A histopathologic, histobacteriologic, and radiographic study of periapical endodontic surgical specimens

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Data available on 230 periapical endodontic surgical specimens were studied. It was found that bacteria occurred in the periapical tissue very infrequently. In addition, there was no correlation between the presence of acute inflammatory cells and the presence or absence of pain. Granulomas with epithelium occurred in 61 out of 230 cases, while 14 of these were cysts. Of the 110 cases with radiographic follow-up data, 67 were classified as successful, 40 were uncertain, and 3 were unsuccessful according to a modification of Strindberg's criteria, whereas 107 would have been successes according to the criteria of Bender and Seltzer and their associates. No valid biologic or clinical basis for endodontic therapy as suggested by Bhaskar was found in this material.

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Numerous investigators have attempted to correlate the histopathology and bacteriology of the pulp and periapical tissues of the human dentition. Henrici and Hartzell recorded only presence or absence of bacteria after splitting teeth with forceps and culturing, whereas Harndt and Kühn attempted to localize the microorganisms in histopathologic studies. Harndt observed microorganisms in some granulomas, whereas Kühn considered this an unsolved question and proposed that bacterial toxins and tissue degradation and disintegration products within the root canal system could be the etiologic agents for the development and continuance of periapical pathosis. It could be extrapolated that the periapical inflammation demonstrated by Torneck and his colleagues was caused by bacterial toxins in the root canals of the involved exposed teeth. Langeland and Langeland supported this idea on the basis of studies in which microorganisms in the superficial end of the dentinal tubules, bacteria in the dentinal tubules adjacent to a filling margin, or iatrogenic dentin changes stimulated a pulpal inflammation without the presence of microorganisms in the pulp. Following the penetration by bacteria to the pulp and consequent breakdown, bacteria appeared in the necrotic part of the pulp, but not in the inflamed, healthy part of the pulp adjacent to the necrosis. Also, when no more than 0.5 mm. of vital pulp remained, bacteria were present in the root canal and in the adjacent dentinal tubules, but they were not seen in the granuloma. On the basis of these findings, it was suggested that the old theory of focal infection was not valid, but the relationship between microorganisms and the types of cells in the pulpal disintegration and inflammation indicated that the pulpal and periapical response was immunologic in nature. This idea was further supported by the demonstration of circulating antibodies in monkeys after introduction of sheep erythrocytes, bovine serum albumin, and human gamma globulin in their pulp chambers and by generally accepted concepts in immunology. This concept, however, was challenged by Stewart and by Winkler and co-authors, who believed that actual bacterial invasion of the periapical tissues was the etiologic factor.

The previous scientific methods used for bacterial analysis of the periapical tissues were (1) periapical surgery with culturing used by Thoma, Kesel, and Birch and associates, (2) intracanal culturing reported by Coolidge, Grossman, Winkler and Van Amerongen, Bender and Seltzer, and Sulzzeanu and colleagues, (3) trocar and canalization demonstrated by Coriell and later used by Grossman, Bernier, Alin and Agren, Burkett, and Grossman and Prinz, and (4) the examination and bacterial analysis of extracted teeth. The basic problem with all of these methodologies was that, even under strictly controlled conditions, the risk of bacterial contamination was a serious problem.

Several investigators have performed bacteriologic and histopathologic studies on extracted human teeth with attached periapical lesions. Turner and Drew stated that sections of dental granulomas, after extraction of human teeth, invariably showed the presence of microorganisms, particularly cocci and the diphtheroids. Specific location of the bacteria was not reported, and there was no indication of any inflammatory response to these microorganisms.

Bulleid found that only 15 per cent of 400 extracted teeth had periapical
lesions. He thought that the periapical pathologic tissue was usually removed with the tooth and that he could sterilize the outside surface of the specimen and culture the internal contents without external contamination. On the basis of this methodology, he concluded that all dental granulomas were infected with bacteria. Boyle examined 63 extracted human teeth histologically. Giemsa, Gram, and eosin-methylene blue stains demonstrated large numbers of gram-positive bacilli within phagocytic cells in the solid portions of attached dental granulomas. Boyle claimed that these microorganisms were not contaminants, since they were intracellular.

