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Histologic features of apical periodontitis in human biopsies

DOMENICO RICUCCI & GUNNAR BERGENHOLTZ

The purpose of this article is to exhibit some of the challenges a clinician may be faced with in the management of apical periodontitis associated with primary infected pulps. Histological sections of selected clinical cases are displayed to show variations in the location of the bacterial mass, features of the microbial–host tissue interface, advanced apical root resorption and radicular cyst formation. While a single case may only give a glimpse of the set of events that may prevail in apical periodontitis, histologic examination of human biopsy specimens, linked to clinical records, has nevertheless served as an important basis for our current understanding of its natural history.

Introduction

The use of histological sections from human biopsy samples to examine the microanatomy of apical periodontitis has a long tradition in endodontology. A prime purpose of such studies has been to weigh the clinical symptoms and the radiographic signs against the nature of the tissue changes that may have taken place periradicularly in response to pulpal infections and endodontic treatment measures. For years, histology has served as the ultimate standard by which the true character of the clinical manifestations of apical periodontitis can be confirmed and distinguished from lesions of other origins. The knowledge generated has also served as an important basis for our current understanding of the natural history of apical periodontitis. Furthermore, it has been highly significant for the development of clinical treatment strategies in endodontics.

It is obvious from numerous studies that the tissue lesion in apical periodontitis may present itself differently depending on the time frame for its progression and the character of the cause (1–19), see also review by Nair (20)). In an early study of extracted teeth to which the soft-tissue lesion was attached, Thoma (1) identified what he termed as 'simple dental granulomata', 'epitheliated dental granulomata', 'dental granulomata showing necrosis and suppuration', 'dental granulomata showing various retrograde processes' implying beginning cyst formation, and 'dental granulomata showing cyst formation'. This classification on the overall microscopic appearance of apical periodontitis has undergone only minor modifications over the years. A sensible simplification, used in many texts, may be to sub-group apical periodontitis into exudative lesions (periapical abscesses), periapical granuloma and periapical cysts. In between these forms are of course a variety of transitional stages and tissue configurations. While these responses may apply to any kind of irritant released from the tooth interior, the response to bioincompatible root filling materials alone may be more specific after the acute toxic phase has subsided. Such lesions may lack the typical variety of leukocytes in inflammatory infiltrates and in stead, next to fragments of root filling material, feature no more than accumulations of macrophages and large phagocytic cells, known as giant cells (21), see also review by Nair (22)). Moreover Simon (10) and Nair et al. (19) have on the basis of serial sectioning of human biopsy samples proposed two forms for periapical cyst. This depending on whether the cyst cavity is in direct continuity with the root canal space or not. Nair et al. (19) designated the term 'true cyst' for a cyst cavity completely surrounded by epithelium and without a direct communication with the root canal. A cyst with the cavity linked to the root canal was termed 'pocket cyst'.

While much of the early histological work was geared to identifying these overall structural frameworks and

to determine their respective prevalence among clinically diagnosed lesions, much of recent studies have been to characterizing the inflammatory infiltrate (e.g. (23–34)). The prime purpose of such studies has been to explore the nature of the involved immune response. By the use of immuno–histochemical markers, the identification of various immuno-competent cells including lymphocyte subsets and their cytokine profiles have been delineated. Findings, thus far, have led to the understanding that in the initial phases of lesion development, pro-inflammatory activities dominate, whereas in more long-standing lesions cytokines for down regulation of the inflammatory process prevail ((35), see also review by Stashenko et al. (36)). In this line of research animal models have played an important role and have contributed considerably to our current understanding of both the innate and the adaptive immune-defense mechanisms occurring in apical periodontitis. These advances will not be reviewed here but elsewhere in this volume of Endodontic Topics (see the paper by Kiss).

The purpose of this communication is to display selected clinical cases to illustrate some of the more outstanding microscopic attributes of apical periodontitis associated with primary infected pulps and make some clinical inferences. Cases are based on extracted teeth with attached soft-tissue lesions allowing a much better overview of the tissue response and the bacterial–host interface than normally possible in regular biopsies including only the soft-tissue portion. In the processing of the tissue samples, traditional histological techniques have been employed including demineralization of hard-tissue structures, embedding in paraffin blocks, taking and staining sections for light microscopy. To visualize the presence and localization of bacterial profiles, the Taylor modified Brown–Brenn staining technique was used.

Features of the host–parasite interaction in apical periodontitis

Alike any inflammatory lesion, periapical inflammatory processes are continuous and active for as long as the causative agents have not been neutralized or eliminated. Apical periodontitis is, however, unique in the sense that often the host organism alone cannot eradicate the cause, especially when an infection has established itself in a necrotic pulp. Defense mechanisms simply cannot prevail very far into an environment such as the one in root canals, where, after the breakdown of the pulp, it will lack support of neurovascular elements. Provided for by entry of inflammatory exudates, proteolytic root canal bacteria are favored in addition, and will undergo rapid growth and multiplication (see e.g. recent review of Sundqvist & Figdor (37)). Host tissue attempts to repair and regenerate the damaged tissue obviously become futile because of the continuous production of bacterial elements. Consequently, in principle, apical periodontitis sustained by a root canal infection will be a never-ending process unless adequate clinical treatment, aimed to eliminate the infectious agents, is instituted.

From a treatment aspect it is of critical importance where the bacterial front line is located and where it meets the host tissue defense. It is commonly believed that in the presence of radiographic signs of apical periodontitis, the pulp tissue is fully necrotic to the exit of the canal at the apical foramen. Therefore, instrumentation and disinfection along the entire length of the root canal space is often thought necessary. Such an approach to endodontic treatment may, however, do more harm than good and cause a detrimental overextension of the apical preparation resulting in a clinical failure to resolve the periapical inflammatory process (21, 38). Also the need for such a measure must be questioned from the aspect that morphological observations have shown that vital tissue, although inflamed, may prevail well inside the root canal, thus safeguarding bacterial advancement into the periapical tissue compartment. This seems be the case even in non-trauma cases, where caries or operative procedure has initiated the inflammatory process (14, 39). Observations by Nair (15)) have indicated that the position of the bacterial front is unpredictable. By examining primary infected root specimens with attached soft-tissue lesion, he noted that the level of the bacterial–host tissue interactions varied and could be well inside the root canal space at some distance from the root tip and at the exit of the apical foramina, but rarely so in the lesion *per se*.

Case 1 is about a series of sections from an extracted lower first molar in a 19-year-old female. Tooth # 36 displayed radiographic signs of apical periodontitis on both the distal and the mesial roots (Fig. 1A). Patient recalled several painful episodes but was not in pain when the tooth was extracted. There were no clinical manifestations of acute inflammation except that the tooth was slightly tender to percussion.

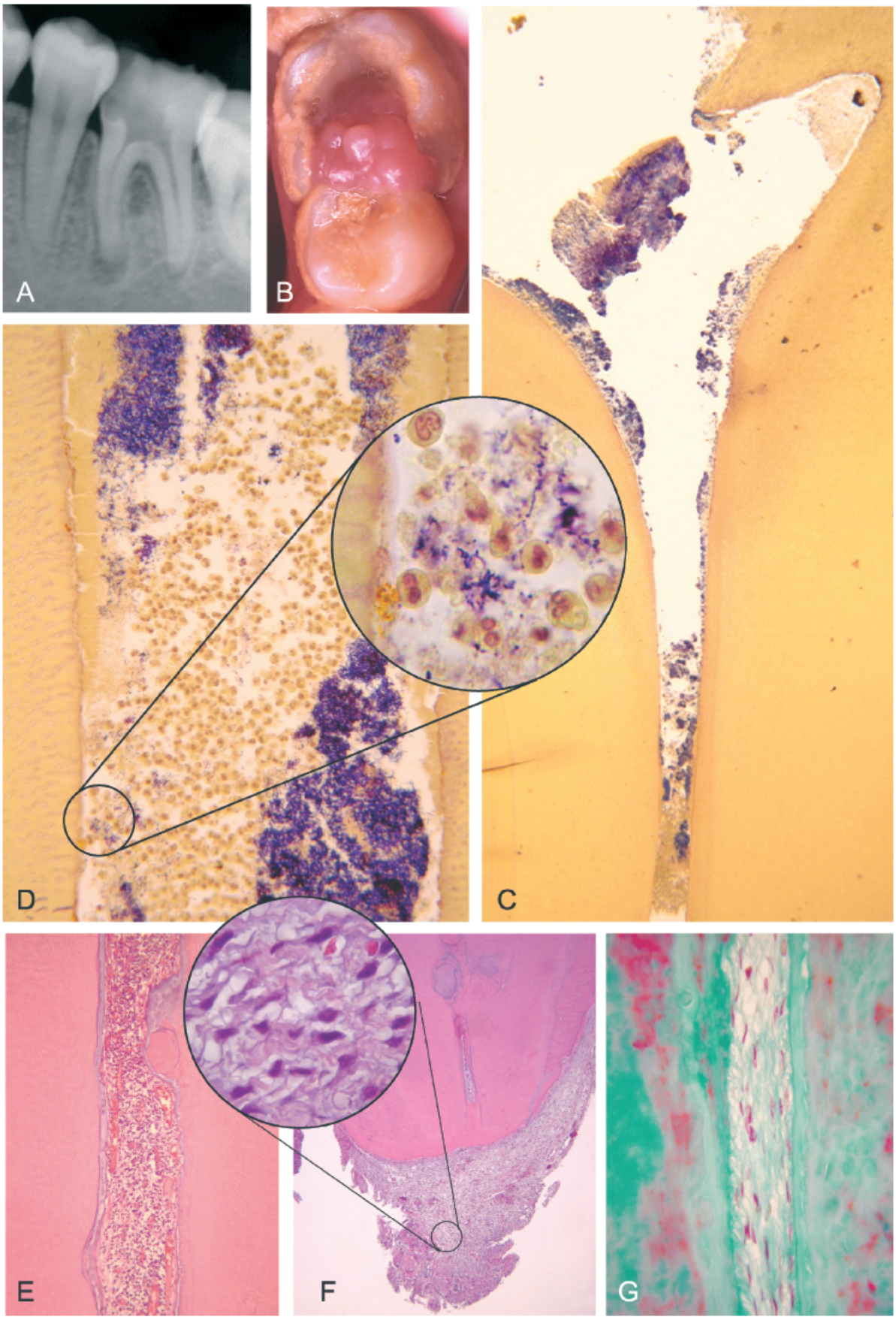


Fig. 1

On the mesial aspect of the tooth, gingival tissue has proliferated (Fig. 1B). On probing, vital pulpal tissue was lacking suggesting pulpal necrosis. Histological examination of the root canal content revealed, however, that pulpal necrosis was only half-ways in both roots. An overview of a bacterially stained section shows bacterial aggregations free from interferences of inflammatory defense activities (Fig. 1C). Further down in the middle portion, numerous polymorphonuclear leukocytes (PMNs) are seen engaged in phagocytic activity (Fig. 1D and insert). In more apical portions of the canal, numerous obviously hyperaemic vessels exist in an infiltrated connective tissue (Fig. 1E). In apical segments note that the pulp displays normal tissue structures (Fig. 1G). The connective tissue attached to the root tip (Fig. 1F), obviously representing the radiographic lesion, is more or less free from inflammatory infiltrates.

