Fate of the Tissue in Lateral Canals and Apical Ramifications in Response to Pathologic Conditions and Treatment Procedures

Domenico Ricucci, MD, DDS, and José F. Siqueira Jr, DDS, MSc, PhD†*

Abstract

Introduction: This article reviews and reports on the histopathologic and histobacteriologic status of the tissue in lateral canals and apical ramifications (LC/AR) in diverse clinical conditions as well as in response to endodontic treatment. **Methods:** In total, serial sections from 493 human tooth specimens obtained by extraction or apical surgery were screened for the presence of LC/AR. **Results:** LC/AR were observed in about 75% of the teeth. In clinically vital teeth, vital tissue was consistently found in LC/AR. In teeth with periodontal disease, the whole pulp became necrotic only when the subgingival biofilm reached the main apical foramen. In teeth with pulp exposure by caries, the tissue in LC/AR remained vital as far as the pulp tissue in the main canal did so. When pulp necrosis reached the level of the LC/AR, the tissue therein was either partially or completely necrotic. Chemomechanical preparation partially removed necrotic tissue from the entrance of LC/AR, whereas the adjacent tissue remained inflamed, sometimes infected, and associated with periradicular disease. Vital tissue in LC/AR was not removed by preparation. In cases in which lateral canals appeared radiographically "filled," they were actually not obturated, and the remaining tissue in the ramification was inflamed and enmeshed with the filling material. **Conclusions:** Overall, the belief that lateral canals must be injected with filling material to enhance treatment outcome was not supported by literature review or by our histopathologic observations. It appears that strategies other than finding a technique that better squeezes sealer or gutta-percha within LC/AR should be pursued to effectively disinfect these regions. *(J Endod 2010;36:1–15)*

Key Words

Apical periodontitis, apical ramification, endodontic treatment, lateral canal, periodontal disease, pulpitis

From *Private practice, Rome, Italy; and the †Department of Endodontics and Molecular Microbiology Laboratory, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, Brazil.

Address requests for reprints to Dr Domenico Ricucci, Piazza Calvario, 7, 87022 Cetraro (CS), Italy. E-mail address: dricucci@libero.it.

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Lateral and apical ramifications of the main root canal are formed after a localized fragmentation of the epithelial root sheath develops, leaving a small gap, or when blood vessels running from the dental sac through the dental papilla persist. Dentinogenesis does not occur in this specific area, giving rise to a canal containing small blood vessels and sometimes nerves. Although ramifications contain connective tissue and blood vessels, this is not usually regarded as collateral blood supply and consequently provides little contribution, if any, to pulp function, except possibly for the ramifications located in the apical 1–2 mm of the canal (1, 2). Ramifications can be observed anywhere along the length of the root, but they occur more commonly in the apical portion and in posterior teeth (3). In 73.5% of the cases, ramifications are found in the apical third of the root, in 11% in the middle third, and in 15% in the coronal third (4). For consistency, we refer to ramifications as furcation canals, lateral canals, and apical ramifications. This article deals with the latter two.

Ramifications comprise potential pathways through which bacteria and/or their products from the necrotic root canal might reach the periodontal ligament and cause disease, and likewise, bacteria from periodontal pockets might reach the pulp. Lateral canals and apical ramifications are arguably difficult to reach, clean, disinfect, and fill during treatment. Their possible clinical significance has long called the attention of clinicians and researchers as to how and whether these ramifications should be approached and what is the fate of the tissue present therein after treatment. The present study reviews and reports on the histopathologic and histobacteriologic status of the tissue in lateral canals and apical ramifications in diverse clinical conditions as well as in response to endodontic treatment. In light of these observations, the influence of lateral canals and apical ramifications on treatment outcome is discussed.

To Fill or Not to Fill: Is This the Question?

The actual need to fill lateral canals has been a bone of contention among endodontists. Schilder (5–7) postulated that the main objective of endodontic treatment procedures should be the cleaning and filling of the root canal in its entire extent, also including all lateral canals and apical ramifications. After him, some advocated that "those who do 3-D obturation techniques have historically claimed technical and even moral superiority over those who do techniques that only fill primary canals" (8). Thus, clinician's skill or the technical ability of filling lateral canals has been considered by many as a measure of endodontic excellence. Personal opinions based on impression left aside, it has been reported that "unfilled lateral canals" might be associated with post-treatment disease (9). It has also been claimed that lateral canals harboring inflamed and/or infected material might cause pain during endodontic treatment (10).

On the basis of these assumptions, the idea that lateral canals and apical ramifications should be filled for successful endodontic therapy has been accepted by many clinicians and researchers in an almost dogmatic manner. In this context, a myriad of in vitro studies have aimed at evaluating the ability of different obturation techniques of filling lateral canals (11–17). Curiously, most of these studies reported that no significant differences were observed for the efficacy of different techniques in forcing sealer into the lateral canal, even though thermoplasticized techniques obviously also tended to force gutta-percha in many specimens. It has also been found that lateral canals are filled less frequently after use of calcium hydroxide medication (18).

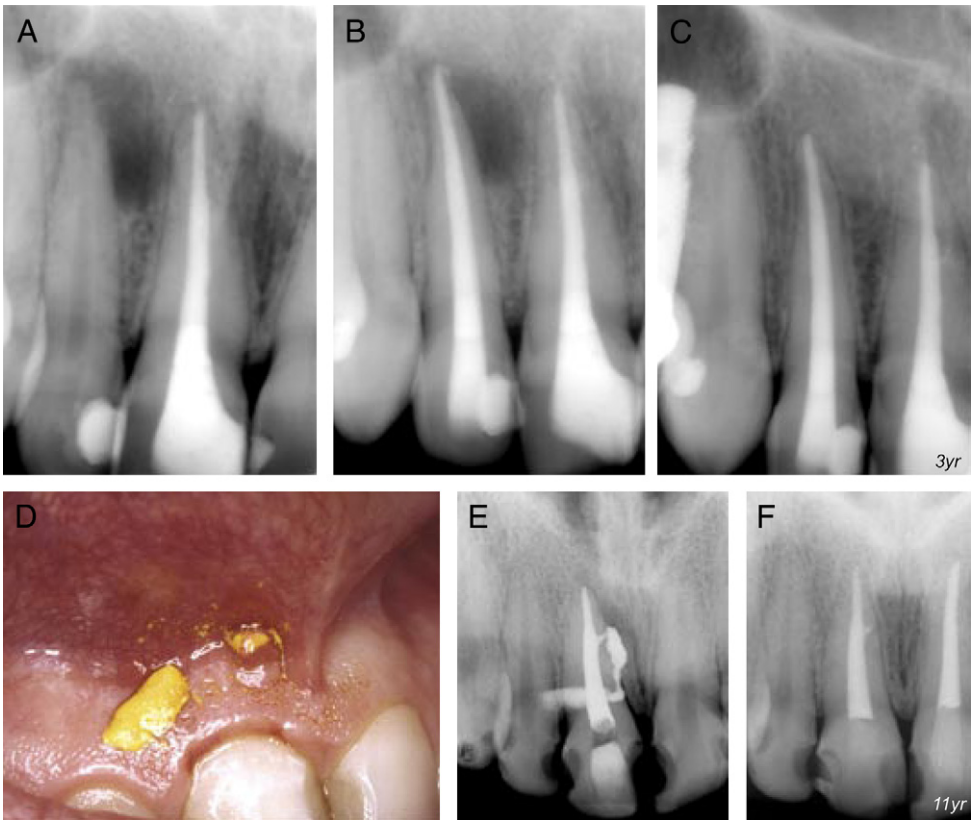


Figure 1. (*A*) Maxillary lateral incisor with necrotic pulp and a large radiolucency involving the apex and the mesial aspect of the root. Patient presented with spontaneous pain. Tooth was tender to percussion. Presence of lateral canals, although not visible in the radiograph, is likely under this circumstance. (*B*) After instrumentation and 1-week calcium hydroxide medication, the canal was obturated with laterally compacted cold gutta-percha and a sealer. Note that no lateral canal/ramifications were injected with filling material. (*C*) Three-year follow-up radiograph. The lateral lesion appears completely healed. (*D*) This is a case in which the canal of a maxillary central incisor with necrotic pulp was instrumented and medicated with an iodoformic paste. After rubber dam removal, some medicament pushed through the sinus tract was seen on the buccal mucosa. (*E*) The material penetrated into a lateral lesion through a large lateral canal and followed the course of the sinus tract. Because of its high radiopacity, it permitted to obtain sort of a "fistulography". The canal was then obturated after 40 days, when the sinus tract appeared clinically healed, with chloroform modified lateral compaction technique and sealer. Postoperative radiograph revealed that the filling material penetrated into the large lateral canal (not shown). No symptoms emerged in the postoperative period. (*F*) Eleven-year follow-up radiograph showing that the lateral lesion healed completely, with the radiographic image of a lamina dura following the entire root outline.

Nevertheless, there is no consensus as to the need to fill lateral canals to optimize the treatment outcome. Weine (10) admitted that although the frequency of lateral canals has been demonstrated to be high, they are not so frequently revealed radiographically after root canal filling. Even so, failure to fill lateral canals has been assumed not to lead to endodontic treatment failure characterized by a post-treatment lateral lesion in the huge majority of cases.

