STUDIES OF TERTIARY DENTIN FORMATION
IN MONKEY TEETH UTILIZING VITAL DYES

By

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The interest and advice of Dr. Paul Starkey, and of the entire Pedodontic staff were a source of help and stimulation.

The author wishes to express a genuine "thank you" to Dr. David F. Mitchell. His encouragement, patience and help made this investigation interesting and rewarding.

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The author wishes to thank his parents for their continued inspiration and faith throughout his academic career. Finer people were never born.

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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF THE LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>STATEMENT OF THE PROBLEM</td>
<td>11</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>12</td>
</tr>
<tr>
<td>DATA</td>
<td>18</td>
</tr>
<tr>
<td>FIGURES</td>
<td>21</td>
</tr>
<tr>
<td>ILLUSTRATIONS</td>
<td>25</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>28</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>33</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>37</td>
</tr>
<tr>
<td>CURRICULUM VITAE</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
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</table>
INTRODUCTION
"... For many years, dentists have regarded dentin merely as a hard tissue that is cut for restorative procedures, not as part of tissue that reacts to injury. Actually cutting of the dentin and the odontoblastic processes which traverse the tubules involves exposure of living pulp cells to irritation.

Moreover, it has been believed by many that, once the root of the tooth is formed, the pulp has served its function and, following the completing of root end formation, no longer serves a useful purpose. In fact, the pulp has been considered to be a nuisance, giving rise to pain, on manipulative procedures, and complications of periapical inflammation when severely injured."

Indeed, few relationships in nature are depicted by a greater attraction; the pulp exists for the dentin and the dentin exists by the gratitude of the pulp. The major role of the dental pulp is the formation of dentin in which there lies such an intimate physiologic and histologic union that some investigators consider them as two parts of the same pulp organ.

The preservation of the tooth and the pulp itself is served basically by the production of new dentin in the face of irritants. The pulp can do this well by generating odontoblasts into action or by creating new odontoblasts to form the needed defense barrier. This dentin possesses its own characteristics. The formation site is localized. The production rate is faster than that seen at areas of nonstimulated secondary dentin formation.

Several factors determine the type and the amount of dentin created during the defense-response period. What is the degree of attack? Is it thermal, chemical, physical or bacterial?
Over what time interval has the irritation existed?

With these points in mind, this investigation was initiated to study the rate and the amount of dentin formation, in relation to various dental filling materials, in relation to cavity depth, and over given periods of time by the use of fluorescent vital dyes. It was hoped to establish the feasibility of using vital dyes for the quantitative measurement of dentin formation. To the author's knowledge the literature contains very little research regarding the subject.
REVIEW OF THE LITERATURE
The review of the literature is divided into four subsections relative to the study of the rate and the amount of tertiary dentin formation. The sub-titles are Classification of Dentin, Tertiary Dentin, Measurement of Tertiary Dentin, and Vital Dyes Used for Making Dentin.

CLASSIFICATION OF DENTIN

According to the classic description of Tomes, the dentin consists of an organic matrix richly impregnated with calcium salts and permeated throughout by parallel tubes which radiate with some minor exceptions from the pulp cavity outward.

In the past, chaos has reigned over the terminology used to describe the various forms of dentin. In an attempt to end the confusion, Kuttler proposed to divide dentin into three types: primary, secondary and tertiary. Primary dentin being the regular, normal dentin, most of which develops before the eruption of the tooth. Secondary dentin he believed is that which develops in response to the mild aggressive effects of normal biologic function, such as mastication, light thermal changes, chemical irritants and slight trauma. It contains fewer and narrower tubules and this characteristic makes it differ from primary dentin. Generally a line or demarcation zone separates the secondary dentin from the primary dentin. The secondary dentin tubules bend more sharply at this line, they may be waxy and many stain somewhat darker.

Tertiary dentin, according to Kuttler, differs from the other two forms in that it is localized exclusively to the irritated zone,
its tubules being very irregular, tortuous, and reduced in number or even absent. Calcification is deficient, cellular inclusions converting into spaces may be numerous, and a different tonality may be apparent.

