POSSIBLE PATHOLOGIC CHANGES ASSOCIATED WITH BLEACHING TETRACYCLINE STAINED VITAL TEETH

by

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ABSTRACT

The purpose of this study was to determine possible pathologic changes that might result from bleaching vital tetracycline stained teeth. Temperatures inside the pulps of three dog teeth were shown to respond to external applications of heat, hydrogen peroxide, and a combination of both. An initial temperature was recorded inside the pulps and temperature rises were noted in response to all of the conditions when applied to the buccal enamel surfaces of the teeth. Hydrogen peroxide was applied at three concentrations and shown to be more effective in raising pulpal temperatures at the higher concentrations. When heat and hydrogen peroxide were applied together, pulpal temperatures were noted to stabilize at four centigrade degrees below the enamel surface temperatures. Fourteen additional teeth, four from the same dog and ten from a second, were exposed to a bleaching procedure that involved application of hydrogen peroxide heated to about forty-one centigrade degrees for one-half hour at one week intervals for four consecutive weeks. Histologic sections of these teeth removed one week following the final bleaching were not suitable for study due to fixation artifact.

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INTRODUCTION

Discoloration of teeth associated with tetracycline drug therapy is frequently encountered by the dental profession. With those individuals severely effected by tetracycline stain, the dentist may be confronted with the challenge of improving esthetics. The exact mechanism by which tetracyclines impart their typical blue-gray color on teeth remains fairly obscure. Treatment in the past has consisted primarily of full coverage of the anterior teeth involved; treatment has not commonly attempted removal of the discoloration until very recently. A technique has been presented by Cohen and Parkins[1] whereby discolored vital teeth may be bleached.

PURPOSE

The purpose of this project was to examine the possibility of pathologic changes in vital dog teeth when exposed to a bleaching procedure similar to that described by Cohen and Parkins[1].

REVIEW OF THE LITERATURE

Tetracyclines were shown by Regna[2] in 1951 to act somewhat like chelating agents in their ability to bind calcium and other metals at physiologic pH. Prolonged retention of tetracyclines was found in the bone marrows of rats by Helander and Bottiger[3] while investigating the distribution of the drug in various body tissues. Milch, Rall, and Tobie[4] in 1957 detected tetracyclines along the periosteal and endosteal surfaces of bones of rats twelve hours after administration. The observation that tetracyclines may retard active calcification in sand dollars and chick embryos was made by Bevelander[5,6].

Milch et al.[7] reported the localization of tetracyclines in bone in its chemically unaltered form; that localization was independent of dose, route of administration, schedule, or sex. They suggested a complex formation between the drug, calcium ions, and protein matrix. Kelly and Buyske[8] concluded from radioactive calcium labeling and chemical tests that tetracyclines enter into a chelation with calcium in the blood which is then deposited in areas of active bone formation in direct contact with blood supply. They demonstrated that tetracyclines will fluoresce when combined with calcium ion but not in the absence of calcium.

Harcourt[9] suggested that tetracycline binding could not be explained simply on the basis of complex formation with calcium. His studies indicated that tetracycline drugs administered to growing children were incorporated into the dentin, but not the enamel of teeth. To explain the preferential incorporation into dentin, he suggested that the drug binds with the organic phase which is present in relatively high concentrations in the dentin as compared with the presence in enamel, rather than the inorganic phase which is relatively dense in the enamel.

Qualitative and quantitative determinations were made by Urist and Ibsen[10] of the chemical reactivity of oxytetracycline in several calcium containing substances both in vitro and in vivo. They presented three possible sites for oxytetracycline binding:

A.	B.	C.
collagen	collagen	collagen
•	•	•
polysaccharide	oxytetracycline	polysaccharide
•	•	•
apatite	polysaccharide	oxytetracycline
•	•	•
oxytetracycline	apatite	apatite

Of the three possible sites, proposal A was suggested to be the most probable on the basis of their studies. They described deposition of the drug in areas on the surface of the apatite microcrystal of high calcium ion concentration.