Admittedly, filling ramifications would be a desirable objective of treatment only when there is a chance for bacterial traffic from the canal to the periodontal ligament, ie, in previously necrotic infected cases in which bacteria were left undisturbed in the main canal or in the ramification. However, clinical experience reveals that lateral lesions might heal after endodontic treatment, even when the lateral canals are not filled (Fig. 1*A–C*). Indeed, Camps and Lambruschini (19) stated that filling of lateral canals is not always necessary for a successful root canal treatment. A study examining root canal–treated teeth from human cadavers reported no relationship between unfilled lateral canals and the status of inflammation at the periradicular tissues (20).

However, it must be stated that lateral canals and apical ramifications have been implicated with endodontic treatment failure when they are sufficiently large to harbor significant numbers of bacteria and to provide these bacteria with frank access to the periradicular tissues (21–26). Therefore, disinfection of lateral canals and apical ramifications in cases of pulp necrosis and apical and/or lateral periodontitis should be considered an important goal of the treatment, although difficult to achieve with current treatment procedures. What remains to be determined is whether forcing the filling material into ramifications exerts any significant influence on the outcome by killing bacteria in these ramifications as a result of the antibacterial effects of the material or by promoting an effective lateral antibacterial (bacteria-tight) seal. It is important to point out that the antibacterial effects of filling materials are usually weak and only transient, reaching a peak of effectiveness before setting (27–33). Moreover, attainment of a predictable antibacterial seal provided by the material injected into the small and usually tortuous ramification is highly unlikely and has not been demonstrated.

As one can infer from this discussion, although the influence of filling lateral canals on treatment outcome has been subject of debate and personal opinion (34), there is no clear scientific evidence as to this matter. Because bacteria located in lateral canals and in apical ramifications can certainly influence the treatment outcome, it seems advisable that efforts should be made to apply therapeutic strategies to reach and incorporate these areas in the disinfection process.

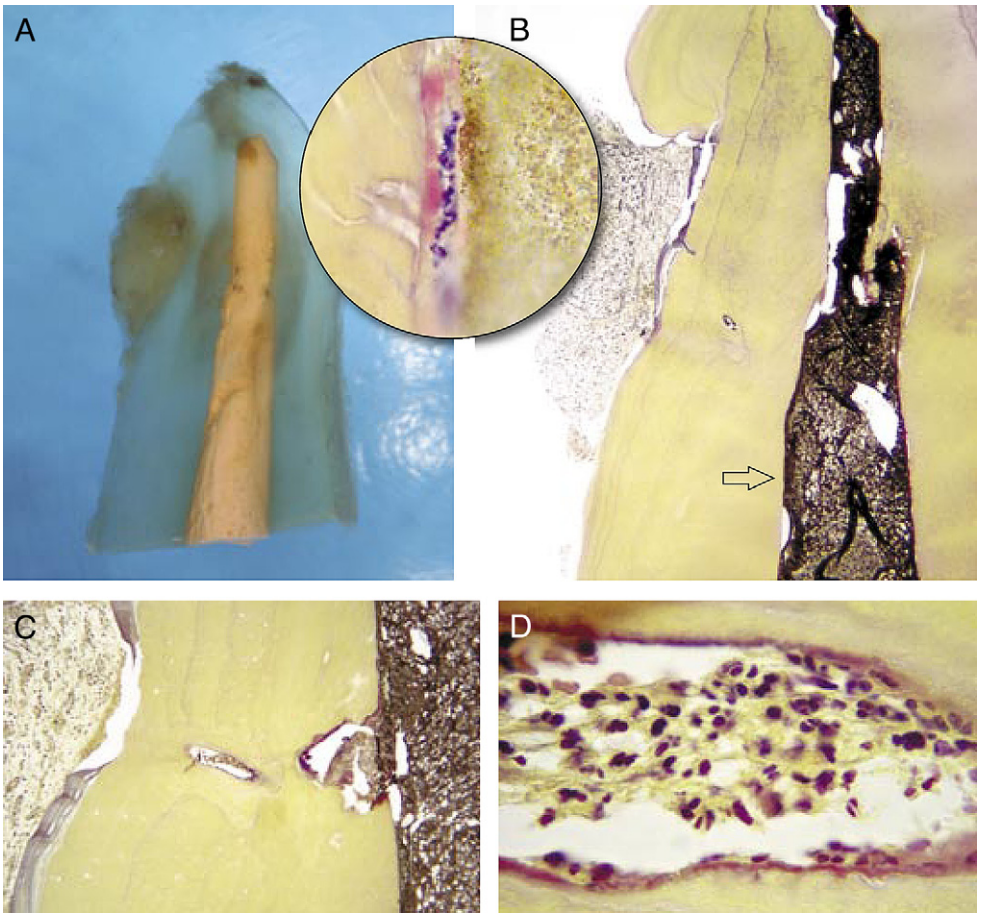


Figure 2. Maxillary first molar in a 35-year-old patient. Tooth had been treated endodontically 19 years before and had remained asymptomatic for the whole period until severe symptoms appeared. Clinical examination revealed an oblique fracture, and the tooth was extracted. (*A*) After extraction pathologic tissue was seen on the lateral aspect of the palatal root apex. This was more evident at the end of the demineralization procedure after clearing of the specimen before embedding in paraffin. (*B*) Histologic serial sections revealed a lateral canal connecting the main canal and the lesion (Taylor's modified Brown & Brenn; original magnification, ×25). Inset shows a higher magnification of the area indicated by the arrow in (*B*). Bacteria are seen at the interface between the root canal wall and the filling material (Taylor's modified Brown & Brenn; original magnification, ×1000). (*C*) Section taken approximately 30 sections from that in (*B*), displaying part of the lateral canal course, and the connection with the main canal (Taylor's modified Brown & Brenn; original magnification, ×100). (*D*) Higher magnification of the lateral canal contents. Inflammatory tissue can be visualized, but bacteria are absent (Taylor's modified Brown & Brenn; original magnification, ×1000).

Whether this can be achieved by forcing gutta-percha and/or sealer into the ramification is highly questionable and remains to be proved.

Considerations Based on Clinical Observations

Lateral canals, with few exceptions, are not visible in the preoperative radiographs (Fig. 1*A*). The occurrence of lateral canals can be usually suspected only when there is a localized thickening of the periodontal ligament on the lateral surface of the root or when a frank lateral periodontitis lesion is present (Fig. 1*A*). After root canal obturation, lateral canals can also be visualized on radiographs when a consistent amount of filling material is forced into the ramifications by compaction (usually sealer but also gutta-percha in thermoplasticized techniques) (Fig. 1*D–F*).

Lateral canals and apical ramifications might be revealed by a variety of filling techniques and are probably more frequently indicated in necrotic cases than in vital pulps; this supposition is based on both clinical impression and the alleged degree of resistance offered by the type of tissue present in the ramification (10). It is salient to emphasize our usage of the terms *revealed*, *indicated*, and *injected* instead of *filled* when referring to the observation of filling material in ramifications. This is because actual filling of lateral canals under in vivo conditions has not been consistently demonstrated. In fact, our histopathologic observations clearly demonstrated that lateral canals and apical ramifications were never completely filled by endodontic materials.

One important question that arises as to the issue of lateral canals is, given their high frequency, why lateral periodontitis lesions associated with these ramifications are not so frequent. There is no clear answer to this question, but it might be related to the size and patency of the ramification as well as the histologic/microbiologic condition of its content at a certain moment of the disease process. A morphologic study of 100 permanent molars revealed that 79% had lateral/accessory foramina with diameters ranging from 10–200 μm (35). The largest diameter was nearly twice to 3 times smaller than the mean diameters reported for the main apical foramen (36–38). These differences in diameters between the main apical foramen and lateral/accessory foramina might help explain why apical periodontitis is observed far more than lateral periodontitis.

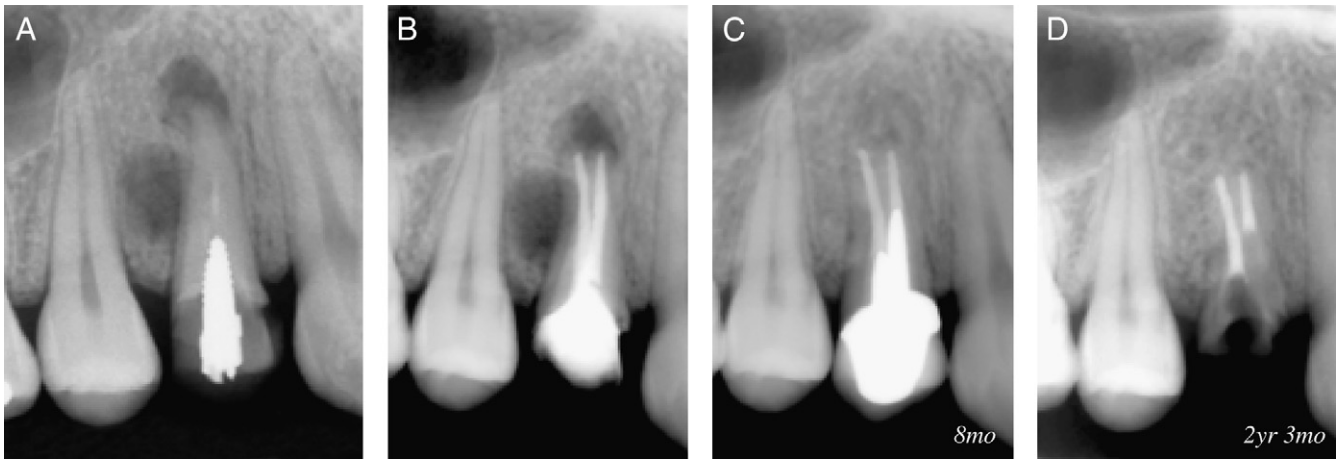


Figure 3. (*A*) Maxillary first premolar in 16-year-old girl. Tooth appears to be treated endodontically and restored with a screw-post and composite. Two large separate radiolucencies, one apical and one lateral, are present. Tooth is asymptomatic. (*B*) Nonsurgical retreatment was performed. Canals were obturated after 2 weeks of calcium hydroxide medication. Note that no lateral canals appeared injected with obturation materials. Tooth was restored with a cast post and core and a ceramo-metallic crown. (*C*) Eight-month follow-up. Considerable bone formation has taken place. (*D*) Patient presented 2 years and 3 months after treatment with the restoration missing. Radiograph shows complete healing of the 2 radiolucencies.

