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Ian C. Bennett and David B. Law

J DENT RES 1965 44: 788
DOI: 10.1177/00220345650440042701

The online version of this article can be found at:
http://jdr.sagepub.com/content/44/4/788
Incorporation of Tetracycline in Developing Dog Enamel and Dentin

IAN C. BENNETT* and DAVID B. LAW
Department of Pedodontics, University of Washington School of Dentistry, Seattle, Washington

Over the last 5 years reports have appeared in the dental literature on the appearance of yellow, brown, or gray discoloration of teeth. The patients had been exposed to extended treatment with antibiotics of the tetracycline group while these particular teeth were calcifying.

There is lack of agreement in the literature between the work of Owen [1] and of Harcourt, Johnson, and Storey [2] concerning the localization of the drug in the enamel and dentin. Owen [1] has reported the results of a study on a normal 8-week-old dog dosed with chlorotetracycline for 1 month daily except on Sundays. When the teeth erupted, they appeared pale yellow in daylight, and there was a yellow fluorescence under ultraviolet light. Histologic examination of ground sections with ultraviolet light showed yellow fluorescence of both enamel and dentin.

Harcourt et al. [2] have reported on their work with extracted teeth from five young patients known to have been extensively treated with tetracycline. They have observed (in ground sections viewed under ultraviolet light) numbers of bright golden-yellow bands in the dentin but no obvious fluorescence in the enamel. In their discussion, Harcourt, Johnson, and Storey have referred to Owen, suggesting that the fluorescence observed by him in enamel could be due to light scattering from the faces of the enamel rods and not to fluorescence of interprismatic substance.

In view of these conflicting reports, it was thought that if the enamel and dentin of dog teeth exposed to tetracyclines during their formation and calcification could be analyzed separately by an appropriate method, then the reflection effect would be eliminated and the presence or absence of the drug in the enamel would be confirmed.

**Materials and Method**

TREATMENT OF EXPERIMENTAL ANIMALS WITH TETRACYCLINE.—A litter of four mongrel dogs was used to obtain material for study. The puppies were weaned at 57 days of age and separated from their mother. The litter was housed in one cage and fed the same diet, which consisted of meal and cooked beef. When they were 60 days old, the dogs were weighed, and three of them selected to receive a daily dose of tetracycline. Each of the dogs selected to receive the drug was given 20 mg. of tetracycline per kilogram body weight per day. Each dog was given the calculated dose for 3 days; then the dogs were reweighed and the dosage was recalculated and given for the next 3 days. The three treated dogs were given the calculated dose every day orally in capsule form. This procedure was repeated at 3-day intervals until the animals were sacrificed. During the course of the tetracycline administration all the dogs, experimental and control, were apparently in excellent health. At 22 weeks of age the four dogs were sacrificed by giving them an intraperitoneal injection of 50 mg. of pentobarbital per kilogram body weight. After sacrifice, the mandibles and maxillae were removed and placed separately in 10 per cent formalin as a preservative.

PREPARATION OF POWDERED TOOTH MATERIAL.—The permanent teeth, both erupted and unerupted, were dissected out and scraped clean of any fragments of bone and

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*Present address: Department of Pedodontics, College of Dentistry, University of Kentucky, Lexington, Ky.

†Tetracycline used in this study was supplied by Lederle Laboratories, Pearl River, N.Y.
periodontal tissue. At this time a gross examination of the teeth was made. The teeth were then immersed in liquid nitrogen (−195° C.) for about 20 minutes and then dropped into hot water. The sudden change of temperature caused the teeth to crack apart, and the dental pulps were scraped out. The freezing and warming procedure was repeated three times until the teeth were in fairly small fragments. The pieces were then washed in chloroform to remove residual fat and dried.

The tooth fragments were then powdered in a hammer-mill which reduced the enamel and dentin to a fine dust. The powder was passed through a 60-mesh brass screen, and all particles of steel (from the hammer-mill) were removed with a magnet.

Separation of enamel from dentin.—The enamel was separated from the dentin by a centrifugal flotation method, similar to the method of Manly and Hodge. A mixture of 10 parts bromoform and 1 part acetone, density 2.7 gm/ml, was used.

After separation the process was repeated with the enamel powder using pure bromoform, density 2.8 gm/ml, as the separating liquid to insure maximum purity of the enamel. Manly and Hodge claimed this method gave enamel of 99.4 per cent purity. At this time the powder samples were examined with ultraviolet illumination.

In order to verify that the process of separation of the enamel from the powdered-tooth substance did not remove the tetracycline, a preliminary spectrographic examination was made. A sample of treated powdered tooth was compared with a sample of treated powdered tooth which had been through the separation process and then recombined. The method of preparing the solution for this check examination was identical to that described in the next section.

Spectrophotometric examination of enamel and dentin.—The method developed by Hilton for analysis of tetracycline in bone was used to determine whether the drug was present in enamel and dentin. Samples of treated enamel, untreated enamel, treated dentin, and untreated dentin were processed. The ultraviolet absorption spectra of the solutions obtained were then measured in a photospectrometer, and the spectra from the treated and untreated samples of enamel and dentin were compared.

Results

The spectrophotometric examination consisted of comparison of four pairs of pigmented extracts dissolved in N hydrochloric acid, as follows:

Pure tetracycline and tetracycline which had been subjected to the process used to extract pigment from the tooth powder.—Readings from the spectrophotometer are expressed graphically in Figure 1. The pure tetracycline showed absorption peaks at 2,300, 2,750, and 3,500 A. In the processed tetracycline, the absorption peak at 3,500 A was not seen. It was considered that this was due to the processing causing some change in the tetracycline and eliminating the absorption peak.