Ibsen[11] demonstrated the absorption spectra of "anorganic" bone labeled with oxytetracycline in vitro to be identical with the absorption spectra of true bone labeled in vivo or in vitro with the same drug. He concluded that oxytetracycline requires only the inorganic phase of bone for incorporation.

Bennett and Law[12] in 1965 demonstrated with spectrophotometric examination the incorporation of tetracycline into enamel and dentin with about three times as much drug incorporated into the dentin as the enamel. Further quantitative studies were done by Bennett[13] measuring fluorescence intensity in powdered tooth samples from dogs having received daily dosages of tetracyclines. He found drug incorporation in dentin about nine times as great as in enamel. He suggested that tetracycline is incorporated on the surface of the apatite crystals recalling that, according to Bale, Hodge, and Warren[14],

the ratio of surface areas of dentin to enamel is about eleven to one.

Perin[15] in 1965 hypothesized a model to explain tetracycline binding onto the surface of hydroxyapatite crystals proposing a three bond attachment of tetracycline to the apatite crystal that could without steric interference provide a strong adhesion of the drug molecule to the bone surface.

Bevelander[16] injected rats with radioactive calcium and tetracycline simultaneously and then traced the progression of the fluorophore into areas undergoing most mineralization as indicated by the uptake of the radioactive calcium.

Gassner and Sayegh[17] in 1968 supported a surface attachment of drug to apatite crystal and further suggested that organic material may even mask the uptake of tetracycline onto the inorganic component. Their in vitro studies indicated that organic dentin takes up about one-half the amount of tetracycline as inorganic dentin. By grinding the dentinal samples into different grades of powder, they demonstrated that by increasing the surface area of dentin, tetracycline incorporation could be increased.

In this same period, Lars Hammerstrom[18,19] published two reports on the incorporation in vivo of tetracyclines with radioactive calcium and proline concluding that complex formation between the drug and protein was responsible for the major uptake of tetracycline by developing teeth. The first study, in 1967, showed different patterns of incorporation of radioactive labeled calcium and tetracycline over a four day period in developing rats teeth. The second study, in 1968, demonstrated similar directional extensions of radioactive labeled proline and tetracycline. This second study also showed an inverse relationship to exist between the mineral content and tetracycline fluorescence in areas of developing enamel.

Complexes of tetracycline with materials other than calcium containing materials have been reported. Kaplan, Yuceoglu, and Strauss[20] determined that about twenty-seven per cent of total circulating tetracyclines in their study were protein bound. Sirota[21] established that about eighty per cent of protein bound auromycin was complexed with albumins. Different tetracycline analogues exhibit different affinities for binding with proteins according to Wozniak[22]. Higuchi and Bolton[23] demonstrated tetracycline binding to fused ring compounds such as DNA, riboflavin, and tryptophan.

Tetracyclines and Teeth:

Schwachman[24,25] reported his findings from two studies on the incidence of tooth discoloration in children having received long term tetracyclines. In his first study, in 1956, he found less than five per cent of a group of three hundred children on tetracycline therapy showed discoloration of their teeth. The second study, in 1959, detected discoloration in forty patients out of a group of fifty having histories of long term tetracycline therapy.

In 1961, Wallman[26] reported seeing discoloration and deformities in the deciduous teeth of many of the babies receiving tetracycline therapy at his hospital. He was not sure if the permanent teeth were involved.

Zegarelli[27] reviewed the histories of fifty-two children between the ages of nine months and sixteen years who had taken "massive doses" of tetracyclines prophylactically for cystic fibrosis. Thirty-eight of the patients demonstrated some degree of tooth discoloration.

Grøn[28] reported on experimental data collected by feeding two groups of rats doses of 10 and 100 mg/kg oxytetracycline. He found tooth discoloration in both groups and tooth hypomineralization in the rats receiving the higher dose. In the same publication, he reported on the microscopic

analysis of deciduous teeth exfoliated from an eleven year old girl having a history of long term tetracycline therapy. All the teeth showed similar patterns of fluorescence but varied in the localization of the stain indicating rates and stages of formation when the drug was administered.

