# A comparative study of tooth apexification in the dog

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Calcium hydroxide, collagen-calcium phosphate gel, and blood clot were compared as inducers of periapical calcification in the immature nonvital teeth of dogs. This investigation showed that calcium hydroxide accelerated hard tissue bridging of the open apex irrespective of complete resolution of initially induced inflammatory state. Collagen-calcium phosphate gel inhibited the reparative process of the initial inflammatory lesion leading to extensive destruction of the periapical tissues with no evidence of apexification. Locally induced blood clot maintained the initial inflammatory state and did not result in hard tissue bridging of the open apex. The investigation confirmed the use of the dog as an effective animal model for meeting the criteria of the experimental design.

Apexification procedures to treat the nonvital immature root canal with a wide open apex have come into common use in the modern clinical practice of endodontics. Although calcium hydroxide pastes are the root canal filling materials most often used to induce apexification,[1-12] one school of thought questions the necessity of placing any filling material in the root canal to fulfill this objective. Nygaard-Ostby[13] reported that the creation of an apical blood clot by over-instrumenting sterilized necrotic roots caused continued apical root closure. Moodnick[11] proposed that removing the bulk of necrotic pulpal tissue and filling with gutta-percha short of the apex could provide healing at the apex. Ham and others[11] have shown that both calcium hydroxide and the induction of a blood clot would lead to apexification of immature root canals in the monkey.

Some investigators recently have introduced new materials to provide more predictable success. Tricalcium phosphate ceramic material used as a resorbable implant material to stimulate apical closure has been successful in experiments on monkeys[15] and equally successful when compared with use of calcium hydroxide in human studies.[16] However, apical closure was not achieved with this material in an investigation in which dogs were used.[17]

Among the newer materials, collagen-calcium phosphate gel appears promising. In 1976, Nevins and others[18] published a report on the use of this gel as a filling material in pulpless teeth with open apexes in monkeys. When compared with a calcium hydroxide paste, this substance promoted a rapid and more physiological apexification. Several of the teeth filled with the collagen gel "appeared to be revitalized with various forms of hard and soft connective tissue, including cementum, bone, and dentin derived from lacerated remnants of uninflamed pulp tissue."[18] The authors concluded that this material may be a step in the direction of revitalizing human pulpless teeth.

Aside from the contention that several different techniques will lead to apexification of wide open root canal apexes, careful perusal of the literature has disclosed several variations in the research methodology relating to experimental design, choice of animal model, and interpretation of the experimental data.

Experimental design includes those conditions to be controlled

before the apexification procedures are performed. Studies have been done on root canals that, before the actual apexification procedures, have ranged from those in intact healthy teeth[9,13,17] to those that have been experimentally infected and made necrotic.[8,10,11] Similarly, studies have been published in which the experimental teeth have contained fully developed root canals in which the apex was perforated to simulate a blunderbuss canal[13,15,16] to immature root canals with naturally occurring wide open apexes.[2,8,9,10,12]

The choice of animal model in these investigations also is controversial. Basically, three species have been used: humans[1,3,13,14,19] monkeys,[8,10,11,15,18] and dogs.[4,12,13,17] Monkeys have been used extensively in these studies because of the evolutionary resemblance to humans, in particular the resemblance anatomically to the human dentition. However, this outward resemblance may be deceiving according to Torneck and others[20] who, in 1973, studied the effect of injury to the pulp with oral contamination on developing monkey incisors. In spite of filing the walls of the root canals to remove residual fragments of pulp tissue and leaving the teeth open to oral salivary contamination for up to 95 days, dentin and cementum were deposited at the periapex leading to apexification of the open apexes. The data suggest that monkeys, when compared with humans, have greater recuperative powers. Specifically, monkey pulpal tissue is highly resistant to the effects of oral contamination; despite the presence of severe inflammatory disease, the periapical tissues of the monkey still allow for repair of hard tissue in the form of dentin, cementum, and bone.

Several investigators have found a close similarity in healing processes of teeth and supporting structures between humans and dogs.[21,22] In 1932, Orban[23] neatly summarized why dogs make ideal animal models: "Dogs' teeth are more sensitive to any kind of injury than human teeth. This may be due to a greater permeability of hard structures, dentin and cementum in the dog than in humans, and that if treatment in the far more sensitive dog proves satisfactory it can be expected to be satisfactory in humans."