Winkler and associates examined fifteen extracted teeth with attached periapical lesions. After extraction, these lesions were found to be both clinically and histologically intact. Using hematoxylin and eosin, as well as the Johns Hopkins modified bacterial tissue (Gram stain), these investigators found bacteria dispersed uniformly throughout the granulomas in slight to moderate concentrations in most cases. Hamed examined forty dental granulomas from extracted human teeth and concluded that all solid granulomas were sterile. On the other hand, granulomas with areas of degeneration were found to contain bacteria as contaminants in gaps and fissures of the tissue. Kühn stated that he could demonstrate only three out of twenty-eight to be "infected with bacteria" which could not be excluded as contaminants caused by tooth extraction. Quoting Kronfeld, Grossman stated "that a tooth with a granuloma may have an infected root canal, but a sterile periapical tissue. In Gram-stained sections through infected pulpless teeth in situ that were examined, bacteria in abundance were always found within the root canal but granulation tissue and cysts attached to the apices of these teeth were often free from microorganisms, and that a granuloma is not an area in which bacteria live, but in which they are destroyed."

Appleton, Blayney, and Burket and Burn pointed out that it was difficult, if not impossible, to extract teeth and remove periapical tissue from the adjoining root surface without risking contamination. Andreasen and Rud and their colleagues examined sixty-six biopsy specimens containing apices and periapical tissue in order to evaluate the relation between bacteria in the dental structures and periapical tissue changes. Using modified gram stain as suggested by Crone, they found bacteria in the root canal and the adjacent dentinal tubules located near the main canal. No relationship was found between the amount and location of the bacteria in the dentin tubules and the quantity of periapical inflammation. It was concluded that these bacteria were of minimal significance. In only three cases were bacteria found in the periapical soft tissues, despite the fact that many of the specimens showed evidence of a severe periapical inflammation.

Although Tagger and Massler did not address themselves specifically to the bacteriologic aspects, bacteria obviously played an important part in their experimentation, since they left rat molars open to the oral cavity after perforation of the pulp. They reported two types of reaction (one suppurative and one reparative) and concluded that the periapical tissue had a good healing potential.

Lundy and Stanley attempted to correlate pulp histopathology and clinical symptoms in human teeth left open to saliva. They reported that the initial pulp
Table I. Clinical and histologic data of 230 cases

<table>
<thead>
<tr>
<th>Histologic</th>
<th>Clinical</th>
<th></th>
<th></th>
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<td>57</td>
<td>47</td>
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<td>230</td>
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<td>185</td>
<td>57</td>
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<td>230</td>
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<td>14</td>
<td>9</td>
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<td>74</td>
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<tr>
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<td>23</td>
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<td>8</td>
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<td>11</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Total No. of cases</td>
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<td>185</td>
<td>57</td>
<td>47</td>
<td>33</td>
<td>39</td>
<td>230</td>
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*Regardless of location.

Table II. Radiographic follow-up classification

<table>
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<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
<th>5 years</th>
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<td>9</td>
<td>4</td>
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<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>40</td>
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<tr>
<td>Unsuccessful</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>Total</td>
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<td>14</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>110</td>
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</table>

responses were quite severe, but if 1.5 mm. or more dentin remained it protected the pulp, that the longer the teeth remained open the greater the amount of bacterial penetration, and that severe clinical responses usually indicated predominance of neutrophilic leukocytes in the pulp. In a study of germ-free rats Kakehashi and co-workers\textsuperscript{10} reported complete healing of pulps exposed to saliva.

PURPOSE

The purpose of this investigation was to study (1) the presence or absence of bacteria within periapical lesions of endodontically treated human teeth, (2) the occurrence and frequency of chronic inflammatory cells found in periapical lesions, (3) the occurrence and frequency of acute inflammatory cells and epithelium in periapical lesions, (4) the possible correlation of clinical signs and symptoms with the specific histologic findings, and (5) the correlation of the clinical and radiographic success of endodontic surgery with specific histologic findings.

