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Protein-Deficient Diet, Fluoride and Amelogenesis

by

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OPSOMMING

Drie groepe albinorotte van die Wistar-tipe is in die ondersoek gebruik. Die een groep het 'n voldoende dieet ontvang terwyl die oorblywende groepe 'n proteïentekort-dieet ontvang het. 'n Maand na die aanvang van die proefneming is natriumfluoried onderhuids aan die rotte toegedien en die veranderinge (indien enige) wat in glasuurvorming en in die ameloblaste na periodes van 6, 24 en 48 uur voorgekom het, is ondersoek. Veranderinge in die mandibulêre snytande het soos volg voorgekom: klein globules of 'n reeks oneweredige uitgroeisels op die vormingsoppervlak van die glasuurmatrys, klein vormingsdefekte in 'n skuins lyn in die glasuurmatrys gerangskik, enkele uitgroeisels van die glasuurmatrysoppervlak en morfologiese veranderinge in die ameloblaste. Uit die groep van 15 rotte wat glasuurafwykinge getoon het, was slegs 2 uit die groep wat op 'n voldoende dieet was terwyl onderskeidelik 4 en 9 uit die ander 2 groepe afkomstig was. Die feit dat 6 rotte afwykinge na 6 uur getoon het, 6 na 24 uur en slegs 3 na 48 uur, laat die vermoede ontstaan dat die nadelige gevolge van 'n enkele toediening van fluoor van tydelike aard is. Bevindinge in die huidige ondersoek dui ook daarop dat 'n geskikte dosis natriumfluoried om 'n uitwerking, deur middel van onderhuidse toediening, op glasuurvorming in rotte te veroorsaak, 17 mg per kg liggaamsgewig is; dat 'n proteïentekort in die dieet die vatbaarheid van ameloblaste vir die skadelike invloed van fluoor verhoog en dat sodanige skade spoedig na die toediening van fluoor sigbaar word.

SUMMARY

Sodium fluoride was administered subcutaneously to Wistar strain albino rats on protein-adequate and protein-deficient diets. Changes in the enamel matrix and ameloblasts of the mandibular incisor teeth take the form of a series of globules or irregular outgrowths on the surface of the matrix, a line of poorly stained defects within the matrix, localised outgrowths of the matrix and morphological changes in the ameloblasts. Of the 15 rats showing abnormalities only 2 were on a protein-adequate diet while 4 and 9 respectively were from the two groups subjected to a protein-deficient diet. Abnormalities of amelogenesis were noticed in 6 rats after 6 hours, in 6 after 24 hours and in 3 after 48 hours. The findings in this study suggest that an adequate dose of fluoride to cause damage in enamel of rats is approximately 17 mg per kg of body weight, that a protein-deficient diet influences the response of the ameloblasts to the injurious action of fluoride and that changes in amelogenesis, in response to a fluoride injection, become apparent very soon after administration.

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INTRODUCTION

It has been shown in humans and in experimental animals that an excessive fluoride intake alters amelogenesis, both microscopically and macroscopically, resulting in the characteristic defect known as mottling (Smith, Lanz and Smith, 1932; Dean *et al.*, 1934; Schour and Smith, 1935, and Cohen, 1954).

The effects on amelogenesis of dietary variations in conjunction with high levels of fluoride intake have been recorded both in population studies and in experimental animals (Smith, 1936; Massler and Schour, 1952; Ockerse, 1935, and Møller *et al.*, 1970).

Murray and Wilson (1948) found severe mottling in Moroccan children from sub-economic groups with deficient nutrition; it was much less marked in children of the same community who enjoyed adequate nutrition. Massler and Schour (1952) reported that the nutritional status of a population, especially the calcium intake, affected the prevalence and the degree of mottling caused by fluoride in the water supply. The poorer the nutritional status and the lower the calcium intake, the more prevalent and severe the mottling. In a survey conducted in Kenya, Ockerse (1953) attributed the greater amount of mottling in Asian children, as compared to African children living in an area with the same water supply, to a possible low protein intake as well as a high carbohydrate intake. Møller *et al.* (1970) stated that Asians in "high fluoride" areas in Uganda had less severe dental fluorosis than Africans living in the same community. These authors were unable to relate this finding to racial differences or to differences in eating and drinking habits. Smith (1936) reported that the growth rates of young rats were materially retarded when fed on diets containing fluoride and low in protein content. No other changes were observed.