A third shortcoming in some of these studies relates to interpretation of the experimental data with respect to the histological evidence which is subjective to one degree or another. Histological evidence of hard tissue deposition around the apex of a root canal can be seen in a section stained with hematoxylin and eosin. However, under these conditions it is impossible to conclude objectively whether this deposition of hard tissue occurred before, during, or after the apexification procedure. The only way to confirm that the deposition of hard tissue observed is the result of the experiment is to label the tissue with a biological marker before and after the apexification procedure is performed. The dye Procion Red, when viewed in unstained decalcified sections under ultraviolet light and with a specific excitation filter, has biologically marked actively calcifying connective tissues.[21]

An analysis of the results obtained in this investigation has been ensured by simulating a typical clinical situation, namely, necrotic immature root canals; using an animal (the dog) that is similar to humans in biological response; and assessing hard tissue repair with Procion dye, a vital marker.

In applying the foregoing principles, the purpose of this investigation was to study histologically the effects of various techniques to induce periapical calcifications in the teeth of dogs with incompletely formed apexes and with necrotic pulps. Specifically, a comparison of the standard material, calcium hydroxide, with collagen-calcium phosphate gel was made. As a control, the induction of a blood clot without any filling materials also was investigated.

## MATERIALS AND METHODS

### Materials

The double-rooted premolars of two 5-month-old beagle dogs were used, giving a total of 20 experimental teeth. Preliminary radiographs showed incompletely formed roots. Intraperitoneal injections of Procion Brilliant Red dye (2% solution, 100 mg/kg body weight) were administered to each animal at four intervals six weeks apart starting three weeks before the pulpectomies, then at three and nine weeks after the experimental procedure, and shortly before the animals were killed. Before the apexification procedures were begun, the pulps were rendered necrotic by performing pulpectomies and leaving them open to oral salivary contamination for a week. The necrotic root canals were then debrided and treated with 1% aqueous parachlorophenol and sealed with amalgam for another week.

### Methods

Treatment of the open apex with the selected procedure was performed with use of rubber dam isolation and sterile techniques. Five of

the prepared teeth were simply instrumented beyond the apex to induce a blood clot (this was verified by blood seeping into the canal); eight of the prepared teeth were filled with a collagen-calcium phosphate gel prepared in the manner described by Nevins and othrs[18] and the remaining seven teeth were filled with a calcium hydroxide-saline paste. All the teeth then were double-sealed with a stop of base plate gutta-percha followed by a Class I amalgam filling.

All treatments were performed with the animals under general anesthesia; injections of sodium pentobarbital were used.

Periapical radiographs of the experimental teeth were taken at regular intervals to monitor clinical progress of hard tissue pathological conditions or repair. The animals were killed at the end of 11 weeks when large radiolucent areas occurred at the apexes of several of the teeth. The tissues were fixed with a saline and Formalin infusion technique according to procedures used by Kozlowski.[24] Block dissections of the jaws were made and decalcified in Perenyi's solution.[25] The specimens were embedded in paraffin. Semi-serial sections were cut into a thickness of 5 $\mu$ at the plane of the root apex. Alternate slides were stained with hematoxylin and eosin. Adjacent alternate slides were left unstained for viewing with ultraviolet light with use of excitation filter BG12* and barrier filter 53* in a Zeiss photomicroscope.*

## Criteria

The criteria of successful treatment were lack of inflammatory cells and edema in the periapical tissues, continuity of the fiber pattern of the periodontal ligament, and no resorption of cementum or dentin.

Adverse periapical reactions were classified as being either mild or severe inflammatory reactions. Mild inflammation was interpreted as being caused by the presence of moderate populations of inflammatory cells with minimal loss of the continuity of the periodontal ligament. Severe inflammation was characterized by the presence of either granulomatous tissues, abscesses, or cysts concomitant with destruction of the periodontal ligament and resorption of cementum or dentin, or both.