MATERIALS AND METHODS

The experimental material comprised 230 biopsy specimens obtained during endodontic surgical therapy. Surgical intervention was performed in the presence of the signs or symptoms of pain, swelling, fistula, or calcified, missed, or perforated canals combined with periapical radiolucenties. In all 230 cases, clinical data were available (Table I). In 110 of these cases the records consisted of radiographic preoperative, postoperative, and follow-up data ranging from $\frac{1}{2}$ year to 5 years (Table II).
CLINICAL INFORMATION

The following pre- and postoperative clinical signs and symptoms were recorded from the patients' records: Occurrence of soft-tissue swelling and/or fistula; previous and present pain elicited by mechanical, cold, and heat, stimuli; response to cold, heat, percussion, and electric test.

The radiographs were evaluated for conditions of previous endodontic treatment, deep cavities, calcified or perforated canals, and periapical radiolucency. Persisting periapical rarefactions existing after treatment were measured in the same manner. The radiographs were classified into the categories of success, uncertain, or unsuccessful, using a modification of the guidelines suggested by Strindberg. In addition, results of the endodontic surgery were evaluated on the basis of the patient's history, clinical signs and symptoms, and a follow-up radiograph.

SURGICAL PROCEDURES

Lidocaine hydrochloride 2 per cent with 1:50,000 epinephrine was used to anesthetize the surgical area. Anterior to the involved tooth a vertical or oblique incision was made with a gingivectomy knife from the gingival crest into the attached gingiva, and a full-thickness flap of tissue was deflected. As much bone as necessary was removed with a 701 surgical-length friction-grip bur to obtain access and visibility of the periapical lesion. A surgical curette was used to carefully remove each specimen, which was immediately placed in a 10 per cent buffered formalin solution.

LABORATORY PROCEDURES

The histologic, histochemical, and histobacteriologic methods used in this investigation can be found in relevant textbooks. Specific application for the study of teeth has been described. After fixation and processing, between 80 and 280 semiserial sections were cut at 5 microns of each of the 230 specimens. The following stains were used for histologic and histobacterial evaluation: (1) hematoxylin and eosin on sections of all 230 specimens; (2) Brown and Brenn on sections of all 230 specimens; (3) the Johns Hopkins modified Gram stain on selected sections to supplement the Brown and Brenn stain; and (4) Masson's trichrome on selected sections for positive identification of epithelium.

CLINICAL CRITERIA

Criteria for clinical and radiographic follow-up classification according to a modification of Strindberg's are as follows:

Successful
1. No signs or symptoms present on follow-up examination.
2. Complete resolution of the lesion with redevelopment of a continuous lamina dura or a normal-appearing periodontal membrane space.

Uncertain
1. No signs or symptoms present on follow-up examination.
2. The initial pathologic lesion on the radiograph did not resolve completely but either stayed the same size or decreased in size.
Unsuccessful
1. Signs or symptoms present on follow-up examination.
2. The radiographic size of the initial lesion increased in size after treatment.
3. Further treatment was necessary on the tooth in question.

Histopathologic Criteria

The sections were evaluated by criteria established in earlier investigations. These were:
1. The presence of chronic inflammatory cells: lymphocytes, monocytes, plasma cells, macrophages, foam cells, mast cells, and foreign-body cells.
2. The occurrence of acute inflammatory cells: neutrophilic leukocytes.
3. Location of bacteria related to the inflammatory cells.
4. Location of bacteria in tooth structure and in or related to periapical tissue.
5. The presence of extravasated erythrocytes in the tissue.
6. The occurrence of brown pigment related to disintegrating erythrocytes.
7. The presence of cholesterol clefts.
8. The occurrence of nonbiologic foreign material.
9. The presence of fibrocytes and fibers.
10. Root and/or bone resorption.
11. The presence of epithelium without fluid or semisolid filled space, a granuloma.
12. The presence of an epithelium-lined space filled with remnants of fluids or semisolids, a cyst.

Observations

Histologic and histobacteriologic

A correlation of the clinical and histologic data appears in Table I. According to the listed criteria, 169 granulomas without conspicuous epithelium and sixty-one granulomas with distinct epithelium were found (Fig. 1). In fourteen cases epithelial enclosed spaces containing disintegrating blood and other tissue occurred. The latter were recorded as developing or true cysts (Figs. 2 and 3).