To add to the confusion, which Kuttler attempted to rectify, tertiary dentin has been known under many different titles, pathologic, irregular-secondary, irregular of the third order, protective, secondary, adventitious, reparatory, amorphous, irritational, physiopathologic, irregular-odontoplastic, irregular, compensating, and others.

Therefore, throughout the remaining portion of this thesis the words tertiary dentin will be employed.

**TERTIARY DENTIN**

Tertiary dentin has long been recognized as being formed in response to more intense pulpal irritants (caries, erosion, cavity and crown preparation, abrasion-mechanical, chemical and thermal).

Tomes at an early date stated that tertiary dentin formation is a protection against the approach of caries.

Hopewell-Smith stated that tertiary dentin is a physiologic response to a pathologic process. He concluded that tertiary dentin is associated with attrition, abrasion, or dentinal exposure by fracture of the teeth when not complicated by caries of the enamel or dentin.

Noyes and Thomas stated that in tertiary dentin the tubules are smaller, fewer, and less regularly arranged than those of primary and secondary dentin, and that the smaller the pulp becomes, the more
imperfect the dentin formation, until only a granular calcified material is formed. 

6 Beust advocated that the classification of dentin be arbitrary; that dentin formation proceeded throughout the life of the pulp without regard to eruption, apex formation, or occlusion. To him the term tertiary dentin applied only to the local hyalin deposits on the wall of the pulp cavity. He stated that deposition of tertiary dentin under fillings were rare, although deep cavities involving or encroaching on the horns of the pulp may cause formation of tertiary dentin.

7 Kronfeld suggested that, since tertiary dentin is formed only at the central ends of those tubules of which the periphery is exposed, the immediate stimulus causing the formation of tertiary dentin is injury to the odontoblastic process in the dental tubules, and that it occurs as a defense process against local influence.

That intermittent deposition of tertiary dentin is dependent on chemical or thermal irritation was voiced by Bodecker, who also regarded its formation as physiological because at first he found it in all teeth of middle and old age. He suggested the interposition of tertiary dentin overlying primary dentin and pulp seals off many of the primary dentinal tubules from their odontoblasts. Later, he recognized that occasionally carious and abraded teeth may show little or no formation of tertiary dentin.

8 Fish has stated that tertiary dentin provides a perfect seal preventing entry of irritants to the pulp. He observed the formation of a calcified barrier under affected primary dentin with the
death of almost all the odontoblasts followed by the formation of tertiary dentin over the calcified areas.  

Orban regarded tertiary dentin as bordering between the normal and pathological, and resulting from surface irritation or pulpal inflammation. His explanation of the fewer tubules found in tertiary dentin was that some of the odontoblasts rest at the time of the irritation, and as a result are trapped in the newly formed dentin. He observed that tertiary dentin contains less organic material than primary or secondary dentin, and therefore is less permeable.

Van Huysen et al., using the soft x-ray technique, showed that tertiary dentin under peripheral lesions is hypocalcified in relation to normal unaltered dentin.

**MEASUREMENT OF TERTIARY DENTIN**

In 1955, Weider, Schour and Mohammed, conducted a study in which experimental cavities were prepared in the upper 1st molars of 88 young adult white rats. The cavities in 68 rats were filled with zinc oxide-eugenol, Aquadont, and Aquadont containing 50 percent tricalcium phosphate. In the other 20 animals the cavities were left open. They observed a daily rate of tertiary dentin formation ranging from 3 to 8 microns. The rate increased with depth of cavity preparation, varied with the type of restorative material but decelerated with time.

James, Schour, and Spence investigated the effect of base plate gutta-percha on the dentin and pulp in human teeth. They
found moderate quantities of tertiary dentin in five of nineteen teeth removed 17 to 36 days after various experimental procedures but did not determine the rate of tertiary dentin formation. 14

Lefkowitz performed a study on the desensitization of dentin with an ionization device (Chayes Siemon Desensitizer) which introduced fluoride ions into the dentin. He observed, using only 2 or 3 teeth, that considerable amounts of tertiary dentin were formed one to ninety days following the application of the ionization device.

