplements at the ages of 12, 24, and 36 months, respectively. Although Richards et al. (65) used an animal model to show that fluorosis can occur when enamel is exposed to fluoride only during maturation, more severe fluorosis occurs when exposure is in both the secretory and maturation stages of formation.

The dose of fluoride at any one time is also important to the formation of enamel fluorosis. Studies by Angmar-Manson and co-workers (71,72) showed that in a rat given a single injection of fluoride, the subsequently erupting enamel continued to show fluorosis after the period of time during which the serum fluoride levels would have reached baseline levels. This study supports the suggestion that fluoride accumulates locally in the surrounding tissues or bony environment of the developing tooth, and that higher than optimal levels may be released beyond the time at which the initial fluoride challenge occurred. Although the specific fluorotic lesion is an event that appears to occur during the early-maturation stage of tooth enamel formation, higher levels of fluoride accumulating earlier in tooth development may result in an increased level of exposure during the time at which enamel fluorosis occurs.

These studies show that although the secretory stage of enamel formation appears to be affected only when exposed to high levels of fluoride, exposure to fluoride during this stage clearly increases the risk for fluorosis. Mineralization of tooth enamel occurs for the most part during the maturation stage of enamel formation. Therefore, to reduce the risk of fluorosis, exposure to systemic fluorides would appear to be most appropriate beginning after the secretory stage of enamel formation and continuing posteruptively to allow a maximum topical effect of fluoride on the developing teeth.

References

1. Newbrun E, Brudevold F. Studies on the physical properties of fluorosed enamel—I. Arch Oral Biol 1960;2:15-20.
2. Fejerskov O, Silverstone LM, Melson B, Moller IJ. The histological features of fluorosed human dental enamel. Caries Res 1975;9:190-210.
3. Sundstrom B, Jongebloed WL, Arends J. Fluorosed human enamel. Caries Res 178;12:329-38.
4. Triller M. Structural and histochemical observation of fluorotic enamel matrix. J Dent Res 1979;87:105-14.
5. Fejerskov O, Thylstrup A, Larsen MJ. Clinical and structural features and possible pathogenic mechanisms of dental fluorosis. Scand J Dent Res 1977;85:510-34.
6. Nanci A, Slavkin HC, Smith CE. Development and calcification of enamel. In: Bonucci E, ed. Calcification in biological systems. Boca Raton, LA: CRC Press, 1992:313-43.
7. Bronckers AL, Woltgens JH. Short-term effects of fluoride on biosynthesis of enamel-matrix proteins and dentin collagens and on mineralization during hamster tooth-germ development in organ culture. Arch Oral Biol 1985;39:181-5.
8. Lyaruu DM, DeJong M, Bronckers AL, Woltgens JH. Ultrastructural study of fluoride induced in vitro hypermineralization of enamel in hamster tooth germs explanted during the secretory phase of amelogenesis. Arch Oral Biol 1986;31:109-17.
9. Holland, RI, Hongslo JK. The effect of fluoride on the cellular uptake and pool of amino acids. Acta Pharmacol Toxicol 1979;44:354-8.
10. Walton RE, Eisenmann DR. Ultrastructural examination of various stages of amelogenesis in the rat following parenteral fluoride administration. Arch Oral Biol 1974;19:171-82.
11. Bronckers AL, Jansen LL, Woltgens JH. A histological study of the short-term effects of fluoride on enamel and dentine formation in hamster tooth-germs in organ culture in vitro. Arch Oral Biol 1984;29:803-10.
12. Kerley MA, Kollar EJ. Regeneration of tooth development in vitro following fluoride treatment. Am J Anat 1977;149:181-96.
13. Nordlund AL, Ekstrand JI, Hammarstrom L. Fluoride-induced cystic changes in the enamel organ of the rat molar. J Oral Pathol 1986;15:87-92.
14. Nordlund AL, Simmelink JW, Henell F, Hammarstrom L. Ultrastructure of fluoride-induced cysts in the rat molar enamel organ. Scand J Dent Res 1986;94:327-37.
15. Monsour P, Harbrow J, Warshawsky H. Effects of acute doses of sodium fluoride on the morphology and the detectable calcium associated with secretory ameloblasts in rat incisors. J Histchem Cytochem 1989;37:463-71.
16. Kruger J. Autoradiographic assessment of the effect of fluoride on the uptake of ^{3}H-proline by ameloblasts in the rat. Arch Oral Biol 1970;15:103-8.
17. Kruger, BJ. Utilization of ^{3}H-serine by ameloblasts of rats receiving sub-mottling doses of fluoride. Arch Oral Biol 1972;17:1389-94.
18. Wiseman A. Effect of fluoride on enzymes. In: Smith FA, ed. Pharmacology of fluorides. Berlin: Springer Verlag, 1970: 48-97.
19. Den Besten PK, Crenshaw MA. The effects of chronic high fluoride levels on forming enamel in the rat. Arch Oral Biol 1984;29:675-9.
20. Smith CE, Nanci A, Den Besten PK. Effects of chronic fluoride exposure on morphometric parameters defining the stages of amelogenesis and ameloblast modulation in rat incisors. Anat Rec 1993;237:243-58.
21. Basford KE, Patterson CM, Kruger BJ. Multivariate analysis of the influence of mottling doses of fluoride on the amino acids of enamel matrix protein of rat incisors. Arch Oral Biol 1976;21:121-9.
22. Aoba T, Moreno EC, Tanabe T, Fukae M. Effects of fluoride on matrix proteins and their properties in rat secretory enamel, J Dent Res 1990;69:1248-50.
23. Bronckers AL, Jansen LL, Woltgens JH. Long-term effects of exposure to low concentrations of fluoride on enamel formation in hamster tooth-germs in organ culture in vitro. Arch Oral Biol 1984; 29:811-19.
24. Den Besten PK. Effects of fluoride on the protein secretion and removal during enamel development in the rat. J Dent Res 1986;65:1272-7.
25. Crenshaw MA, Wennberg A, Bawden JW. Fluoride-binding by the organic matrix of developing bovine enamel. 1978;23:285-7.
26. Crenshaw MA, Bawden JW. Fluoride