The criteria of successful apexification and active cementogenesis at the apex were evaluated by looking for the presence of orange fluorescent bands in the unstained sections under ultraviolet light, because Procion dye labels actively calcifying tissues. As four injections of the Procion dye were administered at six-week intervals, the number of fluorescent lines (to a maximum of four) were recorded as an index of the quantity of cementum laid down.

**Table 1 • Response of the periapical tissue to the experimental procedure.**

| Procedure | Periapical tissue response* None | Mild | Severe |
|---|---|---|---|
| Induction of a blood clot (5)† | 0 | 1 | 4 |
| Collagen (8) | 0 | 0 | 8 |
| Calcium hydroxide (7) | 5 | 1 | 1 |

*Number of teeth showing the response in each experimental group.
†Total number of teeth in parentheses.

**Table 2 • Evidence of apexification and cementogenesis.**

| Specimen | Apexification‡ None | Evident | Cementogenesis* (No. of Procion bands) 0-1 | 1-2 | 4 |
|---|---|---|---|---|---|
| Blood clot (5)† | 5 | 0 | 3 | 1 | 1 |
| Collagen (8) | 8 | 0 | 5 | 3 | 0 |
| Calcium hydroxide (7) | 0 | 7 | 0 | 0 | 7 |

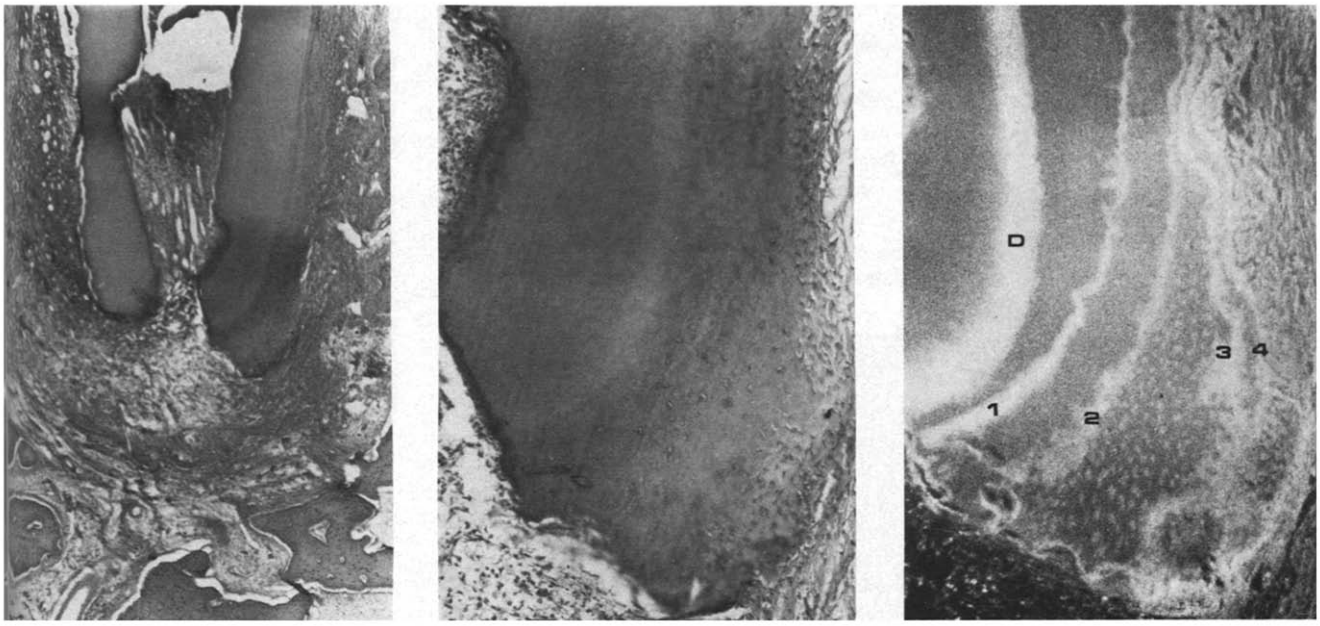
*Number of teeth showing the response in each experimental group.
†Total number of teeth in parentheses.

## RESULTS

The response of the periapical tissues to the experimental procedures with respect to inflammatory changes and hard tissue deposition is shown in Tables 1 and 2.