15 El-Kafrawy and Mitchell prepared Class V cavities in 83 (mixed dentition) teeth in four monkeys. The cavities on one side of the mouth were left exposed to the oral fluids for three months and then restored for either 1 or 2 weeks with silicate cement. The teeth on the opposite side of the mouth were prepared and restored immediately with the same cement and were removed after 1 or 2 weeks. With the use of any eyepiece micrometer, they observed that the average amount of tertiary dentin deposited in 3 months was calculated to be approximately 300 microns beneath deep cavities and 160 microns beneath shallow cavities in both deciduous and permanent teeth. Tertiary dentin formation was observed in teeth restored for 2 weeks but not in similar teeth filled for 1 week. Evidence indicated that the deeper the cavity, the greater the quantity of tertiary dentin.

16 Stanley et al. conducted a histologic study on 108 human teeth. They believed that the more traumatic low-speed operative techniques utilizing diamond stones without a coolant produced a
greater degree of tertiary dentin, as compared with the very mild high-speed operative techniques using an air-water spray and a small carbide bur which produced very little. The milder the insult, the longer the time lapse before tertiary dentin formation. Only twenty-five teeth restored with zinc oxide-eugenol came from the high-speed water-cooled categories. The remaining specimens were prepared with the slow-speed technique and were predominantly restored with zinc oxide-eugenol. The postoperative removal time ranged from 15 to 132 days. Serial sections were prepared, and using an ocular micrometer measurements were obtained. They observed very little evidence of tertiary dentin formation from day one to 30 days. During the 27 to 48 day period the rate of tertiary dentin formation was the greatest; it declined markedly after the forty-eighth day; and it declined further during the 72 to 132 day period. The average rate for the total study time was 1.49 microns per active day. They also concluded that the factor of remaining dentin thickness by itself did not appear to affect tertiary dentin production.

VITAL DYES USED FOR MARKING DENTIN

It is not the intention of the author to review all the literature concerning vital dyes, for hundreds have been tested and only a minority have survived; and even these have their limitations. So only the most widely used (be it past or present) will be mentioned.

Literature stating the vital staining properties of the azo
dyes have appeared sporadically, but the azo group has never been exploited extensively.

Alizarin has been administered for years as a vital dye in its natural or synthetic form for the staining of incremental lines in calcifying tissue: but alizarin lines are removed by decalcification. Therefore, ground sections must be made to study the specimens and this is a more difficult procedure because the sample must be oriented properly or the resultant section is inferior. Also the dosage must be carefully controlled, for alizarin is very toxic and an overdose is harmful and can be lethal.

In the last decade lead acetate has been used to mark incremental lines in calcifying structures, but it is necessary to decalci fy the specimens in a special procedure using H₂S to precipitate and preserve the lead in the tissue.

In the past fluorescein (resorcenolphthalein), a non-toxic acid chromogen dye had been used mainly to measure blood circulation and in the diagnosis and localization of neoplasms. Just recently fluorescein was successfully employed as a vital dye in the marking of mineralizing tissue. The specimens marked with fluorescein cannot be decalcified for the dye is not preserved, hence ground sections must be prepared to study the sample.

The literature shows that trypan blue has been used as a 47-50 vital dye. Auskaps and Shaw observed its ability to mark developing bone and dentin. The staining was not necessarily
lost if the specimens were decalcified, but the dye proved to be critically toxic.

Today, probably the most widely used vital dye is the tetracycline. Johnson and Johnson and Mitchell have made an exhaustive search of the literature on the drug's history, uses and limitations.

