0099-2399/85/1110-0428/$02.00/0
JOURNAL OF ENDODONTICS

Printed in U.S.A.
VOL. 11, NO. 10, OCTOBER 1985

A Histological Study of the Effect of Diameter Enlargement of the Apical Portion of the Root Canal

Estudio Histologico del Efecto del Ensanchamiento del Diametro de la Porcion Apical del Conducto

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The influence of intentional enlargement of the apical foramen in periapical repair was studied in dogs. After biopulpectomy, 134 root canals were overinstrumented 2 mm beyond the apical foramen and the apices enlarged using files #40, 60, or 80. The root canals were filled 1 to 3 mm short of the apex using radiographic measurements.

The animals were killed 3, 7, 30, and 120 days after the endodontic treatment and the teeth were processed for microscopic studies. The results showed the proliferation of periodontal connective tissue into the root canals and morphological changes accompanying maturation of this connective tissue with increasing time. The observations indicated that enlargement of the apical foramina permitted ingrowth of connective tissue into the root canals and the formation of a thick layer of cementum in the apical root canals.

Se estudió en perros la influencia del ensanchamiento intencional del foramen apical en la reparación periapical. Luego de la biopulpectomía, se sobreinstrumentaron 2 mm más allá del foramen apical en 134 conductos, utilizando limas no. 40, 60 y 80. Los conductos se obturaron de 1 a 3 mm antes del ápice utilizando medición radiográfica.

Los animales se sacrificaron 3, 7, 30 y 120 días después del tratamiento endodóntico y cada diente fue procesado para su estudio microscópico. Los resultados mostraron la proliferación del tejido conectivo periodontal dentro del conducto y cambios morfológicos acompañando la maduración de este tejido conectivo con el tiempo. Las observaciones indican que el ensanchamiento del ápice permiten el desarrollo del tejido conectivo dentro del conducto y la formación de una capa gruesa de cemento en la porción apical de la raíz.

Connective tissue ingrowth from the periodontal ligament region into the interior of the canal has been observed by several researchers (1–3).

Such tissue formation has been observed in partially filled canals where the remaining spaces were occupied with blood clots (2, 4–6) or from apical overinstrumentation (7–12).

In the studies mentioned above, little attention was given to the diameter of the apical canal, probably one of the most important factors in periapical repair.

Considering that periapical repair results from connective tissue proliferation, one must reflect about the tubular condition of the root canal and how this could influence the proliferative activity of this tissue.

The pioneer study of the biologist Selye (13) has given us the opportunity to appreciate the considerable potentiality of connective tissue ingrowth into the lumen of a tube implanted into the subcutaneous tissue of rats. Later, Torneck (14) verified that the ingrowth was dependent on the tube's diameter and not on its length. Similar results were achieved by Phillips (15).

Strong evidence, supporting the argument that the apical diameter is important to periapical repair are the favorable results presented by open-apex endodontic therapy. In such conditions, the apical canal is naturally wide and, certainly, this is the major reason for the good results invariably obtained independent of the filling materials applied.

The purpose of this work was to study the effect of canal diameter enlargement in the apical portion of the root as an influential factor in the periapical repair process after endodontic treatment. Features to be evaluated are: (a) the occurrence of connective tissue ingrowth from the periodontal ligament and how this is influenced by varying the diameter of the foramen and the level of the canal filling; and (b) the type of connective tissue resulting from the repair process.

We chose not to use materials or conditioners that stimulate tissue proliferation in order to avoid any bias in the interpretation of the results.

MATERIALS AND METHODS

One-hundred and thirty-four root canals in 13 mongrel dogs 2 to 3 yr old were treated endodontically. Under general anesthesia, with pentobarbital (30 mg/kg), the teeth were isolated by rubber dam. Access openings were made in the crowns, permitting pulp extirpation with barbed broaches or Kerr files, based on radiographic measurements of the root canals.

The canals were instrumented 2 mm beyond the apices, as confirmed by radiograms with files in the root canals. They were enlarged up to #40, 60, or 80 Kerr files. The canals were irrigated frequently during instrumentation with Tergentol, 28% lauryl diethylene glycol ether sodium sulfate (Searle, Chicago, IL), and dried with absorbent paper points.

