
Morphometric analysis of chronic inflammatory periapical lesions in root-filled teeth

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The cell distribution and the tissue composition of chronic inflammatory periapical lesions were analyzed from seventeen biopsies of root-filled maxillary canines and incisors. The specimens, which included the root tip, the soft granulation tissue, and portions of the surrounding alveolar bone, were subjected to a stereologic analysis based on morphometric point-counting procedures. Plasma cells and lymphocytes were found in large numbers (about 40 percent of the tissue volume) in areas close to the apical foramen. The number of fibroblasts and blast cells was comparatively low in this zone. Neutrophils, monocytes/macrophages, and mast cells accounted each for 1 to 2 percent of the infiltrated tissue volume. With increasing distance from the apex, the tissue examined harbored a decreasing number of inflammatory cells but an increased volume of noninfiltrated connective tissue. It was concluded that long-standing periapical lesions of root-filled teeth had many features in common with those of advanced inflammatory lesions of the marginal periodontium.

Inflammatory processes at the apex of a tooth with nonvital pulp often represent a defense reaction of the periapical tissues to a bacterial infection in the root canal system of the affected tooth.¹ Periapical lesions may also appear subsequent to endodontic treatment procedures as a result of the irritation produced by microbial, chemical, and/or mechanical trauma. (For review, see Seltzer.²)

The character of periapical lesions has been extensively studied on a light microscopic level. Most studies have been carried out on human biopsy material,³⁻⁹ but information has also been obtained from observations in animals.¹⁰⁻¹² The lesions are described as richly vascularized connective tissue granulomas which, to varying extents, are infiltrated by inflammatory cells within areas of resorbed alveolar bone. Both acute and chronic forms of periapical lesions have been identified. Occasionally epithelial structures pierce the granulomas and may form a radicular cyst. Efforts have been made to implicate immunologic mechanisms in the development of the inflammatory lesions of the periapical region.^{8,13-16}

Careful analysis of gingival biopsy material from human subjects and various laboratory animals has revealed that different types of inflammatory lesions may be present in plaque-associated periodontal disease. Page and Schroeder,¹⁷ in a review paper,

were able to distinguish between four different categories of inflammatory lesions within the gingiva—the initial, the early, the established, and the advanced lesions. While the initial lesion was characterized by vasculitis, loss of some perivascular collagen, and enhanced migration of neutrophils in the junctional epithelium, the early, established, and advanced types included immunopathologic features such as accumulation, to a varying extent, of lymphoid cells in a connective tissue portion which was poor in collagen and which, in addition, contained fibroblasts with obvious cytopathic alterations.

It is reasonable to assume that lesions in the periapical area may have features in common with those present in the gingiva. The character of the periapical lesion may also vary with the stage of its development and the intensity and quality of the irritants affecting the periapical tissues. However, detailed information on the composition of the lesions in the periapical area is sparse. Recently Stern and associates⁹ analyzed the cell population and the variation in cell distribution in thirty-three granulomas and cysts using a technique for differential cell counting on a light microscopic level.¹⁸ They reported that, irrespective of morphologic type, treated or untreated, occurrence of clinical symptoms, or duration, the lesions showed essentially the

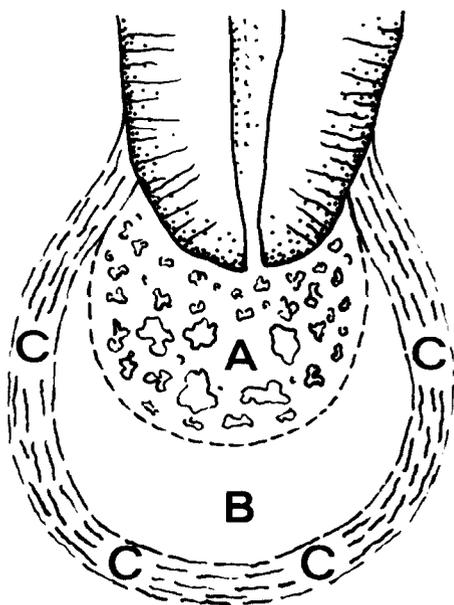


Fig. 1. Schematic drawing of a periapical lesion showing the three zones: *A*, close to apex; *B*, intermediate; *C*, peripheral, from which sections for electron microscopy and stereologic analysis were taken.

same types and distribution of cells. They further reported that macrophages accounted for almost half and lymphocytes for one third of the inflammatory cells.

In the present study an electron microscopic technique combined with a morphometric method, developed by Weibel¹⁹ and modified by Schroeder and Münzel-Pedrazzoli²⁰ to describe the inflammatory lesion within the gingiva, was used to assess the numerical and volumetric densities of various tissue components and cells in a sample of persistent periapical lesions in root-filled teeth.