Large and patent lateral foramina might, however, allow larger numbers of bacteria and bacterial products to reach and contact a larger area of the lateral periodontal ligament to cause disease. The amount of bacterial irritants in small ramifications, with small volume capacity and small exiting foraminal area, might be insufficient to induce significant disease to be discernible radiographically. Thus, a definite lateral lesion often indicates the presence of a significantly large lateral canal with sufficient infected necrotic tissue to give rise to periodontal inflammation.

Given the direct communication to the periodontal ligament, the tissue in lateral canals is nourished by the rich periodontal blood supply, offering a significant resistance against necrosis and further bacterial invasion. It appears that the tissue in lateral canals becomes necrotic and thoroughly colonized by bacteria only in cases with long-standing pulp necrosis (24, 25). The presence of vital, albeit inflamed, tissue into the ramification is also the explanation why a lateral lesion might heal without squeezing materials into any lateral canal. Bacteria in the main canal elicit inflammation in the tissue within the lateral canal. Inflammation might extend to the periodontal ligament, being sustained by bacterial products (not necessarily bacterial cells) that diffuse through the tissue to reach the periodontal ligament. For instance, low-molecular-weight bacterial products, such as polyamines, hydrogen sulfide, butyrate, propionate, and N-formyl-methionyl-leucyl-phenylalanine peptides, might promptly diffuse through the connective tissue and conceivably evoke inflammation at a certain distance from the place where the bacterial cells are located (39). These products might be toxic to host cells and tissues, might stimulate the synthesis and release of proinflammatory cytokines, and might act as chemoattractants to polymorphonuclear leukocytes (PMNs) (39). All these events might give rise to a lateral lesion, which in turn is not associated with infected necrotic tissue in the ramification. In these cases, the lateral lesion simply heals because root canal procedures, removing the bacterial content of the main canal, interrupt both the aggression caused to the tissue in the ramification and the egress of bacterial products through the lateral canals up to the periodontal ligament. Fig. 2 illustrates this situation. A lateral lesion is discernible on the palatal root of a maxillary first molar, and no bacteria are seen in the lateral canal, which contains inflamed tissue. Bacteria indeed occurred in the main canal between the root canal wall and the obturation.

Weine (40) reported on 3 types of lateral lesions that can be radiographically observed. These conditions might represent different stages until the ultimate third type of condition emerges:

(1) Lateral lesion with no apical lesion: as the infection progresses apically, it might reach a sufficiently large lateral canal to allow a substantial amount of bacteria and bacterial products to reach the lateral periodontium to cause inflammation. In these cases, it is possible that the pulp tissue apical to the ramification is still vital, but in some cases it is arguably already necrotic and infected, and the development of an apical lesion is just a matter of time, or it is already established but still cannot be visible on radiographs.
(2) Separate lateral and apical lesions: if the pathologic process advances without professional intervention, an apical periodontitis lesion can also be visible radiographically. This means that sufficient numbers of bacteria and their products are concomitantly egressing from the apical and lateral foramina to cause disease (Fig. 3).
(3) Coalescence of lateral and apical lesions: in some cases, the second condition can progress to this third one, also regarded as a "wraparound" lesion (40) (Fig. 1*A*).

In all these conditions, assuming that the size of the lateral canal is large enough and that it might frequently contain necrotic pulp tissue, there is a high possibility for these canals to be injected with filling materials, regardless of the technique used. As aforementioned, the validity of filling these lateral canals and whether they are actually sealed are only conjectural.

Histopathologic and Histobacteriologic Observations

There are no studies in the endodontic literature consistently showing the fate of the tissue in lateral canals and apical ramifications, as well as the tissue adjacent to these ramifications, in response to different pathologic conditions or treatment approaches. This review takes advantage of the large number of specimens composing the histologic collection of one of the authors (D.R.). In total, serial sections from 493 human teeth subjected to previous histopathologic analysis were screened for the presence of lateral canals and/or apical

TABLE 1. Frequency of Lateral Canals and/or Apical Ramifications (LC/AR) and Distribution of 493 Examined Teeth According to Clinical Conditions

	Untreated teeth with clinically vital pulp	Untreated teeth with clinically necrotic pulp	Root canal-treated teeth classified as success	Root canal-treated teeth classified as failure	Teeth extracted after chemomechanical procedures	Total
Tooth type*						
Maxillary						
Incisors	6 (2, 33%)	12 (3, 25%)	38 (34, 89.5%)	24 (19, 79%)	11 (7, 64%)	91 (65, 71%)
Canines	3 (1, 33%)	9 (9, 100%)	13 (8, 61.5%)	5 (4, 80%)	2 (1, 50%)	32 (23, 72%)
Premolars	12 (8, 67%)	28 (21, 75%)	37 (33, 89%)	17 (13, 76.5%)	4 (4, 100%)	98 (79, 81%)
Molars	12 (11, 92%)	41 (32, 78%)	7 (4, 57%)	7 (6, 86%)	2 (2, 100%)	69 (55, 80%)
Mandibular						
Incisors	2 (1, 50%)	5 (4, 80%)	1 (1, 100%)	2 (1, 50%)	-	10 (7, 70%)
Canines	4 (3, 75%)	2 (1, 50%)	3 (2, 67%)	3 (1, 50%)	-	12 (7, 58%)
Premolars	8 (6, 75%)	16 (12, 80%)	39 (17, 44%)	5 (4, 80%)	1 (1, 100%)	69 (40, 58%)
Molars	34 (28, 82%)	55 (48, 87%)	10 (8, 80%)	12 (8, 67%)	1 (1, 100%)	112 (93, 83%)
Total*	81 (60, 74%)	168 (130, 77%)	148 (107, 72%)	75 (56, 75%)	21 (16, 76%)	493 (369, 75%)
Reasons for extraction/biopsy						
Nonrestorability (recurrent caries or root fracture)	22	125	117	32	—	296
Periodontal disease	13	20	3	—	—	36
Orthodontic or prosthetic treatment plans	13	—	—	—	21	34
Patient accepted no treatment	33	23	28	5	—	89
Need for apical surgery	—	—	—	38	—	38
Status of tissue in LC/AR						
Vital uninflamed	54	—	39	—	7	100
Vital inflamed	6	29	9	12	2	58
Partially necrotic	—	22	35	7	5	69
Necrotic	—	79	24	37	2	142
Injected with filling material	—	—	31	7	—	38
Cementum formation narrowing the lumen	—	—	51	21	—	72
Bacteria present	—	59	—	34	1	94

*Number of teeth examined (number of these teeth with LC/AR - %)

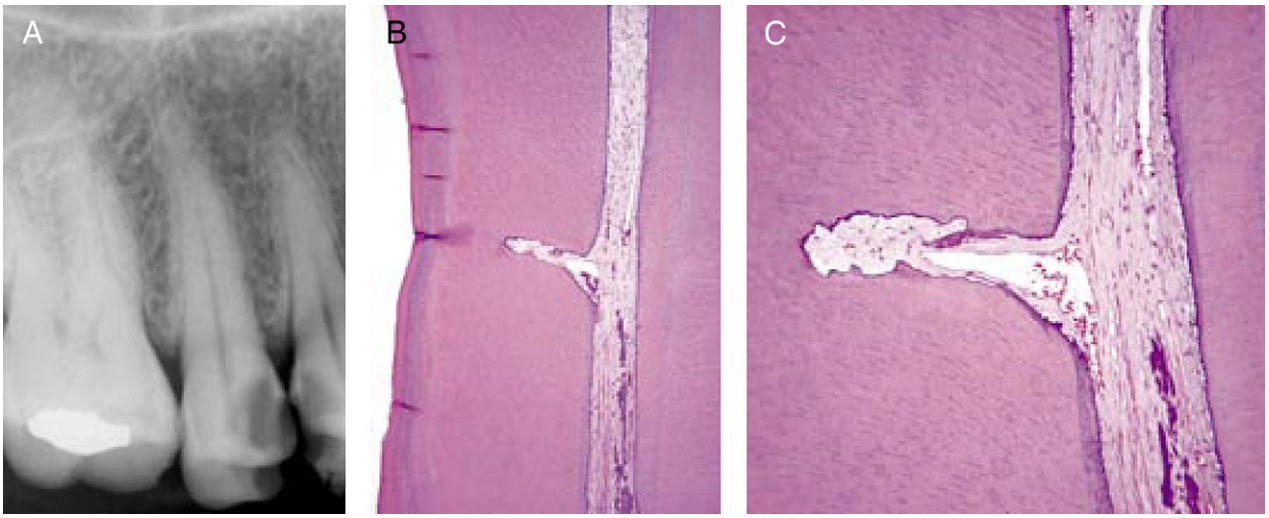


Figure 4. (*A*) Maxillary second premolar with caries penetrating to the pulp. Patient complained of severe pain and did not accept any treatment but extraction. (*B*) Longitudinal serial sections show a lateral canal at the middle portion of the root (hematoxylin-eosin; original magnification, ×50). (*C*) Vital uninflamed pulp tissue can be observed in the main canal as well as in the lateral canal (hematoxylin-eosin; original magnification, ×100).