The case of a ten year old girl was presented in 1962 by DeVeber[29]. She had been on long term declomycin therapy and after over-exposure to sunlight exhibited discoloration of teeth and fingernails. After two weeks, both fingernails and teeth returned to normal color.

Wallman and Hilton[30] in 1959 traced the histories of fifty patients who within a twelve month period had been administered tetracyclines during the first week of life for a variety of reasons. Discoloration of teeth was seen in forty-six of these children, the most severe discoloration appearing in those cases having received the highest doses of tetracycline. They pointed out that discoloration of teeth was due to the drug and not the disease process. To prove their point, they reported spectrophotometric detection of tetracycline in all teeth exhibiting discoloration. They concluded from the case histories that a total dose of 108 mg was sufficient to cause mild discoloration and that the duration of dose was not as important as total dose administered.

Wallman and Hilton[31] also examined forty-six premature babies for tooth abnormalities. They found discoloration or deformities of teeth in thirteen of the babies, all of whom had received tetracyclines except one who had kernicterus.

Zegarelli[32] compared the teeth of cystic fibrosis patients with those of non-cystic fibrosis patients. He found discoloration in the teeth of the cystic fibrosis children only, and concluded the discoloration was disease related as well as drug related.

Hypoplastic teeth were detected in fifteen of seventeen children with histories of tetracycline administration by Witkop and Wolf[33]. Bevelander[34] studied teeth from rats fed daily doses of tetracyclines for five days and found reduced calcification in areas of enamel and dentin formation.

Likins and Pakis[35] reported that no reduction in incorporation of radioactive calcium into molar teeth of rats resulted from administration of graded doses of tetracycline.

Antalovska[36] showed macroscopically visible zones of enamel hypocalcification associated with tetracycline administration in dogs. These zones increased in size as the drug dose was increased.

Omnell and Lofgren[37] in 1970 described an incremental band common to the enamel of all rat incisors having been exposed to a single systemic injection of tetracycline. The band appeared as a hypomineralized line running from the dentino-enamel junction to the enamel surface decreasing in width as it approached the surface.

Goodman and Gilman[38](1955) reported that tetracyclines pass the placental barrier and can be identified in the fetal circulation at a level of one-fourth to three-fourths that of the maternal circulation. Madison[39] reported a case of a thirty-one year old woman who had taken a dose of achromycin totaling about eight grams at sporatic intervals twice each month during pregnancy. The baby developed discolored teeth.

Douglas[40] followed eight women taking tetracycline during pregnancy. All eight gave birth to children who developed discolored teeth. Hochberg and Kutscher[41] reported a case of a young mother who took tetracyclines during her twenty-eighth and thirty-first weeks of pregnancy and gave birth to a child whose teeth were discolored.

An analysis of the histories of 126 children displaying tetracycline staining was made by Frankl and Hawes[42] in 1964. They found the earliest record of drug therapy associated with permanent tooth discoloration was ten months of age. The latest record of drug therapy associated with staining of primary teeth was nine months of age. They concluded from their data that the critical period of discoloration occurs toward the end of crown formation when the enamel and dentin development is taking place at the cervical areas of the tooth.

Cohen and Parkins[1] described a technique in 1971 effective in the removal of tooth discoloration and improvement in esthetics for children exhibiting the typical tetracycline stained teeth. The procedure, which made use of warm Superoxol^{R} solution as the bleaching agent, was tested on the teeth of six children displaying tetracycline stained teeth. Five of the children showed results worthy of the procedure. For all the teeth in the study, electric vitality tests remained unchanged throughout the study period.

METHODS AND MATERIALS

The project consisted of two parts. First, the effect on pulpal temperature of externally applied heat and hydrogen peroxide were studied. Second, a bleaching procedure was applied to teeth using hydrogen peroxide and heat.