Controversy therefore exists in the literature on the effects on enamel formation resulting from variations in the relationship between dietary protein and fluoride. The present investigation was undertaken to determine the effect of fluoride on amelogenesis in albino rats subjected to a protein-deficient diet.

MATERIALS AND METHODS

Thirty-six Wistar-strain albino rats, subdivided in the groups and subgroups as shown in Table I, were used. Group 1 received a standard diet containing 19.8 per cent protein *ad libitum* (Mice biscuits, Vereeniging Consolidated Mills) while Groups 2 and 3 were given a protein-deficient diet containing 4.7 per cent protein.

After one month on these diets sodium fluoride was administered by subcutaneous injections. Groups 1 and 2 were given 3.39 mg F as a 2.5 per cent sodium fluoride solution while Group 3 received 2.54 mg F as a 1.9 per cent sodium fluoride solution. The injections were given as a single dose of 0.3 ml. One animal in each subgroup was used as a control and received no fluoride.

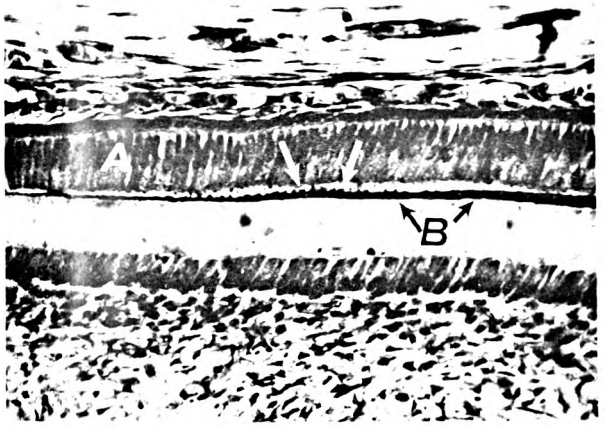
Table I: *One subgroup of 4 rats from each group was sacrificed 6, 24 and 48 hours after fluoride administration.*

Grouping		Number of rats	Age (days)	Mean weight at beginning (g)	FLUORIDE		
					Dosage (mg)	(mg/kg)	Duration of activity (hours)
GROUP 1	A	4	50	117.7	3.39	16	6
	B	4					24
	C	4					48
GROUP 2	D	4	50	121.1	3.39	30	6
	E	4					24
	F	4					48
GROUP 3	G	4	81	152.8	2.54	17	6
	H	4					24
	I	4					48

One subgroup from each group was sacrificed at intervals of 6, 24 and 48 hours after the injection (Table I). Immediately after death the mandibles were removed, cleaned of soft tissue and fixed in 10 per cent buffered formol-saline. After decalcification and embedding in paraffin wax longitudinal sections were cut at a thickness of 6 μm and stained with haematoxylin and eosin or the picro-Mallory method.

RESULTS

Two rats from each of the subgroups E and F died and were not included in the material examined. Most of the demonstrable enamel in decalcified rat incisors was in the form of young enamel, according to the criteria of Schour and Massler (1963) and Scott and Symons (1971). Changes noticed in this enamel and in the ameloblasts were as follows:



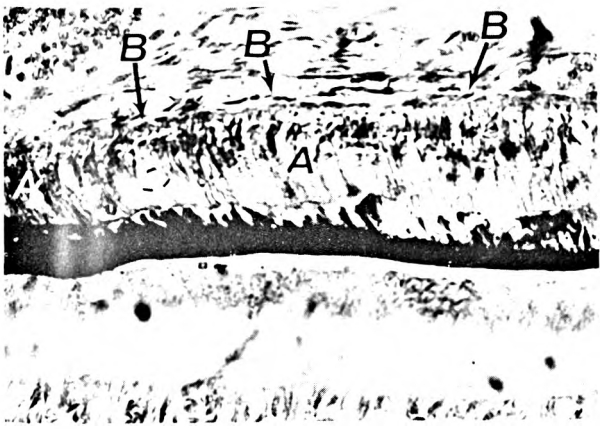


Fig. 2. Irregular outgrowths (white arrow) on the surface of the enamel matrix. Note that the ameloblasts (A) next to the outgrowths have lost their regularity and that the stratum intermedium (B) appears fragmented. Haematoxylin and eosin x 250.

subgroup G. Larger globules and surface irregularities were seen in 2 rats, one being in subgroup E and the other in subgroup H. The globules and outgrowths stained similarly to the rest of the enamel matrix, which appeared normal.