ramifications. Specimens were obtained by extraction or apical surgery. Reasons for extraction are shown in Table 1. When present, apical periodontitis lesions were removed still attached and in the original relationship to the root tip. Specimens included clinically intact teeth; untreated teeth with carious lesions of varying degrees (from shallow to deep caries, including caries penetrating to the pulp) or coronal restorations with diverse materials; untreated teeth affected by varying degrees of periodontal disease; untreated teeth extracted with apical periodontitis lesions attached; teeth extracted after root canal chemomechanical procedures by using different techniques; root canal–treated teeth after long-term follow-up periods; and root canal–treated teeth submitted to apical surgery that presented post-treatment disease. Immediately after removal, specimens were processed for histopathologic and histobacteriologic analyses as reported previously (23, 24, 41–43). Serial sections were examined under light microscopy.

Table 1 displays detailed information on the distribution of teeth and status of the tissue in lateral canals and/or apical ramifications according to several aspects, including tooth type and clinical conditions. The frequency of lateral canals and/or apical ramifications is also shown on the basis of diverse aspects. One should be aware that these calculations might have been underrated because only 1 root was examined for some multi-rooted teeth, and only the root apex was available for analysis of cases subjected to apicoectomy. Discounting these limitations, the overall frequency of lateral canals and/or apical ramifications was about 75%. Higher frequencies (80% or more) were observed for molars and maxillary premolars.

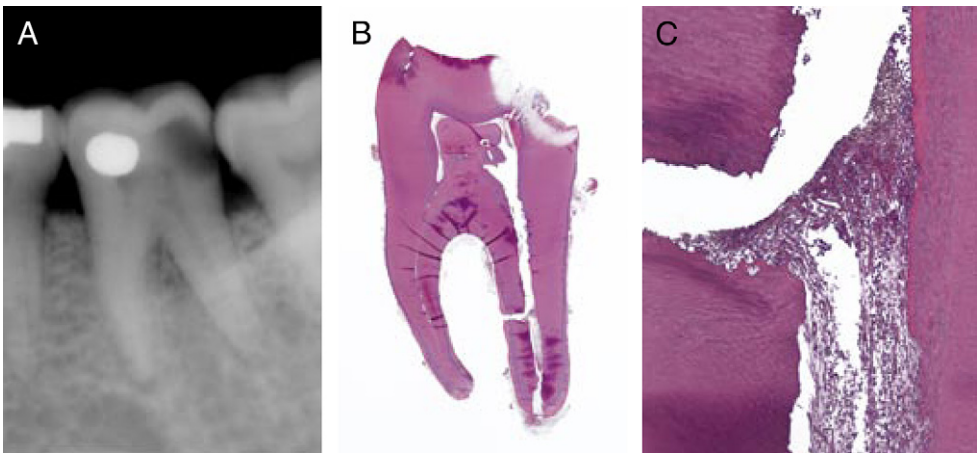
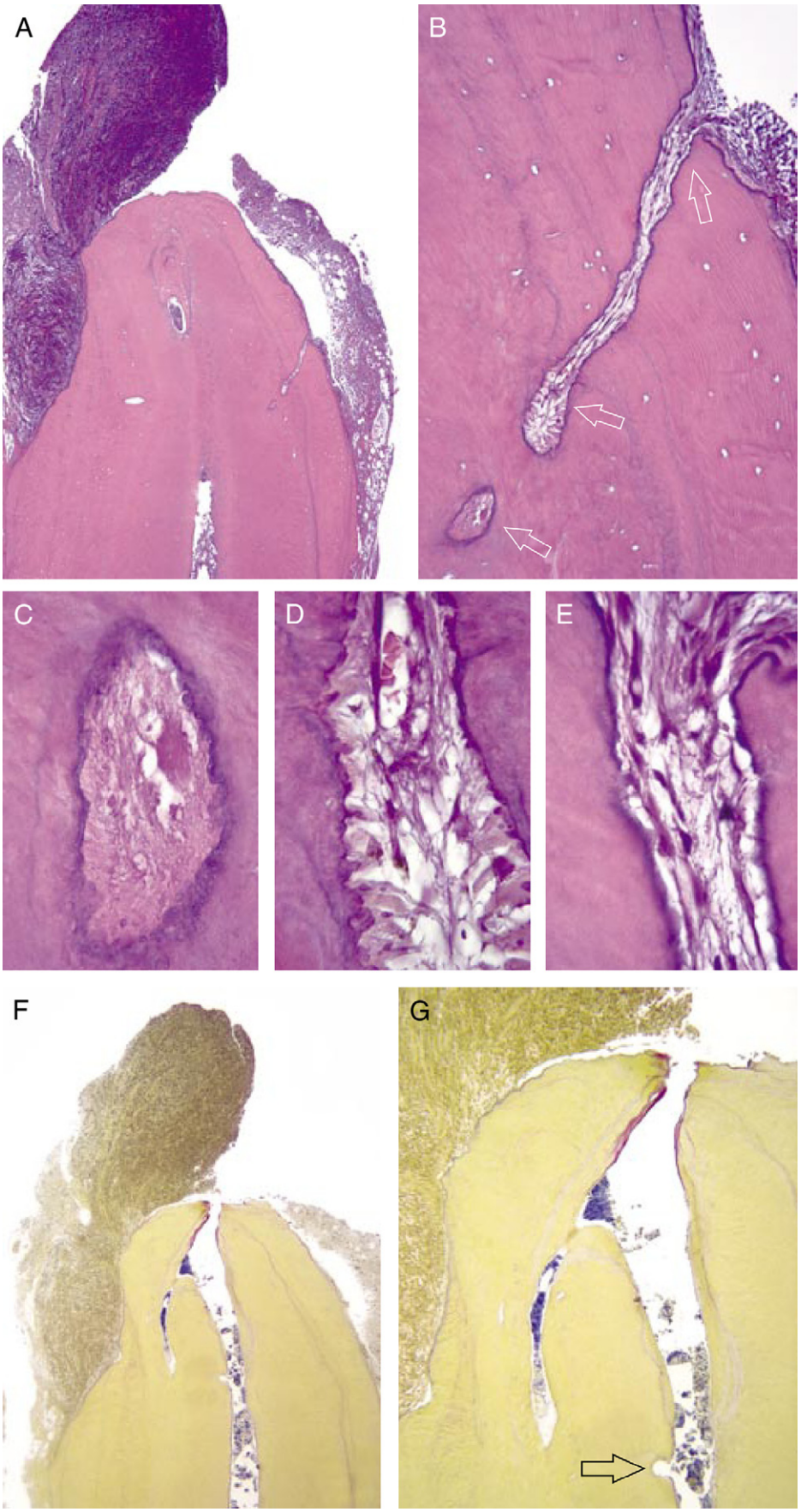


Figure 5. Sixty-year-old woman presenting with an abscess in the mandibular left quadrant. Clinically the mandibular second molar showed a buccal amalgam restoration and a large distal carious lesion. (*A*) Radiographically, the carious lesion appeared proximal to the pulp chamber, which shows extensive calcification. A small radiolucency is present on the mesial root apex, and bone loss is visible on the mesial aspect of the distal root. Patient did not accept any treatment aimed at conservation and requested extraction. (*B*) Tooth was processed for routine histology, and sections were taken on a mesiodistal plane. Overview shows the pulp stone occupying large part of the pulp chamber and a large lateral canal ending on the mesial aspect of the distal root at the transition between the apical and the middle third (hematoxylin-eosin; original magnification, ×6). (*C*) Area where the lateral canal joins the main canal. Inflamed connective tissue is present from this point to an apical direction. Note resorptive defects in dentin. Empty spaces in the pulp tissue are shrinkage artifacts (hematoxylin-eosin; original magnification, ×50).



