An Investigation of Yellow Fluorescent Lines Observed in Human Dentinal Hard Tissues

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Summary: Orally administered tetracycline antibiotics have been known to deposit in mineralized tissues. Tetracycline is readily identified as yellow bands under light microscopy and as fluorescent lines in bone or dentin under the ultraviolet light of fluorescent microscopy. In addition, inhibition by tetracycline of hard tissues formation and calcification has also been demonstrated.

In observations of ground sections of human deciduous teeth in the present study, numerous yellow lines that were deposited along the incremental lines of dentin were detected. All teeth examined were free of remarkable dental caries, dental structural defects, or macroscopic discoloration.

The frequency of appearance of yellow fluorescent lines in the deciduous teeth in the present study was only 27% under light microscopic observation, but was as high as 52% in the deciduous incisors, 62% in the deciduous canines, and 66% in the deciduous molars (an average of 61%), when examined under fluorescent microscopy, indicating a significantly higher frequency of tetracycline deposition than originally evident under light microscopy.

In the present study, in order to clarify nature of the yellow line within the dentin, absorption spectra were measured using a microspectrophotometer in order to measure the characteristics of the wave-length of absorbency. The wave-length characteristics of absorbency of the material deposited in the dentin were found to be identical with those of the tetracycline antibiotics (Vibramycin syrup). The former demonstrated a peak at 260–280 nm, whereas the latter peaked at 370–390 nm. Accordingly, the evidence strongly suggests that the yellow fluorescent lines identified in human dentin in the present study were due to tetracycline.

Moreover, the relationship between the fluorescent and incremental lines largely reflects the observation that tetracycline deposition appears most distinctly in the dentin. When tetracycline is incorporated into the developing dentin, a yellow fluorescent deposition line appears not only in the dentin, but also in the dentin-predentin junction. From an embryological viewpoint, the fluorescent line coincides with the fluorescent line in the enamel as well as the fluorescent line within the cementum at the junction.

These findings illustrate the synchronicity between periodic calcification and tetracycline deposition.

These findings were based primarily upon studies of deciduous teeth, similar finding have been obtained for permanent teeth (upper first premolar and lower third molars).
Orally administered tetracycline antibiotics have been known to deposit in mineralized tissues. Tetracycline is readily identified as yellow bands under light microscopy and as fluorescent lines in bone or dentin under the ultraviolet light of fluorescent microscopy. In enamel, on the other hand, tetracycline has been reported to appear as a diffuse fluorescent area, an appearance different from the fluorescent lines observed in the dentin\textsuperscript{1, 2}). Few reports are available on the behavior of tetracycline in cementum.

In observations of ground sections of human teeth in the present study, numerous yellow lines probably representing tetracycline deposits were detected. Although these deposits were indistinct when viewed under light microscopy, they were distinctive in appearance under fluorescent microscopy using a microspectrophotometer. Accordingly, attempts were made to analyze yellow fluorescent lines that were deposited along the incremental lines of dentin qualitatively in order to clarify the properties of the deposit. Fluorescent lines in the dentin, enamel, and cementum were also reviewed histologically.

Materials and Methods

The investigation was conducted on 200 human deciduous and permanent teeth extracted from children between the ages of 7 and 12 years over a period of 2 years between 1980 and 1982. The study sample consisted of deciduous incisors and molars from both the upper and lower jaws, in addition to one upper first premolar and two lower third molars. All teeth examined were free of remarkable dental caries, dental structural defects, or macroscopic discoloration.

Freshly extracted teeth were fixed in 10% neutral formalin for approximately 1 wk, and sectioned into slices of 200 μm thickness. Following refixation, ground sections of 50 - 80 μm thickness were prepared for histological examination. Wave length characteristics based on distribution of absorbance were evaluated and compared graphically for both the yellow fluorescent lines in dentin and tetracycline antibiotics (Vibramycin syrup) using a microspectrophotometer. The fluorescence microscope, manufactured by Carl Zeiss, and the microspectrophotometer manufactured by Olympus, MMSp-Tu, were used.

The yellow fluorescent line was studied using both electron and scanning electron microscopy.

Observation

Microspectrophotometry revealed absorbance distribution of the yellow fluorescent line in dentin at 280 and 390 nm, and a corresponding distribution for vibramycin syrup at 260 and 370 nm, with 2 peaks. Thus, both the yellow fluorescent lines in dentin and the vibramycin syrup showed identical absorption spectra (Figs. 1, 2).

The frequency of appearance of yellow fluorescent lines was 27% under light microscopy and 61% under fluorescent microscopy (Table 1).