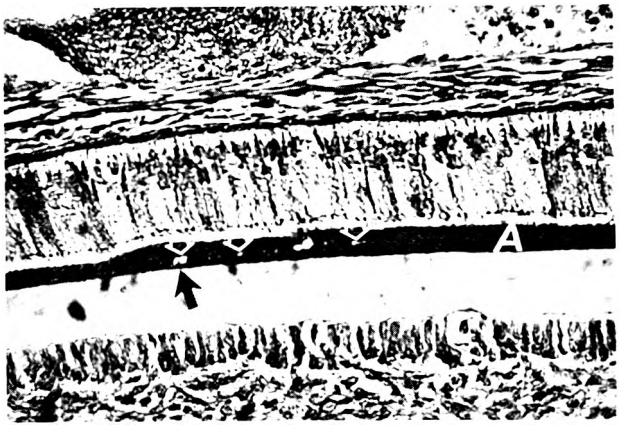


Fig. 3. An irregular line of small unstained areas (small arrows) starts next to the amelodentinal junction (black arrow) on the left and passes through the enamel matrix (A) to the right in an incisal direction. Haematoxylin and eosin x 250.

(b) A linear defect in the enamel matrix. It consisted of small irregular poorly-stained areas which followed the incremental pattern of enamel formation (Fig. 3). Another section of the same specimen (Fig. 4) shows even greater disruption in the matrix. This change was seen in 1 animal from subgroup H.

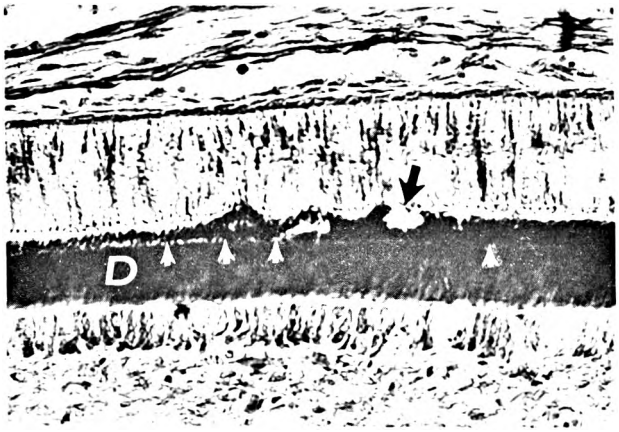


Fig. 4. A different field of the same specimen shown in Fig. 3. One of the defects involves the surface of the matrix, as shown by the black arrow. The dentine (D) appears very dark due to the different stain used and is separated from the enamel matrix by a faint line (small arrows). Picro-Mallory x 250



Fig. 5. An irregular "island" of enamel (A) is present on the surface of the matrix.

Near the middle of the field outgrowths similar to those shown in Fig. 2 are indicated by an arrow. Haematoxylin and eosin x 250.

(c) Localised outgrowths of the surface of the enamel matrix. These were observed in 2 rats and varied in appearance from a fairly small irregular enamel "island" (fig. 5, a rat from subgroup B) to a large outgrowth (Fig. 6, a rat from subgroup C). The latter had the appearance of a "bridge" of sound enamel matrix over a globular non-staining defect.