Case 2 includes sections from an extracted upper second molar in a 25-year-old male. Patient had suffered numerous acute episodes of pain, tenderness and swellings. At the time of extraction patient was, however, asymptomatic and there were no objective clinical signs of ongoing root canal infection.

In the overview, stained with hematoxylin–eosin (Fig. 2A) one observes the lesion attached to the mesio–buccal root. Inflammatory tissue is present within the canal orifice at a short distance from the root tip. At the inflammatory tissue front a disorganized mass of what appears to be debris can be seen (Fig. 2B). The nearby sections (Fig. 2C and D) stained for bacteria show, however, that the major bulk of it is made up of stainable bacteria. Higher magnifications (insert in Fig. 2D) suggest primarily colonization with filamentous organisms. PMNs are in close contact and in other sites an obvious phagocytic activity is present.

The lesion at the palatal root is shown in Fig. 2E. Here the soft-tissue lesion is much further inside the apical foramen and even some vascularity (arrows) can be observed suggesting an attempt to repair and tissue regeneration (Fig. 2F). The soft-tissue lesions outside the root tip show typical signs of the tissue response of a periapical granuloma with some inclusion of epithelial (ep) proliferations (Fig. 2G). The ep strands appear organized in islands surrounding inflamed connective tissue. Higher magnifications (Fig. 2H and I) of areas indicated by arrows in Fig. 2G show ep strands infiltrated by neutrophils. Mononuclear leukocytes reside in the rich vascular network.

A nearby section stained for bacteria (Fig. 2J and K) demonstrates, apical to the soft tissue front in the canal, an area with filamentous organisms attached to the root canal wall. While PMNs line this microbial condensation, some reasonably well-organized connective tissue is seen in a more central location.

From a somewhat obliquely cut area of the disto-buccal root canal, a similar feature of microbial condensation on the canal walls, surrounded by an infiltrated connective, can be observed (Fig. 2L–N).

Bacterial organisms were not possible to identify in the soft-tissue lesions *per se* except for the very periphery (Fig. 2O). The position of these rather small bacterial aggregates at the external surface of the lesion, where inflammatory infiltrates are virtually absent, suggests contamination from the oral environment during the extraction procedure.

Case 3 shows the tip of a palatal root of a maxillary first molar in a 37-year-old male. There have been several flare-ups, but at the time for extraction patient was asymptomatic. The interesting feature of this case is that there are two exists of the main canal. One (Fig. 3A–D) is not as clearly displayed but harbors bacterial organisms near its exit into the periapical tissue compartment (Fig. 3D). The other portion is severely curved (Fig. 3C and E), in which the bacterial front is at the end of the canal (Fig. 3E and F).

Case 4 demonstrates microbial presence in a totally different position than the ones displayed in cases 2 and 3. Longstanding caries has resulted in extensive destruction of the clinical crown of tooth # 25 and an obvious large exposure of the root canal space (see radiograph taken from a orthopantomogram in Fig. 4A). The tooth is from a 63-year-old male who had suffered repeated abscess formations in the area. At the time of extraction there is a sinus tract exiting in the vestibule associated with the swelling. Tooth is only slightly mobile with shallow pocket probing depths of ca. 3 mm excluding marginal periodontitis as a contributing cause of the inflammatory condition.

On macroscopic inspection of the root tip, the bacterial mass appeared to be partly mineralized (Fig. 4B) suggesting calculus formation. Stainable bacterial organisms occupy the root canal space at the canal exits and are also colonizing the outer root surface (Fig. 4C and D).

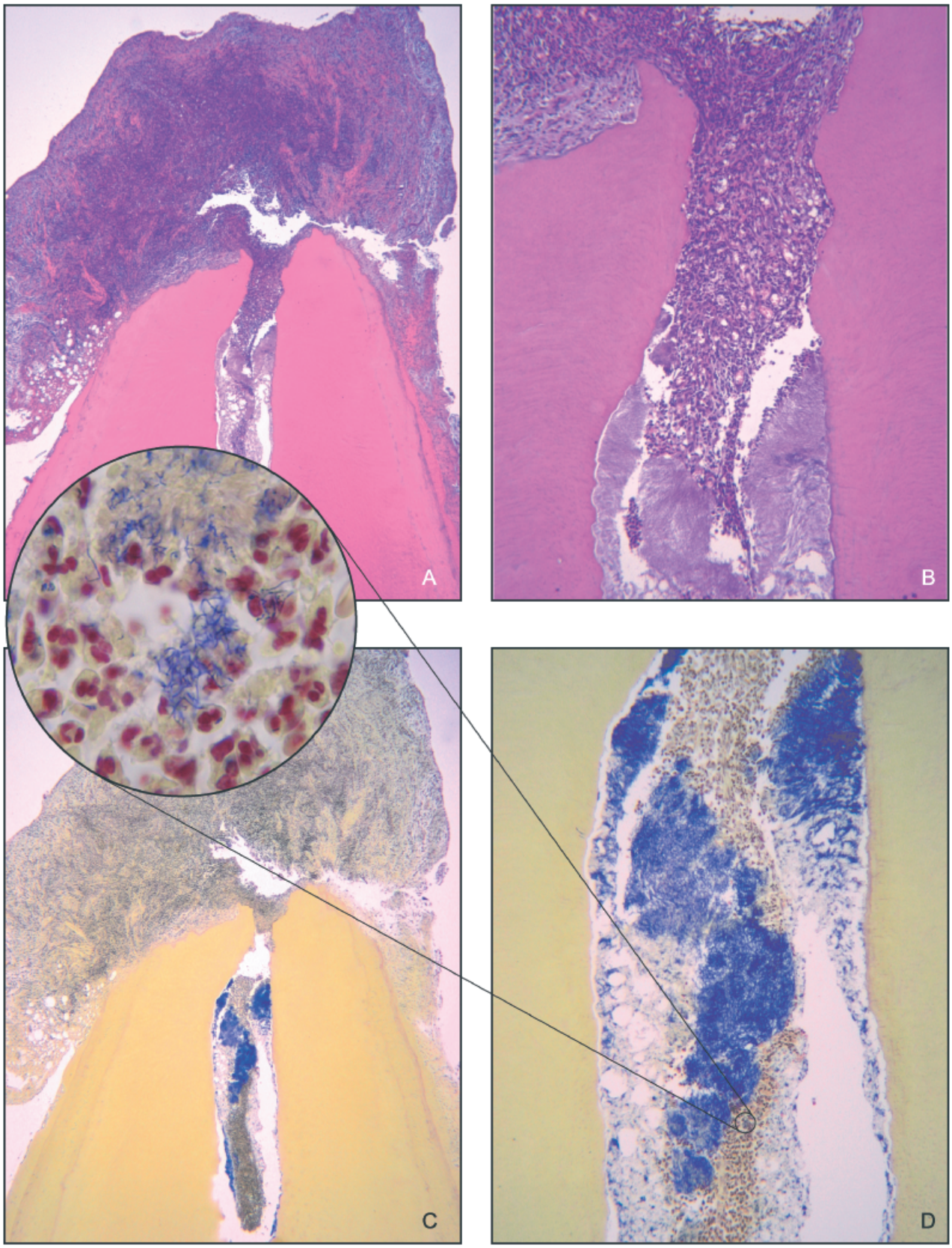


Fig. 2

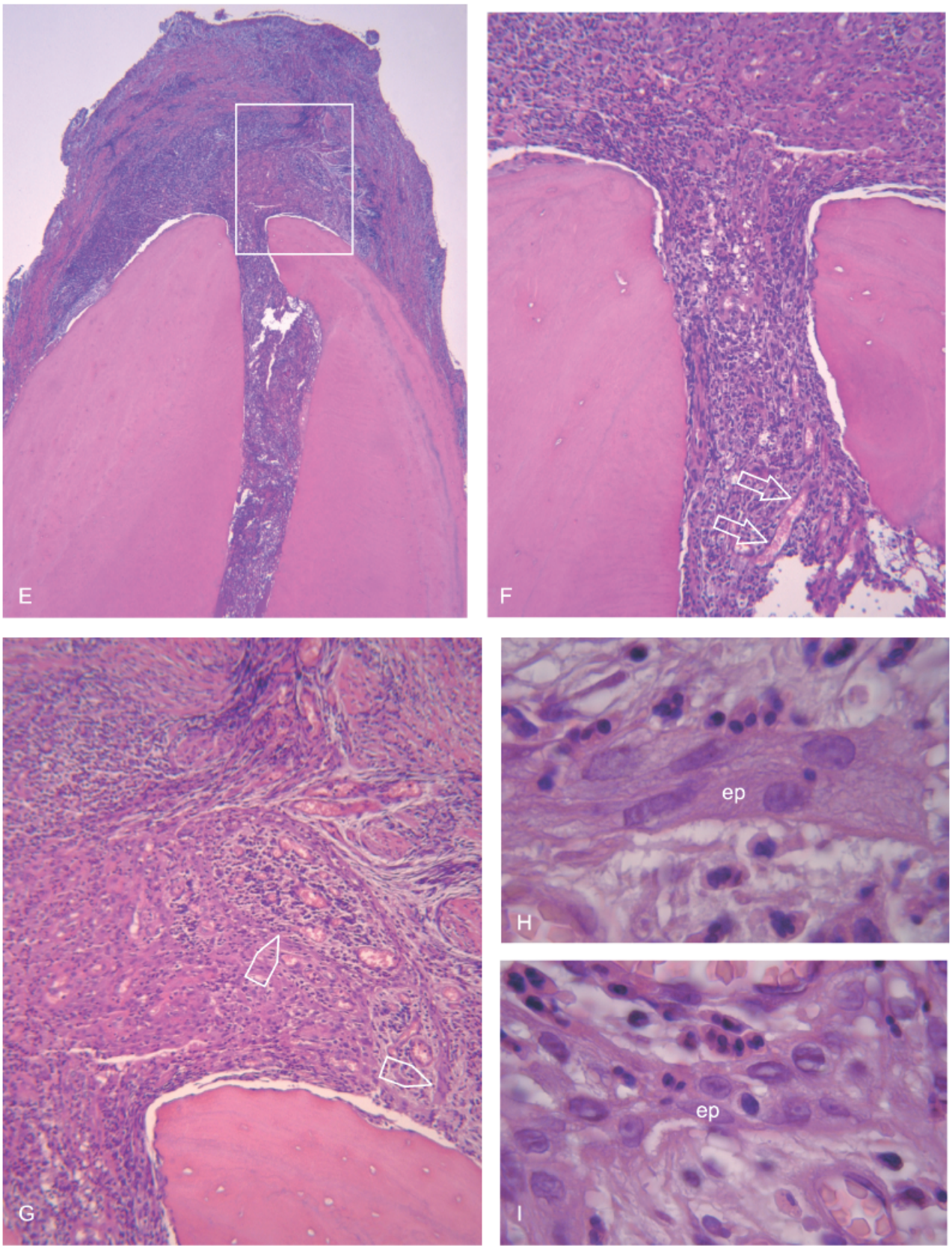


Fig. 2 (continued)