Connective tissue surrounded by epithelium was infiltrated by numerous chronic inflammatory cells mixed with considerable numbers of neutrophilic leukocytes (Fig. 1, B and C), which were also observed in the surrounding epithelium (Figs. 1, B, 2, B, and 4, A). When this occurred, empty spaces were present in the epithelium, epithelial cells lining the area of extravasated erythrocytes appeared swollen, and brown pigment was observed among the erythrocytes (Fig. 2, C). With further development of this condition, a space was developed (Fig. 3, A). In the surrounding tissue brown pigment which was refringent in polarized light was a common occurrence (Fig. 3, B E, 204 of 230 cases). All types of chronic inflammatory cells were present in varying numbers and distribution, either alone or mixed with neutrophilic leukocytes. The chronic inflammatory cells were small and large lymphocytes, plasma cells,
Fig. 1. Case 214, Tooth No. 2 had a history of root canal therapy 16 years earlier. Currently there was a generalized periodontal disease with acute pain in the region of this tooth. There was a 1 mm. area of apical rarefaction on the mesial root. A hemisection was performed and the mesial root was removed. A 2-year follow-up indicated an asymptomatic tooth with uncertain radiographic status (Fig. 10). A, Granuloma with epithelium, but no sacs with liquefied or semisolid tissue. B, From area indicated by arrow in A. Epithelium enclosing loose connective tissue with acute and chronic inflammatory cells. Neutrophilic leukocytes also among epithelial cells. C, From concentration of cells in B. Neutrophilic leukocytes (nl) lymphocytes (ly), macrophage (m) with inclusion in lacuna, plasma cell (p) with two nuclei. (Original magnifications: A, ×25; B, ×320; C, ×1,000.)
Fig. 2. Case 248. Teeth 7 and 8 (Fig. 9) presented with a history of trauma in which the teeth were displaced lingually and repositioned several years previously. At the time of endodontic intervention there was moderate swelling but no pain. There was a 4 mm. apical area radiographically. At the initial appointment both teeth were débrided and totally instrumented, and 4 days later they were filled with gutta-percha. Three days after obturation, curettage was performed on both teeth, and an apical amalgam was placed on No. 8. A 4-year clinical and radiographic follow-up indicated healing of periapical tissue of tooth 8, and a considerable reduction of the size of the lesion above tooth 7. A, Conspicuous epithelial sheaths and disintegrating blood. B, From area indicated by arrow in A. Fingerlike epithelial projection with numerous neutrophilic leukocytes and adjacent extravasated blood. C, Neutrophilic leukocytes adjacent to empty spaces (s), degenerating epithelial cell (e), and extravasated erythrocytes (er) with small particles of brown pigment (p). (Magnifications: A, x32; B, x400; C, x1,000.)
Fig. 3. A, C, and E, Case 23. Tooth No. 10 presented with moderate swelling, pain, and a 6 mm. area of rarefaction radiographically. Root canal therapy entailed gutta-percha and curettage. B and D, Case 214 (same case as shown in Fig. 1). A, Epithelium surrounding central cavity which is partly empty, partly filled with disintegrating tissue. B, From area indicated by arrow in A. Epithelial lining and adjacent inflamed tissue with small particles of brown pigment. C, Exactly same area as shown in C, taken in polarized light. The brown pigment is birefringent, indicating breakdown of blood in the tissue. Lack of particles in empty space adjacent to the epithelium is evidence that the pigment is located within the tissue. B and D, From Fig. 1, photomicrographs taken in the exact same manner as C, and E. Aggregates of brown pigment birefringent in polarized light. These blood-disintegration products can easily be distinguished from the sealant particles demonstrated in Fig. 8. (Original magnifications: A, x25; B and D, x1,000; C and E, x252.)
macrophages, mast cells, foreign-body cells, foam cells, and mesenchymal cells. They were found throughout the connective tissue of the granuloma and the tissue surrounding the cyst (Figs. 4 and 5). Root resorption was also a common occurrence (seventy-four of 230 cases) when the tissue involved root ends (Fig. 6).

Bacteria were recorded as present regardless of their location. They were found in twenty-three of the specimens. However, in only one case were these bacteria located in the disintegrating tissue of the root canal and the periapical tissue (Fig. 7). In the remaining twenty-two cases they were present in bacterial plaque attached to tooth substance, in the tubules of root dentin or fragments of this, in artificial voids, and as a contamination on the surface of the tissue.