Two dogs, a german shepard and a beagle, both one year old males, were used. The dogs were given general anesthetic for each procedure. Surital (1cc/5 lb) and Atropine (2.5cc) were the pre-anesthetic agents. Intubation was oral and light anesthesia was maintained with Fluothane^{R} and oxygen mixture. Body temperature and heart activity were monitored throughout all procedures.

Part I: The teeth used were from the german shepard. They were the upper right canine, upper right fourth premolar, upper left fourth premolar, and lower right first molar (see Fig. 1). The teeth were isolated with a rubber dam. Using high speed hand piece with water spray coolant, a #4 carbide round bur was inserted into the lingual of each tooth until the pulp chamber was visible through a thin remaining barrier of dentin. The pulp was then entered as atraumatically as possible with a #35 carbide inverted cone bur to avoid injury to peripheral blood vessels of the pulp chamber. A thermistor measuring forty mils (about one millimeter) in diameter at the tip was inserted through the opening into the pulp chamber. It was sealed in position with zinc-oxide and eugenol cement. The thermistor was previously connected to a calibrated ammeter for convenient monitoring of intra-pulpal temperatures. After ten minutes, the physiologic temperature of each tooth was recorded. To each tooth, different influences were applied to the buccal enamel surfaces and changes in pulpal temperatures were recorded.

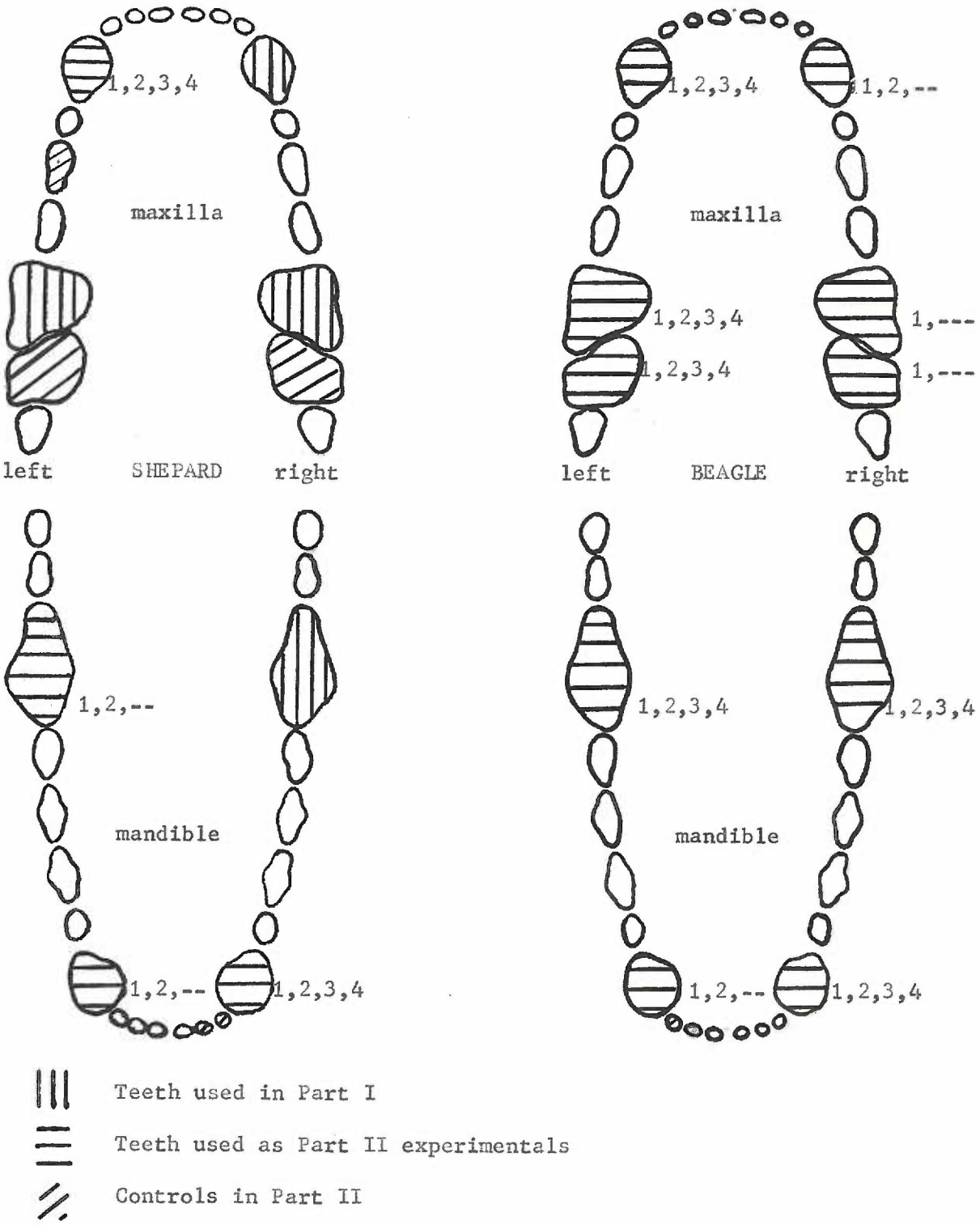
External heat of temperatures 43°C, 44°C, and 45°C were applied to the upper right canine and upper left fourth premolar using a Fluro-Ted Bleaching Tool. Intra-pulpal temperature responses were recorded after stabilization.