The root filling consisted of gutta-percha cones and Endomethasone (powder: zinc oxide, bismuth subnitrate, dexamethasone, hydrocortisone, diiodotymol, paraformaldehyde; liquid: eugenol (Septodon, France)), as a sealer. The single cone method was used, varying the obturation distance between 1 and 3 mm short of the foramen as confirmed by radiographs. All access openings were sealed with reinforced zinc oxide-eugenol (IRM; L.D. Caulk, Milford, DE).

The animals were killed after 3, 7, 30, and 120 days by perfusion with a solution of formalin following the technique of Bramante et al. (16). Segments of the jaws containing the teeth were then blocked and submitted for roentgenographic examination. The specimens were fixed in 10% neutral buffered formalin solution for 48 h and decalcified in formic acid and sodium citrate, according to the method of Morse (17), for a period of 20 to 30 days. The roots were separated one by one and prepared for histological examination in the usual manner. The specimens were embedded in paraffin and serially sectioned to an average thickness of 7 μm. They were than stained with hematoxylin and eosin.

Distribution of the number of canals relative to the experimental time period, filling distance, and canal diameters are shown in Table 1.

RESULTS

Radiographic Observations

The radiographs were mainly used to control root length measurements and to observe the apical limits of filling.

One major observation was the verification of two cases showing periapical lesions, later confirmed by histological examination as periapical chronic inflammation.

Histological Observations

The microscopic observations revealed, initially, that the instrumentation perforated the thick base of cementum and established a vast communication between the canal and the periodontal ligament. The overinstrumentation also caused a rupture in the periodontal ligament and alveolar bone. In several cases fragments of cementum or dentinal chips were scattered into the periapical area, detached by the mechanical action of the instruments.

More detailed descriptions of the relevant repair aspects found in the various time periods follow.

AT 3 DAYS

In all 16 cases disorganization of the periodontal ligament occurred with an intense inflammatory reaction. Blood clots and scattered fragments of mineralized tissue in the apical canals were caused by the trauma of mechanical instrumentation (Fig. 1).

TABLE 1. **Distribution of the number of canals relative to the experimental time period, filling distance, and canal diameter**

Diameter of Instrument Used	Filling Distance Relative to the Apical Foramen (mm)	Experimental Time Period (days)				
		3	7	30	120	Total
40	1–2	2	2	4	12	20
	3	2	3	6	10	21
60	1–2	3	3	4	11	21
	3	3	3	4	10	20
80	1–2	3	3	4	16	26
	3	3	4	6	13	26
Total		16	18	28	72	134

FIG 1. Three days after endodontic treatment. The root canal was overinstrumented with file #60. The periapical region is disorganized and shows an intense inflammatory reaction due to the mechanical trauma (hematoxylin and eosion; original magnification ×30).

AT 7 DAYS

In all 18 cases a reorganization process of the periodontal ligament projected loosely constituted granulation tissue into the interior of the apical canal space.

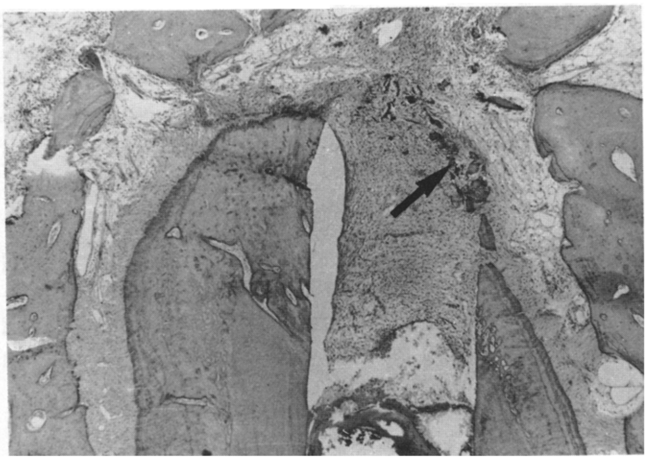


FIG 2. Seven days after endodontic treatment. The root canal was overinstrumented with file #40. Incipient granulation tissue is proliferating in the periapical region. The *arrow* points to tissue fragments created by instrumentation (hematoxylin and eosin; original magnification ×30).

In 10 cases the granulation tissue presented a chronic inflammatory infiltrate, alternating with various degrees of neutrophilic exudation.

In the remaining eight cases, the proliferative granulation tissue became more fibrotic even though still incipient, yet, free of inflammatory infiltration (Fig. 2).