Of the five blood clot controls, the predominant lesion was granulomatous. One specimen showed only mild inflammation, and one showed cementogenesis (Fig 1).

All eight collagen-calcium phosphate gel specimens disclosed severe inflammatory lesions (Fig 2). Acute abscesses, granulomas, and cysts causing extensive destruction of the periapical tissues were characteristic responses. Resorption of both cemen-

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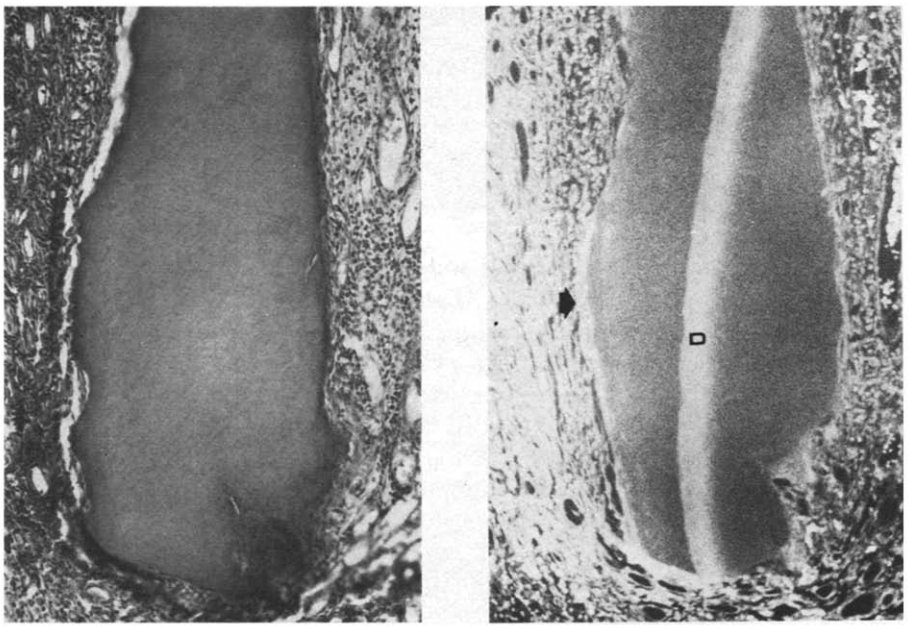



D E

*Fig 1—A, blood clot specimen showing cementum on lateral aspect of one side of root, as well as internal resorption of dentin on its medial aspect. Other side of root shows resorption of cementum and dentin. Notice cellular invasion of root canal space (H&E, orig mag ×35). B, higher magnification of lateral aspect of root in Figure 1, A, showing detail of cementum (H&E, orig mag ×140). C, corresponding unstained section of Figure 1, B. Notice four bands of fluorescence in cementum confirming active cementogenesis (1, 2, 3, and 4), and only one fluorescent band in dentin (D), confirming that no new dentin has been deposited after experimental procedures (ultraviolet light, orig mag ×140). D, higher magnification of other side of root in Figure 1, A, showing resorption of hard tissues (H&E, orig mag ×140). E, corresponding unstained section of Figure 1, D, confirming resorption of cementum. Only one partial band of fluorescence can be seen (arrow). Also notice one fluorescent band in dentin (D), which was present in all experimental teeth (ultraviolet, orig mag ×140).*