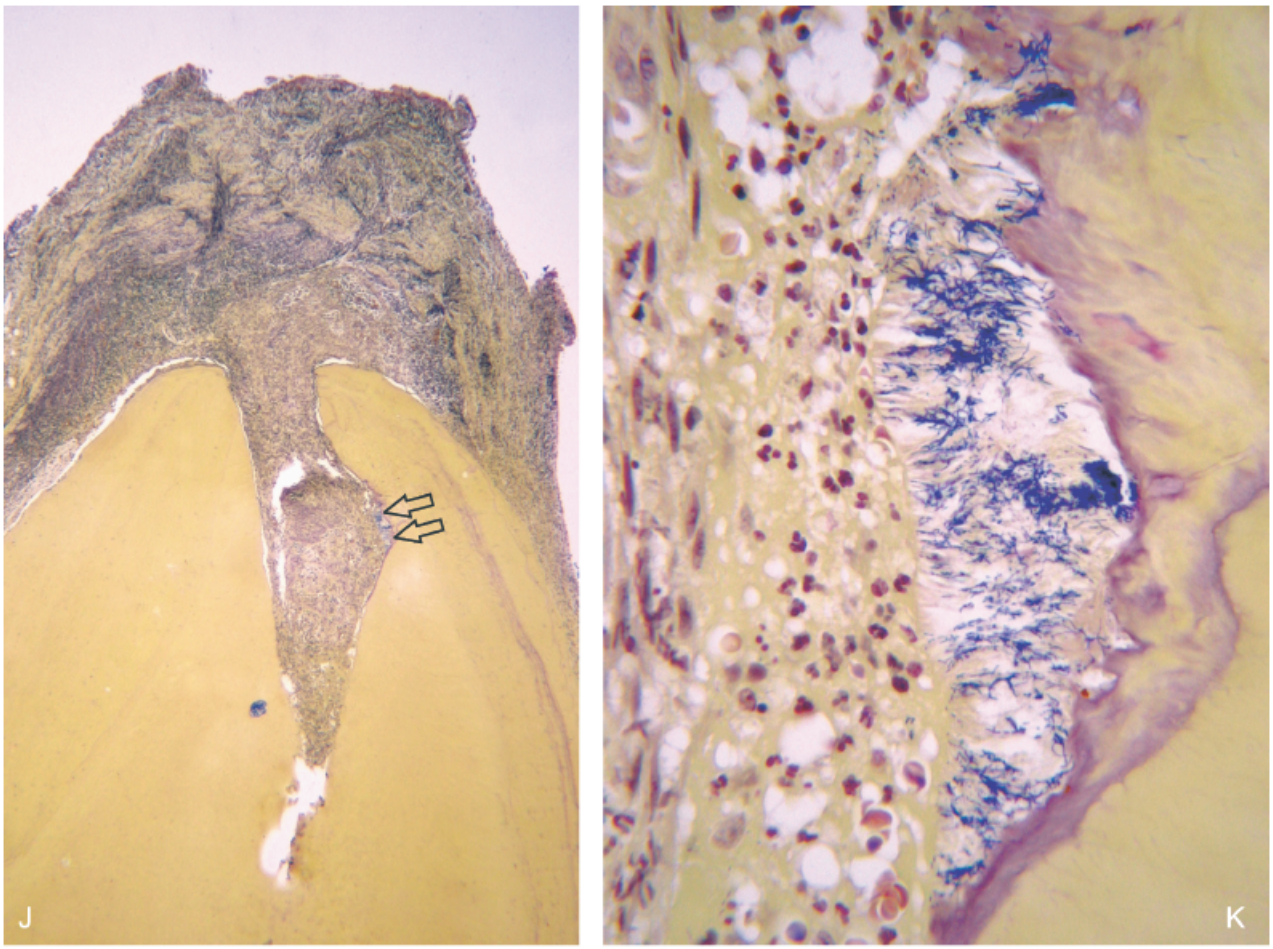
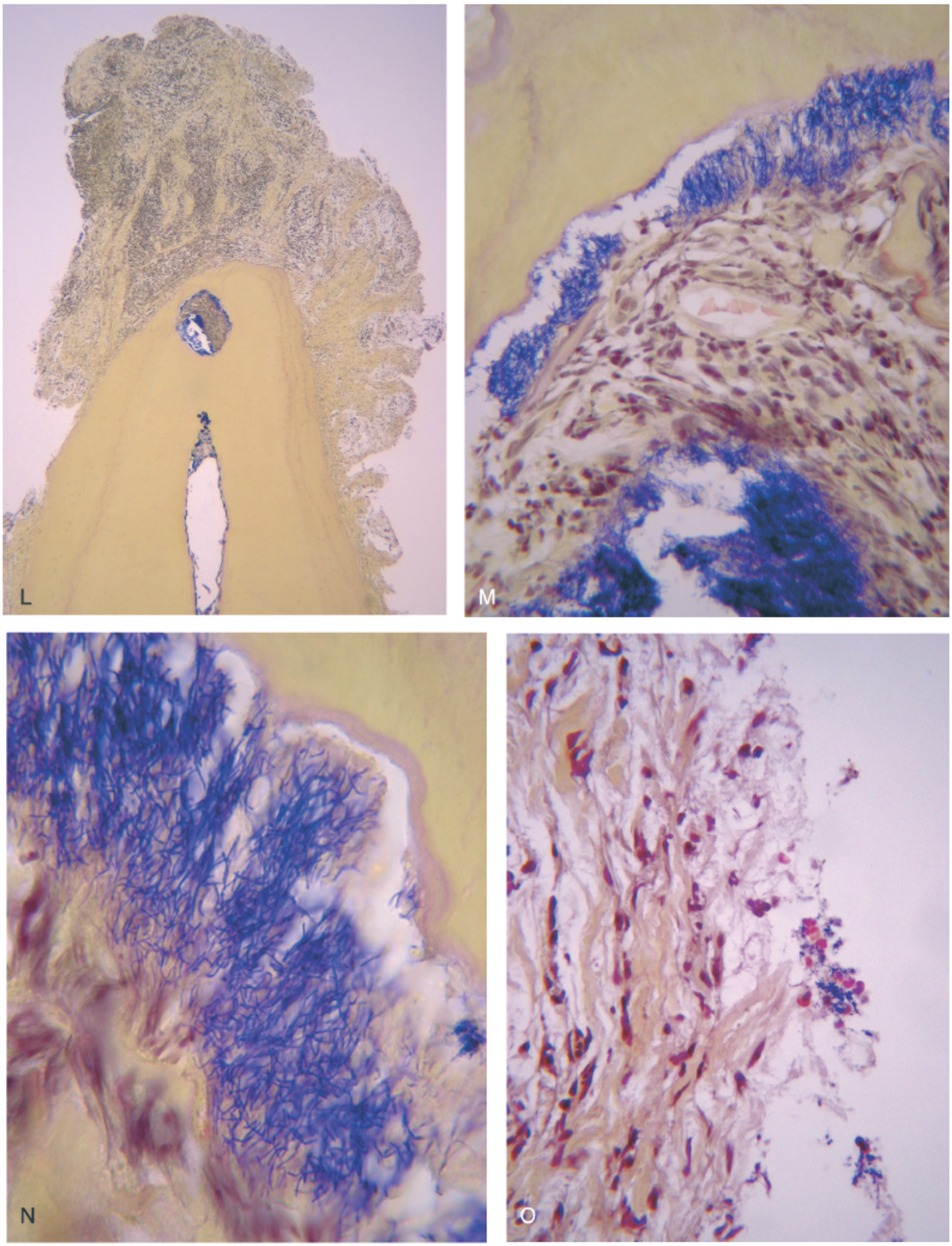


Fig. 2 (continued)

Clinical inferences of cases 1–4

The findings in these cases support the view that the exit of the root canal is not necessarily a natural demarcation line for host tissue – bacterial interactions in apical periodontitis. Indeed, Langeland has pointed this out repeatedly on the basis of analyses of similar types of cases (see review by Langeland (39)). It is, thus, obvious that the host tissue is able to mobilize barriers anywhere inside the root canal space against the penetration of bacterial organisms. It is reasonable to assume that the level observed in a single case may just be temporary and that the time from the onset of bacterial invasion of the pulpal tissue will determine where it finally is going to end up. Hence, the more long-standing the lesion is, the greater the likelihood may be for the bacterial front to gain terrain. This means that the foramen and even the outer external root surface could be an end point for the bacterial advancement in a very long-standing lesion as the fourth case may exemplify. Another factor for the bacterial colonization on the root tip could be the heavy bacterial load that was precipitated in this case by virtue of the direct exposure of the root canal to the oral environment by the caries lesion (see further below).

Indeed, there are several publications describing bacterial biofilms on the external root surface (see review by Tronstad & Titterud-Sunde (40). Tronstad et al. (41), on examining root tips of teeth in some treatment resistant cases with scanning electron microscopy (SEM), was early to alert to the possibility that bacterial plaque may form on root tips and in resorption crypts. Several other reports testify to similar findings (e.g. (42–45)). By contrast, Siqueria & Lopes (46), on observing the root ends of 27 extracted teeth with extensive carious lesions, necrotic pulps and attached soft-tissue lesion by SEM, failed to observe such bacterial aggregations in more than one case. Also Nair (20) in his light and transmission electron microscopic analysis of 31 teeth with apical