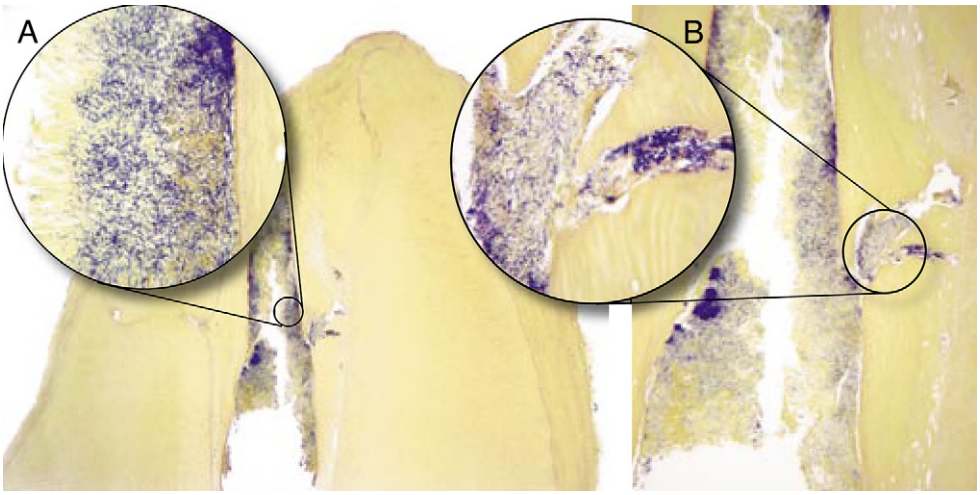


Figure 7. (*A*) Section passing through the main canal of a maxillary incisor with necrotic pulp. Lateral canal is present (Taylor's modified Brown & Brenn; original magnification, ×25). (*B*) Detail of the main canal with the lateral canal entrance. The lumen of both is filled with a dense bacterial biofilm (Taylor's modified Brown & Brenn; original magnification, ×100). Insets show higher power view of the bacterial biofilm adhered to the walls of both main and lateral canals (Taylor's modified Brown & Brenn; original magnification, ×400).

Considerations Based on Histopathologic Observations

Teeth with Different Preoperative Conditions

In teeth with clinically vital pulps, vital tissue was consistently found in lateral canals and apical ramifications. In cases of carious pulp exposure, the tissue in lateral canals and apical ramifications remained vital as far as the pulp tissue in the main canal did so (Fig. 4).

The pulp tissue reacts promptly to caries disease, and accumulations of inflammatory cells can be seen in histologic sections as soon as the enamel is penetrated and bacteria reach the peripheral end of the dentinal tubules. In fact, at an early stage when a carious lesion can hardly be diagnosed clinically, chronic inflammatory cells accumulate in the subodontoblastic pulp area subjacent to the affected dentinal tubules, in response to bacterial products traveling the tubules the whole way from the front of caries lesion to the pulp. These early reactions will intensify in severity with the progression of the carious process, often in the absence of clinical symptoms, and are reversible, provided bacteria are confined to the dentin. If the carious lesion remains untreated, invariably bacteria penetrate the pulp, and a minor area of necrosis with bacterial colonization is established in a pulp horn. It must be stressed that the surrounding tissue remains relatively unaltered. The time elapsed between pulp exposure and infection of the entire canal is unpredictable, but it is usually a slow process that very often occurs by tissue increments (44–48) (Fig. 5).

Exposure of the pulp to bacteria aggravates inflammation in the area subjected to aggression. Typical vascular events take place, including vasodilation and increased vascular permeability, resulting in exudation. This leads to edema formation, with consequent increase in tissue pressure, which might be critical to such a tissue encased in a low compliance environment. If the aggression is severe, tissue pressure might exceed that of thin-walled venules, which are compressed and collapse. Consequently, drainage is impeded, and stagnation of blood flow not only promotes increased blood viscosity but also causes impaired removal of waste products (47). This might lead to cell death and tissue necrosis. In addition, several bacterial products are directly toxic to host cells and might contribute to necrosis in the area. PMNs that are attracted to the area might also contribute to tissue damage by releasing enzymes and oxygen-derived products that degrade tissue components. The intensity and duration of the aggression will influence the severity of the tissue response.

All this sequence of events occurs in the tissue area adjacent to the infection front and not in the entire extent of the pulp. Tissue pressure near the site of inflammation is almost normal and shows no signs of severe inflammation, indicating that tissue pressure changes do not spread rapidly (47). The difference in pressure between inflamed and uninflamed areas can be a result of several edema-preventing mechanisms. For instance, the increase in tissue pressure might, in turn, force fluid back into lymphatics and capillaries in the nearby uninflamed tissue, consequently lowering the tissue pressure (48–50). Resilience of the ground substance of the pulp tissue might also help prevent spread of the pressure throughout the pulp (51).

It is currently recognized that total pulp necrosis is resultant of the gradual accumulation of local necrosis (2, 47, 52). After necrosis of a pulp tissue compartment, bacteria advance in the front of infection. Consequently, the tissue immediately adjacent to the infected region will be insulted and respond with the same inflammatory vascular events discussed above. In brief, after pulp exposure to caries, pulp tissue compartments are sequentially subjected to bacterial aggression and

Figure 6. (*A, B*) Distobuccal root of a maxillary molar extracted with an apical periodontitis lesion attached. Section passing through a lateral canal (arrows) (hematoxylin-eosin; original magnification, ×25 and ×100, respectively). (*C*) Higher magnification of the area indicated by the lower arrow in (*B*). Lateral canal displays necrotic tissue at this level (hematoxylin-eosin; original magnification, ×1000). (*D*) Higher magnification of the area indicated by the intermediate arrow in (*B*). Transition from necrotic to vital tissue (hematoxylin-eosin; original magnification, ×1000). (*E*) Higher magnification of the area proximal to the periodontal ligament indicated by the upper arrow in (*B*). Vital tissue free from inflammatory cells (hematoxylin-eosin; original magnification, ×1000). (*F, G*) Section taken approximately 60 sections distant from that shown in (*A, B*). Second ramification is present on the opposite root canal wall (arrow), with the lumen completely filled by a bacterial biofilm (Taylor's modified Brown & Brenn; original magnification, ×25 and ×50, respectively).

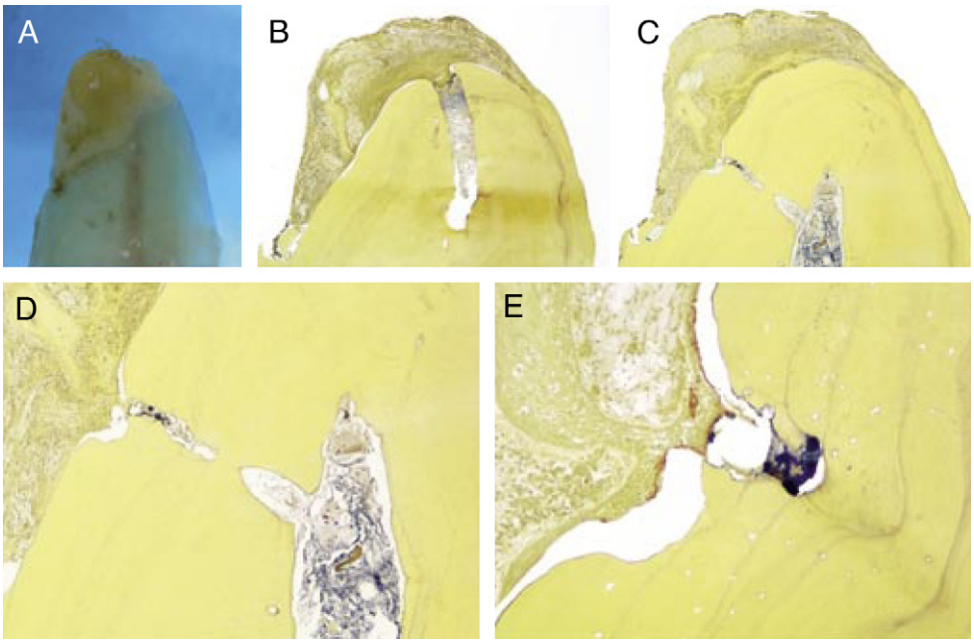


Figure 8. (*A*) Root apex of a maxillary second premolar extracted with an apical periodontitis lesion. Specimen is cleared before embedding in paraffin. (*B*) Section passing through the main canal. Note the apical root canal clogged with a bacterial biofilm (Taylor's modified Brown & Brenn; original magnification, ×16). (*C*) Section taken approximately 80 sections distant from that shown in (*A*), displaying a lateral canal that connects the main canal and the lesion (Taylor's modified Brown & Brenn; original magnification, ×16). (*D*) Magnification showing bacterial content of the lateral canal (Taylor's modified Brown & Brenn; original magnification, ×25). (*E*) Section taken 30 sections after that shown in (*D*), passing through the foramen of the lateral canal, also clogged with a dense bacterial biofilm. Empty spaces are shrinkage artifacts (Taylor's modified Brown & Brenn; original magnification, ×100).

become inflamed, necrotic, and eventually infected. These events occur by increments that coalesce and migrate apically until the entire pulp is necrotic and infected. Therefore, in a given moment, different stages of the disease process can be observed throughout the pulp. For instance, necrosis can be present at the area of pulp exposure, the coronal pulp can be severely inflamed in response to bacterial invasion, but the radicular pulp might remain uninflamed (45).