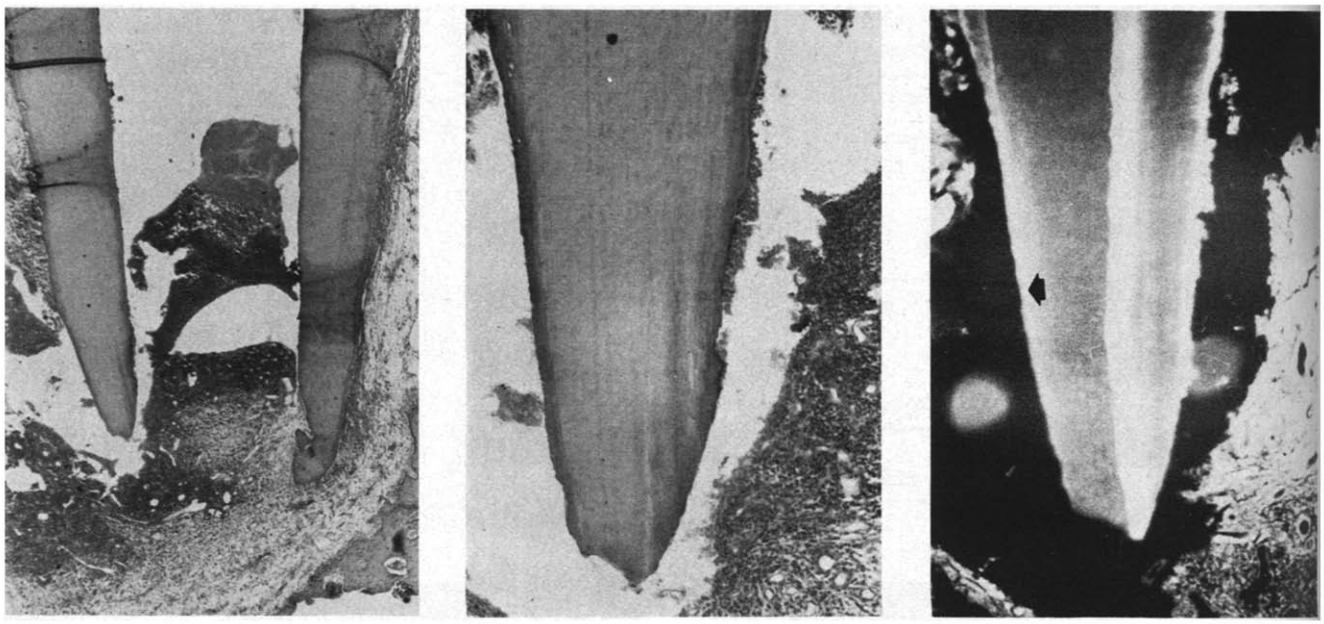


*Fig 2—Left: collagen-calcium phosphate gel specimen showing granuloma at apex and early soft tissue invasion of root canal space (H&E, orig mag ×35). Center, higher magnification of left side of root in Figure 2, left, showing extensive resorption of cementum and destruction of attachment apparatus (H&E, orig mag ×100). Right, corresponding unstained section of Figure 2, center, showing only one band of fluorescence in cementum (arrow), thus indicating resorption of cementum or lack of cementogenesis taking place (ultraviolet light, orig mag ×100).*

tum and dentin occurred frequently. Destructive granulomatous soft tissue invasion of the root canal space extended to a few millimeters from the apex of the root. No evidence of apexification or cementogenesis was observed (Fig 2).

Five of the seven calcium hydroxide specimens showed no evidence of inflammation and destruction of the periodontal supporting apparatus. Two specimens showed inflammatory changes. All specimens had apexification and apical cementogenesis (Fig 3, 4). The hard tissue bridge was identified as cementum or amorphous calcified matter, or both.

