EXPERIMENTAL PRODUCTION OF MOTTLED ENAMEL

By
MARGARET CAMMACK SMITH AND EDITH M. LANTZ

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NO MOTTLLING

MILD MOTTLLING

MODERATELY SEVERE MOTTLLING

SEVERE MOTTLLING
EXPERIMENTAL PRODUCTION OF MOTTLED ENAMEL

By
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INTRODUCTION

Mottled enamel in humans is a serious defect of the enamel of the permanent teeth which may be described as follows. It is chiefly characterized by the presence of dull, chalky-white areas distributed more or less irregularly over the surface of the tooth. Enamel which is mottled does not have the translucent, glassy luster of the enamel of normal teeth. Sometimes the whole tooth is dull white and opaque in appearance. The enamel is structurally weak and in severe cases is pitted, corroded, and tends to chip off. According to the histological examination of Black,(1) the inter-cementing material which is normally present between the enamel rods is lacking, at least in the outer fourth of the enamel. In severe cases even the enamel rods themselves appear to be imperfectly calcified.

In all but the mild cases, as pictured in Plate I, there is a subsequent infiltration of stain into the mottled areas which varies in color from dark brown (almost black) through orange to yellow. This discoloration is a secondary phenomenon and in general tends to be more pronounced on the upper central incisors and to follow the lip line whereas the lower teeth and back teeth, though mottled, are usually free from discoloration.

Plate I shows types of mottled enamel found among the native-born inhabitants of certain sections of Arizona. Mottled enamel of the very mild and mild types is distinguished by dull, opaque, paper-white areas only. The teeth are normal in form and shape but not normal in color. In the more severe type, a greater proportion of the surface of the tooth is affected and there is an accompanying discoloration, especially noticeable on the teeth most exposed. Mottled enamel which is severe in type is further characterized by pitting and corrosion.
Plate II.—Mottled teeth of Pima Indians showing the opaque white and corroded areas typical of this dental defect.
Plate III.—Normal rat incisors. Showing the typical translucent appearance of the incisors of rats raised on Sherman's Diet B and distilled water. The anterior surfaces are normally tinged orange yellow.
Plate II shows some extracted mottled teeth of the Pima Indians. Though not stained, they show the abnormal whiteness and loss of luster characteristic of mottled enamel and areas in which the several layers of the enamel are corroded may be readily seen.

In an investigation into the cause of mottled enamel, experimental production of the defect in laboratory animals was attempted. A preliminary report (2) of the findings has been published in Technical Bulletin No. 32 of this Station. Since that time, mottled enamel has been produced in dogs and guinea pigs as well as in rats. It is the purpose of this brief report to set forth the methods used in the experimental production of mottled enamel in the different types of laboratory animals.

EXPERIMENTAL PRODUCTION OF MOTTLED ENAMEL IN ALBINO RATS

Mottled enamel in rats’ teeth has been produced in this laboratory by several methods.

In the first series of experiments of the earlier work, an effort was made to establish the suspected relationship between mottled enamel of human teeth and the drinking water of the afflicted persons. Accordingly, water was given to the rats to drink, which was obtained from St. David, Arizona, a community where all the native inhabitants have mottled enamel of the moderately severe types. Other rats received rations in which the residue resulting from the evaporation of this same water was incorporated.

In the second series of experiments, mottled enamel has been produced by feeding sodium fluoride at different levels, namely, 0.025 percent, 0.05 and 0.1 percent of the basal ration. This is equivalent to 0.0116, 0.0226, and 0.0452 percent of fluorine respectively. The sodium fluoride was incorporated directly into the basal ration, (Sherman’s Diet B) which was composed of two-thirds ground whole wheat, one-third powdered whole milk and sodium chloride, equal to 2 percent of the weight of the wheat.

In the third series of experiments, mottled enamel was produced by the subcutaneous or intramuscular injection of fluorides.

In all three series of experiments the rats were taken from the stock colony bred on Sherman’s Diet B and placed upon the experimental regime at the time of weaning, which is at 4 weeks of age in this laboratory. They were kept in individual round metal cages with raised bottoms to prevent access to the excreta and were given the adequate
basal diet (Sherman Diet B) and distilled water *ad libitum*, except in Series 2 in which St. David drinking water was given.