In general, there was a vestige of fibrin on the upper surface of the granulation tissue in the canal; however, in the apical periodontal regions there appeared to be a great number of mineralized tissue fragments detached during instrumentation (Fig. 2).

There was no apparent difference between the underfilling cases of 1 to 3 mm or in the variation of widening with files #40, 60, and 80.

AT 30 DAYS

The microscopic analysis disclosed that invariably the connective tissue ingrowth into the interior of the apical canal formed an artificial "pulp stump." This tissue was characterized by an increased cellularity and intense fibroblastic and angioblastic proliferation free of inflammatory infiltration. An incipient fibrous density can be seen in Fig. 3.

A thin cellular cementum coat occurred next to the internal face of the canal wall, beginning at the foramen and extending in a coronal direction.

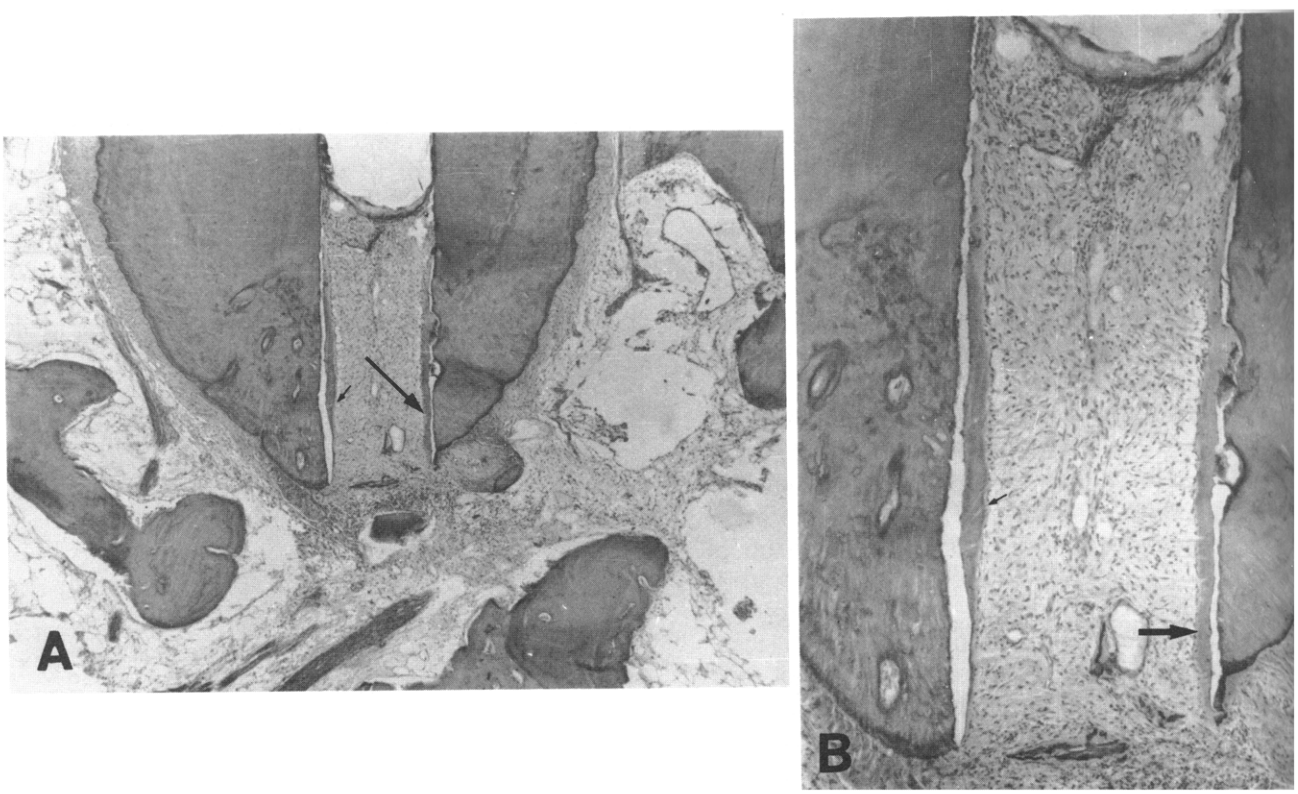


FIG 3. *A*, Thirty days after endodontic treatment. Overinstrumentation was made with file #40. Periapical connective tissue has grown into the root canal. Observe the formation of a thin cellular cementum layer on the internal face of the canal wall (*arrows*) (hematoxylin and eosin; original magnification ×30). *B*, Higher magnification of *A* to show the connective tissue ingrowth rich in fibroblasts and angioblasts and free of inflammatory infiltrate. *Arrows* show the cellular cementum coat (hematoxylin and eosin; original magnification ×50).