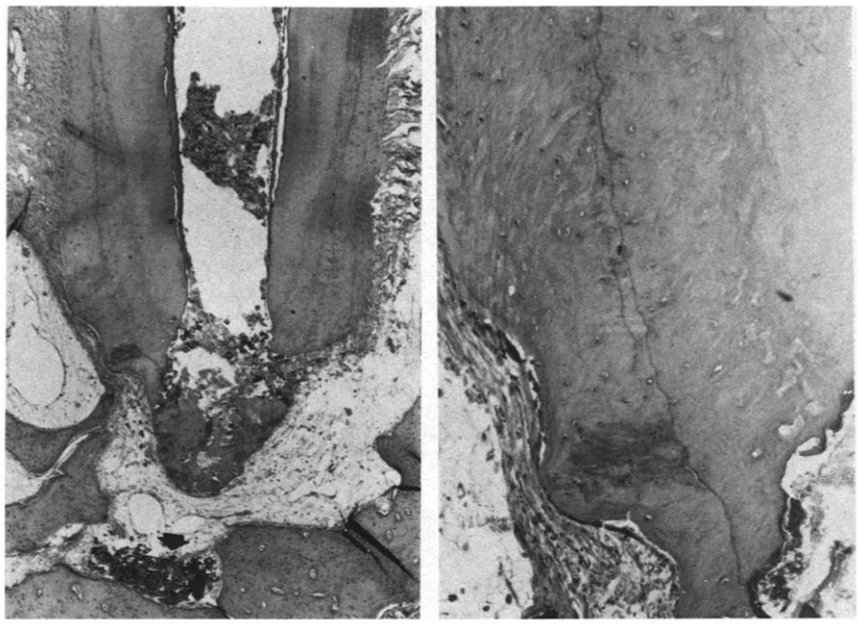
## DISCUSSION

To confirm that the cementogenesis and apexification that were observed were newly deposited, unstained sections were examined under ultraviolet light. All of the calcium hydroxide specimens showed evidence of four fluorescent bands indicating active cementogenesis (Fig 4, C). As the Procion dye was injected at four intervals (the first injection was administered three weeks preoperatively), an untreated control tooth should show four fluorescent bands in both the cementum and the dentin (Fig 5). A comparison of the calcium hydroxide sections with the unoperated control tooth, as viewed under ultraviolet light, showed two important features. First, the bands of ultraviolet fluorescence in the calcium hydroxide sections were much more widely spaced than in the unoperated control specimen. This confirmed that a quantity of cementum, greater than normal, was being deposited between each injection interval in the calcium hydroxide-treated teeth compared with the unoperated control teeth. The second important feature was that all the experimental teeth showed only one fluorescent band in the dentin corresponding to the first preoperative injection of the dye. This showed that the experimental teeth were made necrotic; no viable odontoblasts were left to produce dentin.

The hard tissue bridging at the apexes of the teeth showed under ultraviolet light as a mass of orange fluorescence with no neat bands demarcating the different periods of injection (Fig 4, D). It can be inferred that the hard tissue deposition in this area was laid down rapidly and in a disorganized fashion. Corresponding sections stained with hematoxylin and eosin reflected the disor-

*Fig 3—Calcium hydroxide specimen showing hard tissue bridging in presence of a periapical granuloma. Notice presence of calcium hydroxide crystals in root canal space (H&E, orig mag ×35).*

*Fig 4—A, calcium hydroxide specimen showing both cementogenesis and apexification. Notice presence of calcium hydroxide crystals both in root canal space and in periapical area (arrow). Also notice absence of inflammation (H&E, orig mag ×35). B, higher magnification of left side of root in Figure 4, A, showing detail of cementogenesis (H&E, orig mag ×140). C, corresponding unstained section of Figure 4, B, showing four widely separated bands of fluorescence (1, 2, 3, 4), confirming continued active cementogenesis (ultraviolet light, orig mag ×140). D, higher magnification of hard tissue bridging in Figure 4, A, showing detail of calcified tissue (H&E, orig mag ×140). E, corresponding unstained section of Figure 4, D, showing mass of fluorescence confirming active calcification in area occurring within relatively short time (ultraviolet light, orig mag ×140).*