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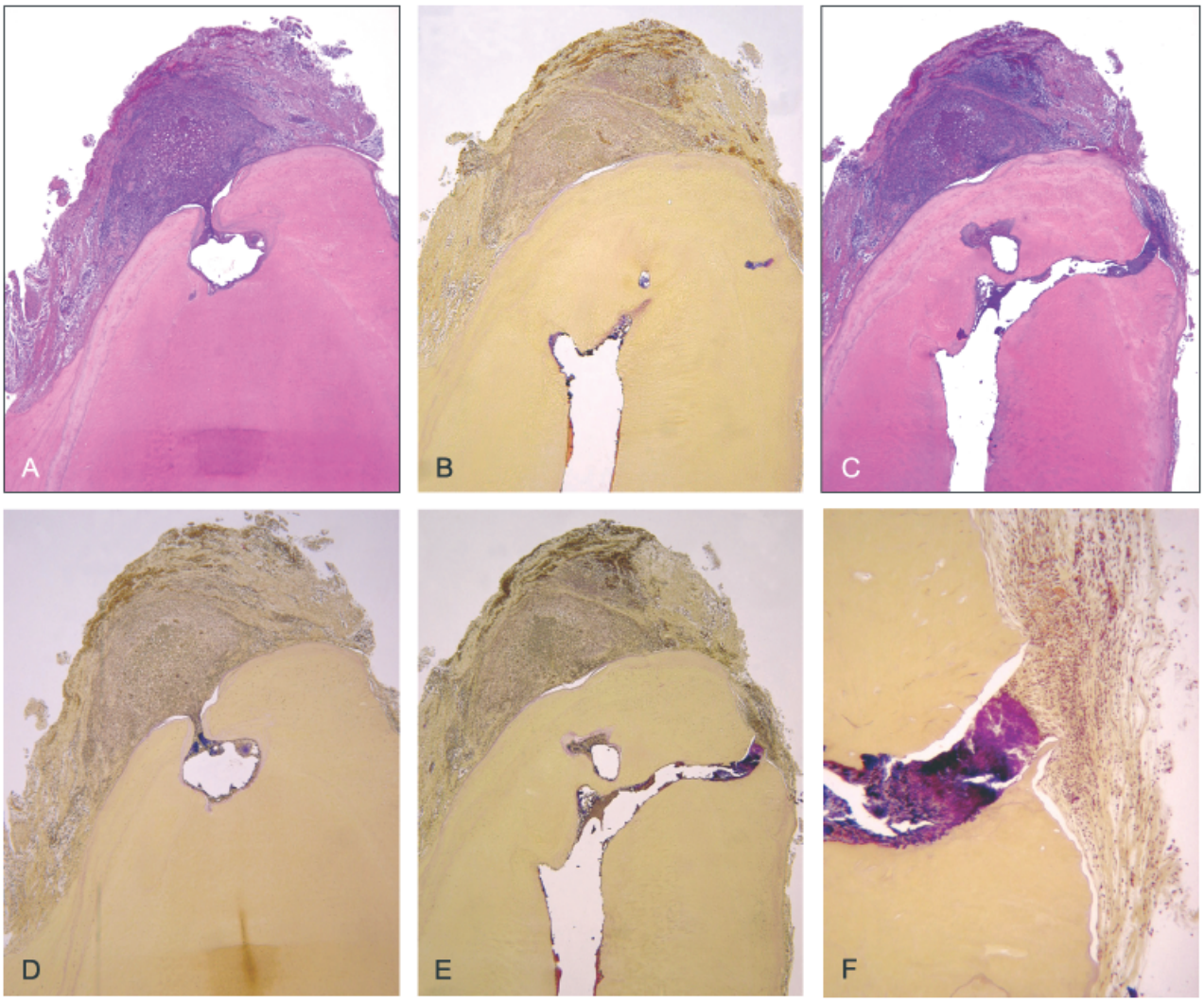


Fig. 3

periodontitis and grossly affected by caries, gave no report of such bacterial condensations. Consequently, the frequency and under which conditions bacterial biofilms may develop on the external surface of roots with infected necrotic pulps is uncertain. Such knowledge is, of course, of great clinical importance as bacterial aggregations externally on root tips are likely to pose a significant clinical treatment problem that is likely to be non-treatable by orthograde endodontics. Rather endodontic surgery (retrograde endodontics) seems to be the logical treatment measure in such cases.

Thus far, reports on apical biofilm formations are based on limited case series and there is a paucity of analyses of large and well-defined clinical samples, where both the preconditions and the prevalence of these bacterial condensations are determined. The question of the origin of the organisms in apical biofilms is also unresolved. It is indeed possible, but remains to be shown, that in the acute, exudative phase of apical periodontitis, bacterial organisms present in the lesion *per se* as planktonic organisms may attach and establish themselves in a biofilm environment on the root surface. As already brought up, a gross bacterial infection of the pulpal space, as promoted by a direct exposure to the oral environment by caries as in case 4, may be an important prerequisite. Yet, the observation that often sinus tracts are associated with these bacterial aggregations suggests that such a pathway is also potentially significant (47). Bergenholtz & Spångberg (48), in a review, argued that the distance along a fistulous tract to the root-tip may only be a couple of mm. Therefore it can not be excluded that the

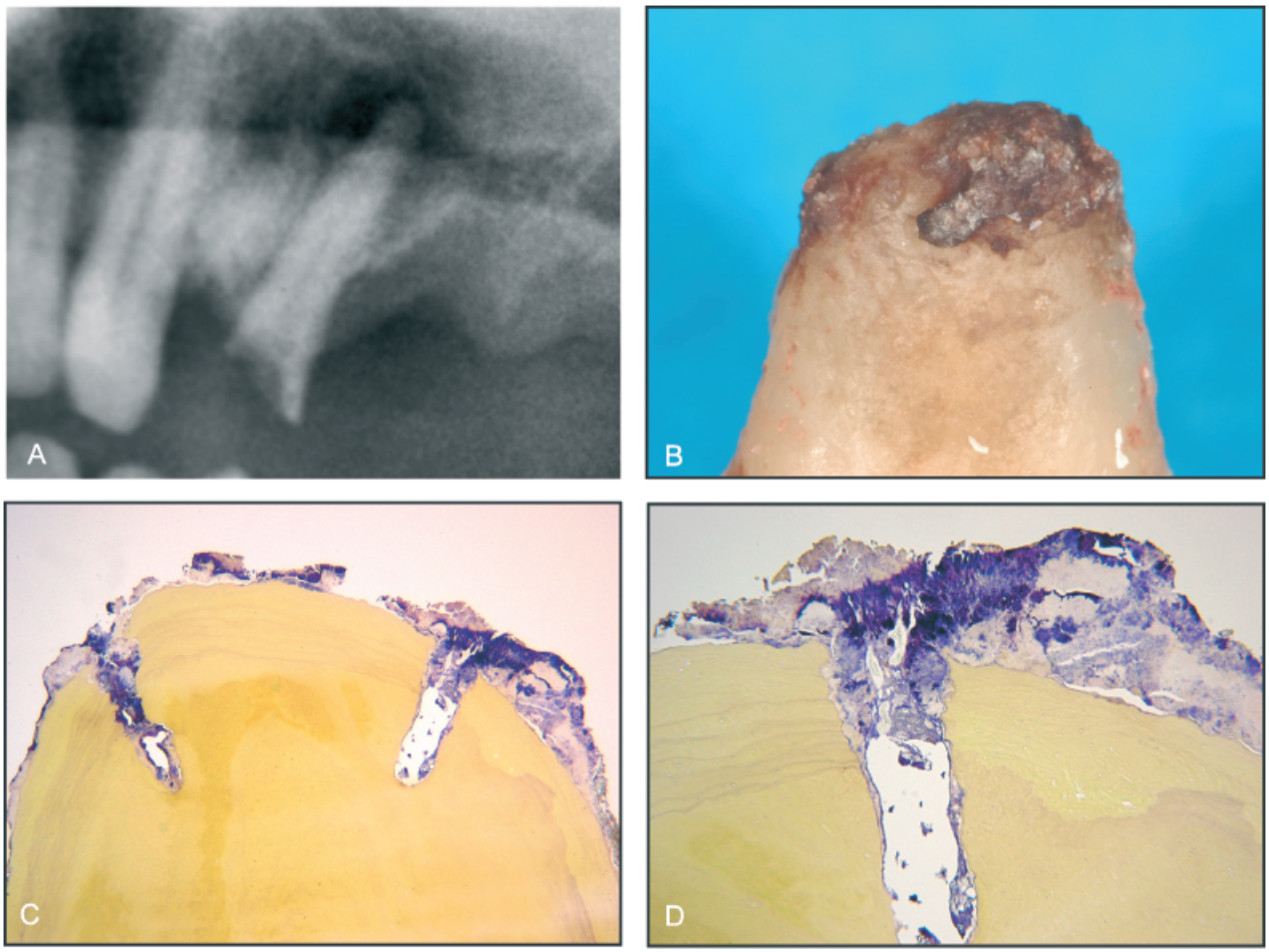


Fig. 4

mechanism for apical biofilm formation, at least after the development of a sinus tract, is similar to the one for plaque and calculus formations in marginal periodontitis.

While the issue of external biofilms on root tips of endodontically involved teeth has attracted considerable attention in recent years, the fact that such bacterial condensations also may occur on the walls of root canals, has more or less escaped notice (see review by Svensäter & Bergenholtz, (49)). Case 2 (Fig. 2J and K) demonstrates that such aggregations may prevail in pockets, clefts or other irregularities of the root canal. It is of particular interest that in this case, the bacterial condensations have not resulted in complete soft tissue necrosis. In fact, while attracting numerous PMNs, more lateral in the root canal, a functional and organized soft tissue seemed to be present suggesting that the tissue destructive effect of the biofilm in this case was moderate.

From a treatment aspect one shall not draw too far-reaching conclusions from the observation of a single case. Yet, if a root canal instrument were to penetrate areas such as those in case 2, bleeding reactions are likely to be evoked and may be interpreted to suggest vital tissue with no need for further instrumentation. Even if instrumented, the bacterial aggregations may have been left behind and, possibly cause a treatment failure in a fashion similar to that in some of the cases reported by Nair et al. (16). Kerekes & Tronstad (50–52) observed in extensive examinations of the root canal diameters of various types of extracted teeth, that large preparations are usually necessary to accomplish a circular canal shape of the most apical portion, whereby the elimination of soft tissue and infected bacterial aggregations on the canal walls are facilitated. However, in oval shaped canals, including certain roots of molar teeth, such preparations can only be a rare outcome without jeopardizing apical over-preparation