Observations of our specimens demonstrated that when pulp necrosis reached the level of the entrance of lateral canals and apical ramifications, the tissue therein was partially or completely necrotic (Fig. 5). In some necrotic cases associated with lateral lesions, the tissue within ramifications was partially necrotic, bacteria were seen colonizing the ramification, and inflamed tissue was also observed adjacent to bacteria. In other cases, the ramification lumen was virtually

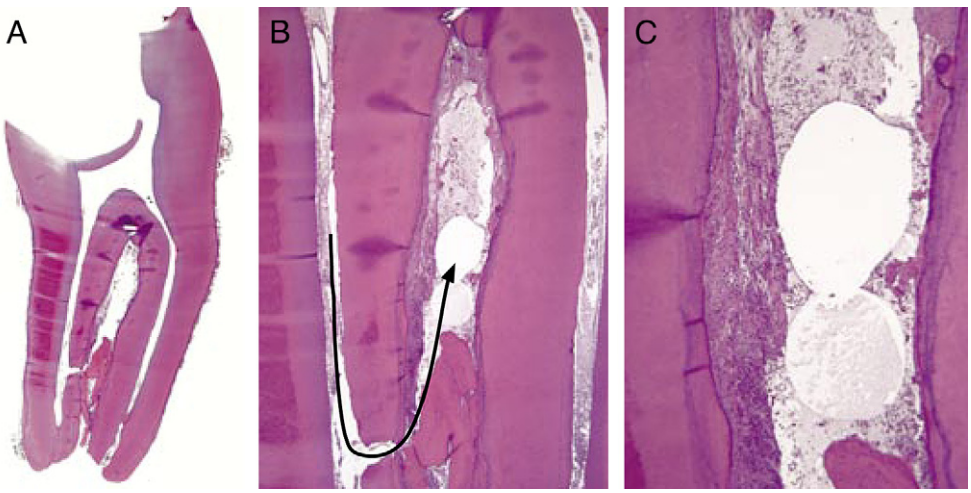


Figure 9. Mandibular second molar with necrotic pulp in a 45-year-old woman. Patient presented complaining of pain at chewing. Tooth did not respond to sensitivity tests and was tender to percussion. Radiograph showed small apical radiolucencies on both roots. Patient opted for no treatment, and the tooth was extracted. Tooth was processed for routine histology, and sections were cut on a mesiodistal plane. (*A*) Overview shows a lateral canal in the distal root ending in the interradicular area where bone loss is visible (hematoxylin-eosin; original magnification, ×6). (*B*) Detail of the interradicular area (hematoxylin-eosin; original magnification, ×25). (*C*) Magnification from (*B*). Cavities occupied by necrotic debris in the center. Vital inflamed tissue on the mesial aspect of the distal root (hematoxylin-eosin; original magnification, ×50).

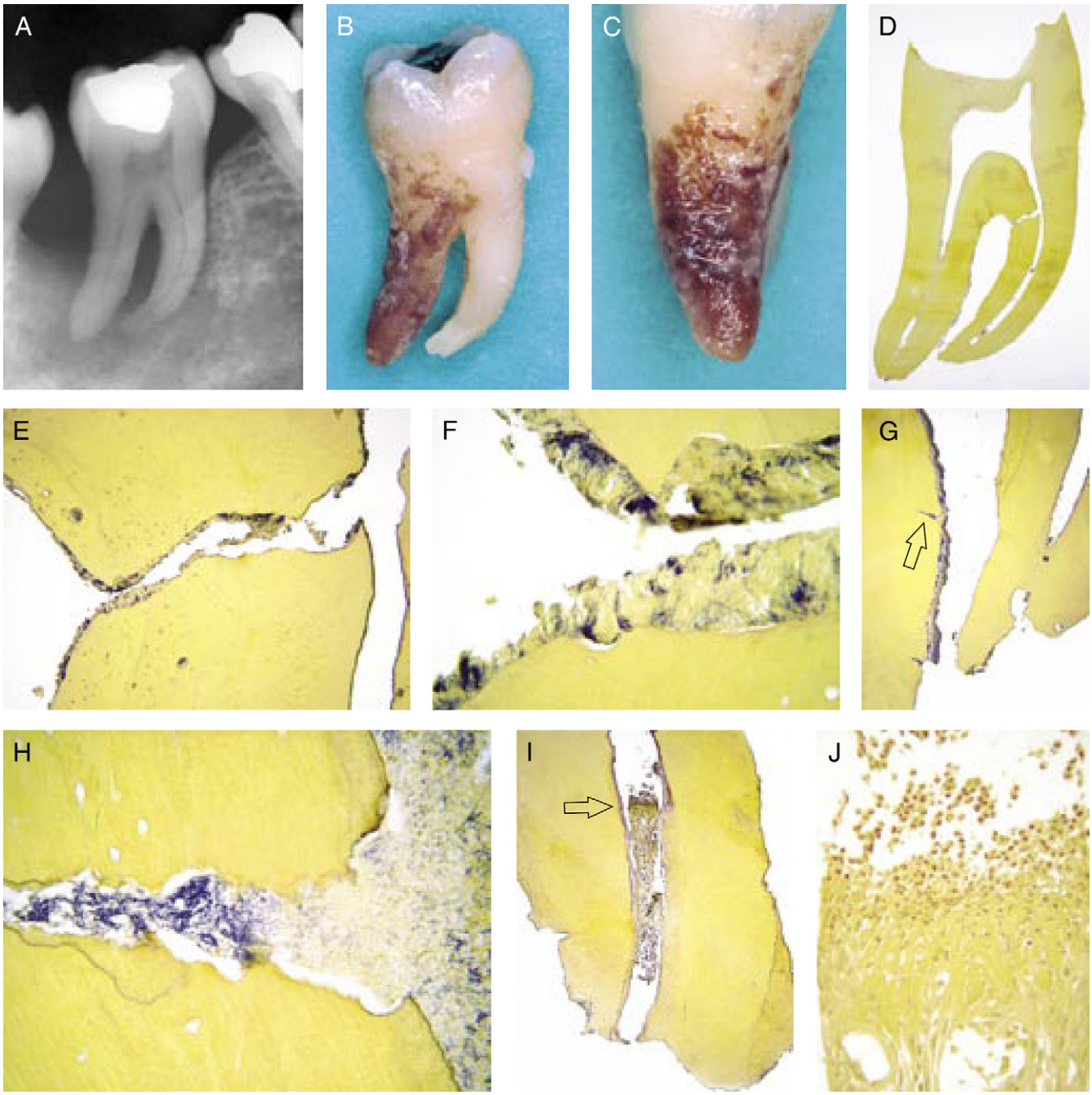


Figure 10. (*A*) Maxillary second molar in a 38-year-old woman causing a periodontal abscess. Pulp did not respond to sensitivity tests. Tooth was deemed untreatable and extracted. (*B, C*) After extraction, calculus is seen covering the entire distal root up to the apex. (*D*) Histologic sections showed a wide lateral canal occupied by a biofilm in the mesial root at the furcation area (Taylor's modified Brown & Brenn; original magnification, ×6). (*E*) Detail of lateral canal (Taylor's modified Brown & Brenn; original magnification, ×100). (*F*) Higher magnification of exit of lateral canal in the furcation area. Walls of the ramification are covered by a dense bacterial biofilm (Taylor's modified Brown & Brenn; original magnification, ×400). (*G*) Section taken at a certain distance from that in (*D*). Ramifications are present in the distal root (Taylor's modified Brown & Brenn; original magnification, ×25). (*H*) Higher magnification of lateral canal indicated by arrow in (*G*), with its lumen filled with bacteria (Taylor's modified Brown & Brenn; original magnification, ×400). (*I*) Section passing through the mesial root apex. Contrary to what was observed in the distal root where the entire pulp was necrotic, there appears to be some vital pulp in the apical segment of the mesial root (Taylor's modified Brown & Brenn; original magnification, ×25). (*J*) Magnification of area indicated by arrow in (*I*). Transition from necrotic to vital tissue (Taylor's modified Brown & Brenn; original magnification, ×400).

clogged with bacteria, very often arranged in biofilms. The case illustrated in Fig. 6 exemplifies these 2 different situations. It is a maxillary molar with the crown totally destroyed by a carious process, deemed nonrestorable. After extraction, the apical periodontitis lesion remained attached to the root apex of the distobuccal root. Serial sections demonstrated that the pulp in the main canal was necrotic, and several ramifications were present. In one of these ramifications, necrotic tissue was observed until approximately halfway of the