In 181 cases, small particles of root canal sealer were observed and could be distinguished from bacteria and the brown pigment of the disintegrating blood by the evenness of the particles (Figs. 3, 7, and 8).

**Correlation of clinical and histopathologic observations**

In the 230 cases in which histologic and clinical data were available, chronic and acute inflammatory cells were present in all the periapical specimens. This includes all clinical categories: in all forty-five patients who presented with pain and in each of the 185 patients without pain, in all fifty-seven cases with swelling, each of the forty-seven fistula cases, all thirty-three percussion-sensitive teeth, and each of the thirty-nine teeth that were opened for relief. Bacteria were present in ten patients who had pain and thirteen patients without pain (Table I).

One hundred-ten of these 230 cases were further evaluated by a follow-up radiograph after periods ranging from $1/2$ year to 5 years’ duration. These cases were classified within the three categories of success, uncertain, and unsuccessful utilizing a modification of Strindberg’s criteria. With this method of classification, there were sixty-seven successful cases, forty uncertain cases, and three unsuccessful cases (Table II). The forty uncertain cases included thirty-three which had decreased in size and seven in which the initial lesion had stayed the same.

Some typical radiographs of the three categories showed a lack of correlation between the histopathologic, clinical, and radiographic findings. Thus, the periapical tissue taken 4 days after endodontic obturation, following physical trauma several years earlier, had all the cellular components of a granuloma with a minimal area which could be recorded as a developing cyst. One of the teeth which had an apical amalgam filling was considered successful 4 years postoperatively, whereas the neighboring tooth was considered uncertain (Figs. 2, 4, B, 5, A, and 9).

Another patient who had acute pain and periodontal involvement 16 years following root canal treatment which had failed because a fourth canal had been left untreated showed both acute and chronic inflammatory cells and the presence of epithelial tissue. The radiograph taken 2 years postoperatively indicated a lack of complete healing, although the tooth was asymptomatic. Because of these factors this tooth was placed in the uncertain category (Figs. 1, 4, E, 6, 8, and 10).
Fig. 4. A, C, and D, Case 257. Tooth No. 7 presented with sensitivity to hot and cold. Root canal therapy included gutta-percha and curettage. B, Case 248 (same case as in Fig. 2). E, Case 214 (same case as in Fig. 1). F, Case 8. Tooth No. 6 presented with severe pain and a fluctuant swelling perforating the labial plate. Root canal therapy included gutta-percha and curettage. A, Epithelium (between arrows) with neutrophilic leukocytes (nl). B, Intermixed neutrophilic leukocytes (nl) and chronic inflammatory cells, such as plasma cells, (p) and macrophages (m), with inclusion in the cytoplasm. C, Plasma cells (p), lymphocytes (ly) with coffee bean-shaped nucleus, and inconspicuous cytoplasm. Macrophage with vacuoles in cytoplasm. Light cells in lower part of photograph are mesenchymal cells. D, Mesenchymal cells alongside blood vessels with mostly small lymphocytes (ly). E, Mast cell from periapical region of apposition and resorption shown in Fig. 6. D (arrow). F, Multinucleated (arrow) foreign-body giant cell within periapical granuloma. (Original magnifications: A, x800; B-F, x1,000.)
Fig. 5. A, Case 248 (same case as in Fig. 2). B and C, Case 56. Tooth No. 10 needed to be opened for relief because the patient had acute pain. The tooth had a +1 mobility, and there was a 1 cm. area on the radiograph. Root canal therapy included gutta-percha and curettage. A, Typical mixture of acute and chronic inflammatory cells: plasma cells (p), neutrophilic leukocytes (nl), some lymphocytes, and, in the lower left-hand portion of the photograph, mesenchymal cells surrounding a blood vessel. B, Granuloma with numerous light areas (arrow). C, Cluster of foam cells with typical granulated cytoplasm intermixed with plasma cells, lymphocytes, and a mesenchymal cell. (Original magnifications: A and C, x1,000; B, x25.)
Fig. 6. Case 214 (same as in Fig. 1). A, Mesial root with attached granuloma and lacuna along the root surface; remaining endodontic material in part of the canal visible in this section (arrow). B, Resorption of periapical root with clastlike cell in lacuna and on its margin. C, Clastlike cell in lacuna in apical area. Attached granuloma with neutrophilic leukocytes, foam cells (arrow), and lymphocytes. D, Upper lacuna with clastlike cell in periapical region; lower lacuna with apposition and inflammatory cells. Mast cell (arrow), also shown in Fig. 4, E. (Original magnifications: A, x16; B, x1,000; C and D, x630.)
In one case (tooth No. 29) in which there was a 9 mm. mesial periodontal pocket reaching the apex, the patient presented without pain. The tooth had previously been treated with a silver cone. The curettage was unsuccessful and the tooth was removed 2 months later. The complication in this case was the periodontal condition with a pocket and plague extending to the apex (Figs. 7 and 11).