In all cases the first indication of dental abnormality noted was the lack of pigment followed by a loss of luster and a resulting dull, chalky-white appearance. The incisors of the control rats were normally translucent and pigmented. The anterior surfaces of the lower incisors are tinged yellow and the upper incisors are usually even darker and more orange in color. In the severe cases of mottled enamel in rats, as in human teeth, the enamel became corroded and pitted and whole sections of enamel tended to chip off. The time of appearance and severity of these symptoms in rats varied with the amount of fluorides given. Upon discontinuing the feeding or injection of fluorides, the teeth became normal in appearance as they continued to grow. They regained both their luster and pigmentation, indicating that there had been no permanent injury to the enamel germ cells.
When the experimental rats were given water for drinking purposes, which had been obtained from St David, Arizona, a community in which all the native inhabitants had mottled enamel, no effect upon the rats' teeth was noted. However, when this same water was evaporated to one-tenth of its original volume, its dissolved material thereby concentrated, and then used as drinking water, the rats' teeth assumed the appearance seen in Plate IV. The incisors lost their luster and became opaque white in appearance but in most cases were otherwise normal in appearance and form. No record of water intake was kept. Control animals which were fed the same basal diet but given, in some cases, distilled water to drink and, in other cases, drinking water from a non-endemic community, did not show this change in enamel.
Plate V shows the teeth of a representative animal fed Sherman's Diet B in which the residue from the drinking water of St David, Arizona, had been incorporated. Distilled water was given to the animals to drink. In this case the incisors not only lost their luster and pigment and became chalky white, but the enamel corroded and chipped off in places. The relationship of water to the defect was thus established. With the subsequent findings of the toxic factor to be fluorides, the residue was incorporated in the diet at such a level as would give theoretically 0.0226 percent fluorine, based upon analysis of the water for fluoride content. As before, control animals received Sherman’s Diet B but without the addition of the water residue.
When sodium fluoride was incorporated in Sherman's Diet B at such a level as to provide 0.0113 percent fluorine, (0.0250 percent sodium fluoride) and distilled water was given the animals to drink, a dental defect such as appears in Plate VI resulted. Two weeks after the animals had been placed on this diet, a difference between their teeth and the teeth of the control animals was noted. Whereas the incisors of the control animals deepen in color with age, there was a corresponding loss in pigment in the teeth of the animals on the experimental ration, accompanied by some loss in luster and translucency. The enamel, however, never became corroded or as opaque as resulted when the higher levels of sodium fluoride were fed. Plate VII shows a higher magnification of the incisors of another animal fed sodium fluoride at the same level. Here it is possible to see rather indistinct rings on the enamel which probably correspond to the intermittent eating habits of the rat. Most of the food is consumed at night in spite of the fact that the animals are fed ad libitum. To the naked eye, the incisors appeared to have lost their pigment and their translucency throughout the whole length of the tooth. It was interesting to find that with the aid of a hand lens, it was possible to see intermittent variations in severity of the defect. At the higher level of fluoride feeding this was not evident.
Animals fed Sherman's Diet B in which sodium fluoride was incorporated at the 0.05-percent level giving a fluorine content of 0.0226 percent showed the dental abnormality shown in Plate VII. After about 2 weeks on this ration the first difference in the incisors of the experimental and control animals was noted. At this time, the teeth of the sodium fluoride-fed rats appeared to have lost some of their luster and to contain less pigment than did their controls on Sherman's Diet B alone. The teeth became duller and more opaque and in most cases began to show pitted areas with sections from which the enamel chipped off between the third and fourth weeks. In some cases this could best be noted with a hand lens but was usually quite obvious to the naked eye.