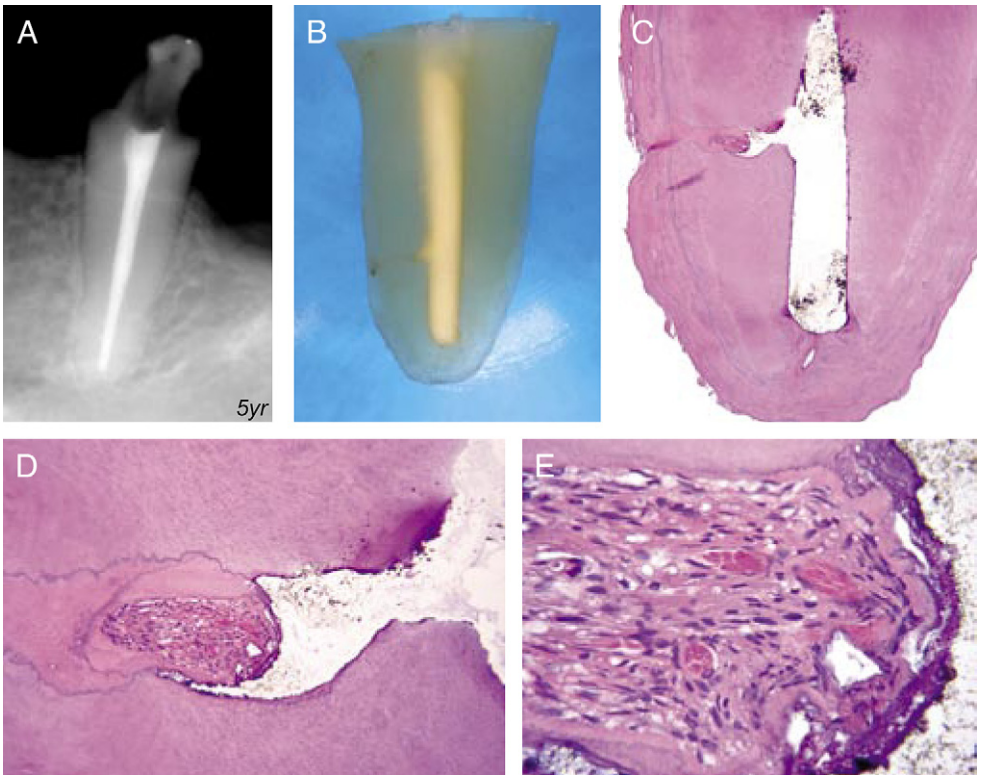


Figure 11. Mandibular premolar with previously vital pulp. (*A*) Patient presented after 5 years with loss of coronal restoration and an oblique fracture. Radiograph showed normal periradicular conditions. Note that no lateral canals appear in the postoperative radiograph. Tooth was no longer restorable and was extracted. (*B*) Clearing of tooth demonstrated a lateral canal with some obturation material at its entrance. (*C*) Section passing approximately at center of the canal. A lateral canal is visible (hematoxylin-eosin; original magnification, ×25). (*D*) Higher magnification of lateral canal shows loss of tissue for some distance and then a material/tissue contact area (wound healing) (hematoxylin-eosin; original magnification, ×100). (*E*) Higher magnification shows vital tissue relatively uninflamed; some hyperemic vessels. Note the limited zone of necrosis at the surface (hematoxylin-eosin; original magnification, ×400).

lateral canal course (Fig. 6*B, C*) followed by a transition with vital inflamed tissue (Fig. 6*D*). It is interesting to note that at the exit of the lateral canal in the periodontal ligament the tissue was free from inflammation (Fig. 6*E*). A totally different condition was observed in a second ramification present in the opposite root canal wall. It was a recurrent canal departing coronally from the main canal and returning to the main canal immediately before the foramen. Its lumen was completely filled by a dense bacterial biofilm (Fig. 6*F, G*). The clinical implications of the 2 conditions are clear. In the first condition, necrotic tissue and bacterial colonization are located only at the entrance of the ramification; irrigating solutions might have a chance to remove the disintegrated tissue. In the second condition, elimination of the bacterial biofilm in the ramification is virtually impossible with the substances and techniques currently available. Another example of necrotic tissue and bacterial biofilms both in the main canal and in lateral canals is shown in Fig. 7.

In summary, our observations for untreated teeth are in agreement with the observations of Langeland (45) in that the histologic condition of the tissue contained in a lateral canal and in apical ramifications reflects the condition of the pulp in the main canal. When a lateral canal is present in an area in which healthy pulp tissue is present in the main canal, healthy tissue is found throughout the lateral canal; when a lateral canal is found adjacent to an area of pulp inflammation, the tissue therein is also inflamed; and when a lateral canal is present in an area in which there is necrosis in the main canal, necrotic tissue is also observed in the adjacent part of the lateral canal. In the latter situation, the necrotic tissue might be followed by a transition zone of necrosis/PMNs and then inflamed tissue connected to a lateral inflammatory lesion. There are also cases in which necrotic tissue and bacteria occupy all the extent of the lateral ramification up to the periodontal ligament, which is usually inflamed and associated with bone resorption (Fig. 8).

In cases with periodontal disease, when the subgingival biofilm reached a lateral canal, the corresponding microcirculation was severed, but inflammation of the adjacent pulp tissue was minimal. When the subgingival biofilm reached the main apical foramen, however, the whole pulp became necrotic. In multi-rooted teeth, when only 1 root was involved by periodontal disease up to the apex, vital tissue was still observed in the pulp chamber.

Sometimes a lateral periodontitis lesion associated with a lateral canal in the presence of pulp necrosis might simulate a marginal periodontitis lesion or give rise to a typical secondary periodontal involvement (Fig. 9). Nonetheless, there has been no consensus as to whether the opposite is true, ie, whether subgingival biofilms associated with periodontal disease can directly cause pulp disease. Our findings confirm previous observations that although degenerative and inflammatory changes of different degrees might occur in the pulp of teeth with associated marginal periodontitis, pulp necrosis as a consequence of periodontal disease only develops if the periodontal pocket reaches the apical foramen, leading to irreversible damage to the main blood vessels that penetrate through this foramen to irrigate the pulp (53). Fig. 10 depicts a case in which the pulp of a mesial root canal was necrotic in the coronal segment as a result of bacterial aggression coming from both the infected necrotic distal canal and the lateral canal. However, there was still vital tissue in the apical segment of the

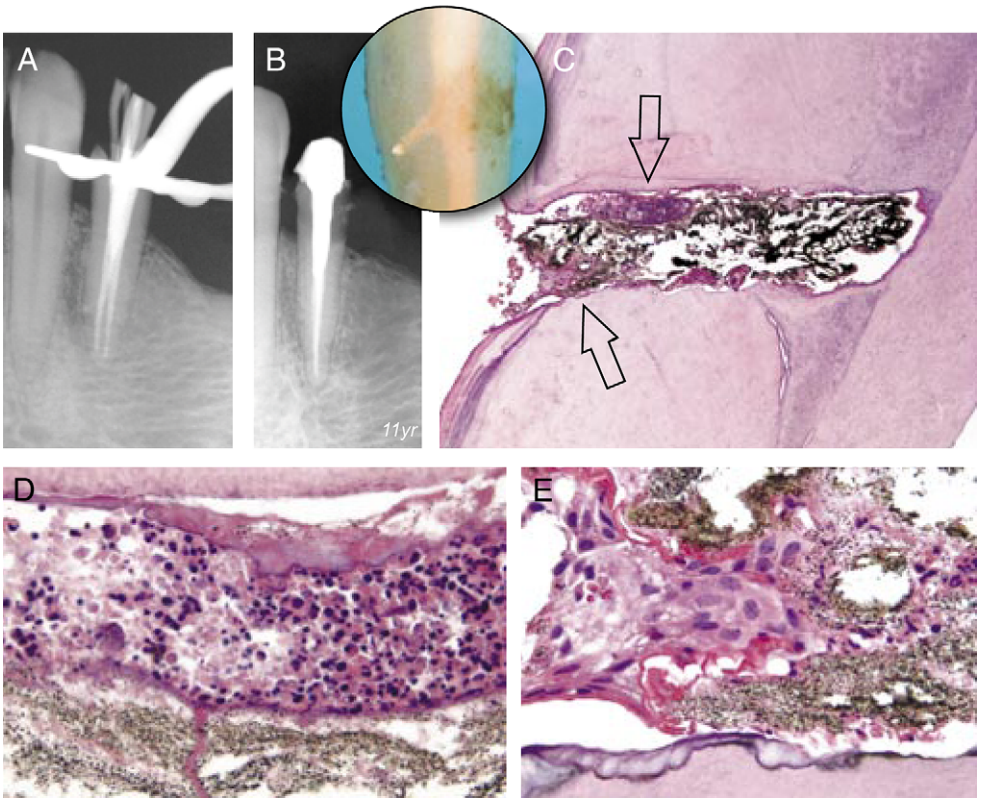


Figure 12. (*A*) Mandibular premolar with necrotic pulp. Postoperative radiograph showed a lateral canal revealed by filling material. (*B*) After 11 years, the injected lateral canal is no longer visible, and periradicular conditions are normal. An extensive caries lesion was now present on the distal aspect; the tooth was deemed no longer restorable and was extracted. After clearing, the injected lateral canal can be seen (inset). (*C*) Section passing through the lateral canal, demonstrating that the canal is not actually filled. Tissue is present intermixed with the obturation material (hematoxylin-eosin; original magnification, ×50). (*D*) Magnification of area indicated by upper arrow in (*C*). Accumulation of inflammatory cells (hematoxylin-eosin; original magnification, ×400). (*E*) Magnification of area indicated by lower arrow in (*C*). Mononuclear inflammatory cells in a tissue engulfed by obturation material (hematoxylin-eosin; original magnification, ×400).

mesial canal, where the main circulation had not been significantly affected by the periodontal pocket biofilm.

Teeth Subjected to Endodontic Treatment Procedures

Specimens subjected to different treatment procedures were also examined for the presence of lateral canals and apical ramifications and the fate of the tissue therein in response to treatment. Chemomechanical preparation partially removed necrotic tissue from the entrance of ramifications, whereas the adjacent tissue remained inflamed and associated with lateral and/or apical disease. In cases with long-standing pulp necrosis and complex internal anatomy, necrotic tissue and bacteria in ramifications remained untouched.

As for vital cases, although tissue in lateral canals and ramifications was never removed by chemomechanical preparation, most of the time it appeared free from inflammation. It appeared on histologic observations of other cases that forcing obturation material into this vital tissue might give rise to physical damage and chemical toxicity that result in unnecessary inflammation. The long-term outcome of this inflammatory response as it relates to treatment outcome is unknown.

Our observations are in consonance with experimental studies that demonstrated that lateral canals are rarely, if ever, completely cleaned after preparation, regardless of the instrumentation technique and irrigants used (54–57).