MATERIALS AND METHODS

The present sample consisted of periapical tissue preparations harvested from thirteen patients (four female and nine male) with an average age of 49.8 years. The patients were selected by chance among those referred to the Department of Oral Surgery, University of Göteborg, Sweden, for periapical surgery.

A total of seventeen biopsy specimens were collected from upper canines and incisors. Periapical lesions that measured more than 3 mm. in diameter in the roentgenogram were not included in the analysis. Cases with clinical symptoms of acute inflammation and teeth treated endodontically within a period of 2 years prior to operation were excluded from the material.

The biopsy specimens were obtained in conjunc-

tion with an apicoectomy procedure, the operation being carried out in accordance with routines used at the Department of Oral Surgery.²¹ After the raising of a mucoperiosteal flap and the removal of the cortical bone of the apical region, the apex area of the tooth was exposed and the pathologic process was identified. Specimens including the root tip, surrounding granulation tissue, and portions of surrounding alveolar bone were obtained by means of a trephine bur with an inside diameter of 3.0 mm. The tissue preparations were immediately fixed in a Karnovsky fixative for at least 48 hours. After initial fixation the specimens were transferred to a mixture of 0.25 percent EDTA and 4 percent glutaraldehyde for demineralization. The end point of demineralization was determined by radiographic examination.

Each biopsy specimen was cut into two blocks by an incision made longitudinally through the root canal and the apical tip of the root. The blocks were processed for light and electron microscopic examination and embedded in Epon according to a procedure described by Schroeder.²² From the cut surface of each block two cross sections, 2 to 3 μm thick, were prepared and stained with periodic acid-Schiff (PAS) and toluidine blue.²³ The stained sections were used to obtain a general impression of the histologic appearance of the lesion and to provide orientation for preparation of sections for electron microscopy.

In the cross sections the periapical tissue specimen was divided into three zones (*A*, *B*, and *C*, Fig. 1). Zone *A* represented a part of the lesion close to the apical foramen, Zone *B* an intermediate area, and Zone *C* the periphery of the lesion. From each of the three zones (*A*, *B*, and *C*) a standardized series of nine electron micrographs was prepared (fifty-four from each biopsy). The electron micrographs were used for a stereologic analysis based on a morphometric point counting procedure developed by Weibel¹⁹ and with the use of a sampling design described by Schroeder and Münzel-Pedrazzoli²⁰ as modified by Schroeder and Lindhe.²⁴ Hence, the entire infiltrate was included in the analysis. From the electron micrographs (Level V; Schroeder and Münzel-Pedrazzoli²⁰; magnification $\times 3,400$; ratio 0.33; grid P 42) representing infiltrated connective tissue (ICT), the volumetric density (V_v) was determined for fibroblasts, neutrophilic granulocytes, monocytes/macrophages, mast cells, small- and medium-sized lymphocytes, immunoblasts/x-lymphocytes, plasma cells, collagen fibers, vascular structures, and residual tissue (epithelium, matrix, lymph vessels, nerves, etc.). The number of the various cell types (N_v) was also assessed. All stereologic parameters were expressed in terms of volume density (cubic