The teeth were structurally weak, as evidenced by the fact that the incisors tended to wear down excessively. (Note the bluntness of the lower incisors in Plate IV.) As the animals grew older, in some of the cases an overgrowth and excessive curvature of the upper incisors as first reported by McCollum have been noted. (See Plate IX.) In some cases the lower incisors became unduly elongated as shown in Plate VIII. The opaque white appearance and pitting were, however, the most characteristic and consistently observed dental abnormalities.
When 0.1 percent of sodium fluoride (or 0.0452 percent fluorine) was incorporated, the characteristics of the dental defect produced were the same, although a more severe abnormality was effected in a shorter time than when the lower levels of sodium fluoride were fed. The incisors showed the first indication of abnormality between the first and second weeks on this ration and became progressively more severely dull and chalky white with marked pitting of the enamel occurring about the third week. At this level of fluoride feeding there was marked interference with the food intake and growth rate of the animal.
Dental abnormalities of the same kind as those obtained by the feeding of fluorides were also produced by the subcutaneous or intramuscular injection of sodium fluoride into albino rats. With daily injection of 1 cc. of a 0.1-percent solution of sodium fluoride, the incisors gradually lost their luster, becoming characteristically dull and opaque white. When stronger solutions were used a local irritation was produced which resulted in severe sores. This was avoided to some extent by cleansing the place of injection. A mottled-enamel effect which is perhaps more typical of human mottled enamel was produced by the intermittent injection of fluorides. By this means, incisors with both normal translucent areas and abnormal white and pitted areas were obtained. By varying the length of the interval between injections and the concentration of sodium fluoride solution injected, enamel effects differing in outward appearance have been obtained. Plate X shows the incisors of an adult rat which has been given eight injections of a 2% -percent sodium fluoride solution. The injection of 0.3 cc. of the solution was made every other day. Eight rings of abnormally dull white and corroded areas alternating with normal pigmented areas may be noted. It was interesting to note that no outward change in the enamel of the already erupted portion of the incisors was produced by the injections. As in the case of human teeth, normally formed enamel did not become mottled upon fluoride intake. As the incisors grew, however, a loss in pigment in the newly erupted portion was noted and about 2 weeks after the cessation of the injection or, in this case, 27 days after the first injection, the first ring was apparent. Evidently the most sensitive part of the enamel organ in the basal portion of the tooth was affected by the fluoride injection. The rat incisor renews itself in about 30 days. Histological examination of the effect of fluoride injections upon enamel germ cells and the enamel in all stages of development is being made by Dr. Isaac Shour.

Plate XI shows the effect produced by injections of 0.6 cc. of the same solution into an adult rat every fourth day. Evidently injecting twice as much fluoride has caused an interference with enamel development over a greater period of time in enamel growth, the defective bands in the enamel being much wider than the normally pigmented areas. As before, the alternate rings of normal and abnormal enamel were not apparent until the fourth week after the first injection.

Two other cases of the results produced by intermittent fluoride injections are shown in Plates XII and XIII. The animals in the cases pictured here were younger and the effect produced was less regular. In each case, normal areas on the enamel may be seen as well as the white and corroded areas of defective enamel. Plate XII shows severely corroded areas due to 0.6 cc. injections of 2% -percent solution.

The animal shown in Plate XIII was given a 0.3-cc. injection on 2 successive days with a 2-day interval between injections. A considerable irregularity in enamel formation is shown which is characteristic of mottled human teeth.
PLATE X
EXPERIMENTAL PRODUCTION OF MOTTLED ENAMEL
IN DOGS

Following somewhat the same procedure as has been used with rats, the experimental production of mottled enamel in dogs has been accomplished. Rats have only one set of molars and incisors which grow continuously and wear down by attrition, but dogs are like humans in that they have both a temporary and a permanent set of teeth.

Litter-mate puppies, taken shortly after weaning (about 5 weeks of age) were placed upon purified rations containing sodium fluoride at the same levels as were fed to rats, namely 0.025 and 0.05-percent sodium fluoride and continued on the ration until the eruption of the permanent teeth. The basal diet was composed of casein 192 parts, sucrose 207, Osborne and Mendel salt mixture 10, agar 4, yeast 12, and butterfat 60. Forty-seven and five-tenths grams of this ration per kilo body weight were fed daily.

No change in the temporary teeth which were partially erupted at the beginning of the experiment could be noted as a result of sodium fluoride feeding. The chief characteristic of the enamel defect of the permanent teeth as produced by fluoride feeding in dogs was the corroded appearance and chipping off of layers of enamel. As the teeth of dogs do not have the yellow-orange pigment normal to rats and are naturally more opaque and less glassy in appearance than human teeth, corroding of the enamel and not abnormal whiteness is the most noticeable characteristic of the dental defect produced.
The upper photograph in Plate XIV shows the characteristic front view and the lower photograph the side view of the permanent teeth of Dog No. 11 fed an adequate basal ration in which sodium fluoride had been incorporated at the level of 0.025 percent. The dog was placed upon the ration at the age of 5 weeks and fed 475 grams of the basal diet including 0.118 gram of sodium fluoride per kilo of body weight daily during the period of body growth and calcification of the second set of teeth, which erupted between the fourth and sixth months. An initial body weight of 22 kg with a body weight of 72 kg at the age of 20 weeks with corresponding increase in daily food intake from 104 to 347 grams resulted in an average total daily intake of 0.057 grams of sodium fluoride in this period for this dog.