To the lower right first molar, heat was applied at a temperature of 44°C and the pulpal temperature recorded after stabilization. The tooth was then allowed to return to physiologic temperature and a guaze strip saturated with 30% hydrogen peroxide was placed over the buccal surface of the tooth. Temperature responses were recorded after stabilization. After again allowing the pulp to return to physiologic temperature, both the heating tool and the hydrogen peroxide saturated guaze were applied to the enamel surface. The pulpal response was recorded.

The effect of applying varying concentrations of hydrogen peroxide to the buccal surface of the upper right fourth premolar was studied. Concentrations used were 30%, 20%, 10%, and 0% (water). Temperature changes were recorded after application of each of these solutions. In every case, the temperature was given time to stabilize before recording. An intra-pulpal temperature was recorded fifteen minutes after application of the 30% hydrogen peroxide saturated guaze and the heating tool (the saturated guaze and the heating tool were held on the tooth for a fifteen minute period) at a surface temperature determined by removing the thermistor from the pulp and inserting the heat sensitive tip under the guaze strip in direct contact with the enamel surface. An accurate temperature differential was established between the pulp temperature and the surface temperature when exposed to 30% hydrogen peroxide and heat.

All four teeth were restored with formocresol pulpotomies after completion of the procedure.

Figure 1: A diagrammatic view of the teeth used in the study.



Note: The numbers associated with Part II experimental teeth refer to the number of treatments applied to each tooth.

Part II: Fourteen teeth were used for the experimental procedure; eight teeth were used as controls (see Fig. 1). The experimental teeth were treated in pairs. The teeth were prepared for the procedure by removing all calculus, drying, and isolating with rubber dam. Guaze strips were cut to fit over the buccal and occlusal surfaces of the teeth, saturated with 30% hydrogen peroxide, and placed on the teeth. The heating tool was placed on the guaze strip with pressure directed against the buccal surface. Two heating tools were used for the pair. Temperatures were monitored at five to seven minute intervals at the enamel surface directly under that portion of guaze contacted by the heating tool. The temperatures were recorded and an attempt was made to hold temperatures constant at 41°C. The guaze strip was kept saturated at all times. The procedure lasted thirty minutes. The teeth were rinsed with water and the rubber dam removed. The treatment was then repeated on another pair of teeth until all the experimental teeth included in the study were treated. The treatment was done once each week for each dog for four consecutive weeks.