The procion dyes are new and are very promising in the area of vital staining having the distinct advantage of remaining in decalcified sections. Golard and Prescott, Oehler and Mitchell have carried on the initial research with these dyes showing that the procion group possesses many different colors, and its toxicity seems to be a relatively minor problem.
Since the dental profession has become so interested in the biologic potential of the dental pulp, it is important that standards be established regarding tertiary dentin formation. This investigation was designed to study the rate and the amount of tertiary dentin formation, in relation to cavity depth, and over given periods of time, by the use of fluorescent vital dyes. It was hoped to establish the feasibility of employing vital dyes for the quantitative measurement of dentin formation.
EXPERIMENTAL PROCEDURE
Preparation of the Animals for Surgery

Twenty deciduous and fifty permanent teeth in three Macaca speciosa monkeys ("stumptailed maceques") were selected, using as a criteria, the complete eruption of the teeth, and no gross amount of abrasion. In regard to the deciduous teeth, sufficient root structure was required so that the teeth would not be exfoliated before sacrifice.

With the help of laboratory assistants, each animal was removed from its cage and anesthetized. Nembutal sodium was injected intraperitoneally by means of a 20 gage short needle. The recommended dosage was used; 1.00 ml. (10 mg.) of Nembutal sodium for every three pounds of body weight. It was necessary in several instances to administer an additional .5 ml. to obtain a more profound level of anesthesia.

After anesthesia was obtained, an orientated lateral headplate radiograph was taken by personnel in the orthodontic department. This radiograph was another aid in the selection of teeth. The animal was then placed on the operating table in a supine position with the head tilted back to provide an open airway. To facilitate movement of the tongue for ease of operation and visibility, it proved advantageous to clamp the tip of the tongue with a hemostat. This also aided in keeping a clear airway by preventing ingress of the tongue.
Surgical Procedures

The gross outline of a Class V (labial - buccal) cavity was prepared on each tooth with a high-speed rotary instrumentation, using a No. 35 carbide friction grip bur rotating at maximum speed (approx. 200,000 r.p.m.), under 30 pounds of air pressure. A water spray was not used in the cutting procedure. An assistant intermittently administered short blasts of air to clear the cavity of debris. The final preparation depth was ascertained by conventional speed (approx. 7000 r.p.m.) instrumentation using a No. 35 carbide bur.

The preparations were made deep (trying to establish minimal floor thickness - without pulp exposure, with the prime intention being to stimulate the laying down of tertiary dentin.

Materials

After the desired cavity depth was established, three filling materials were placed in the preparations. Through previous work it had been established that tertiary dentin was laid down in proportion to cavity depth. For this study it was hypothesized that the rate of apposition would vary proportionally with the irritational properties of a filling material. Therefore, three materials were chosen on the basis of their known irritational qualities.
The materials were:

A. ZINC OXIDE-EUGENOL
   Mixed in the conventional manner and placed with a TP3 plastic instrument (Tanno).

B. *CROTON OIL
   With a small pellet of cotton the cavity floor was painted with it and then the preparation was restored with zinc oxide-eugenol.

C. ** DYCAL
   The cavity floor was covered with it and then the preparation was restored with zinc oxide-eugenol.

D. CAVITY REMAINED OPEN
   This was used as a control.

To measure the rate of tertiary dentin two fluorescent vital dyes were employed. Procion red (H8BS) and Achromycin (tetra-

53-55 cycline antibiotic) were the marking agents. Goland and Prescott have demonstrated the ability of the procion dye to mark calcifying structures such as bone and dentin. Johnson

51 and Johnson and Mitchell , likewise showed the effectiveness of the tetracyclines as vital fluorescent markers.

The recommended dosages were administered intraperitoneally, procion red (H8BS), 300 mg. per Kg. of body weight and 250 mg.


** Commercial calcium hydroxide preparation manufactured by the Gaulk Co., Milford, Del.

- I.C.I. Organics Inc., Providence, R.I.

- Product of Lederle Laboratories (A Division of American Cyanamid Co.) Pearl River, N.Y.
of the tetracycline. The time intervals of administration were day one, fifteen days, thirty days, and sixty days. Combinations of vital dyes were utilized on two monkeys while the third animal received only procion red.