Root Canal–Treated Teeth

An important question related to the influence of lateral canals on treatment outcome is what happens to the ramification contents and adjacent periodontal tissues after obturation. The tissue within lateral canals is left relatively undisturbed by chemomechanical procedures. Therefore, 4 possible situations arise: vital or necrotic tissue present in the lateral canal, into which the filling material was forced or not.

In vital cases in which the filling material was not seen within lateral canals, the tissue therein usually remained vital, and there was no significant influence on the outcome (Fig. 11). In some cases, part of the tissue proximal to the main canal was damaged by the cumulative effect of the treatment procedures (instrumentation, irrigation, obturation), but the bulk of the tissue in the ramification remained undisturbed and uninflamed in the absence of bacteria (Fig. 11). This is because tissue vitality and relative normality are maintained by the periodontal ligament blood circulation.

When endodontic filling materials were seen in lateral canals or apical ramifications containing vital tissue, the ramification was not actually filled (despite the radiographic appearance), with damaged and inflamed tissue being histologically observed around and adjacent to the filling material.

Similar histologic conditions were observed when the filling material was forced into lateral canals of teeth with preoperative necrotic pulp. Fig. 12 clearly illustrates a situation in which the postoperative radiograph showed a lateral canal revealed after treatment of a mandibular premolar with necrotic pulp. The tooth was extracted after 11 years because of recurrent caries, and despite radiographic normal periradicular conditions, histologic sections demonstrated that the lateral canal was actually not filled, with islands of residual tissue showing inflammation of varying degrees. Therefore, whenever a lateral canal

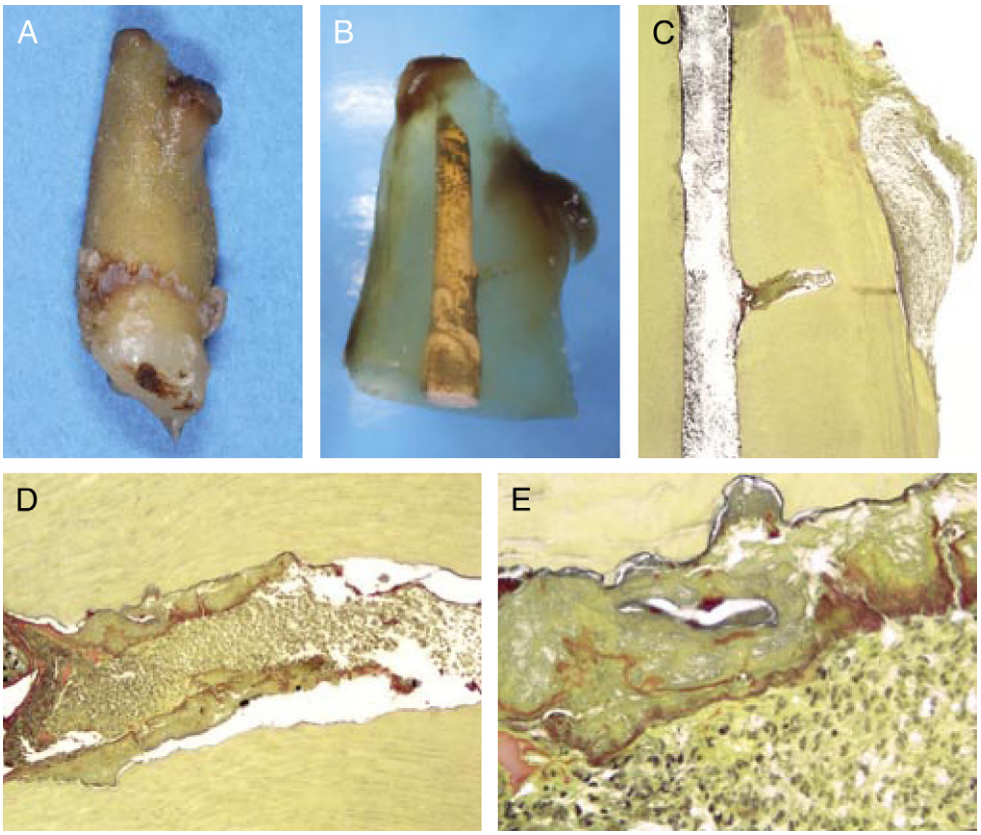


Figure 13. (*A*) Maxillary incisor of a 56-year-old man extracted 16 years after endodontic treatment. Root filling had been exposed for long time to the oral environment. A lateral lesion remained attached to the root. (*B*) The apical third was dissected free and processed separately from the coronal two thirds. After clearing, a lateral canal, apparently empty, can be observed centering the lesion. (*C*) Section passing through main canal and part of lateral canal (Taylor's modified Brown & Brenn; original magnification, ×16). (*D*) Detail of lateral canal. An amorphous structure is layering the lateral canal walls, faced with an inflammatory reaction (Taylor's modified Brown & Brenn; original magnification, ×100). (*E*) At higher magnification, host cells are recognized as PMNs, but no bacterial cells are seen (Taylor's modified Brown & Brenn; original magnification, ×400).

was revealed radiographically and histologic evaluation was made possible because of tooth loss, the lateral canal was shown to be never cleaned and filled. Necrotic debris and/or tissue at varying degrees of inflammation were commonly present along with filling materials.

In some cases of failure after treatment, retreatment, or surgery, lateral canals and/or apical ramifications not revealed radiographically were found to contain bacteria along their entire extent. Bacteria were sometimes forming biofilms on the ramification walls and were always associated with inflammation in the periodontal ligament immediately adjacent to the exit of the ramification. This confirms that infected lateral canals and apical ramifications might be related to the failure of the endodontic treatment, regardless of the presence or not of filling material injected therein. Located in these areas, bacteria are likely to escape the effects of instruments (because of physical limitations) and irrigants (because of inactivating chemical reactions and time constraints) (23–25, 58). Although an intracanal medication with calcium hydroxide might theoretically display the potential to reach these areas to exert disinfecting effects, failure to eliminate bacteria in ramifications has also been observed for this medicament (24). This might be related to its low solubility and inactivation by dentin, tissue fluids, and organic matter, all of which can hamper the pH-dependent antimicrobial effects of calcium hydroxide (59, 60).

In some other cases of treatment failure associated with a lateral canal or apical ramification, bacteria were seen colonizing the walls of the main root canal but not within the ramification, which contained inflamed tissue (Fig. 2). In these cases of persistent or secondary intraradicular infection, bacteria and their products reached the vicinity of the ramification, and the tissue therein became or remained inflamed. Extension of the inflammatory response to the periodontal ligament can induce or maintain a lateral lesion. Also, low-molecular-weight soluble bacterial products can diffuse through the tissue in the ramification, reach the periodontal ligament, and induce/sustain inflammation with consequent release of cytokines involved with activation of bone resorption mechanisms. In other words, the front of infection is within the root canal and not necessarily reaches the boundary between the foramen (apical or lateral) and the periodontal ligament. Indeed, many studies revealed that apical periodontitis develops before necrosis and infection reach the apical foramen (42, 61–64). Therefore, it becomes apparently clear that for a lateral lesion to develop, the lateral canal must not necessarily be necrotic and infected.

An intriguing situation was observed for the case depicted in Fig. 13. That tooth was extracted 16 years after completion of the endodontic treatment. It was always asymptomatic, and the root canal filling had been exposed for a long time to the oral environment. At extraction, a lateral lesion was observed and subjected to histopathologic and histobacteriologic processing. PMNs accumulated in the lateral canal, facing a noncollagenous amorphous structure that was layering the ramification walls. No bacterial cells were detected therein. Instead, bacteria were found in the coronal segment of the main root canal between the canal wall and the obturation. The characteristics

and the way the amorphous material related to the ramification walls resembled those from the biofilm extracellular matrix. The great accumulation of PMNs adjacent to the amorphous material suggests bacterial involvement. The possibility exists that the histobacteriologic technique used failed to detect bacteria, or they were lost during processing (the technique is more sensitive for gram-positive bacteria, and sometimes gram-negative bacteria might pass unnoticed). However, there is also the possibility that this amorphous material, being a "biofilm carcass," remained sufficiently antigenic and/or entrapped enough residual bacterial virulence factors (structural components and products) to sustain inflammation even in the absence of bacterial cells. This statement, however, is speculative and needs to be confirmed by experimental data.

Our observations confirm the statement that the ability to inject filling material into lateral canals is not an indication of superiority of a given obturation technique over another (34), because this approach, in addition to being unpredictable, exhibited virtually no therapeutic benefit.

Concluding Remarks

Histopathologic and histobacteriologic observations of untreated teeth revealed that the conditions of the tissue within lateral canals and apical ramifications reflect the conditions of the pulp in the main canal. Observations from treated teeth in turn allowed some practical conclusions: (1) tissue within ramifications remain relatively unaffected by instruments and irrigants after chemomechanical preparation, regardless of the preoperative pulp conditions; (2) in cases with vital pulp, forcing obturation materials into lateral canals caused unnecessary damage to the tissue, with consequent inflammation; (3) material that radiographically appears in the lateral canals and apical ramifications was forced into these areas, but this by no means indicates that the ramification is sealed or disinfected; and (4) because bacteria located in large ramifications might reach sufficient numbers to cause or maintain disease, strategies other than finding a technique that better squeezes sealer or gutta-percha within ramifications should be pursued to effectively disinfect these regions and optimize the treatment outcome.

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