One week following the final treatment, the dogs were sacrificed by bilateral perfusion of the carotid arteries with normal saline and heparin followed by 10% buffered formalin. This technique minimizes fixation artifact. Tooth bearing blocks of full thickness mandible and maxilla were further fixed for one week and then decalcified with formic acid. Paraffin sections were cut at seven microns and stained with hematoxylin-eosin. Qualitative determination of effects of the treatment were determined against controls.

RESULTS

Part I: Insertion of the thermistor tip into the pulp chamber of the cuspid tooth was not possible. All results from this part of the experiment pertain to the three posterior teeth.

The results are shown in Table I. The initial physiologic pulp temperatures were 35.5°C for two of the teeth; 35.0°C for the third. In the

External Condition		Pulpal Temperature (°C) A*	B*	C*
None (initial temp.)		35.5	35.5	35.0
Heating Tool Applied	43°C	39.0		
	44°C	39.8	39.5	
	45°C	40.5		
Hydrogen Peroxide	30%		38.5	36.5
	20%			36.0
	10%			35.0
	0%			33.5
Heating Tool (44°C) + 30% H_2O_2			42.0	40.0

* A -- maxillary right fourth premolar
B -- mandibular left first molar
C -- maxillary left fourth premolar

Table I: External conditions applied to the buccal enamel surface with resultant pulpal temperatures.

range of heat applied to the buccal surface, pulpal temperatures did not rise above values within four to four and one-half centigrade degrees of the external temperature. Pulpal temperatures were always four centigrade degrees or greater below the enamel surface temperature when heat alone was the external condition applied. When 30% hydrogen peroxide was applied alone to the enamel surface, rises in pulpal temperature of

one and one-half to three centigrade degrees were noted within ten minutes and stabilized. Reduced concentrations of hydrogen peroxide resulted in reduced thermal responses inside the pulp. Water caused the pulp temperature to drop. Ten per cent hydrogen peroxide caused no temperature change. All solutions, when applied to the buccal enamel surface, caused initial cooling of one to one and one-half cdntigrade degrees immediately, all solutions being at room temperature when applied.

When 30% hydrogen peroxide and heat were applied in combination, the effect on the pulpal temperature was greater than when either of them was used alone. Temperatures rose to within two and four centigrade degrees of the surface temperatures. A temperature differential of four centigrade degrees between enamel and pulp temperatures was found in the maxillary left fourth premolar (tooth C) when the hydrogen peroxide and heat combination was applied. In this case, the thermistor was used to measure both surface and pulpal temperature.

Part II: The pulpal tissues of all teeth, both experimental and control showed fixation artifact and could not be used for reliable experimental data. Therefore, the histologic effects of the bleaching procedure could not be ascertained.

The electrocardiogram revealed current leakage from one of the heating tools amounting to at least one milliamp. The tool was repaired to correct for the defect.

DISCUSSION

The normal physiologic pulp temperature of dogs in this project compare with a physiologic temperature range of 28°C to 34.9°C found in the incisors, cuspids, premolars, and molars of four dogs by Bhaskar and Lilly[43].

Pulpal temperature could be increased by applying heat to the enamel surface of the tooth as expected. Within the range of temperatures applied, pulpal temperatures rose to within four centigrade degrees of the surface enamel temperature. The enamel and dentin apparently acted to buffer external heat. Pulpal vascularity must also account for some temperature stability in the pulp when unusual thermal conditions are present externally.

The highest pulp temperature recorded during the application of heat alone was 40.5°C, five centigrade degrees above initial temperature. Zach and Cohen[44] have shown that increases in pulpal temperature of 10°F(5.5°C) were sufficient to cause irreversible necrotic changes in the pulps of fifteen per cent of the teeth he studied. Heat rises of 20°F (11°C) almost invariably destroyed the pulps.

The suggestion can be made from the results of part I that hydrogen peroxide has some thermal activity in the pulp. The results indicate that inert room temperature solutions such as water will lower pulp temperature; that hydrogen peroxide at room temperature will initially lower pulp temperature and then cause it to rise to levels above the physiologic temperature. That hydrogen peroxide may possess thermal activity might explain in part why Cohen and Parkins[1] detected pain responses at external temperatures as low as 88°F (31°C), six centigrade degrees below body temperature, and four centigrade degrees below the normal external temperature of the tooth[45].