The animals received:

**10 lb. Monkey (Animal 1)**

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<td>&quot;</td>
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<td>60 days</td>
<td>16 cc</td>
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The drugs were administered intraperitoneally by means of a large gage needle. The dye was introduced on day one directly after completion of the surgical procedures, hence the animal was still anesthetized. For the subsequent marking periods, the animal was not anesthetized but was restrained by laboratory assistants while the vital dye was administered. No immediate or subsequent side effects were noted; although, the animal's body did turn red for a period of time with the procion red dye.
To minimize the abrasion of the restorations the diet of the animals was altered. A soft diet was furnished consisting mainly of apple sauce and water soaked Purina's monkey chow.

After ninety days, conventional surgical procedures were used to remove the teeth. Removal of the labial plate of bone with the automatic bone impactor (Dudley) facilitated the extractions. Pedodontic forceps No. 101 proved to be of considerable value as they were readily adaptable to the anatomical form of the teeth. Immediately after extraction, to facilitate fixation of the pulps, the teeth were ground down on the mesial or distal surface with a rotating stone wheel under a water spray until the pulpal outline was visible. The teeth were then placed in the 10 percent formalin for complete fixation.

Decalcification and slide preparation was performed by a laboratory technician. Serial paraffin sections seven microns thick were made through the cavity preparations and underlying pulp. Approximately fifteen slides with 2 or 3 sections from each tooth were stained with hematoxylin and eosin in the conventional manner. Two or three slides with sections from the center of the cavity preparation were mounted but not stained for fluorescence microscopy.

From the 10 lb. monkeys the first bicuspids were selected for ground sections. After fixation in 10 percent formalin, the teeth were dehydrated with ascending grades of alcohol. The specimens were then placed in styrene for three days, 50 percent styrene and 50 percent Bioplastic for three days, and
in Bioplastic for three days. Then they were embedded in Bioplastic and ground sections approximately 50 microns thick were made of each tooth. The sections were dehydrated in ascending grades of alcohol, cleared in xylene and mounted in canada balsam.

Slides

The stained decalcified serial sections were used to determine the total amount of tertiary dentin formed over the ninety day period. Measurements were made at a magnification of X100 with a Bausch & Lomb ocular micrometer. Two measurements were recorded, the thickest portion of the tertiary dentin formed, and the thinnest region of primary dentin of the pulpal floor of the cavity.

The unstained decalcified serial sections were used to make observations on the rate of tertiary dentin formation with a Leitz fluorescent microscope. The microscope settings were:

Barrier filter K530
Exciter filter BG12
200 watt mercury lamp
Light field

The ground sections were utilized for study of the lines of the procion dye and the tetracycline.
The following three tablets represent the information gathered from the teeth of the research animals.
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Croton oil - C. oil
Zinc Oxide-Eugenol - ZnOE
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FIGURES
FIGURE 1  (Animals 1 & 3 - anterior & posterior permanent teeth) Represents the relationship observed when the floor thickness (after cavity preparation) is plotted against the tertiary dentin formed.
FIGURE 2  (Animals 1 & 3 - anterior permanent teeth) Depicts a picture similar to figure 1 regarding the relationship observed when the floor thickness (after cavity preparation) is plotted against the tertiary dentin formed.
FIGURE 2