The portions of the canal lined by cementum sheltered the pulpal components which appeared to be quite normal. The periodontal ligament was normal and the alveolar bone, touched by instrumentation, had developed new bone (Fig. 4).

The dentin fragments detached during instrumentation were involved by the mineralizing process similar to that of the cementoid tissue (Fig. 5). In one case the fragment did not constitute a core for further precipitation of minerals, but was involved by an exudative inflammation rich in neutrophils (Fig. 6). There were no confirmed differences in the connective tissue ingrowth relative to the variation in the caliber of apical canal.

AT 120 DAYS

Evidence of tissue maturation, expressed by a reduction of cellularity, a greater fibrous density, and well-differentiated vascular walls, was independent of the space size created by underfilling short of the apical foramen (Figs. 7 and 8). A substantial increase in the cemental coat thickness had formed on the internal surface of the canal walls. They showed incremental and lamellar deposition characteristics, linking and establishing a continuity with the lateral root cementum.

In the posttreatment period, it was possible to verify some structural tissue differences in the reparative process among the different sizes of canal enlargement. This was reflected particularly in the alveolar bone growth, with projections into the interior of the apical canal, especially in the cases enlarged up to file size 80 (Fig. 9).

The regenerated periodontal ligament appeared to be normal. The cemental or dentinal fragments scattered in the interior of the tissues became coated by a matrix material looking like cementoid (Fig. 8).

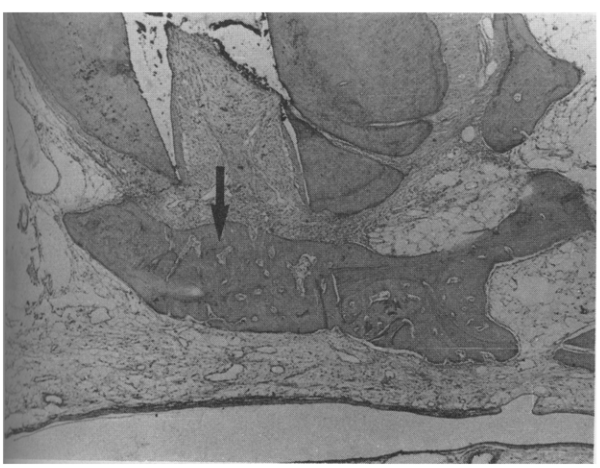


FIG 4. Thirty days after endodontic treatment. Overinstrumentation was made with file #60. The periapical region shows the ingrowth of connective tissue into the apical canal. The periapical periodontal ligament and bone that were destroyed during overinstrumentation are now rearranged and new bone has formed (*arrow*) (hematoxylin and eosin; original magnification ×30).

FIG 5. Thirty days after endodontic treatment. Overinstrumentation was made with file #80. The *arrow* shows fragments resulting from overinstrumentation that served as nuclei for further mineralization. Note the cementoid tissue next to the internal surface of the canal (hematoxylin and eosin; original magnification ×30).

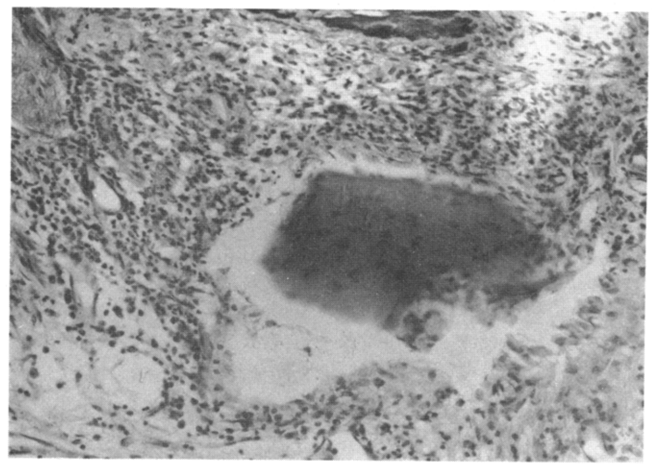


FIG 6. Higher magnification of Fig. 3*A* to show one fragment detached during instrumentation involved by an inflammatory reaction rich in neutrophils (hematoxylin and eosin; original magnification ×120).