Treated, unseparated powdered-tooth material and treated, separated, and recombined powdered-tooth material.—The two curves (Fig. 2) are closely parallel and show absorption peaks at 2,750 A. The high optical density at the low wavelength end of the spectra effectively masks any absorption peak at 2,300 A. Since the two results were parallel but not identical, it was considered that the separation process did not remove the tetracycline but that there had been some loss of pigment in the process.

Untreated dentin and treated dentin.—The untreated dentin showed a regular fall of optical density from the shorter wavelengths to the longer (see Fig. 3). The treated sample showed a marked peak at 2,750 A. This supported the other observations which indicated that tetracycline was present.

Untreated enamel and treated enamel.—Readings from the spectrophotometer are expressed graphically in Figure 4. The untreated enamel showed optical densities very similar to those of the untreated dentin. The treated enamel showed an absorption peak at 2,750 A, but this was only about 25 per cent of the height of the absorption peak of the treated dentin. This was interpreted as showing that the treated enamel did contain tetracycline but in reduced amount compared with treated dentin.

* Model DU, Beckman, South Pasadena, Calif.
Discussion

GROSS EXAMINATION OF THE TEETH.— The permanent teeth of the dogs that had been given the tetracycline were a yellow color. This was anticipated from the reports of Owen. The primary teeth were not discolored, and this also was expected from knowledge of the chronology of the development of the dogs dentition and the time of administration of the drugs. Enamel hypoplasia was also seen in the treated animals, which correlated with the observations of Witkop and Wolf on human teeth.

EXAMINATION OF WHOLE AND POWDERED TEETH BY ULTRAVIOLET ILLUMINATION.— The treated permanent teeth, both whole and powdered, fluoresced under ultraviolet light. This was similar to the results of Owen and others. When separated, the dentin powder fluoresced bright yellow. The enamel, although it fluoresced, was not a convincing yellow color and therefore this test alone was not considered indisputable evidence for the presence of tetracycline.

SPECTROPHOTOMETRIC EXAMINATION OF PIGMENTED EXTRACTS OF POWDERED TEETH. —The method used was based on the work of Hilton and Wallman and Hilton. The analysis of the powdered dentin showed that tetracycline was present. This was expected since there is ample evidence that tetracycline is incorporated in dentin. In the enamel the analysis showed that tetracycline was present. These findings have been sup-

Fig. 1.—Ultraviolet absorption spectra of pigment from pure tetracycline (continuous line) and tetracycline which had been subjected to the process used to extract pigment from the powdered tooth material (broken line).
ported by the histological studies of Bevelander, Rolle and Cohlan, by Owen, and by Wallman and Hilton. The investigation was set up to avoid the problems associated with histological studies, therefore no reluctance was felt in disagreeing with the work of Harcourt et al., or of Brottman and Kutscher, who have denied the presence of tetracycline in enamel.

It might be possible to assume that, since the absorption peak in the enamel spectrum was about one-quarter of the height of the peak in the dentin, there was about four times as much tetracycline in a given weight of dentin than in the same amount of enamel. This might help to elucidate the problems of the mechanism of tetracyclines’ affinity for calcifying tissue.

The mechanism of tetracycline incorporation in calcifying tissue is not clearly understood. Albert has reported on the avidity of tetracyclines for metallic cations. Finerman and Milch have thought that a chelate of calcium and tetracycline may form and that this would explain the presence of the drug in calcified tissue. However, in this case it was expected that more tetracycline would be found in a highly calcified tissue. The smaller amount of tetracycline in enamel as found in this study does not support this theory. Bevelander has shown that when excess calcium ions were added to sea water in which echinoderm embryos were growing while exposed to tetracycline there was no protection from inhibition of calcification. This suggested that the formation of che-

![Fig. 2.—Ultraviolet absorption spectra of pigment extracted from unseparated powdered tooth material (continuous line) and separated and recombined powdered tooth material (broken line).](image-url)
lates was not solely responsible for the inhibition. Milch, Rall, and Tobie\textsuperscript{13} have suggested a complex of ground substance, collagen, and mineral as the possible mechanism for tetracycline incorporation in bone. This might explain the relative proportion of tetracycline in enamel and dentin since the lesser amount of organic material in enamel would lead to reduced tetracycline incorporation.

Urist and Ibsen\textsuperscript{14} have suggested that the tetracycline is adsorbed onto the surface of apatite crystals. The larger crystal size in enamel and consequent reduced surface area could explain the relative difference between the amount of tetracycline incorporated in enamel and dentin.

Another possible explanation for the relative variation in the amount of tetracycline in enamel and dentin is the origin of enamel from ectoderm and dentin from mesoderm. This may lead to a different mechanism of tetracycline uptake and consequent disparity in the amounts of tetracycline incorporated. Much further work is needed to elucidate the answers to these questions.

**Summary**

The purpose of the study was to confirm or deny the presence of tetracycline in the enamel of dogs' teeth which, during the period of amelogenesis, had been exposed to tetracycline. The enamel was obtained from dogs that had been dosed daily with tetracycline from weaning (at 2 months) to sacrifice at 5 months. The dogs' teeth were powdered and the enamel separated from the dentin by flotation. A spectrophotomet-

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**FIG. 3.**—Ultraviolet absorption spectra of the pigment extracted from treated powdered dentin (continuous line) and untreated powdered dentin (broken line).
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A microscopic analysis was made of the enamel, with untreated enamel as a control, and the presence of tetracycline demonstrated. The presence of tetracycline in the dentin was also confirmed. Enamel hypoplasia was observed in the teeth of the animals who received the tetracycline.

References