binding by organic matrix from early and late developing bovine fetal enamel determined by flow rate dialysis. Arch Oral Biol 1981;26:437-76.
27. Aoba T, Collins J, Moreno EC. Possible function of matrix proteins in fluoride incorporation into enamel mineral during porcine amelogenesis. J Dent Res 1989;68:1126-68.
28. Lussi A, Fridell RA, Crenshaw MA, Bawden JW. Absence of in vitro fluoride-binding by the organic matrix of developing bovine enamel. Arch Oral Biol 1988;33:531-3.
29. Den Besten PK, Thariani H. Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. J Dent Res 1992; 71:1238-43.
30. Guy WS, Taves DR. Relation between fluoride in drinking water and human plasma [Abstract]. J Dent Res 1973;52: 238.
31. Paez D, Dapas O. Biochemistry of fluorosis. X. Comparative study of fluoride levels in biological fluids. Fluoride 1982;15:87-96.
32. Singer L, Ophaug R, Harland BF. Fluoride intake of young male adults in the United States. Am J Clin Nutr 1980;33: 328-32.
33. Den Besten PK, Crenshaw MA, Wilson MH. Changes in the fluoride-induced modulation of maturation-stage ameloblasts in rats. J Dent Res 1985;64: 1365-70.
34. Yaeger J. The effects of high fluoride diets on developing enamel and dentin in the incisors of rats. Am J Anat 1966;118:665-83.
35. Shinoda H. Effect of long-term administration of fluoride on physico-chemical properties of the rat incisor enamel. Calcif Tissue Res 1975;18:91-100.
36. Sundstrom B, Jongebloed WL, Arends J. Fluorosed human enamel, a SEM investigation of the anatomical surface and outer and inner regions of mildly fluorosed enamel. Caries Res 1978;12: 329-38.
37. Triller M. Structural and histochemical observation of fluorotic enamel matrix. J Dent Res 1979;87:105-14.
38. Shinoda H, Ogura H. Scanning electron microscopical study on the fluorosis of enamel in rats. Calcif Tissue Res 1978;25: 75-83.
39. Thylstrup A. A scanning electron microscopical study of normal and fluorotic enamel demineralized by EDTA. Acta Odontol Scand 1979;37:127-35.
40. Bhussry BR. Chemical and physical studies of enamel from human teeth: density and nitrogen content of mottled enamel. J Dent Res 1959;38:369-73.
41. Shinoda H. Effects of long-term administration of fluoride on the enamel formation in rats. In: Suga S, ed. Mechanisms of tooth enamel formation. Tokyo: Quintessence, 1983:273-84.
42. Giambro N, Prostak K, Den Besten PK. Characterization of fluorosed human enamel by color reflectance, ultrastructure, and elemental composition. Caries Res 1995;29:251-7.
43. Dajean S, Menanteau J. A western-blotting study of enamel glycoproteins in rat experimental fluorosis. Arch Oral Biol 1989;34:413-18.
44. Suga S. Histochemical observation of proteolytic enzyme activity in the developing dental hard tissues of the rat. Arch Oral Biol 1970;15:555-8.
45. Den Besten PK, Heffernan LM. Enamel proteases in secretory and maturation incisor enamel of rats ingesting 0 and 100 PPM fluoride in drinking water. Adv Dent Res 1989;3:199-202.
46. Overall CM, Limeback H. Identification and characterization of enamel proteinases isolated from developing enamel. Biochem J 1988;256:965-72.
47. Carter J, Smillie AC, Sheperd MG. Purification and properties of a protease from developing porcine dental enamel. Arch Oral Biol 1989;34:195-202.
48. DenBesten PK, Heffernan LM. Separation by polyacrylamide gel electrophoresis of multiple proteases present in rat and bovine enamel. Arch Oral Biol 1989; 34:399-404.
49. Smith CE, Borenstein A, Fazek A, Nanci A. In vitro studies of the proteinases which degrade amelogenins in developing rat incisor enamel. In: Fearnhead RW, ed. Tooth enamel. V. Yokohama, Japan: Florence Publishers, 1989:286-90.