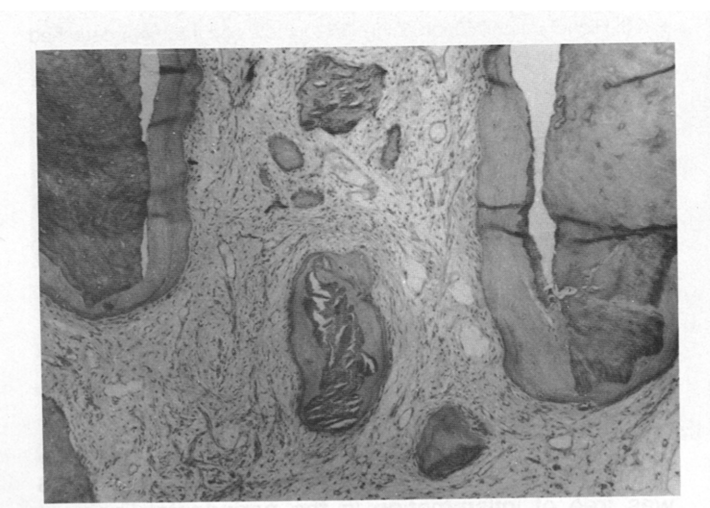
Ingrowth of mature connective tissue was observed in 70 of the treated canals, but two cases showed an intense inflammatory reaction (Fig. 10).

RESULTS

These results convincingly confirm the evidence described by Davis et al. (7). They showed that overinstrumented premolar canals of dogs, filled with gutta-percha and Rickert cement 3 mm short of the foramen, demonstrated excellent healing. The repair process was free of inflammation in the periodontal ligament space and extended inclusively into the prepared and unfilled part of the canal. The present study also confirmed the connective fibrous tissue ingrowth, the cemental formations on the internal walls of the canals, and the occurrence of osteogenesis in the axial part of

FIG 7. One-hundred and twenty days after endodontic treatment. Overinstrumentation was made with file #40. The periapical region shows an advanced degree of maturation of the connective tissue ingrowth. Observe a substantial increase of the cementum coat thickness formed in the internal surface of the canal wall (*arrows*) (hemotoxylin and eosin; original magnification ×50).

FIG 9. One-hundred and twenty days after endodontic treatment. Overinstrumentation with file #80, showing the growth of alveolar bone (*arrows*) into the apical canal (hematoxylin and eosin; original magnification ×30).



Overinstrumentation with file #80. Fragments of dentin are coated with a matrix tissue similar to a cementoid tissue. On the internal surface of the canal wall, the cemental coat is prominent (hematoxylin and eosin; original magnification ×50).

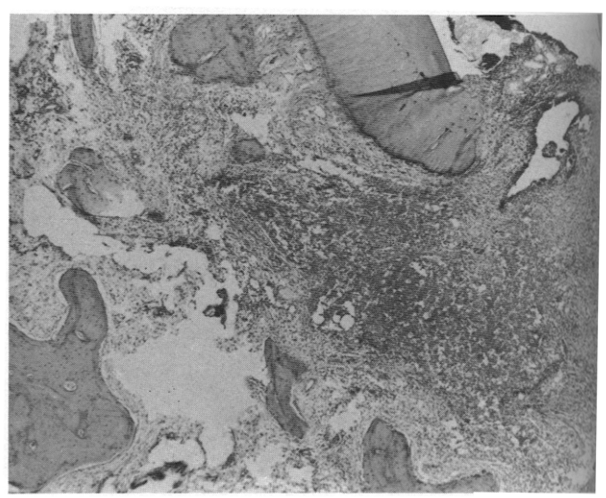


FIG 10. One-hundred and twenty days after endodontic treatment. Overinstrumentation with file size 80. The periapical region is intensely inflamed and there is no ingrowth of connective tissue into the root canal (hematoxylin and eosin; original magnification ×30).

the ingrowth tissue. In spite of the considerable quantity of hard tissue formation, it was not verified that total recovery of the vacant space by mineralized tissue was always coated by a fibrous connective tissue capsule, free of inflammation.

The phenomenon of osteogenesis and cementogenesis in the apical unfilled space of the canal, in the absence of stimulating materials for mineralization, was previously described by Ostby (4). He studied the influence of blood clots in the posttreatment periapical healing of root canals in human and dog teeth.