Tetracycline staining was most distinctly observed in the dentin. In the dental crown, this line started at the dentinoenamel junction, ran approximately parallel to the outline of the dental crown, and finally terminated at the dentinoenamel junction of the contralateral side, with the general appearance of a line corresponding to the incremental line of dentin (Figs. 3, 4). When several fluorescent lines were found in one tooth, the configuration of the lines was parallel within the matrix, despite some differences in fluorescence intensity.
Table 1. The frequency of appearance of fluorescent line within the dentin of deciduous teeth.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of the teeth</th>
<th>The frequency of appearance</th>
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<tbody>
<tr>
<td>Deciduous incisors</td>
<td>60</td>
<td>6%</td>
</tr>
<tr>
<td>Deciduous canines</td>
<td>50</td>
<td>12%</td>
</tr>
<tr>
<td>Deciduous molars</td>
<td>90</td>
<td>60%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>27%</td>
</tr>
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and line thickness. Some of these lines corresponded to interglobular dentin (Fig. 5). In teeth with more than two occlusal cusps, separation at the central groove in the dentin immediately below the enamel without contiguous communication often occurred, as a result of calcification starting from the tip of each occlusal cusp (Fig. 6). Between 10 and 20 fluorescent lines of tetracycline were often found in parallel in one tooth. Dentinal tubules surrounding the lines also exhibited fluorescence, appearing as fine fibers (Fig. 7).

Fluorescent lines within the enamel were distinctly connected with those within the dentin at the dentoenamel junction at the edge of the tooth (Figs. 8, 9, 10). These fluorescent lines also corresponded to some of the incremental lines of Retzius, but the fluorescence was slightly less intense and the lines narrower than those in the dentin. It was possible to clearly distinguish them from other lines of Retzius that did not have deposits.

In the cervical areas of the dentin, fluorescent lines parallel to the incremental lines were connected with each of the fluorescent lines within the cementum at the cemento-dentinal junction.

Some of the lines exhibiting brilliant fluorescence in the dentin gradually formed one bundle in the cementum as it reached the cemento-enamel junction (Figs. 11, 12, 13).

No remarkable changes were noted in either the electron or the scanning electron microscopic photographs of the matrix surrounding the fluorescent lines in the dentin.

Results and Discussion

Characteristic yellow-gold fluorescence has been reported to appear upon examination under ultraviolet radiation of tetracycline incorporated and deposited in bone and teeth. In addition, inhibition by tetracycline of hard tissue formation and calcification has also been demonstrated. According to several reports, hypoplasia did not occur when the dose of tetracycline administered was clinically appropriate. Among several studies conducted on the effects of tetracycline, Kutscher (1963)⁴, Douglas (1963)⁵, and Nishino (1965)⁶ examined the relationship between the time factors of tetracycline administration and tooth discoloration. According to these authors, the faintly fluorescent line in the outermost layer of dentin observed under fluorescent microscopy represents tetracycline that had been administered to the mother and transferred to the developing dental germ of the embryo via the placenta. Accordingly, the fluorescent line in the dentin was believed to represent the time of tetracycline administration. The frequency of appearance of tetracycline discoloration in teeth has been reported to be 21.2% in American children by Witkop et al. (1965)⁶, and 24.2% in Melbourne children by Brearley et al. (1968)⁷. Possible tetracycline deposition has been demonstrated in 55% of first permanent molars and 72% of second primary molars based on histological examinations of extracted teeth by Stewart (1968)⁸, in 82% of both first permanent molars and second primary molars examined by Brearley et al. (1968), and in 87% of permanent teeth and 74.5% of deciduous teeth examined by Ebihara (1974)⁹.

The frequency of appearance of yellow fluorescent lines in the deciduous teeth in the present study was only 27% under light microscopic observation, but was as high as 52% in the deciduous incisors, 62% in the deciduous canines, and 66% in the deciduous molars (an average of 61%), when examined under fluorescent microscopy, indicating a significantly higher frequency of tetracycline.
cycline deposition than originally evident under light microscopy.

One to two fluorescent lines per tooth were frequently observed in the deciduous incisors. In most of the deciduous molars, more than 3 lines were common, with a maximum of 15 lines observed in some specimens. The frequent occurrence of fluorescent lines in the deciduous incisors may be explained by the reliance upon the number of fluorescent lines in the dental crown alone to calculate the frequency rate, due to frequent encounters of dental root resorption in the specimens examined.

No consistent trends in tetracycline distribution at the site of deposition was noted, due to the irregular time factors of tetracycline administration. The appearance of several to twenty fluorescent lines within a tooth suggested successive or repeated administration of tetracycline antibiotics.

Soentgen et al. (1965)\(^{10}\) have cited hereditary, metabolic, chemical, and medicinal factors as well as dental trauma as endogenous causal factors in tooth discoloration, and dental caries, poor oral hygiene, chromogenic bacteria, and external contact with chemical and medicinal agents as exogenous factors. Kurihara (1974)\(^{11}\) found fluorescent lines in ground sections of primary teeth of children with neonatal jaundice, children administered corpus luteum hormone, children with Crouzon syndrome, and children administered the tetracycline antibiotics. In order to identify the nature of the substance deposited in the teeth, Wallman and Hilton (1962)\(^{12}\) treated discolored teeth with hydrochloric acid and measured the ultraviolet spectra of the elution. The investigators demonstrated a peak at 270 \(\mu\)m, identical to that of tetracycline. Nishino et al. (1965)\(^{5}\) further confirmed the role of administered tetracycline antibiotics in the development of yellow fluorescent lines evoked by blue light around 4047 – 4348 \(\AA\) wavelength under fluorescent microscopy.