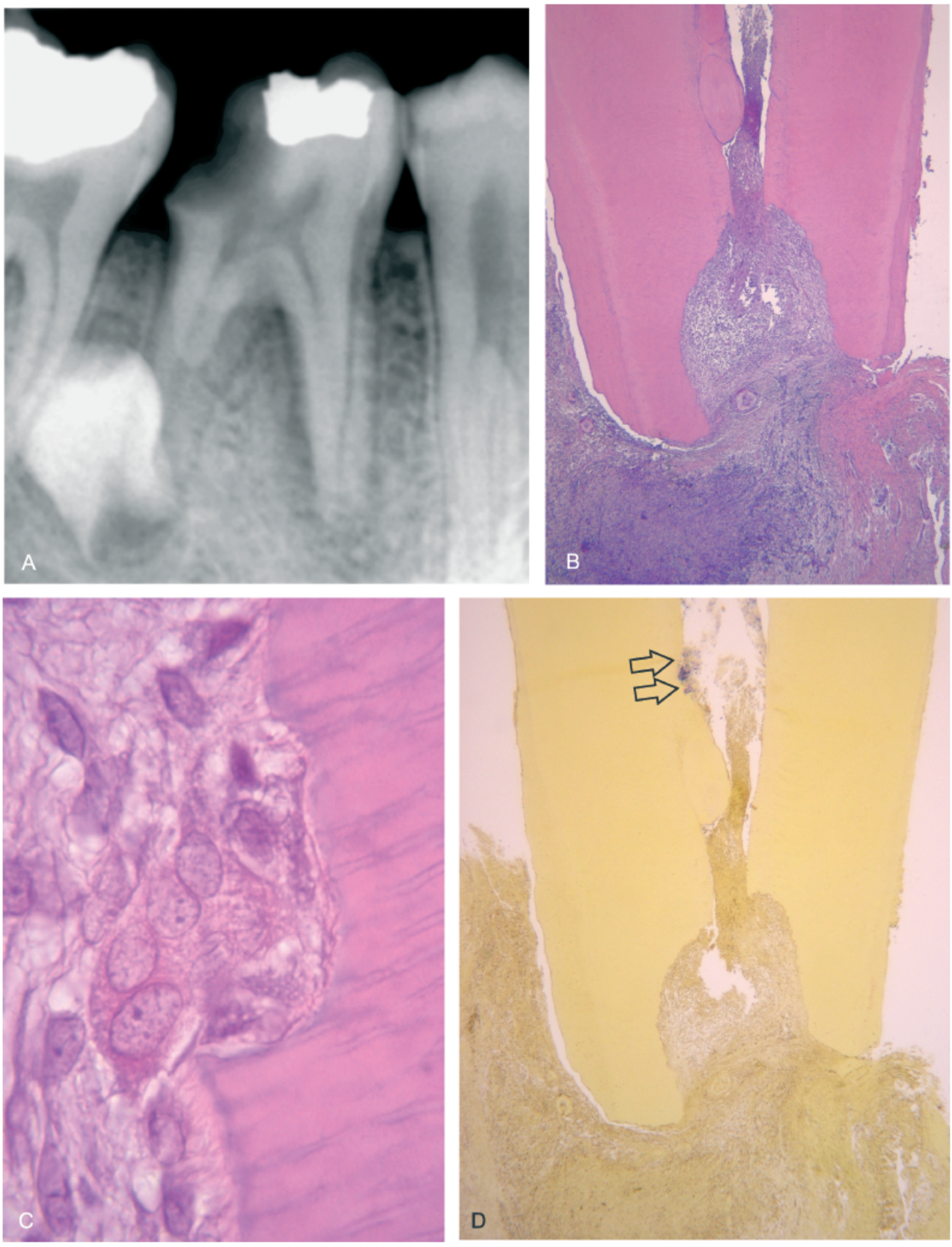


Fig. 5

and lateral perforation (52, 53). Consequently, in the treatment of teeth with infected pulp necrosis, clinicians are faced with the dilemma of balancing under-preparations against over-preparations. It is obvious that in a given case an ideal shape of the canal in its apical portion cannot simply be predicted and especially not when resorptive defects, induced by the inflammatory lesion, have altered the original canal anatomy (see case 5 below). Hence, the clinician must take a decision on how wide and how long the canal should be prepared in each individual case (48). An overwhelming documentation in the endodontic literature, however, suggest that, as a rule of thumb, the clinician is well advised to avoid gross over-preparations and maintain a short safety distance to the anatomical apex ((54–59), for reviews see e.g. (60, 61)).

Root resorption in apical periodontitis

Resorptions that may have altered the original anatomy of the root apex represent a distinct treatment challenge in endodontics. It is probably a more common event in apical periodontitis than usually anticipated, because conventional radiographs often are unable to provide useful diagnostic signs (62, 63, 18). While not being invariably present, apical root resorptions are indeed frequently occurring in clinical cases with pulp necrosis and apical periodontitis (63, 18). Experimental studies in the mouse have confirmed that apical root resorption leading to root shortening is a consistent finding on teeth subjected to pulpal infection (64). Root resorption involving the root tip both internally and externally may also occur as a sequel to mechanical trauma and tissue repair in conjunction with a pulpectomy procedure (65).

Root resorptions associated with apical periodontitis may show different configurations and destroy the apical root structure more or less extensively. They may involve the periforaminal root structures and the inner root canal walls or both (63, 18). In a sample from the study of Nair et al. (19), comprising 114 teeth with attached periapical inflammatory processes, Laux et al. (63) identified 30 specimens with severe defects occurring both externally and internally of the observed root tips. In some of the cases, the resorptive process had opened up the foramen in a basin like fashion and undermined the root structure to the extent that pieces of the root tip became detached and lodged into the periapical inflammatory lesion. In only a few of the cases, with severe root resorption, were radiographs diagnostic. In an analogous sample of 104 root apices, examined by SEM, Vier & Figueiredo (18) reported frequent resorptions of a similar appearance and rate of severity as that in the report by Laux et al. (63). No correlation to presence or extension with the character of the periapical inflammatory process could be confirmed, although most of the lesions in the sample were non-cystic periapical abscesses.

Case 5 relates to a 15-year-old boy. Extensive distal caries penetrating the pulp is visible in the radiograph, together with a radiolucency surrounding the mesial root (Fig. 5A). Radiograph also shows that an extra-numerous premolar is under development. The patient had suffered spontaneous pain. Histologic sections of the mesial root demonstrated an extensive area of resorption in the most apical area not discernible in the radiograph (Fig. 5B). A mineralization process is displayed more coronally. Inflamed tissue is present in the apical portion of the root canal. High magnification ($\times$ 1000) of a resorptive defect shows lacunae in the dentin walls, with typical multinucleated clastic cells (Fig. 5C). Figure 5D demonstrates that the bacterial front is well inside the canal space (arrows).

It seems that root resorption in apical periontitis is initiated simultaneously with the triggering of the bone resorptive activities in the early inflammatory stages (64). Along with the destruction of the periodontal ligament, cementum becomes denuded and potentially exposed to the action of clastic cells. The extent to which root resorption, once initiated, is a progressive process has been a matter of debate. Little data exists, thus far, to support such a notion and, alike root resorptions in trauma cases, both transient and progressive types of apical root resorptions may be identified. Hence, histological examinations of root specimens may reveal areas of clastic activity as well as sites where a previous resorptive defect may have been partly or completely repaired with cementum (1, 66, 67). Why the tissue destruction in certain cases becomes extensive and much less pronounced or non-existing in other cases of apical periodontitis, where all other potentially causative factors appear similar, is poorly understood. Alike other root resorptive processes infection is implicated as a crucial stimulus (68). Wedenberg & Lindskog (69) for example, in an experimental series in the monkey observed the development of internal root resorption by challenging pulps with Freund's complete adjuvant

after either closing up the access opening or leaving it open to the oral environment. Over the 10-week observation period, teeth with open access cavities showed more extensive and lasting clastic activity than non-infected teeth. However, in the tissue samples observed in the studies by Laux et al. (63), Vier & Figueiredo (18), the infection parameter must have been present in all the cases as suggested by the attached periapical inflammatory lesions. Yet, there were varying degrees of hard tissue destruction.

It seems that root resorptions may become less active and may even enter a resting stage after the termination of the initial bone destructive phase in apical periodontitis and even in the presence of an active root canal infection. Maybe the increased release of anti-inflammatory cytokines in the subsequent process plays a role (see the paper by Kiss in this issue of Endodontic Topics). It is obvious that more research is needed to clarify the conditions under which apical root resorption develops and progresses in apical periodontitis.

Clinical inferences of case 5

The existence of apical root resorption may escape detection unless the resorptive defect is large enough to allow radiographic recognition. Bleeding tissue on probing the apical portion of the root canal may be indicative. The dilemma facing the clinician is then to decide if the tissue should be subject to an attempt for removal or whether the working length ought to be taken at the level of the bleeding tissue. Of course in a gross resorption of the nature displayed in case 5, attempt for soft tissue elimination is futile. The extent to which apex locators are helpful in determining the soft tissue level is not well studied, but is likely to work if the resorptive defect is sufficiently large.

Moreover, trying to remove the tissue in an attempt to reach the vicinity of the apical foramen may inadvertently result in an overfilling in the resorptive area, which certainly would not benefit a successful outcome of the treatment. Cotti et al. (70) presented a clinical case of a lower second molar with extensive root resorption mimicking incomplete root development in a 20-year-old patient. Instrumentation to the resorption level and dressing the canal subsequently with calcium hydroxide successfully managed the case. More case studies ought to be to carried out to document the efficacy of such a treatment mode.

Transformation to periapical cyst in apical periodontitis

The development of apical periodontitis to periapical cyst may be seen as an end stage of an ongoing host-defense response to root canal infection. Yet no data are available to suggest that an established periapical lesion such as a periapical granuloma invariably turns into a cyst. Careful examinations of human biopsy samples (8, 19) give rather little indication that this, indeed, is the case as often the specimens in such studies display different categories of tissue responses including periapical abscesses, periapical granuloma with and without proliferating epithelium and fully developed cysts. As a matter of fact cyst formations are often in the minority and may constitute less than 15% of the lesions observed (8, 19). One theory for the development of periapical cysts states that a pre-existing space is required for example an abscess, which may serve as a mould for the ep proliferations (71, 72), see also (22)). Consequently, if this assumption is true, cyst development would be threatening only when the periapical response involves a localized periapical abscess. In general, however, little is known about the process of cyst transformation in apical periodontitis. For an account of existing theories the excellent recent reviews by Nair are suggested (73, 20, 22).

A most conspicuous feature of periapical cysts, but equally poorly understood, is that the process appears able to expand, although slowly, and does not seem to ever arrive at a steady state. Eventually, if left untreated, a considerable portion of the alveolar bone may be consumed. This contrasts the established lesion in apical periodontitis, the periapical granuloma, where the process, after the acute phase, appears to find a balance between repair/regenerative and tissue destructive forces. Valderhaug (72) noted on observing periapical tissue reactions to experimentally induced pulpal infections in the monkey that considerable time is required before the typical configuration of a cyst lumen, partly or fully lined with epithelium, takes form. Of 16 induced pulpal lesions observed after 200 days, 11 developed periapical cyst characteristics, whereas none of the 23 specimens examined by less than 200 days, displayed cyst formation. It should be noted that in all instances, root canals were left open to the oral environment for the entire observation period, thus enabling a gross bacterial exposure of the periapical tissue. In all instances of a fully developed cyst, there was attachment of epithelium to