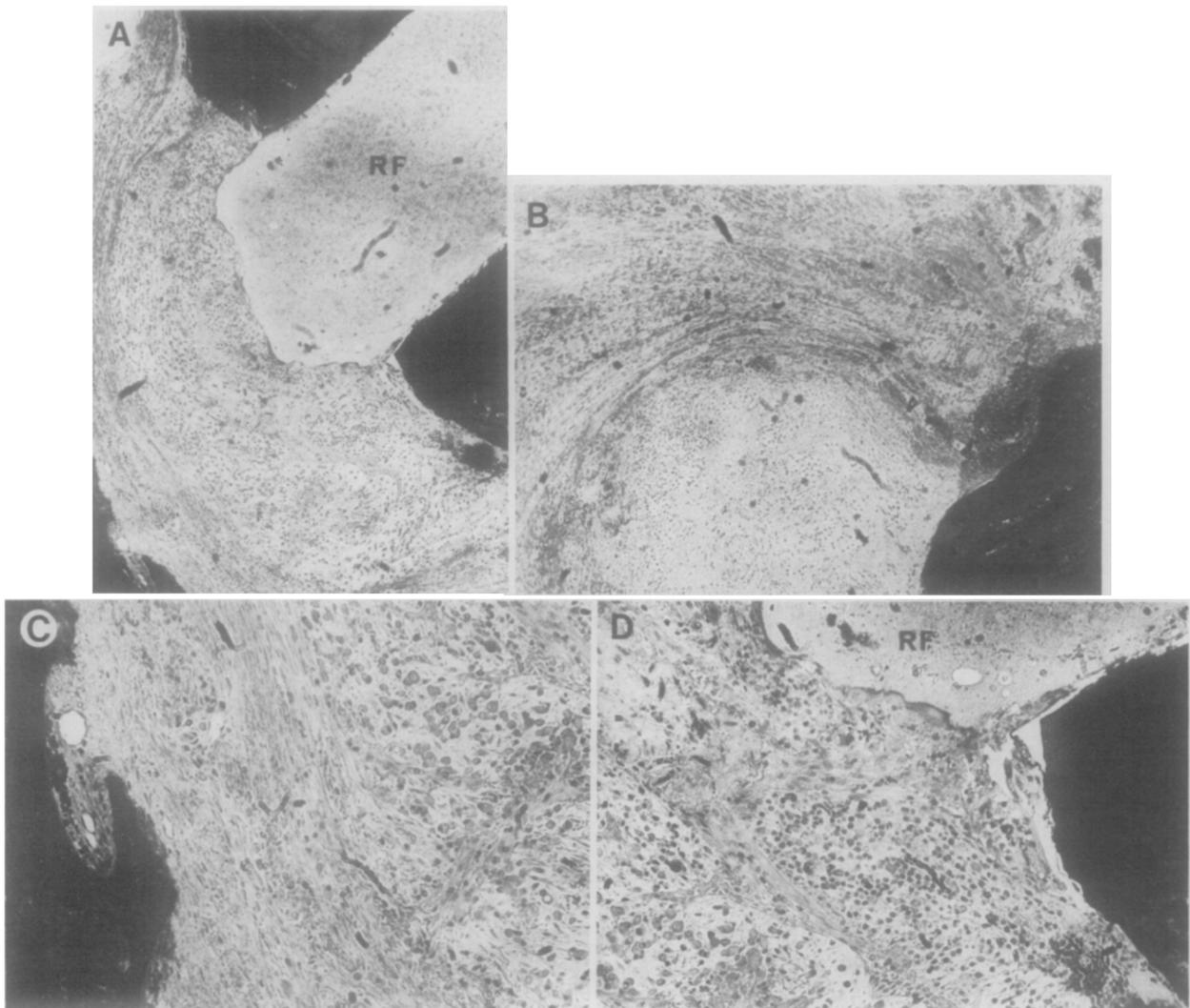


Fig. 2. Light microscopic demonstration of periapical lesion. **A**, Apical section showing the infiltrated connective tissue around a root filling (*RF*). (PAS–toluidine-blue stain. Magnification, $\times 30$.) **B**, Higher magnification of the apical section in *A*, showing sparsely infiltrated connective tissue in zone *C*. (PAS–toluidine-blue stain. Magnification, $\times 300$.) **C**, Higher magnification of the apical lesion in *A*, showing connective tissue (zone *C*) toward the surrounding bone containing resorption lacunae. (PAS–toluidine-blue stain. Magnification, $\times 300$.) **D**, Higher magnification of the apical section in *A*, showing the inflammatory cell infiltrated connective tissue close to the apex and the root filling (*RF*), Zone *A*. (PAS–toluidine-blue stain. Magnification, $\times 300$.)

millimeters per 100 mm.²) and numerical density (percent) of ICT.

The results representing Zones A, B, and C were analyzed with Student's *t* test.

RESULTS

Histologic observations

All biopsies taken were diagnosed as chronic inflammatory lesions. Representative pathologic changes observed are illustrated in Fig. 2. The lesions examined occupied an area of 1 to 3 by 2 to 3 mm. around the apices of the teeth.

The root surface facing the lesions consistently showed the absence of a connective tissue attachment (Fig. 2, *A*). Large resorption lacunae were observed, sometimes bordered by a layer of epithelium of varying thickness. This epithelium was continuous with strands or bands of epithelium piercing the lesion. In areas lateral to the lesion there was a normal attachment between the periodontal ligament and the root cementum.