50. Aoba T, Fukae M, Tanabe T, Shimizu M, Moreno EC. Selective adsorption of porcine amelogenins onto hydroxapatite and their inhibitory activity on hydroxapatite growth in supersaturated solutions. Calcif Tissue Int 1987;41:281-9.
51. Doi Y, Eanes ED, Shimokawa H, Termine JD. Inhibition of seeded growth of enamel apatite crystals by amelogenin and enamel proteins in vitro. J Dent Res 1984;63:98-105.
52. Robinson C, Shore RC, Kirkham J, Stonehouse NJ. Extracellular processing of enamel matrix proteins and the control of crystal growth. J Biol Buccale 1990;18: 355-61.
53. Hiller CR, Robinson C, Weatherell JA. Variations in the composition of developing rat incisor enamel. Calcif Tissue Res 1975;18:1-12.
54. Robinson C, Kirkham J. Enamel matrix components, alterations during development and possible interactions with the mineral phase. In: Fearnhead RW, Suga S, eds. Tooth enamel. IV. Amsterdam: Elsevier Science Publishers, 1984:261-5.
55. Robinson C, Kirkham J. The dynamics of amelogenesis as revealed by protein compositional studies. In: Butler WT, ed. The chemistry and biology in mineralized tissues. Birmingham, AL: Ebsco Media, 1985:248-63.
56. Weatherell J, Deutsch D, Robinson C, Hallsworth AS. Fluoride concentrations in developing enamel. Nature 1975;256: 230-2.
57. Robinson C, Fuchs P, Deutsch D, Weatherell JA. Four chemically distinct stages in developing enamel from bovine incisor teeth. Caries Res 1978;12:1-11.
58. Robinson C, Kirkham J, Hallsworth AS. Volume distribution and concentration of protein mineral and water in developing dental enamel. Arch Oral Biol 1988; 33:159-62.
59. Bawden JW, Crenshaw MA, Takano Y, Hammarstrom L. Ion transport through the enamel organ-an update. J Dent Res 1982;61:1552-4.
60. Kanwar KC, Singh M. Zinc, copper and manganese levels in various tissues following fluoride administration. Experientia 1981;37:1328-9.
61. Zipkin I, McClure FJ, Lee WA. Relation of the fluoride content of human bone to its chemical composition. Arch Oral Biol 1960;2:190-5.
62. Robinson C, Kirkham J. The effect of fluoride on the developing mineralized tissues. J Dent Res 1990;69:685-91.
63. Pendrys DG, Stamm JW. Relationship of total fluoride intake to beneficial effects and enamel fluorosis. J Dent Res 1990; 69(Spec Iss):529-38.
64. Burt BA. The changing patterns of systemic fluoride intake. J Dent Res 1992; 1:1228-37.
65. Richards A, Kragstrup J, Josephsen K, Fejerskov O. Dental fluorosis developed in postsecretary enamel. J Dent Res 1986; 65:1406-9.
66. Suckling GW, Thurley DC, Nelson DGA. The macroscopic and scanning electron-microscopic appearance and microhardness of enamel, and the related histological changes in the enamel organ of erupting sheep incisors resulting from a prolonged low daily dose of fluoride. Arch Oral Biol 1988;33:361-73.
67. Ishii T, Nagaki H. Study of the correlation between the degree of dental fluorosis and the duration of fluoride present in drinking water. In: Fearnhead R, Suga S, eds. Tooth enamel. IV. Amsterdam: Elsevier Science Publishers, 1984:338-41.
68. Ishii T, Suckling G. The appearance of tooth enamel in children ingesting water with a high fluoride content for a limited period during early tooth development. J Dent Res 1986;65:974-7.
69. Pendrys DG, Katz RV. Risk of enamel fluorosis associated with fluoride suplementation, infant formula, and fluoride dentifrice use. Am J Epidemiol 1989;130: 1199-208.
70. Holm AK, Anderson R. Enamel mineralization disturbances in 12-year-old children with known early exposure to fluorides. Community Dent Oral Epidemiol 1982;10:335-9.
71. Angmar-Mansson B, Whitford GM. Single fluoride doses and enamel fluorosis in the rat. Caries Res 1985;19:145-52.
72. Angmar-Mansson B, Lindh U, Whitford GM. Enamel and dentin fluoride levels and fluorosis following single fluoride doses: a nuclear microprobe study. Caries Res 1990;24:258-62.