The Effect of Sodium Fluoride on the Calcification of the Enamel and Dentin of the Rat Incisor

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According to Schour and Smith (1934), in ground sections viewed microscopically with transmitted light, enamel and dentin showed light (disturbed) and dark (recovery) incremental layers for each injection of sodium fluoride. Irving (1943) injected fluoride in different doses in an attempt to estimate its effect on the basis of the amount per kilogram of body weight of the animal. Both hypocalcification and hypercalcification of the dentin occurred. When the phosphorus or calcium in the diet was reduced, only hypercalcification of the dentin occurred following fluoride injection.

Yaeger and Eisenman (1963) have reported that the injection of fluoride induced both hypomineralized and hypermineralized increments in forming dentin. It was observed by Allan (1963) that the enamel of young dogs injected with smaller doses of fluoride showed hypercalcified lines only. Weber and Yaeger (1964), on the other hand, induced a pair of lines in the enamel when they gave parenteral doses of fluoride to rats. The pair of lines showed up in the historadiographs as hypomineralized and hypermineralized lines.

The purpose of this study was an investigation of the response of the enamel and dentin in the incisor teeth of rats injected with sodium fluoride.

Materials and Methods

Young adult rats averaging 150g were injected intraperitoneally with a 2.5% solution of sodium fluoride. Each rat was injected several times, i.e., at 48 hour intervals so that it received 25 mg of sodium fluoride per kg of body weight at each injection. The rats were sacrificed with chloroform inhalation two days after the last injection. Immediately after decapitation, the maxillae of each animal were separated through the palatal suture, and both maxillary incisors with the surrounding tissues were fixed in 10% formalin for 24 hours.

The right maxillary incisors were prepared for ground sections, while the left maxillary incisors were prepared for decalcified, paraaffin embedded sections. The right maxillary incisors were embedded in Bio-plastic* resin and cut transversely about 100 micron thickness with a Thin Sectioning Machine.** The sections were further reduced to 20—30 micron final thickness by surface grinding, using finger pressure with a slurry of oxide of alumina and a glass plate. The ground sections

were attached to a 10 mm diameter metal ring or washer having an open center of 2 mm in diameter. The specimen on the ring was put in a camera in contact with the film emulsion.

Contact historadiographs were made with a Philips GMR Unit with Eastman Spectroscopic Photographic Emulsion Type 649–0. All exposures were at 4.5 kv and 2 m amp, the exposure times varying from 60 seconds to 6 minutes. The film was developed in Kodak D-158 developer for 5 minutes at 20°C. The density of the historadiographs was measured with a densitometer (MP-3 Type Microphotometer, Rigaku Denki Co., Japan).

On the other hand, the left maxillary incisors with their surrounding tissues were demineralized in 5% nitric acid and embedded in paraffin. Transverse serial sections of 8 micron thickness were made and stained with hematoxylin and eosin. The ground sections, historadiographs and paraffin sections of dentin, immature and mature enamel, were observed under the microscope.

Observations

Enamel Each of the historadiographs showed two wide lines of hypercalcification and two of hypocalcification in the immature enamel (Fig. 1). The hypercalcified lines were closer to the dentino-enamel junction than their associated hypocalcified lines. The hypercalcified lines were not sharply demarcated.

When the two hypocalcified lines were compared, the external was narrow and the internal was wide. There was no detectable hypercalcified line in the enamel matrix between the external hypocalcified line and the enamel surface, but there was a hypermineralized zone on the enamel surface. This zone was believed to be normal or structure at least not concerned with the fluoride induced hypo- and hypercalcified lines.

Notwithstanding the fact that the historadiograph (Fig. 1) showed hyper- and hypocalcified lines, one could see only two enamel matrix deficient lines in the photomicrograph of the ground section (Fig. 2). It was obvious that the two wider lines in the decalcified section of the enamel coincided with the two wider hypocalcified lines seen in the historadiograph. The response observed in the two matrix deficient lines was represented by the diminution in diameter of the enamel rods. The enamel rod matrix along the rod margins was recognized to be first affected by the fluoride. Apparently either the original ameloblast cell wall, or the rod sheaths, or the cementing substance between the rods because of the juxtaposed alignment of rods

![Fig. 1. Historadiograph of the transverse section of immature enamel of the upper incisor. 20μ thick, 4.5 KV., 2 m. A., 4 minutes](image_url)
first became deficient. A higher concentration of the fluoride affected the entire ameloblast. This matrix defect was seen in the internal and external lines (Fig. 3).