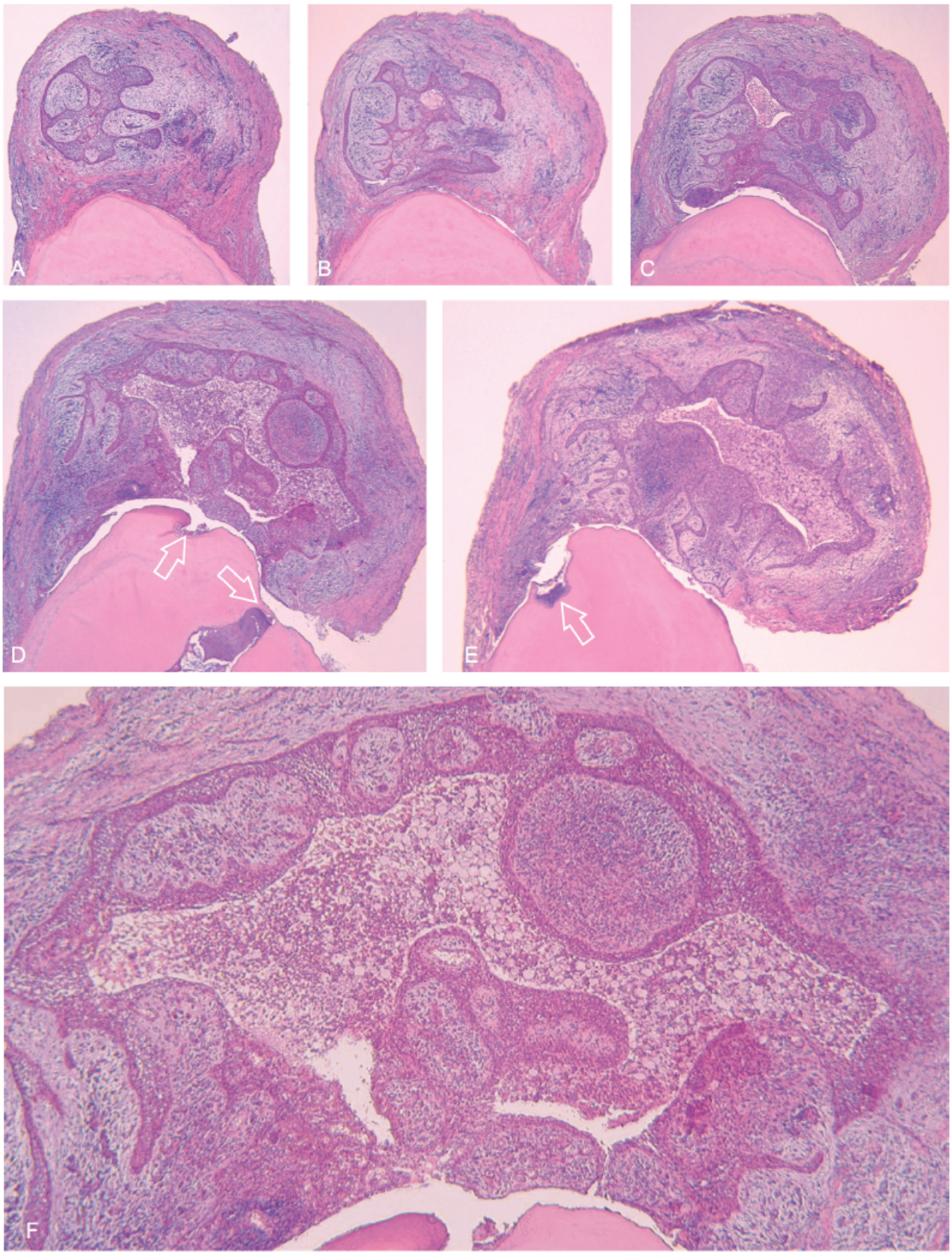
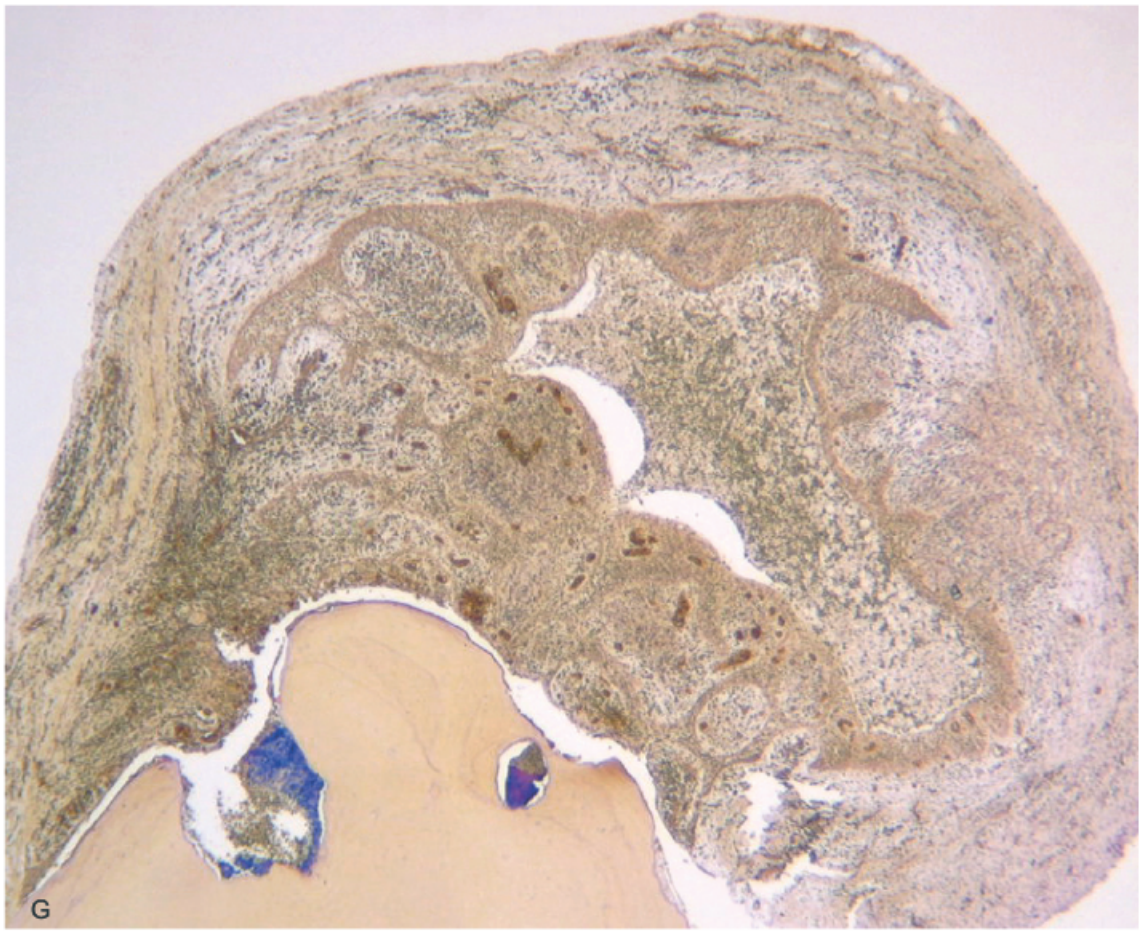


Fig. 6



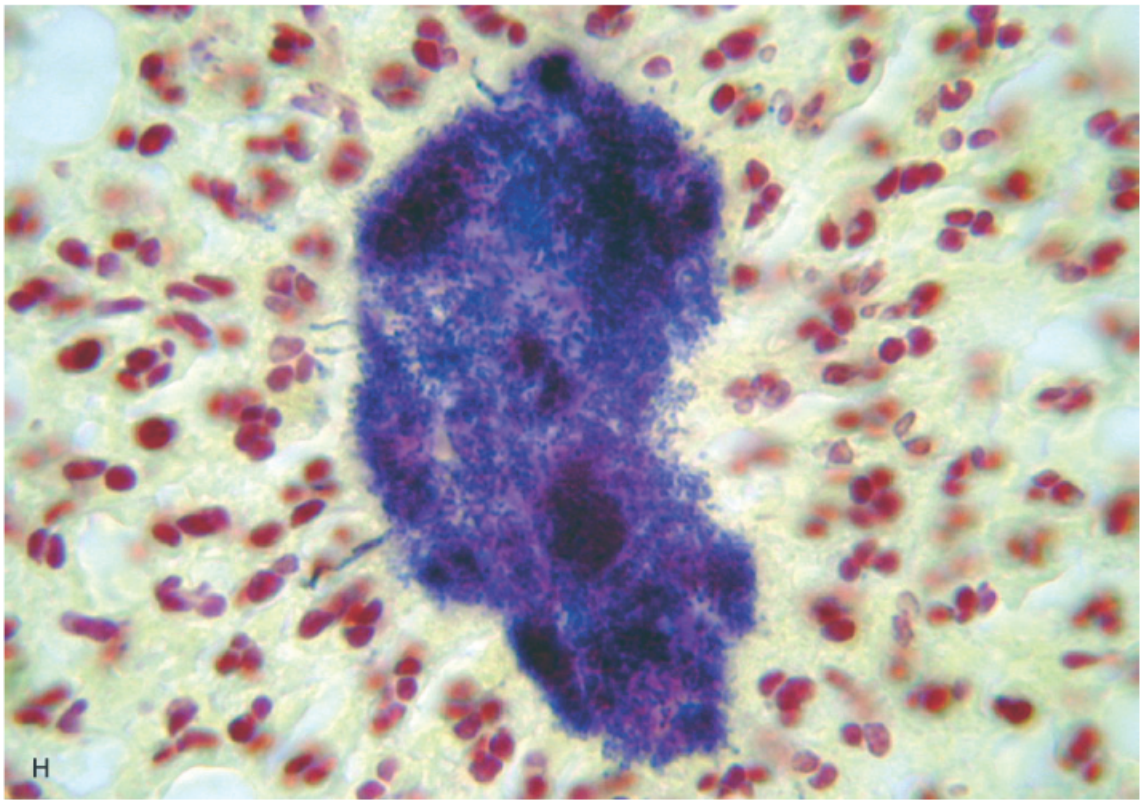


Fig. 6 (continued)