DISCUSSION

The cause and persistence of periapical inflammation may be due to several factors: nonspecific injury with direct insult via host tissue and/or bacterial breakdown products, specific immunologic response to bacterial antigens, or possible altered host tissue antigens. With one exception in which a periodontal plaque reached the apex of the involved tooth (Figs. 7 and 11), bacterial cells were not observed in the periapical tissue. This corroborates our earlier findings in the study of periodontal, pulpal, and periapical interrelationship.13 The absence of bacteria in the granulomas does not rule out the possibility of the presence in the tissue of bacterial components such as cell walls. Such components would not be seen with the technique used in this study.

The absence of bacteria in our material should be considered relative to their presence in the material of other investigators. The adequacy and interpretation of the bacterial stains are important factors. It should be noted that a number of the stains used, in addition to bacteria, also stain the chromatin of normal cells. However, there are variations in the way sections take stain despite the use of identical reagents and methods. Thus, chromatin may stain in some sections and not in others, but if the chromatin stains it does so also in areas where no bacteria are present. In control material of intact teeth, the chromatin may stain and could appear as engulfed bacteria, such as reported by Boyle.31 The finding by Winkler and associates32 of bacteria evenly dispersed throughout the granuloma is not corroborated in our study, although we have used the identical reagents and the same staining methods. The only particles which were evenly dispersed in our sections were brown pigment and particles of the endodontic sealant (Fig. 8).

More important, in cases where root remnants were enclosed in the specimen, bacteria did stain in the necrotic tissue but not beyond this in the remaining vital part of the pulp or in the periapical granuloma or cyst. Therefore, the demonstration of bacteria in the necrotic part of the canal, in adjacent dentinal tubules, and in bacterial plaque shows the efficacy of the staining method used. The absence of bacteria in the periapical tissue of the same sections can therefore not be a false negative. This corroborates the findings of a number of other investigators.1, 2, 4, 38-46

The location of bacteria related to specific cells is an important consideration. The fact that bacteria appear in the necrotic region of the pulp tissue only (Fig. 7) and that neutrophilic leukocytes gather in a heavy concentration next to this area indicates that these leukocytes operate as microphages. Furthermore, the alteration in the cell picture as one observes in the apical direction—the decrease
Fig. 7. Case 155. Tooth No. 29 had a 9 mm. mesial periodontal pocket, pain, and a fistula. The tooth had previously undergone root canal therapy with a silver cone and curettage. Two months later the tooth was extracted. (Modified Brown and Brenn stain.) A, Root tip with bacterial plaque (arrows) and remaining pulp and periapical tissue. B, Bacterial on coronal root surface (right arrow in A). C, Bacteria in disintegrated pulp tissue within root canal mixed with sealer. D, Totally disintegrated tissue and bacteria periapically mixed with root canal sealer. E, Bacteria and root canal sealer periapically. (Original magnifications: A, x16; B-E, x1,000.)
in number of neutrophilic leukocytes, the appearance of lymphocytes, plasma cells, macrophages, foreign-body cells, and mast cells in relatively unaltered remaining pulp tissue—indicates that the acuteness of the inflammation decreases in the apical direction. The appearance of a considerable periapical involvement under these conditions in the presence of a remaining vital root pulp has been the subject of considerable disagreement, but the explanation seems simple in terms of inflammation and immunology. It is the tissue-disintegration products, the bacterial toxins, and the cell walls that stimulate the periapical response.\textsuperscript{54-66} One could assume that the accumulated disintegration products and bacterial toxins travel from their place of origin through the lymph vessels of the remaining pulp and gather in the periapical tissue. This would explain the seemingly illogical appearance of a remaining nearly unaltered pulp tissue between two areas of severe inflammation.