Historadiographs of mature enamel showed two wide hypocalcified lines. These lines were similar to the hypocalcified lines described above in the immature enamel. Again, the external line was narrower than the internal line. Each hypocalcified line was also wide at the cementum-enamel junction, becoming narrower towards the dorsal enamel (Fig. 4).

A curve obtained by a densitometric technique (Fig. 5) denoted three peaks in the mineralization of the enamel. The highest mineral density was observed in the enamel near the dentino-enamel junction (Z). A high mineral density was also observed in the enamel between the two hypo-
calcified lines (Y) and the surface of the enamel (X).

**Dentin** Three hypercalcified lines were observed in the historadiograph of the dentin. The lines were from six to eight microns in width, and diminished in clearness from the pulp cavity to the dentin surface (Fig. 6). Because of this, the graphic registration made by the recording densitometer seemed to show that the mineralization of the dentin increased from the pulp side to the dentin surface (Fig. 7). The curve of the graph also showed that the outer hypercalcified line was highest in intensity. The inner line was low and the middle line was of intermediate intensity. Along the entire lengths of the calcified lines, the dentinal tubules were narrowed by the hypercalcification which occurred in the matrix between the tubules. It is believed that the hypercalcified lines had

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**Fig. 4.** Historadiograph of mature enamel. A and B: Hypocalcified lines. X, Y and Z: Normal enamel parts. Density was measured with densitometer along the line XYZ.

**Fig. 5.** Densitometric graph measured along the line XYZ in Figure 4.
**Fig. 6.** Historadiograph of dentin. $A, B,$ and $C$: Normal dentin parts. $X, Y$ and $Z$: Hypercalcified lines. Density was measured with densitometer along the line $XYZ$.

**Fig. 7.** Densitometric graph measured along the line $XYZ$ in Figure 6.
been developed by the hypermineralization of the intertubular matrix.

**Discussion**

The forming rat incisor enamel in this study showed hyper- and hypocalcified lines in the historadiographs in the same manner as the rat enamel observed by Weber and Yaeger (1964) who injected sodium fluoride doses of 50 mg/kg of body weight. A hypercalcified line appeared in the enamel near the dentinoenamel junction. However, the hypercalcified lines in the historadiographs could not be found in the ground and paraffin sections. Along the line of hypocalcification the enamel matrix was missing in the paraffin sections. This indicated a narrowing of the enamel rod matrix which seemed to separate each of the approximating enamel rods. If organic fibrils play an important role in enamel maturation (Nylen and Scott 1960), it is thought that the hypocalcified line was the result of diminution and separation of enamel rods during enamel matrix formation. In all probability, the ameloblastic cytoplasm was reduced by the toxic fluoride.

The historadiograph showed many fine short radiolucent lines running at right angles to the external hypocalcified line of the maturated enamel. This was probably a processing artifact due to the splitting of the enamel.

One could recognize two hypocalcified lines in the historadiograph of the mature enamel (Fig. 4). However, the width of both lines became narrower in the enamel near the cement-enamel junction (a). In the dorsal enamel (b) they were wider and the lines at c were again narrower. The dorsal enamel was thicker than that on the sides of the tooth. This may imply that the dorsal enamel formed faster than that on the lateral surfaces, therefore the deleterious effects would prove more severe on the dorsal than near the cementum.

It was also remarked that the outer line was narrower than the inner line in the paraffin section of the immature enamel (Fig. 3). This may imply that the enamel matrix as it approaches the surface matured less rapidly.

In the past the dentin response to a sodium fluoride injection was described as hyper- and hypocalcified lines. Yaeger (1963) recognized hyper- and hypocalcified lines in rat dentin when he injected sodium fluoride in doses of 50 mg/kg of body weight. In this study, when the sodium fluoride was injected into rats at levels of 25 mg/kg only hypercalcified lines were seen. Probably, this was due to the difference in the quantity of sodium fluoride injected.