In the present study, in order to clarify the nature of the yellow line within the dentin, absorption spectra were measured using a microspectrophotometer in order to measure the characteristics of the wavelength of absorbency. The wavelength characteristics of absorbency of the material deposited in the dentin were found to be identical with those of the tetracycline antibiotics (vibramycin syrup). The former demonstrated a peak at 260 – 280 nm, whereas the latter peaked at 370 – 390 nm. Accordingly, the evidence strongly suggests that the yellow fluorescent lines identified in human dentin in the present study were due to tetracycline.

Moreover, the relationship between the fluorescent and incremental lines largely reflects the observation that tetracycline deposition appears most distinctly in the dentin. When tetracycline is incorporated into the developing dentin, a yellow fluorescent deposition line appears not only in the dentin, but also in the dentin-predentin junction. From an embryological viewpoint, the fluorescent line coincides with the incremental line of dentin, and is contiguous with the fluorescent line in the enamel as well as the fluorescent line within the cementum at the junction. These findings illustrate the synchronicity between periodic calcification and tetracycline deposition.

In investigations with tetracycline uptake in dental hard tissues, Bevelander et al.\(^{13}\) (1961), Owen (1964)\(^{14}\), and Suga and Murayama (1965)\(^{15}\) demonstrated that deposition of tetracycline at highest concentration occurs in areas that correspond to regions of recent inorganic salt deposition in portions of dentin and cementum newly formed at the time of tetracycline administration. According to reports by Suga and Musashi (1963)\(^{16}\), and Suga, Murayama
diffuse tetracycline uptake at low density occurs over the entire layer of developing enamel, characteristic of the mechanism of enamel calcification. Although the mode of uptake of tetracycline by the calcifying hard tissue and the nature of the tissue component responsible for uptake is unknown, Suga (1965) suggested that a purely chemical or physico-chemical process is likely to be responsible rather than a process dependent upon a biological environment with direct cellular participation. Suga further reported that their histological studies had not determined whether or not the site of tetracycline labeling actually coincided with sites of current calcification. According to a report by Ebihara (1974), the line of tetracycline labeling did not completely correspond to any of the incremental lines. The demonstration of continuity of fluorescent lines in this study with the dento-enamel junction in the early stages of development represents proof of cellular tetracycline uptake. Moreover, correspondence between tetracycline deposition and development of incremental lines of dentin appears to be definite from an embryological point of view.

During enamel formation, tetracycline is not incorporated into the apatite crystals but rather coexists with an organic matrix. With the disappearance of the organic component as a secondary consequence of calcification, tetracycline also disappears or degenerates, which is consistent with the disappearance of fluorescent lines from the enamel as has been reported recently. In the present study, the presence of a fluorescent line in the enamel was also confirmed, and the correspondence of this line with the parallel line of Retzius (incremental line of enamel) was also confirmed. If incorporation into the calcified matrix actually occurred, then enamel rods should be found at least in some areas from an embryological viewpoint. If they had been taken up by the dentin matrix, then they should also be taken up by calcoglobules, and a globular fluorescent structure would be expected to appear. Evidence for either of these cases, however, was completely lacking. Based upon these findings, it is clear that cells participate in tetracycline deposition in the hard tissues, and that the fluorescent line of tetracycline antibiotics appears to be intimately related with cells directly participating in dental growth and development. While these findings were based primarily upon studies of deciduous teeth, similar findings have been obtained for permanent teeth.

References

Explanation of Figures

Plate I

Fig. 3. The fluorescent line in the dentin of deciduous molar. X 10.

Fig. 4. The same specimen as in Fig. No. 3. X 10.

Fig. 5. The fluorescent line within the dentin of lower third molar. X 10.

Fig. 6. The same specimen as in Fig. No. 4. X 10.

Fig. 7. Same of the fluorescent lines corresponded to interglobular dentin. Carbol-fuchsin stain. X 40.

Fig. 8. The same specimen as in Fig. No. 5. X 40.
Plate 1

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8
Plate II

Fig. 9.  The fluorescent line in the central groove in the dentin immediately below the enamel. × 40.

Fig. 10. Many fluorescent lines were often found in one tooth. × 40.

Fig. 11. The fluorescent lines within the enamel of the deciduous molar (dentoenamel junction). × 40.

Fig. 12. The same specimen as in Fig. No. 11. × 40.

Fig. 13. The fluorescent lines within the enamel of the lower third molar (dentoenamel junction). × 40.

Fig. 14. The same specimen as in Fig. No. 13. × 40.
Plate III

Fig. 15. The fluorescent lines within the enamel of the upper first premolar (dentoenamel junction). × 40.

Fig. 16. The same specimen as in Fig. No. 15. × 40.

Fig. 17. The fluorescent lines within the cementium (cement-dentin junction). × 10.

Fig. 18. The same specimen as in Fig. No. 17.

Fig. 19. The fluorescent lines within the radical cementum. × 40.

Fig. 20. The fluorescent lines within the cementum (cement-enamel junction). × 40.