The inferior condition of the enamel of the permanent teeth can be readily seen in Plate XIV. The enamel is badly chipped, several layers of enamel being exposed on all of the teeth. The corroded areas became somewhat yellow as the dog grew older but at the time the photograph was taken the yellow to black stain characteristic of many cases of human mottled enamel had not developed.

Note: This dog was given distilled water to drink from a crockery jar. However, the teeth of another fluoride-fed dog with mottled enamel, given water in a rusty tin pan, became decidedly stained, the orange-red color deepening as time went on. On the other hand, the normal teeth of its litter mate which had not received any fluorine, did not become stained under the same conditions.
Plate XV shows the front and side view of the permanent teeth of a dog (Dog No. 6) fed a low calcium ration, in which 0.025 percent of sodium fluoride had been incorporated. The basal diet was the same as for the control dog except that a low calcium salt mixture was substituted for the adequate salt mixture of Osborne and Mendel. With an initial body weight for this dog of 1.5 kg, which increased to 5.2 kg at the age of 20 weeks, there was an average daily food intake of 172 g of the basal ration including 0.043 gram of sodium fluoride. The enamel of the teeth of this dog was obviously defective for it was severely chipped and corroded. In the same period of time, the control dog, No. 7, on the low Ca ration without the addition of fluorides, gained 3.8 kilos and had an average food intake of 178 grams. Unfortunately this animal was stolen just before it was to be photographed. However, no similar enamel imperfection had been noted in the case of this dog which had received the same ration without the addition of sodium fluoride.
The addition of viosterol to the low calcium ration in which sodium fluoride had been included at the level of 0.025 percent did not prevent the occurrence of the characteristic enamel defect typical of mottled enamel produced by the sodium fluoride. In Plate XVI may be seen the front and side views of Dog No. 12 which was given one-fifth drop (increasing with age) of 250 D. viosterol daily in addition to the low calcium ration containing 0.025 percent of sodium fluoride. The enamel imperfection was of the same degree of severity or even more severe than that resulting from the action of sodium fluoride without the addition of viosterol. The teeth of this dog showed marked malocclusion as well as the corroded enamel.

The body weight of 1.6 kg. at the beginning of the experimental feeding (5 weeks of age) increased to 4.5 kg at the age of 20 weeks and the food consumed increased from 75 to 210 grams with an average daily intake of sodium fluoride of 0.0387 grams. There were no outward indications of rickets or abnormal bone structure in dogs Nos. 6, 12, or 7, all of which were on a ration which was sub optimum in calcium content. Growth of all of these dogs was somewhat less than that of the controls receiving the adequate salt mixture either with or without the addition of sodium fluoride.
PLATE XVI
EXPERIMENTAL PRODUCTION OF MOTTLED ENAMEL IN GUINEA PIGS

More difficulty has been met with in the experimental production of mottled enamel in guinea pigs because of their greater aversion to certain foods and the difficulty in getting them to eat foods of unaccustomed flavor and consistency. That the teeth of guinea pigs, however, respond in the same way as the teeth of rats and dogs to the feeding of sodium fluoride has been proved by the same methods as were used with the other experimental animals, namely, by the feeding or subcutaneous injection of fluorides. Five-hundredths percent sodium fluoride has been incorporated in the dry basal ration composed of 59-percent mixture of equal parts of rolled oats and bran, 39 percent whole milk powder, and 1 percent salt. In addition, one tablespoon of tomato juice was given daily as an antiscorbutic. In some cases, the sodium fluoride has been dissolved in the tomato juice and in other cases it has been necessary to introduce the solution directly into the throat of the pig with a medicine dropper.

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BIBLIOGRAPHY

PLATES XVII AND XVIII

Plate XVII shows the effect produced in the incisors of the guinea pigs by the feeding of sodium fluoride. In this case, 1 cc. of a 2-percent solution of sodium fluoride was mixed with the tomato juice which was given daily in addition to the basal diet of whole milk, whole rolled oats, bran, and salt. The guinea pig was placed on the diet when it weighed 250 grams (at about 6 weeks of age) and in 2 weeks the incisors showed the first indication of abnormality. The incisors of normal guinea pigs are less pigmented than the normal incisors of albino rats, so that there is a less striking difference in color between the incisors of the fluoride-fed pig and the controls than is evident in the case of rats. The corroded appearance of the incisors of the fluoride-fed pigs, however, is typical of the mottled enamel resulting from the action of fluorides in both rat and human teeth.

The subcutaneous injection 0.6 cc of a 2.5-percent solution of sodium fluoride into guinea pig No. 16 resulted in the mottled teeth shown in Plate XVIII.