The vast disagreement in frequency of occurrence of periapical granulomas and cysts\textsuperscript{67-70} should be considered related to the criteria used by each investigator. Dorland\textsuperscript{71} defines a cyst as "Any sac, normal or abnormal, especially one which contains a liquid or semisolid material." According to this original Greek definition, the appearance of epithelium alone in the absence of a lumen filled with a disintegrated liquefied tissue could not be recorded as a cyst. It should be noted that epithelium in the form of Malassez rests appears in the normal periapical tissue and that proliferation is a normal occurrence during periapical inflammation, such as, for example, that caused by irritant endodontic procedures or materials.\textsuperscript{72}

A definition that is specifically relevant to this investigation is given by Shafer, Hine, and Levy\textsuperscript{72}: "A cyst is defined as a pathologic epithelium-lined cavity usually containing fluid or semisolid material." Using this specific definition in our investigation, only fourteen (Figs. 2 and 3) out of 230 cases could be recorded as cysts. Forty-seven additional cases had epithelium present, but no discernible fluid-filled space. Accordingly, our observations are in disagreement with Bhaskar,\textsuperscript{68} who found 42 per cent of all periapical lesions to be cysts, whereas Sommer and Kerr\textsuperscript{70} found an incidence of cysts similar to ours. Other investigators have reported percentages ranging between these figures.\textsuperscript{67} Some of the reported discrepancies may be related to the surgical technique. If, as in most periapical surgery, fragments rather than a whole continuous lesion are removed, fluid-filled spaces could collapse and the fluid escape. This is, however, considered in our laboratory evaluation. Particular attention is directed toward epithelial cells adjacent to an empty space. Any alteration in the cell morphology or in adhering tissue remnants, such as observed in Figs. 2 and 3, permits classification as a developing or a true cyst, whereas a condition such as demonstrated in Fig. 1 would be classified as a granuloma with epithelium. It should be specifically noted that this is representative of the entire series of sections in each case and that the taking of further sections in any investigation could alter the percentages of occurrence.

The possible mechanism of the development of cystic cavities may be anticipated on the basis of the cells present, particularly the neutrophilic leukocytes.
Fig. 3. Case 214 (same as in Fig. 1). A, Root canal sealer in periapical tissue. B, Exactly same area as in A, but photographed in polarized light. Root canal sealer is evenly dispersed, not aggregated together like the brown pigment in Fig. 3, B-E, and easily distinguishable from the bacterial clusters shown in Fig. 7. C, Apical dentin fragment with sealer particles and resorbing cells. The location of the particles is evidence that this is their location in vivo since the particles are not on the dentin. (Original magnifications, x1,000.)
Fig. 9. Case 248 (same as in Figs. 2, 4, B, and 5, A). A, Immediate postoperative radiograph following obturation with gutta-percha of teeth 7 and 8 and curettage of the apical areas with an apical amalgam placed in tooth No. 8. B, Follow-up radiograph taken 4 years postoperative showing complete healing of tooth No. 8 and considerable reduction in the size of the lesion around tooth No. 7.

Fig. 10. Case 214 (same case as in Figs. 1, 4, E, 6, and 8). A, Tooth No. 2 endodontically treated and filled with gutta-percha 16 years previously. This tooth presented with periodontal disease and acute pain. B, Immediate postoperative radiograph after mesial buccal root was amputated and the area curetted. C, Radiograph taken 2 years postoperatively. Although the tooth is asymptomatic, healing is uncertain according to a modification of Strindberg’s criteria.

which are present in all sections in varying concentrations (Table I, Figs. 1, 2, 4, and 5). When they disintegrate they release enzymes capable of dissolving cells and ground substance. Confluence of the microsacs occurs and small fluid-filled spaces are formed. When these occur in or adjacent to the epithelium, a cyst has started to develop.