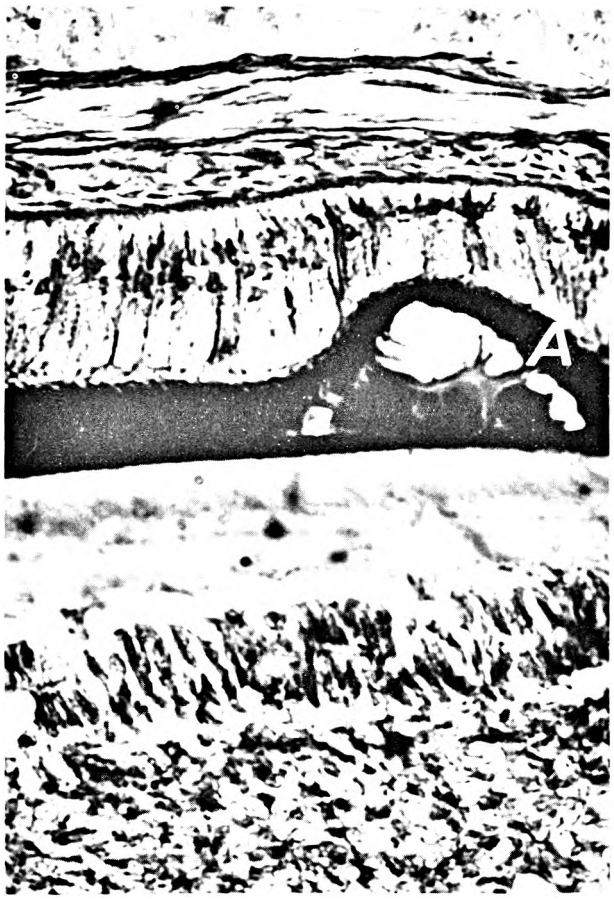


Fig 6. A "bridge" of normal enamel matrix (A) covers a globular unstained defect. This gives the impression of repair. Haematoxylin and eosin x 250.

(d) Morphological changes in the ameloblasts. Changes in the morphology of ameloblasts were observed in 6 rats, 2 each from subgroups G, H and I. The changes were of a fairly mild nature in only 2 animals, both from subgroup I (Fig. 7). In these specimens the ameloblasts showed shortening and retraction from the underlying matrix in localised areas. In the remaining 4 specimens changes were of a more severe nature and manifested as shortening of the cells, complete loss of cellular detail and separation from the matrix over wide areas.

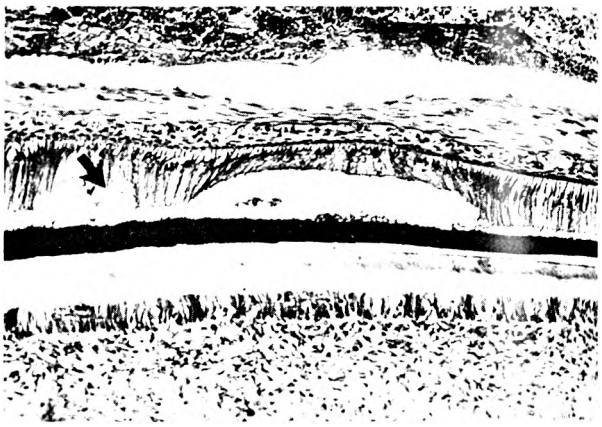


Fig. 7. Morphological changes in ameloblasts. The ameloblasts have retracted from the dark-staining enamel matrix and (on the left of the field) contain vesicles (arrowed) which appear to be coalescing. Haematoxylin and eosin x 100.

In none of the control animals was any amelogenic abnormality observed. The appearance of the dentine was normal in all specimens.

DISCUSSION

The continuously growing rat mandibular incisor is widely used in experimental studies on the effects of fluoride on amelogenesis (Smith, Lanz and Smith, 1932; Dean *et al.*, 1934 and Schour and Smith, 1935). Hence this tooth was used in this study.

De Eds (1941) supported the generalization that young animals respond more rapidly to toxic agents than mature animals and this was confirmed in the case of fluoride by Wallace-Durbin (1954). The rats used in the present experiment belonged to the age group described as young adults by Wallace-Durbin.

The mean weight of animals in Group 1 at the start of the experiment was 117.7 g (Table I). They had a mean weight gain of 92.6 g after 1 month on a standard diet and were given an injection of 3.39 mg fluoride which is equivalent to approximately 16 mg F/kg body weight. Rats in Group 2 received the same amount of fluoride but, because no allowance was made for their loss of weight on the protein-deficient diet, they received a dose of 30 mg F/kg body weight. As a result 4 of the animals died. In

the third Group a loss of weight also occurred after the dietary period; the dosage was however, adjusted to 17 mg F/kg which is approximately equal to the dosage in Group 1.

According to some authorities the dosage of fluoride required to produce changes in amelogenesis in rats is 0.1 mg to 7.0 mg F/kg by subcutaneous injection (Kruger, 1970) and 18 mg F/kg by the intraperitoneal route (Holck, 1963). Review of the literature and the author's own experience in the present investigation showed that 16 mg to 17 mg F/kg by subcutaneous injection can be safely used for experimental purposes.

In the present study rats on an adequate diet presented with only 2 abnormalities of amelogenesis while Groups 2 and 3 presented with 4 and 9 respectively. There does therefore appear to be relationship under experimental conditions between protein deficiency in the diet and the harmful effects of fluoride.