Henschel[46,47] established a "temperature zone of pain tolerance" for human dentin. Temperatures of dentin within a range of 29°C to 54°C will elicit no pain response from the pulp. Temperatures outside of this "zone" will elicit a pain response. The temperature of surface enamel that elicited a pain response in the Cohen and Parkins[1] study was well within this range. Again, it is reasonable to expect possible thermal activity of the hydrogen peroxide within the pulp.

The results also indicate that hydrogen peroxide at higher concentrations affect the pulp temperature most, and that temperature rises in the pulp stabilize after only ten minutes.

Hydrogen peroxide may affect a temperature rise within the pulp by reacting chemically at the surface of the enamel with some portion of the enamel, perhaps the organic component. Such a chemical reaction could be of an exothermic nature and liberate sufficient heat to warm the pulp. More likely, however, is the possibility that hydrogen peroxide penetrates enamel and dentin and reacts directly with pulpal tissue. Permeability of teeth was studied by Atkinson[48] who demonstrated enamel to act as a semi-permeable membrane that would permit water to pass but restrict other substances in solution. Wainwright[49,50] and Bergman[51,52] have studied rates and patterns of enamel permeability. Since most of the tetracycline is bound in the dentin[9,12,13], it seems reasonable that the bleaching agent must permeate the enamel and reach the dentin within a fairly short period of time in order to be effective. There is no deirect evidence from this project that hydrogen peroxide reaches the pulp, but the suggestion can be made that hydrogen peroxide is eliciting an effect on the pulp tissue that may be damaging.

Attention should be drawn to the difference in pulp temperatures recorded

between tooth B and tooth C when the heating tool and hydrogen peroxide were applied together. Pulp temperature recorded in tooth B was two centigrade degrees higher than that in tooth C. A two centigrade degree difference was also noted when hydrogen peroxide was applied to teeth B and C. The difference may be attributed to morphology difference between the two teeth. It is important to point out, however, that the temperatures at the tips of the heating tools were difficult to measure and maintain stable. Only for tooth C was the enamel surface temperature under the guaze measured accurately with the same thermistor used to measure the pulp temperature.

Difficulty in maintaining a constant enamel surface temperature became most apparent in part II of the experiment. With the thermistor placed at the enamel surface, temperature fluctuations were recorded as high as four centigrade degrees. In one case, the surface temperature rose to 48°C. Since the teeth were bleached in pairs, it was impossible to monitor both teeth simultaneously. It became obvious that without constant vigilance of enamel surface temperature, fluctuations in temperatures could exceed values within a reasonably valid range. The range of temperature thought valid for this project was 41°C through 44°C. This was established by accepting a four degree temperature difference between enamel surface and pulp under the conditions of the experiment, and assuming any pulp temperature rises exceeding five centigrade degrees would cause damage as shown by Zach and Cohen[44]. This temperature range is far above the temperature applied by Cohen and Parkins[1] to human teeth, but chosen only on the basis of pathology and not pain response. If no pathological changes resulted from the experimental procedure at this temperature range, then it could be safe to assume that no pathological changes should occur at temperatures applied by Cohen and Parkins[1]. Unfortunately, no conclusions

can be formulated from this project with respect to pulpal changes at a histologic level.

SUMMARY AND CONCLUSIONS

The pulp temperatures of dog teeth were studied in response to external application of heat, hydrogen peroxide, and a combination of both. The study demonstrated that temperatures within the pulp rise in response to each of these external conditions. It can be concluded that hydrogen peroxide has some thermal effect on pulp tissue when applied to the enamel surfaces of normal healthy teeth. It is suggested that this thermal effect be considered a sign of possible pathologic changes occurring within the pulp when hydrogen peroxide is used for bleaching tetracycline stained teeth.

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