TERTIARY DENTIN

FLOOR THICKNESS
FIGURE 3  (Animal 2 - deciduous teeth plus 4 permanent molars) Represents the picture observed when the floor thickness (after cavity preparation) is plotted against the tertiary dentin formed. Note the encircled coordinates, they are the small anterior deciduous teeth.
FIGURE 3
FIGURE 4. Shows the haphazard collection of coordinates observed when the three filling materials of varying irritational quality and the cavities left open are plotted.
ILLUSTRATIONS
ILLUSTRATION 1  (Animal 1) Represents tertiary dentin beneath a cavity preparation. The animal received four doses of procion red, but only three are visible.
ILLUSTRATION 2  (Animal 3) Represents tertiary dentin beneath a cavity preparation. In an attempt to locate the fourth line absent in illustration 1, the animal received four doses of a combination of vital dyes (procion red and tetracycline). Refer to page 15 for the sequence of vital dye administration. Note that again only three lines are visible.
ILLUSTRATION 3  (Animal 3) Represents tertiary dentin stimulated by occlusal abrasion. This picture was obtained from the same slide used for illustration 2. Therefore, the same sequence of marking should be present. Note the presence of the first red line which is absent in illustration 2.
DISCUSSION
OBSERVATIONS ON THE RESEARCH ANIMAL

In the author's opinion the *Macaca speciosa* monkey ("stump-tailed macaque") is an excellent dental research animal. It is unlike the rhesus in that it is more tranquil and much easier to handle. Before attempting any research on a monkey, it cannot be stressed enough to read the literature pertinent to the animal. A great deal of time and unnecessary effort can be eliminated by doing so.

If a study involves preparation of teeth, the anatomy of monkey teeth should be reviewed. It cannot be recommended too strongly, that if deep preparations are to be cut, an operating microscope should be utilized. Granted it takes time, but in this instance time must be sacrificed in order to obtain good results. In this way, much of the "guess work" (floor thickness) will be eliminated; the preparation depths will be standardized, and the results will be superior.

SLIDE PREPARATION

As stated previously, preparation of ground sections is a difficult procedure because the specimen must be oriented properly or the resultant section is inferior. In this study the first bicuspids were arbitrarily selected for ground sections. This was a poor decision for these teeth are multi-rooted and therefore very difficult to mount for sectioning. A better choice would have been an anterior tooth such as a central incisor. In these
teeth the cavity depths were more standardized and the specimens could be oriented properly, thereby producing better ground sections.

**SLIDE INTERPRETATION**

After the ninety day study period there was little evidence of pulpal inflammation, despite the irritation caused by cavity preparation, introduction of the (irritating) filling materials and exposure to the oral fluids.

When there was an intact cavity floor, there was tertiary dentin formation. The deposit corresponded in position to the tract of dentinal tubules injured during cavity preparation, (Illustration 2). This is in agreement with the feeling that tertiary dentin is a specific reaction of the dental pulp to peripheral exposure or irritation of the tubuli.

The tertiary dentin formation was, with few exceptions, regular and well mineralized. This observation is in disagreement with those of Schour et al. who found that tertiary dentin formation was irregular, severely hypocalcified, and contained a large number of inclusions.

Cavity depth was shown to be important in determining the amount of tertiary dentin formation. As the cavity floor thickness decreased, the amount of tertiary dentin deposited increased, (Figures 1 & 2). But this is not the complete picture, the anatomy and physiology of the tooth also must play an important role.
Note Figure 3, (the encircled coordinates) these are deciduous anterior teeth. Observe that the floor thickness is minimal, yet the tertiary dentin formation is no greater than that of the deciduous molars (remaining coordinates on the Figure 3) even though the floor thickness of the latter is greater. This holds true for the permanent anterior teeth, (Figure 2). They have greater floor thickness, but the amount of tertiary dentin formation is equal and in the majority of cases greater. So it appears that a large tooth possesses the capacity and definitely has the space to produce more tertiary dentin, regardless of floor thickness. The results indicate that the smaller the pulp chamber (tooth size) the less tertiary dentin formation occurs, no matter how thin the cavity floor might be.

Histologic observations showed no difference in response between deciduous and permanent teeth. This is in agreement with the findings of El Kafrawy and Mitchell. Both types of teeth reacted to cavity preparation, irritating filling materials and exposure to the oral fluids by forming tertiary dentin to protect the pulp.