Otherwise the results of the present investigation are in discordance with those of Seltzer et al. (8). They verified a persistent inflammatory reaction in the periapical region, after 6 months and 1 yr, in human and monkey teeth whose canals were overinstrumented and filled short of the conventional limit. Also, the results are not in accordance with the conclusions of Myers and Fountain (18), who stated that overinstrumentation with widening of the apical canal did not aid the connective tissue ingrowth and periapical repair after endodontic treatment. They studied pulpless teeth previously contaminated, which was similar to a previous study (1).

Myers and Fountain (18) also verified that the connective tissue ingrowth into the interior of the apical canal was not activated by a blood clot matrix or its artificial substitutes, as had been concluded by Ostby (4). Horsted and Ostby (6) reconfirmed the importance of the clot as a matrix for the proliferation of connective tissue. They considered contamination to be an adverse factor and denied any importance of the widening of the apical canal. For them, the normal diameter would be enough to permit the tissue ingrowth, which would be in agreement with Strindberg (19). He found a significantly higher percentage of success in cases of widened apical canals with Hedstrom #1 files when compared with cases where the canals were widened with greater caliber files.

However, some support for the relevance of widening of the apical canal is found in the studies of Holland et al. (9–12) in monkey and dog teeth with overinstrumentation using files 40, 60, and 80. They found that the apical foramen sealed with mineralized tissue or connective tissue ingrowth, even after the pulp stump had been eliminated by instrumentation. These authors used different mixtures of calcium hydroxide, as filling materials, today known as mineralization and proliferation conditioners of connective tissues. This provided an additional factor for the analysis of influence of widening the canals. In cases in which zinc oxide-eugenol (11) was used, the results were adverse, usually with the presence of periapical inflammation, frequently characterized by microabscesses in the periodontal ligament.

The cemental or dentinal fragments scattered in the apical tissues, resulting from instrumentation, probably constitute mineralized tissue nuclei, which confirms previous reports (7, 8, 20, 21). Paradoxically, when calcium hydroxide is used, these dentinal chips appear to be unfavorable factors for connective tissue repair (22–25).

It seems that radiographic analysis is not adequate to evaluate mineralized tissue deposition in the unfilled apical part of the canal. This is true at least in dogs that have thick cortical bone.

The favorable influence of widening the caliber in the apical third of the canal, found in this research, must be confirmed in future investigations in cases of pulpal necrosis or infection.

CONCLUSIONS

Under the conditions of this experiment with dogs, the following conclusions were made:

1. The pulp stump, destroyed by instrumentation beyond the apical foramen, within certain conditions, can be reconstituted by the ingrowing proliferation of connective tissue from the periodontal ligament.
2. Widening the diameter of the apical portion of the root canal creates conditions which allow for connective tissue ingrowth into the unfilled apical canal space during the posttreatment reparative process, even when the filling is short of the apical limit (underfilling up to 3 mm).
3. There were no significant differences in reparative processes among the three diameter sizes of the apical third of the canals (with files 40, 60, or 80). The exception was alveolar bone growth into the interior of the canal; in some cases enlarged to size 80 file, in 120-day observation periods.
4. Excluding the inflammatory reaction resulting from the overestimation trauma, verified in the first week posttreatment, the connective granulation tissue was free of inflammation. In time, a progressive maturation process, characterized by reduced cellularity, greater fibrous density, and well-differentiated vascular walls, occurred.
5. Even in the absence of filling material tissue conditioners mineralization, of a cementum type, was deposited on the internal wall surfaces of the apical canals. A progressive increase at thickness occurred with time.
6. In addition to cementum formation, bone formation occurred with the ingrowth of alveolar bone into the interior of the apical canal. Particles of dentine and cementum resulting from instrumentation were scattered in the periodontal ligament and became coated with a cementoid-type matrix.

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Erratum

In the July 1985 issue, the article by Jacobson et al., The Effectiveness of Apical Dentin Plugs in Sealing Endodontically Treated Teeth, Vol. 11, No. 7, pp. 289–293, the authors' affiliations were inadvertently omitted:

Dr. Jacobsen, Dr. Bery, and Dr. BeGole are affiliated with the Department of Endodontics, College of Dentistry, University of Illinois, Chicago, IL. Address requests for reprints to Dr. Jacobsen.

We regret any confusion this may have caused.