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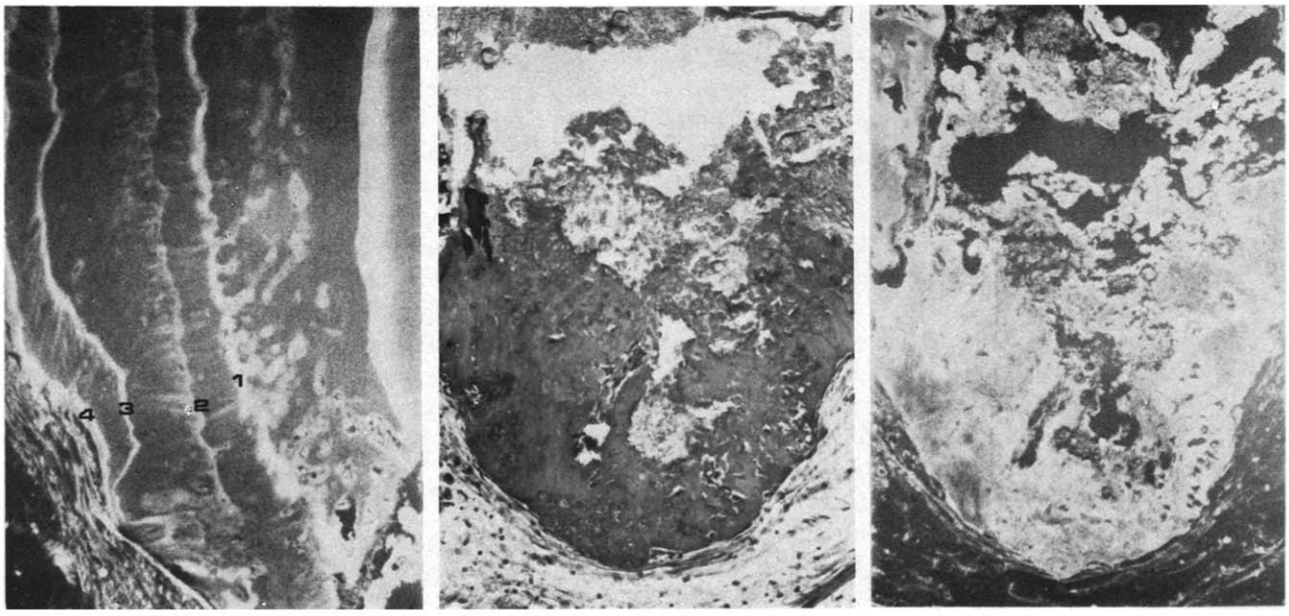


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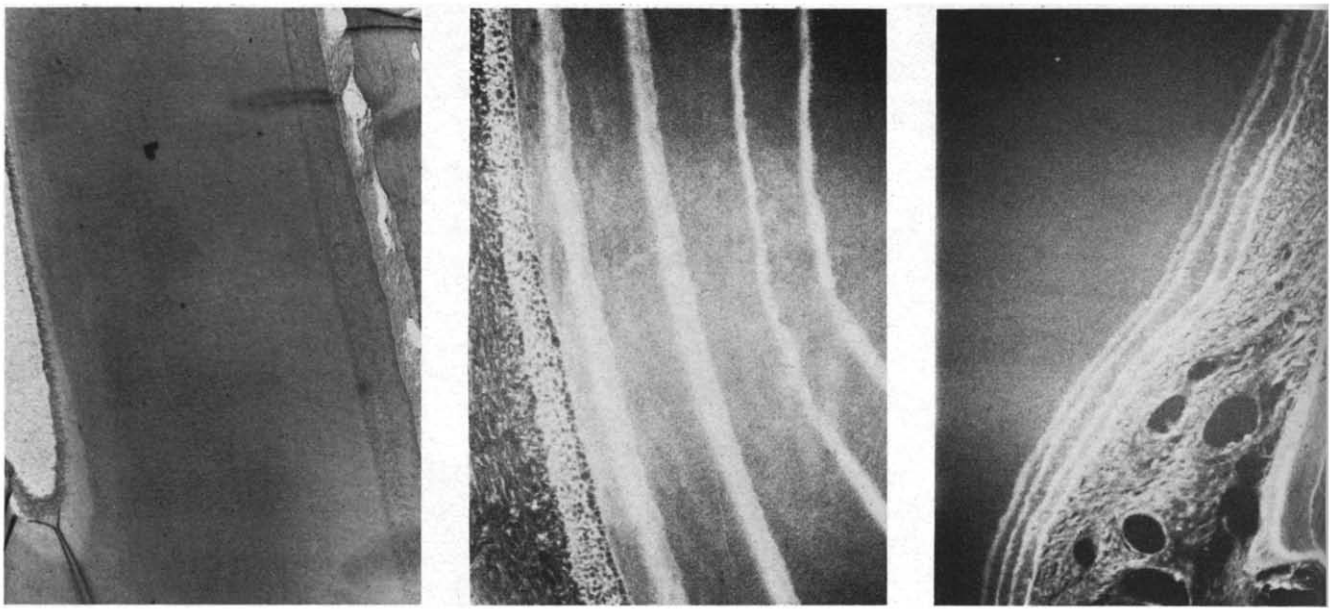

*Fig 5—Left, unoperated control specimen showing intact pulp (left) and intact cementum and periodontal ligament (right) (H&E, orig mag ×35). Center, unstained section at a higher magnification of Figure 5, left, showing four bands of fluorescence in the dentin, confirming that dentinogenesis had not been impaired in the unoperated control specimen (ultraviolet light, orig mag ×140). Right, unstained section shown in Figure 5, center, showing four narrow bands of fluorescence in the cementum as well as intact periodontal ligament (ultraviolet light, orig mag ×140).*

ganized arrangement of the tissue (Fig 4, D).