Three filling materials of varying irritational quality were chosen for study, anticipating that their ability to stimulate tertiary dentin would vary accordingly. Figure 4, demonstrates that there appears to be no such correlation. A haphazard collection of coordinates is illustrated. This conclusion is questionable, for the floor thickness varied greatly and hence a distorted picture might have been observed.
For this study it was hypothesized that the rate of apposition would vary proportionally with the irritational properties of a filling material; this was found to be false. Observations made on the unstained decalcified sections and ground sections (distance between the vital dye markings) under the light and fluorescent microscope indicate that there is no significant difference between the materials (and cavity remaining open) in their ability to stimulate tertiary dentin. This is also in disagreement with Schour et al., who observed that the rate varied with the type of restorative material used.

Investigators in medicine and dentistry have gained considerable knowledge from the experimental utilization of vital staining materials; but in the past the vital dyes have had their limitations. The procion dyes seem to be an improvement; they are non-toxic in proper dosage. The animals in this study gained weight over the three month period, which is a good indication that the dye did not cause serious physical injury. There are many different colors in the procion group, unfortunately the author was unable to take advantage of them for at the time of this study only one dye (H8BS - red) was demonstrating good, detectable staining. But now there are a number that can be used. Probably the most distinct advantage of the procion red dye is its ability to remain in decalcified tissue and be apparent under either the light or the fluorescent microscope. This is not true of other vital dyes (e.g. alizarin, fluorescein, trypan blue and the tetracyclines).
The tetracycline was introduced into the study in an effort to clarify a conclusion. The first animal received four doses of procion red dye (H3BS). When it was sacrificed and sections were prepared of the teeth, it was found that only three lines were visible, (Illustration 1). In an attempt to locate the fourth line the tetracycline and procion red was administered to the second and third animals, (refer to Illustration 2, combination of vital dyes). It was felt that the day one marking was missing. Stanley et al had observed that very little, if any, tertiary dentin is deposited prior to the thirtieth post-operative day. If their conclusion was correct, then the first (red line) procion dye marking should not be present, for the incremental line will occur only when there is mineralization of tissue. This might be explained by saying, due to deep cavity preparation there is a disruption of the odontoblastic layer. Hence, there is a pause in predentin formation; therefore, the absence of the day one marking. This can be demonstrated very nicely by comparing two areas of the same tooth (Illustration 2 & 3). One shows tertiary dentin caused by abrasion (four lines: 1 red, 2 yellow and 1 red), while the other is caused by deep cavity preparation and the filling material (three lines: 2 yellow and 1 red).
SUMMARY AND CONCLUSIONS
SUMMARY AND CONCLUSIONS

"...... At this time, when the biologic potential of the human dental pulp is receiving so much attention from the profession, it is essential that studies be made in an attempt to establish some guidelines for the rate of tertiary dentin formation. The absence of such guidelines is likely to lead to the publication of erroneous and misleading statements concerning the rate of tertiary dentin formation."4

With these thoughts in mind, this study was designed to measure the rate and the amount of tertiary dentin formation, in relation to filling materials of varying irritational qualities; in relation to cavity depth; and over definitive periods of time using fluorescent vital dyes. It was hoped to establish the feasibility of employing vital dyes for the quantitative measurement of dentin formation.

Twenty deciduous and fifty permanent teeth in three monkeys were utilized for the study. A Class V preparation was made in each tooth employing both high and low speed rotary instrumentation. The preparations were made deep (trying to establish minimal floor thickness without pulp exposure), with the prime intention being to stimulate the laying down of tertiary dentin.

For this study it was hypothesized that the rate of apposition would vary proportionally with the irritational properties of a filling material. Therefore, three materials were selected and placed in the preparations, croton oil, Dycal and zinc oxide-eugenol. A certain number of the preparations were left open as controls, since information in this regard was available from a previous study.
To measure the rate of tertiary dentin formation two fluorescent vital dyes were employed. Procion red (HEBS) and Achromycin (a tetracycline antibiotic) were the marking agents.