According to Figure 6 the least calcification was shown in the dentin near the pulp cavity and the density increased toward the dentin surface. A densitometric graph (Fig. 7) also demonstrated the increased calcification in the specimen of Figure 6. In all three hypercalcified lines (X, Y, Z) and the three areas of the dentin (A, B, C), the calcification density in the outer line and outer part of the dentin was higher than in the inner line and area. It may be assumed that the calcification or maturation of the dentin increased from the pulp chamber towards the superficial dentin after each hypercalcified line of dentin was formed. This calcification included not only the normal dentin but also the dentin in the hypercalcified lines.

In the historadiographs one could not see clearly whether or not the dentinal tubule walls showed any hypermineralization response. Shimoyma (1967) reported that peritubular hypercalcified zones were not found in rodent incisor dentin histo-
radiographically. ISOKAWA and SERA (1967) also studied the rodent dentin with a
historadiographic technique and said that the evidence of the peritubular calcification
failed to show in the incisor and molar dentins of 26 rodents. It was concluded
from the present study that the hypercalcified lines came into being by the inter-
tubular calcification of the dentin matrix.

Summary

Responses of rat enamel and dentin were investigated by means of sodium fluo-
ride injections. The rats used weighed about 150 gr. The rats were given several
injections of 25 mg/kg sodium fluoride intraperitoneally. After the rats were sacri-
ficed, the right maxillary incisors were prepared for paraffin sections which were
stained with hematoxylin-eosin. Ground sections and their historadiographs were
made from the left maxillary incisors. The film density of some of the historadio-
graphs were also measured with a densitometer.

The following conclusions were obtained from this study.

1. Hyper- and hypocalcified lines were shown in the historadiographs of imma-
ture enamel. However, only hypoplastic and hypocalcified lines were shown in the
respective paraffin and ground sections.

2. The paraffin sections showed that in the hypoplastic lines the approximating
enamel rods appeared to be separated and thinner. It seemed as though the enamel
matrix formation had been severely disturbed in the hypoplastic lines. In all prob-
ability the ameloblasts were injured by the fluoride and the keratin matrix was
deficient.

3. In some parts of the hypocalcified lines in the immature enamel, it appeared
as though the outer line was narrower in width than the inner line.

4. On the other hand, the hypocalcified line was visible in the historadiograph
in the mature enamel but the hypercalcified line was not.

5. No hypocalcified lines of matrix hypoplasia appeared in the dentin; however,
hypercalcified lines were observed. This showed that calcification of the dentin
matrix unlike the enamel matrix was not disturbed by the fluoride.

6. X-ray absorption of the hypercalcified lines in the dentin near the pulp cavity
was less than that near the enamel surface. It was, therefore, implied that the lines,
were like the dentin under the influence of a process of slowly increasing calcification
or maturation. This would indicate that the gradient of flow of the dentin tubule
fluids which contained the inorganic maturation salts was from the pulp towards the
dentinal surface.

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Frization na triumu no nezumi no kauu shite kari no jikusu no kankei no hasu kosyo (nurasu dego)
Frization na triumu o 25 mg/kg no kake de nezumi no fukuucu ni shoshite, kikai no shite kuru seido no
yokusa tamako to tori kira tamako, oto historadiography ni yotte kanseki, katu historadio-
graphy のあるものは densitometer によって X 線の吸収度を測定した。その結果ネズミの切歯に起こる変化は次のように要約することが出来る。

1. 未熟エナメルでは 高石灰化線と低石灰化線の 2 つが生じることが historadiography によって観察された。しかし 脱灰切片標本と研磨標本では 低石灰化線と一致するものが認められた。

2. 未熟エナメルの低石灰化線はエナメル小柱の離間と直径の減少による線状の細隙であることが 脱灰切片標本で観察された。これは 弗化ナトリウムが基質の形成に障害を与ええたことによって起こったものと考えられる。

3. 成熟エナメルでは 低石灰化線は認められたが、高石灰化線は認めることができなかった。

4. 象牙質では高石灰化線だけが認められた。これは 弗化ナトリウムが象牙質の石灰化に障害をおよぼさなかった ためであろうと考えられる。

5. 象牙質に認められた高石灰化線のうち、歯軸に近いものはほど密度が低く、それから離れるほど高くなっていた。

References