Nevins and others[5] have demonstrated so-called "revitalization" of pulpless monkey teeth. The results reported in this investigation showed that, in the canine species, the collagen-calcium phosphate gel led to severe inflammatory lesions that resulted in extensive loss of periodontal ligament as well as resorption of hard tissues. Also, under ultraviolet light, unstained sections showed an average of only one cemental band in the periapical area instead of the normal four as seen in the unoperated control root. This confirms that the active cemental resorption observable in sections stained with hematoxylin and eosin was taking place. Seven of the eight specimens showed tissue invasion of the root canal space. Nevins and others[5] found this ingrowth was an important constituent in the revitalizing of the pulpal tissues but this revitalized tissue did not show any odontoblastic activity. Thus, they could not show whether this tissue was reparative or degenerative. However, in this investigation, the soft tissue ingrowth in all but one specimen was granulomatous—therefore, reactive or degenerative in character.

As the results show, the blood clot control specimens showed histological changes comparable, albeit not as severe, with the collagen-calcium phosphate gel specimens. One root, however, showed evidence of periapical calcification (Fig 1A, B). Under ultraviolet examination, four bands of fluorescence confirmed the increased activity in the cementum (Fig 1C). The other side of the same root, however, showed the typical resorptive process seen in the other specimens (Fig 1D, E).

One of the criteria in the design of this investigation was to make the experimental teeth necrotic. As these teeth were left open to the oral environment after the pulpectomy procedures and before the filling procedures, it is assumed that the resultant bacterial contamination had caused an initial severe inflammatory reaction in the periapical areas of the affected teeth.

In this context, the results of this investigation have shown that the response to induction of a blood clot (no filling material in the root canal) resulted in maintaining the initial inflammatory state. However, one specimen showed only a mild inflammatory response, whereas another demonstrated early evidence of active cementogenesis. Perhaps if this experiment had been performed for a longer time, the control specimens with blood clots would have shown evidence of some resolution of the initial inflammatory process.

However, the specimens of collagen-calcium phosphate gel not only maintained the initial inflammatory state but, in addition, inhibited any reparative process from occurring, as seen in seven of the eight specimens. All specimens were characterized by large inflammatory lesions causing widespread destruction of both hard and soft tissues. As a result of the tissue destruction, the animals had to be killed at an early date based on radiographic evidence of large radiolucent areas surrounding the roots of some of these specimens.

In comparison, calcium hydroxide caused an acceleration of the reparative process in the periapical area, and, in most specimens, complete resolution of the initial inflammatory lesions occurred.

## SUMMARY AND CONCLUSIONS

The comparative effects of calcium hydroxide, collagen-calcium phosphate gel, and the formation of a blood clot as inducers of periapical calcifications in the immature nonvital teeth of dogs were investigated.

Central to this investigation was the evaluation of the suitability of the dog as an ideal animal model in this field of study. In addition, the vital dye Procion Brilliant Red was evaluated as to its effectiveness as a temporal marker to identify postoperative calcifications.

The following conclusions were made from this investigation:

–Calcium hydroxide accelerated hard tissue bridging of the open apex whether the initial inflammatory state in the periapical area was completely resolved or was still in evidence.

–Collagen-calcium phosphate gel inhibited any reparative process of the initial inflammatory lesion, leading to extensive destruction of the periapical and supporting tissues, with no evidence of apexification.

–Induction of a blood clot resulted in maintaining the initial inflammatory state and did not result in hard tissue bridging of the open apex.

–Procion Brilliant Red dye was an effective temporal marker of hard tissue calcifications.

–The dog was an effective animal model for meeting the criteria of the experimental design including the availability of open apexes and a necrotic and sensitive biological response to the effects of oral contamination.

*Carl Zeiss, Overkocken/Wurtt, West Germany.

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