The recommended dosages were administered intraperitoneally, on day one, after fifteen, thirty, and sixty days. Combinations of vital dyes were utilized on two monkeys while the third animal received only procion red.

After ninety days, the teeth were removed and decalcified. Serial paraffin sections seven microns thick were made through the cavity preparations and underlying pulps. Approximately fifteen slides with 2 or 3 sections from each tooth were stained with hematoxylin and eosin. Two or three slides with sections from the center of the cavity preparation were mounted but not stained for fluorescence microscopy. From two animals (10 lb. monkeys) the first bicuspids were selected for ground sections.

The stained decalcified serial sections were used to determine the total amount of tertiary dentin formed over the ninety day period.

The unstained decalcified serial sections were used to make observations on the rate of tertiary dentin formation.

The ground sections were utilized for comparison study of the lines of the procion dye and the tetracycline.

The following conclusions were drawn from the results obtained from this study:

1. Tertiary dentin formation is a desirable response which protects the dental pulp against permanent injury.
2. Tertiary dentin is a specific reaction of the dental pulp to peripheral irritation of the dentinal tubules.

3. Under the conditions of the study, the tertiary dentin formed, with few exceptions, was regular and well mineralized.

4. Pulpal inflammation was insignificant after three months, despite the irritation caused by cavity preparations, introduction of the (irritating) filling materials or exposure to the oral fluids.

5. Cavity depth is a significant factor in determining the amount of tertiary dentin formed -- the deeper the cavity, the greater the amount of tertiary dentin formed. However, the size of the tooth seemed influential. The smaller the pulp chamber (tooth size) the less the tertiary dentin formed, no matter how thin the cavity floor might be.

6. Histologic observations showed no other difference in response of deciduous and permanent teeth. Both reacted to cavity preparations, filling materials and/or exposure to the oral fluids by forming tertiary dentin to protect the pulp.

7. Tertiary dentin formation was minimal from day one to the fifteenth day. Maximum formation occurred from the 15th to the 60th day. It tended to decrease after 60 days.

8. Due to deep cavity preparation there is a disruption of the odontoblast layer. Hence, there is a pause in pre-
dentin formation. This was demonstrated by the absence of the day one marking with the vital dyes.

9. The vital dyes proved to be a definite aid. The tetracyclines have proven themselves a success in the past, but the procion dyes are relatively new and are in their infancy as research agents. The procion dyes have the distinct advantage of remaining in decalcified tissue and being apparent under the incandescent light or the fluorescent microscope. This is not true of other vital dyes.


55. Grand, N. G., and Goland, P.: The Fixation of Cells and Tissues by the Use of Reactive Dyes. (Unpublished)


STUDIES OF TERTIARY DENTIN FORMATION
IN MONKEY TEETH UTILIZING VITAL DYES

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Indiana University School of Dentistry
Indianapolis, Indiana

The purpose of this investigation was to study various factors and conditions influencing the rate and the amount of tertiary dentin formation by the use of vital dyes. Attempting to establish minimal floor thickness, Class V cavity preparations were made in 70 monkey teeth (3 animals). Three materials possessing different irritational properties were sealed in the preparations, croton oil, Dycal and zinc oxide-eugenol. Procion red (H8BS) and Achromycin (a tetracycline antibiotic) were the marking agents. The dye was administered intraperitoneally, on day one, after fifteen, thirty and sixty days. After ninety days the teeth were removed. The specimens were prepared for histologic observations, either as decalcified sections or ground sections. Measurements of the thickness of remaining dentin and the tertiary dentin formation were made with an ocular micrometer. Observations were made on the rate of tertiary dentin formation with a fluorescent microscope. The different irritational materials were associated with approximately the same amount of tertiary dentin. Tertiary dentin was minimal from day one to the fifteenth day. Maximum formation occurred from the fifteenth to the sixtieth day. It tended to decrease after 60 days. The procion dyes have the distinct advantage of remaining in decalcified tissue and being apparent under the incandescent light and fluorescent microscope. This is not true of other vital dyes.
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