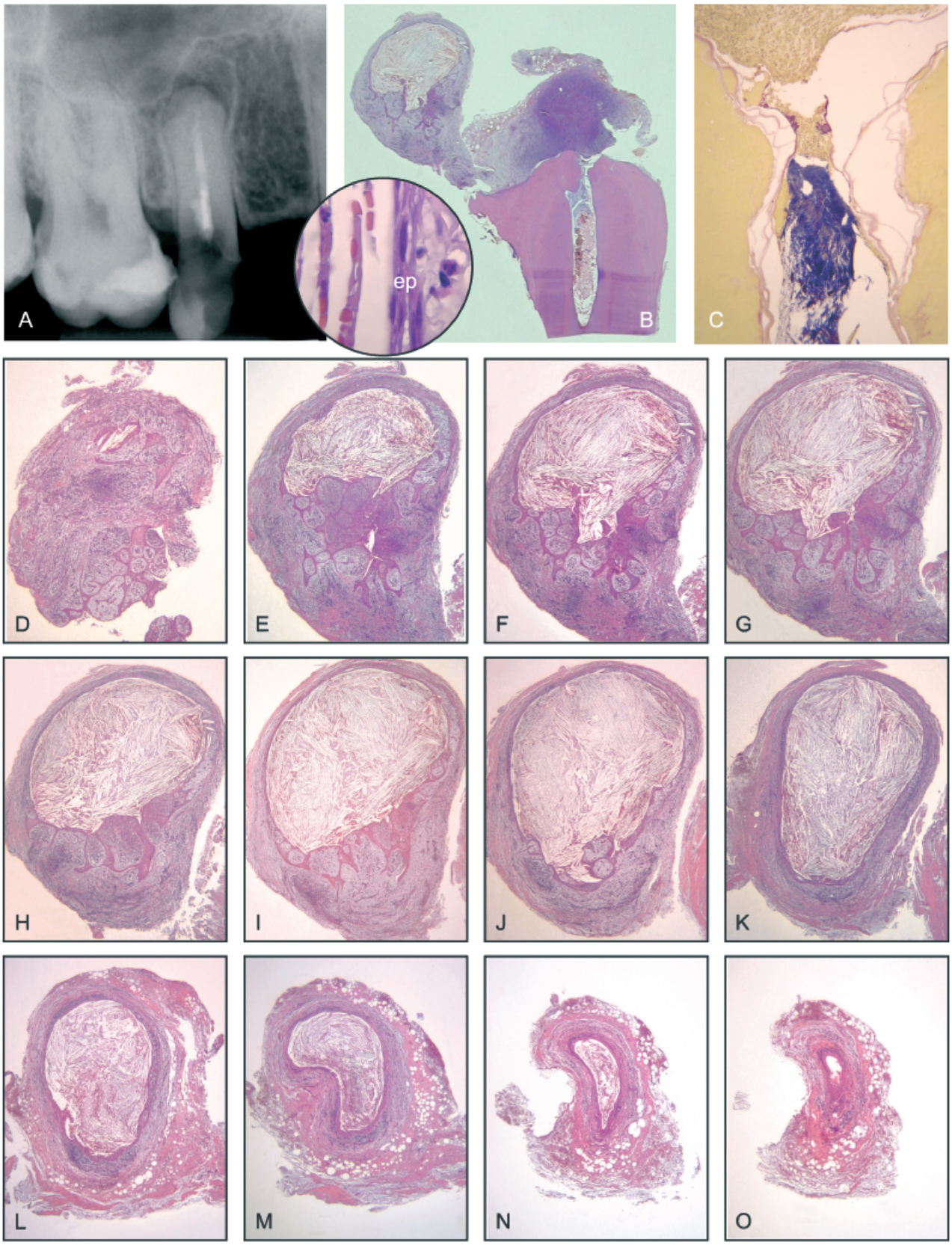


Fig. 7

the root surface. That study did not recognize the configurations described later by Simon (10) and Nair et al. (19) of bay or pocket cyst and true cyst.

Case 6 is an example of what may be termed a true cyst. The lesion was associated with the disto-buccal root of a maxillary first molar in a 36-year-old man, who had a history of repeated flare-ups. Sections taken at different distances show that the lesion could have been histologically diagnosed as an epithelialized granuloma. In Fig. 6A no cyst cavity is present. However from 6B to 6E it is clear that there is a cyst cavity, which is independent of the three foramina appearing in the sections (arrows in D and E). Fig. 6F is the $\times 50$ magnification of the tissue section in D showing complete ep lining of the cavity containing debris and foam cells. Neutrophilic leukocytes are also present but cannot be identified in this magnification.

In sections stained for bacteria, heavy bacterial condensations are seen in the foraminal areas (Fig. 6G) as well as within the cyst lumen (Fig. 6H). The high magnification in Fig. 6H demonstrates a cluster of bacteria surrounded by neutrophilic leukocytes.

Case 7 is another example of what maybe termed a true cyst with the lumen filled with cholesterol crystals. The specimen is from a previously treated second premolar in a 28-year-old man (Fig. 7A). There was a periapical radiolucency but no clinical symptomatology. Figure 7B shows the overview of the sectioned and hematoxylin–eosin stained specimen. The tissue lesion consisted of two distinct entities; one typical periapical granuloma and one comprising the cystic lesion. The inset in Fig. 7B magnifies the stratified ep wall and empty clefts previously occupied by cholesterol. The foraminal area stains for bacteria (Fig. 7C). Serial sections (Fig. 7D–O) demonstrate no communication between the cyst lumen and the root canal. This lesion was cut from one pole to the opposite until the specimen was exhausted.

These two cases confirm the importance of serial sectioning for the establishment of a cyst diagnosis (19, 22). Serial sectioning in addition is required to determine whether or not the cyst cavity has a direct communication with the root canal system and is, thus, important to discriminate a true cyst from a pocket cyst (19, 22).

Clinical inferences of cases 6 and 7

Of distinct clinical importance is the extent to which periapical cysts pose a treatment problem. Nair (74) surmised based on morphological observations (19) that so called true cysts are resistant to conventional, orthograde endodontics. That may be a reasonable assumption given the lack of direct communication of the cyst lumen with the root canal space. Treatment resistance suggests that the process is self-sustaining and thus able to continue and expand regardless of influences of inflammatory mediators released in its vicinity by microbial elements in the root canal space. However, whether this is the situation with true cysts has so far escaped confirmation.

The pocket cyst could also be a challenging treatment problem. As the cyst fluid may be under pressure and continuously wet the canal upon instrumentation, conventional endodontic treatment may be precarious. Yet, calcium hydroxide dressing may offer remedy of the problem. Very recently Caliskan (75) reported on the results of treating teeth with large 'cyst-like' periapical lesions by conventional endodontics. He followed for over 2–10 years the treatment of 42 teeth, which had cholesterol crystals in clear exudates released upon access to the root canal or in fluid aspirated from the root canal space after piercing the apical foramen. Canals were instrumented and filled with calcium hydroxide in glycerin only following the cessation of the discharging exudates. In most cases this took several appointments to drain and up to 1 month in some cases before the treatment could be completed. The calcium hydroxide dressing was changed twice at 3-week intervals and maintained in place for 3 months before the final fill. Complete healing of the periapical lesions occurred in near 75% of the cases. It needs to be pointed out that the cases observed may not necessarily represent cystic lesions as no histology was possible in order to confirm the diagnosis.

Apical surgery is a potentially more rational approach to manage teeth with continuous release of fluid from the root canal space, at least in those cases where exudation is overwhelming and seemingly never ceasing. However, surgery cannot be regarded indicated simply on the basis of lesion appearance in radiographs for example by size, round shape and sharply defined margins. Although, for long, such signs have been thought to be diagnostic for cysts, no clinical data exist, thus far, in support of the view that they are relevant to clinical therapy in more than those exceptional cases, where the lesion has consumed a huge portion of the alveolar bone. Recommendation for apical surgery based upon a 'cystic' appearance in the radiograph must

be regarded a treatment planning error (see further the paper by Abbott in this issue of Endodontic Topics.) It deserves to be reiterated that clinical follow-up studies of endodontically treated teeth have confirmed that conventional root canal treatment in the presence of a periapical lesion has a very high success rate, if properly conducted (e.g. (55–57, 59)). Furthermore, in biopsy examinations of failed endodontic treatments, no documentation has yet been presented to indicate that cysts are overrepresented, a finding suggesting that many radicular cysts may heal after root canal treatment (73). Hence, current understanding suggests that most lesions of apical periodontitis are treatable by a conventional orthograde approach provided the microbial irritants in the root canal system can be controlled. In the case lesions do not resolve after periodic radiographic recall, or if the exudative process along the root canal space is abundant, a surgical treatment may an be excellent adjunct, provided the root tips are resected and the apical portion of the root canal retro-filled to eliminate potential bacterial condensations inside or outside the root canal space.

Concluding remarks

On observing cases of the nature described here it needs to be realized that although interesting clues can be obtained as to the dynamic events that may take place in apical periodontitis, the study of human biopsy materials have some obvious limitations. This is especially true for non-treated teeth, where the case history will only be vaguely known and reliant on the patient's report. Thus, the time span from the early onset to the point in time at which the biopsy is obtained is usually not possible to define. Therefore a single biopsy can only give a snapshot of the condition that existed at the time the biopsy was taken. Consequently it will not reflect much of the set of events that has either preceded or is about to follow. As remarked by Walton & Garnick (76) another limitation of the biopsy technique employed, in the kind of cases displayed here, is that only adherent soft tissue and tooth structure are included, whereas study of the adjacent bone is left out. Thus, the extent of active bone resorption surrounding the inflammatory process will escape assessment. Only animal study or block sections, which include the root portion, the soft-tissue lesion and the adjacent alveolar bone would allow such a study. In terms of human biopsies only small lesion would be possible (58) as otherwise a non-defensible, large surgical defect would result. Histologic examination of human biopsy specimens has nevertheless served as an important basis for the development of clinical treatment strategies in endodontics and for our current understanding of the natural history of apical periodontitis (1, 8, 16, 74).

References

1. Thoma KH. A histo–pathological study of thee dental granuloma and diseased root apex. *J Nat Dent Assoc* 1917: **4**: 1075–1090.
2. Hill TJ. The epithelium in dental granulomata. *J Dent Res* 1930: **10**: 323–332.
3. Bhaskar SN. Periapical lesion: types, incidence, and clinical features. *Oral Surg Oral Med Oral Pathol* 1966: **21**: 657–671.
4. Sonnabend E, Oh C-S. Zur Frage des Epithels im apikalen Granulationswebe (Granulom) menschlicher Zähne. *Dtsch Zahnärztl Z* 1966: **21**: 627–643.
5. Brynolf I. A histological and roentgenological study of the periapical region of human upper incisors. *Odontol Revy* 1967: **18**(Suppl 1l).
6. Lalonde ER, Luebke RG. The frequency and distribution of periapical cysts and granulomas. *Oral Surg Oral Med Oral Pathol* 1968: **25**: 861–868.
7. Mortensen H, Winther JE, Birn H. Periapical granulomas and cysts. An investigation of 1600 cases. *Scand J Dent Res* 1970: **78**: 241–250.
8. Block RM, Bushell A, Rodrigues H, Langeland K. A histologic, histobacteriologic, and radiographic study of periapical endodontic surgical specimens. *Oral Surg Oral Med Oral Pathol* 1976: **42**: 656–678.
9. Langeland K, Block RM, Grossman LI. A histopathologic and histobacteriologic study of 35 periapical endodontic surgical specimens. *J Endod* 1977: **3**: 8–23.
10. Simon JHS. Incidence of periapical cysts in relation to the root canal. *J Endod* 1980: **6**: 845–848.
11. Yanagisawa S. Pathologic study of periapical lesions 1. Periapical granulomas: clinical, histopathologic and immunohistopathologic studies. *J Oral Pathol* 1980: **9**: 288–300.
12. Weiner S, McKinney RV Jr, Walton RE. Characterization of the periapical surgical specimen. A morphologic and histochemical study of inflammatory patterns. *Oral Surg Oral Med Oral Pathol* 1982: **53**: 292–302.
13. Bergenholtz G, Lekholm U, Liljenberg B, Lindhe J. Morphometric analysis of chronic inflammatory periapical lesions in root-filled teeth. *Oral Surg Oral Med Oral Pathol* 1983: **55**: 295–301.
14. Lin L, Shovlin F, Skribner J, Langeland K. Pulp biopsies from the teeth associated with periapical radiolucencies. *J Endod* 1984: **10**: 436–448.