Other fluid-filled spaces may appear where there is an accumulation of foam cells (Fig. 5, B and C). When these cells disintegrate and their high lipid content is released into the tissue, a semisolid filled sac occurs. Only if surrounded by epithelium does such a space fit the definition of a cyst, although the deteriorating masses might later form a part of the contents of a cyst. The disagreement between investigators can be resolved only by a comparison of photomicrographs at sufficiently high magnification and quality to allow exact differential diagnosis.

This histologic term *acute inflammation* is based on the presence of considerable numbers of neutrophilic leukocytes in the tissue and in afferent vessels. However, it is a common misunderstanding to associate the presence of the neutrophilic leukocytes with pain. This investigation confirms our earlier finding of
nonecorrelation between neutrophilic leukocytes which appear in about equal numbers and distribution in tissue taken from patients in pain (Figs. 1, 4, A, and 6, C) and those without pain (Figs. 2, 4, B, and 5, A). A better association could possibly be found, provided an actual count of cells was performed in all serial sections taken through the entire lesion. However, since most laboratories take only very few semiserial sections through what is considered a representative part of the lesion, such counts would be meaningless. Even with the relatively high number of sections taken in this study (80 to 280 sections from each specimen), a quantitation of the results would be misleading.

Other clinical signs and symptoms, such as swelling, fistulas, percussion sensitivity, or teeth needing to be opened for relief, could not be correlated to a specific histologic picture (Table 1). It is confirmed in this study that histologically diagnosed acute inflammation may frequently occur in the total absence of pain.

On the basis of the presence of neutrophilic leukocytes in all specimens, and in equal amounts regardless of pain or other clinical conditions, there is nothing in our material which supports Bhaskar’s recommendation of instrumentation beyond the apex with the intent of causing an acute inflammation such as discussed by Morse. The neutrophilic leukocytes are already there, and their life cycle is known. To instrument beyond the apex causes tissue damage in addition to that which already exists. The inflammatory cells are there as an inflammatory and immunologic response to the various noxious products and toxins deriving from the disintegration occurring in the root canal. Instrumentation beyond the apex will push these toxic products out into the periapical tissues, as demonstrated by Ingle, and aggravate the tissue disintegration, compounding the damaging effect of the mechanical irritation. Clinically, this is known as a flare-up of iatrogenic etiology.

A biologic approach to endodontic therapy dictates the removal of the source of the periapical inflammation, that is, the noxious products gathered in the root canal. When these are successfully removed, the periapical lesion in most cases heals without surgical intervention. If, on the other hand, surgery is performed without re-treatment of the canal, or apical closure of the canal, the periapical
lesion persists or recurs. Thus, there is neither theoretical nor practical support for Bhaskar's theory.

The radiographic follow-up information, which ranges from $\frac{1}{2}$ to 5 years, gives no additional information correlating the clinical and histologic findings. However, it is interesting to note the difference between investigators when evaluating success. Thus, our success rate when using a modification of Strindberg's criteria is considerably lower than if we had used the criteria of Bender and Seltzer and their colleagues in which case our forty uncertain cases would have been recorded as successes. This emphasized the statement by Goldman and co-authors that "success depends upon who is reading the radiograph." This statement is equally true in distinguishing between a granuloma and a cyst; success depends upon who reads the histologic sections and which specific criteria are used. The diagnosis of cyst or granuloma depends on those factors, and the therapy is dependent upon the knowledge of the pre-existing biologic condition, the host's healing response, and the clinician's treatment procedures.

CONCLUSIONS

1. Whole bacterial cells occurred in twenty-three specimens.
2. In only one case, where a bacterial plaque reached the root apex, were bacteria present in the periapical tissue.
3. Both acute and chronic inflammatory cells occurred in all periapical lesions.
4. Epithelium occurred in sixty-one out of 230 periapical lesions.
5. There were fourteen cysts.
6. There was no correlation between the presence of inflammatory cells and the clinical signs and symptoms.
7. The addition of radiographic findings did not reveal any additional information which could be correlated.
8. Instrumentation beyond the apex will force highly toxic material into the periapical tissue and aggravate the inflammatory response.

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