Schour and Smith (1935) stated that the enamel organ was disturbed primarily in those regions which could be readily associated with the enamel-forming epithelium which was in a highly functional state at the time of fluoride administration. These authors, as well as Irving (1943), felt that the ameloblasts were much more sensitive to sodium fluoride than the odontoblasts. Irving also found that changes in the enamel organ and in the organic enamel were present 6 hours after an injection of sodium fluoride while the dentine was still unaffected. These views were supported by Schour and Massler (1963) who stated that the growing tooth in the rat was the first organ to react to fluoride and that the growing enamel and enamel-forming cells gave the most marked response. The normal appearance of the dentine in all specimens of the present investigation is in agreement with these findings.

Schour and Massler (1963) suggested that the earliest effect of an intraperitoneal injection of sodium fluoride on rats was a disturbance in the ameloblasts which could be recognized a few hours after administration. The cytoplasm of these cells showed an abnormal character and distribution of globules, while the surface of the affected organic matrix was covered with globules which stained deeply with haematoxylin. The present findings (Fig. 1) are similar except that intracellular globules could not be demonstrated. It is noteworthy that small surface globules (Fig. 1), as described by Schour and Smith (1935) were present in animals sacrificed after 6 hours and larger irregular outgrowths (Fig. 2) in animals killed after 24 hours. No surface globules were found after 48 hours and this appears to indicate that the reaction of the ameloblasts is limited to approximately the first 24 hours after a single administration of fluoride. This confirms the view of Jenkins (1970) who stated that such basophilic globules form within a few hours after the injection of fluoride but disappear within 24 hours.

The linear defect (Figs. 3 and 4) observed in one rat sacrificed after 24 hours was similar to, but more pronounced than, that described by Schour and Smith (1935). These authors stated that such a layer consisted of both hypoplastic and hypocalcified matrix. The findings in the present experiment support those of Jenkins (1970) who stated that the enamel formed at the time of the injection may show interference with matrix formation and is poorly calcified.

Localised outgrowths of enamel matrix were seen in two animals from Group 1. These outgrowths are similar to the enamel "isles" and "peninsular" projections described by Schour and Smith in 1935.

The fact that the outgrowth was small (Fig. 5) in a rat sacrificed after 24 hours, while it was large and bridged a defect (Fig. 6) in the second specimen sacrificed after 48 hours, suggests repair of the defect in matrix formation by normal enamel. This implies that the fluoride injury is of a transient nature and that recovery of normal function of ameloblasts may be achieved within 48 hours after a single administration of fluoride.

In the present investigation, out of a total number of 15 changes observed, 6 were found after 6 hours, 6 after 24 hours and 3 after 48 hours.

Morphological changes in the ameloblasts ranged from some loss of cellular detail, localised shortening of the cells and retraction away from the underlying matrix (Fig. 7) to complete loss of cellular detail and retraction from the matrix over wide areas. A few intracellular vesicles can be seen in the partly retracted ameloblasts in Fig. 7. Irving (1943) described a similar retraction and distortion of the ameloblast layer, as is shown in Fig. 7, as an artefact.

The mechanism by which fluorides adversely affect amelogenesis are not yet fully understood. Schour and Smith (1935) were of the opinion that the amount of fluoride required to produce dental changes was very small when compared to the amount of calcium in the blood. It was unlikely that the combination of fluoride with calcium in the blood would be sufficient to disturb general calcium metabolism. These authors also stated the probability that the fluoride in the bloodstream unites with calcium to form calcium fluoride which may be taken up by the ameloblasts and injure them by acting as a foreign body. They also felt that the ameloblasts, which normally might have a high selectivity for large amounts of soluble calcium, could be disturbed by receiving calcium fluoride at the expense of calcium carbonate and calcium phosphate.

De Eds (1936) suggested that the toxic effects of fluoride on enzymes possibly interfered with metabolism and the enzymatic processes concerned in bone and tooth development. This author later observed (De Eds, 1941) that the action of fluoride on teeth was not due to calcium deprivation as it could not be counteracted by the administration of calcium but that the toxic action of fluoride may be associated with its inhibitory effect on the enzyme phosphatase.

Schour and Smith (1935) and Schour and Massler (1963) suggested that fluoride exerted a direct local action on the enamel-forming cells.

Jenkins (1970) summarised the uncertainty about the mechanism of the action of fluoride when he stated that doubt existed whether fluorosis resulted from direct action of fluoride on the ameloblasts or whether the cells behaved abnormally because they were receiving unusual concentrations of the raw materials of calcification from the tissue fluid.

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