15. Nair PNR. Light and electron microscopic studies on root canal flora and periapical lesions. *J Endod* 1987: **13**: 29–39.
16. Nair PNR, Sjögren U, Kahnberg KE, Krey G, Sundqvist G. Intraradicular bacteria and fungi in root-filled asymptomatic human teeth with therapy resistant periapical lesions: a long-term light and electronmicroscopic follow-up study. *J Endod* 1990: **16**: 580–588.
17. Pascon EA, Leonardo MR, Safavi KE, Langeland K. Tissue reaction to endodontic materials: methods, criteria, assessment, and observations. *Oral Surg Oral Med Oral Pathol* 1991: **72**: 222–237, Erratum in: *Oral Surg Oral Med Oral Pathol* 1992; **73**: 347.
18. Vier FV, Figueiredo JA. Prevalence of different periapical lesions associated with human teeth and their correlation with the presence and extension of apical external root resorption. *Int Endod J* 2002: **35**: 710–719.
19. Nair PNR, Pajarola G, Schroeder HE. Types and incidence of human periapical lesions obtained with extracted teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996: **81**: 93–102.
20. Nair PNR. Apical periodontitis: a dynamic encounter between root canal infection and host response. *Periodontology 2000* 1997: **13**: 121–148.
21. Ricucci D, Langeland K. Apical limit of root canal instrumentation and obturation, part II. A histological study. *Int Endod J* 1998: **31**: 394–409.
22. Nair PNR. Non-microbial etiology: periapical cysts sustain post-treatment apical periodontitis. *Endod Topics* 2003: **6**: 96–113.
23. Stern MH, Dreizen S, Mackler BF, Selbst AG, Levy BM. Quantitative analysis of cellular composition of human periapical granuloma. *J Endod* 1981: **7**: 117–122.
24. Stern MH, Dreizen S, Mackler BF, Levy BM. Isolation an charterization of inflammatory cells from the human periapical granuloma. *J Dent Res* 1982: **61**: 1403–1412.
25. Matthews JB, Mason GI. Immunoglobulin producing cells in human periapical granulomas. *Br J Oral Surg* 1983: **21**: 192–197.
26. Perrini N, Fonzi L. Mast cells in human periapical lesions: ultrastructural aspects and their possible physiopathological implications. *J Endod* 1985: **11**: 197–202.
27. Skaug N, Johannessen A-C, Nielsen R, Matre R. In situ characterization of cell infiltartees in human dental periapical granulomas 3. Demonstration of T lymphocytes. *J Oral Pathol* 1984: **13**: 120–127.
28. Johannesen AC, Nilsen R, Skaug N. Enzyme histochemical characterization of mononuclear cells in human dental periapical chronic inflammatory lesions. *Scand J Dent Res* 1984: **92**: 325–333.
29. Torabinejad M, Kettering J. Identification and relative concentration of B and T lymphocytes in human chronic periapical lesions. *J Endod* 1985: **11**: 122–125.
30. Lukic A, Arsenijevic N, Vujanic G, Ramic Z. Quantitative analysis of the immunocompetent cells in periapical granuloma: correlation with the histological characteristics of the lesions. *J Endod* 1990: **16**: 119–122.
31. Piattelli A, Artese L, Rosini S, Quarenta M, Musiani P. Immune cells in periapical granulomaa: morphological and immunohistochmical characterization. *J Endod* 1991: **17**: 26–29.
32. Babal P, Soler P, Brozman M, Jakubovsky J, Beyly M, Basset F. In situ characterization of cells in periapical granuloma by monoclonal antibodies. *Oral Surg Oral Med Oral Pathol* 1987: **64**: 348–352.
33. Marton IJ, Kiss C. Characterization of inflammatory cell infiltrate in dental periapical lesions. *Int Endod J* 1993: **26**: 131–136.
34. Takahashi K, MacDonald DG, Kinane DF. Analysis of immunoglobulin-synthesizing cells in human dental periapical lesion by in situ hybridization and immunohistochemistry. *J Oral Pathol* 1996: **25**: 331–335.
35. Walker KF, Lappin DF, Takahashi K, Hope J, MacDonald DG, Kinane DF. Cytokine expression in periapical granulation tissue as assessed by immunohistochemistry. *Eur J Oral Sci* 2000: **108**: 195–201.
36. Stashenko P, Teles R, De Souza R. Periapical inflammatory responses and their modulation. *Crit Rev Oral Biol Med* 1998: **9**: 498–521.
37. Sundqvist G, Figdor D. Life as an endodontic pathogen. Ecological differences between untreated and root-filled root canals. *Endod Topics* 2003: **6**: 3–28.
38. Trope M, Bergenholtz G. Microbiological basis for endodontic treatment: can a maximal outcome be achieved in one visit? *Endod Topics* 2002: **1**: 40–53.
39. Langeland K. Tissue response to dental caries. *Endod Dent Traumatol* 1987: **3**: 149–171.
40. Tronstad L, Titterud Sunde P. The evolving new understanding of endodontic infections. *Endod Topics* 2003: **6**: 57–77.
41. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. *Endod Dent Traumatol* 1990: **6**: 73–77.
42. Lomcali G, Sen BH, Cankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. *Endod Dent Traumatol* 1996: **12**: 70–76.
43. Ferreira FBA, Ferreira AL, Gomes BPF, Souza-Filho FJ. Resolution of persistent periapical infection by endodontic surgery. *Int Endod J* 2004: **37**: 61–69.
44. Noiri Y, Ehara A, Kawahara T, Takemura N, Ebisu S. Participation of bacterial biofilms in refractory and chronic periapical periodontitis. *J Endod* 2002: **28**: 679–683.
45. Leonardo M R, Rossi MA, Silva LAB, Ito IY, Bonifácio C. EM evaluation of bacterial biofilm and microorganisms on the apical external root surface of human teeth. *J Endod* 2002: **28**: 815–818.
46. Siqueira JF, Lopes H P. Bacteria on the apical root surfaces of untreated teeth with periradicular lesions: a scanning electron microscopic study. *Int Endod J* 2001: **34**: 216–220.
47. Ricucci D, Martorano M, Bate AL, Pascon EA. Calculus-like deposit on the apical external root surface of teeth with post-treatment apical periodontitis: report of two cases. (Submitted).

48. Bergenholtz G, Spångberg L. Controversies in endodontics. *Crit Rev Oral Biol Med* 2004: **15**: 99–114.
49. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. *Endod Topics* (in press).
50. Kerekes K, Tronstad L. Morphometric observations on root canals of human anterior teeth. *J Endod* 1977: **3**: 24–29.
51. Kerekes K, Tronstad L. Morphometric observations on root canals of human premolars. *J Endod* 1977: **3**: 74–79.
52. Kerekes K, Tronstad L. Morphometric observations on the root canals of human molars. *J Endod* 1977: **3**: 114–118.
53. Wu MK, van der Sluis LW, Wessenlink PR. The capability of two hand instrumentation techniques to remove the inner layer of dentin. *Int Endod J* 2003: **36**: 218–224.
54. Ketterl W. Histologische Untersuchungen über die Behandlung der Pulpitis mit Hilfe der Quersnitt-Mess Technik nach A. Mayer. *Dtsch Zahnärtzl Z* 1955: **10**: 773–783.
55. Strindberg LZ. The dependence of the results of pulp therapy on certain factors. *Acta Odontol Scand* 1956: **14**(Suppl 21).
56. Grahnén H, Hansson L. The prognosis of pulp and root canal therapy. A clinical and radiographic follow-up examination. *Odontol Rev* 1961: **12**: 146–165.
57. Kerekes K, Tronstad L. Long-terms results of endodontic treatment performed with a standardized technique. *J Endod* 1979: **5**: 83–90.
58. Bergenholtz G, Lekholm U, Milthon R, Engström B. Influence of apical overinstrumentation and overfilling on retreated root canals. *J Endod* 1979: **5**: 310–314.
59. Sjögren U, Hägglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. *J Endod* 1990: **16**: 498–504.
60. Ricucci D. Apical limit of root canal instrumentation and obturation. Part I. Literature review. *Int Endod J* 1998: **31**: 384–393.
61. Kirkevang L-L, Hörsted-Bindslev P. Technical aspects of treatment in relation to treatment outcome. *Endod Topics* 2002: **2**: 89–202.
62. Malueg, Wilcox LR, Johnson W. Examination of external apical root resorption with scanning electron microscopy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996: **82**: 89–93.
63. Laux M, Abbott PV, Pajarola G, Nair PNR. Apical inflammatory root resorption: a correlative radiographic and histological assessment. *Int Endod J* 2000: **33**: 483–493.
64. Balto K, White R, Mueller R, Stashenho P. A mouse model of inflammatory root resorption induced by pulpal infection. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002: **93**: 461–468.
65. Engström B, Spångberg L. Wound healing after partial pulpectomy. A histologic study performed on contralateral tooth pairs. *Odontol Tidskr* 1967: **75**: 5–18.
66. Andreasen JO. Cementum repair after apicectomy in humans. *Acta Odontol Scand* 1973: **31**: 211–221.
67. Andreasen JO, Rud J. Modes of healing histologically after endodontic surgery in 70 case. *Int J Oral Surg* 1972: **1**: 148–160.
68. Tronstad L. Root resorption, etiology, terminology and clinical manifestation. *Endod Dental Traumatol* 1988: **4**: 241–252.
69. Wedenberg C, Lindskog S. Experimental internal resorption in monkey teeth. *Endod Dent Traumatol* 1985: **1**: 221–227.
70. Cotti E, Lusso D, Dettori C. Management of apical inflammatory root resorption: report of a case. *Int Endod J* 1998: **31**: 301–304.
71. Forsberg A, Hägglund G. Den radikulära tandcystans genes och fysikaliska expansion. *Sven Tandlak Tidskr* 1959: **52**: 223–244.
72. Valderhaug J. A histologic study of experimentally induced radicular cysts. *Int J Oral Surg* 1972: **1**: 137–147.
73. Nair PNR. New perspectives on radicular cysts: do they heal? *Int Endod J* 1998: **31**: 155–160.
74. Nair PNR, Sjögren U, Schumacher E, Sundqvist G. Radicular cyst affecting a root-filled human tooth: a long-term post-treatment follow-up. *Int Endod J* 1993: **26**: 225–233.
75. Caliskan MK. Prognosis of large cyst-like periapical lesions following nonsurgical root canal treatment: a clinical review. *Int Endod J* 2004: **37**: 408–416.
76. Walton RE, Garnick JJ. The histology of periapical inflammatory lesions in permanent molars in monkeys. *J Endod* 1986: **12**: 49–53.