Fourteen of the seventeen lesions examined were separated from the surrounding bone by a sparsely infiltrated connective tissue (Fig. 2, *B*), the width of

Table I. Morphometric parameters describing the tissue composition (N_v = numerical density percent; V_v = volumetric density percent) of the infiltrated connective tissue of the periapical lesions.*

	Zone A				Zone B				Zone C			
	N_v		V_v		N_v		V_v		N_v		V_v	
	\bar{X}	S.E.	\bar{X}	S.E.	\bar{X}	S.E.	\bar{X}	S.E.	\bar{X}	S.E.	\bar{X}	S.E.
Fi	7.9	2.0	4.5	1.1	32.8	8.3	14.8	4.1	92.5	4.1	10.8	4.1
Pc	52.1	7.1	30.7	3.2	41.1	8.6	21.4	4.3	—	—	—	—
B	8.6	2.7	3.0	0.8	2.1	1.1	0.9	0.5	—	—	—	—
NG	3.5	1.4	1.5	0.6	6.8	3.9	3.0	1.9	—	—	—	—
Mo/Mc	1.8	0.7	1.7	0.6	1.4	0.7	1.2	0.7	1.5	0.4	—	—
L	23.4	5.9	9.0	2.4	13.6	4.3	4.4	2.0	1.1	0.2	—	—
Mast	1.9	0.7	1.2	0.3	1.7	1.1	0.6	0.4	2.1	0.7	—	—
DC	0.8	0.6	—	—	0.4	0.4	—	—	0.9	0.4	—	—
			<u>51.6</u>				<u>46.3</u>				<u>10.8</u>	
Co			15.6	1.8			26.3	4.9			80.8	5.9
R			30.8	1.6			25.4	3.3			5.8	6.6
V			<u>2.0</u>	<u>0.5</u>			<u>2.1</u>	<u>0.8</u>			<u>2.6</u>	<u>3.7</u>
			48.4				53.8				89.2	

*Zone A, close to apex; Zone B, intermediate; Zone C, peripheral (Fig. 1). Fi, fibroblast; Pc, plasma cells; B, blast cells; NG, neutrophils; Mo/Mc, monocytes/macrophages; L, lymphocytes; Mast, mast cells; DC, dead and unidentified cells; Co, collagen; R, residual tissue; V, vessels.

which varied between 60 and 150 μm (\bar{x} = 90 μm ; S.E. = 19 μm). In the remaining three cases, the inflammatory cell infiltrate was not encapsulated at the periphery. In two biopsies the marrow spaces of the adjacent alveolar bone were infiltrated by inflammatory cells. In five of the biopsies there were signs of bone resorption in the compartments immediately lateral to zone C (Fig. 2, C).

The lumen of the root canals contained, besides root-filling material, nonvital connective tissue cells and/or microorganisms of varying morphologic forms. In some cases microorganisms were also detected at the outer surface of the root.

The inflammatory cell infiltrate (Fig. 2, D) contained plasma cells in large numbers and lymphocytes. Fibroblasts occurred to a varying extent. Monocytes/macrophages and polymorphonuclear cells were also present. Neutrophils were often observed within the portion of the lesion that was occupied by epithelium.

Stereologic findings

The over-all composition of the ICT of zones A, B, and C is presented in Table I and in Fig. 3. In zones A and B cellular and extracellular structures occupied an almost equal portion of the ICT. Collagen fibers accounted for 15.6 percent (S.E. = 1.8 percent) and 26.3 percent (S.E. = 4.9 percent) of the ICT volume in zones A and B, respectively. The corresponding figures for residual tissue components (epithelial cells, matrix, lymph vessels, and unidentified noncellular structures) were 30.8 percent

(S.E. = 1.6 percent and 25.4 percent (S.E. = 3.3 percent)).

The cellular infiltrate of zone A was dominated by plasma cells (N_v = 52.1 percent, V_v = 30.7 percent) and lymphocytes (N_v = 23.4 percent, V_v = 9.0 percent). Thus, on the average, every second cell could be identified as plasma cell. Fibroblasts and blast cells occupied 4.5 percent (S.E. = 1.1 percent) and 3.0 percent (S.E. = 0.8 percent) of the ICT volume, respectively, whereas neutrophils, monocytes/macrophages, and mast cells each accounted for around 1 to 2 percent of the volume in this zone (Table I).

Also, in zone B plasma cells dominated (N_v = 41.1 percent, V_v = 21.4 percent) the infiltrate. In comparison to zone A, the numerical density in zone B of fibroblasts (N_v = 32.8 percent; S.E. = 4.3 percent) significantly ($p > 0.001$) increased and of lymphocytes (N_v = 13.6 percent; S.E. = 4.3 percent) significantly ($p > 0.05$) decreased (Table I). The numerical density (N_v , percent) of blast cells was significantly ($p > 0.05$) lower in zone B than in zone A. The volume (V_v , percent) of zone B occupied by neutrophils, monocytes/macrophages, and mast cells varied between 3.0 and 0.6 percent.

Fibroblasts made up around 4.5 percent (Zone A) and 14.8 percent (Zone B) of the ICT volume (Table I), thus giving a fibroblast/plasma cell ratio of 1/7 (Zone A) and 2/3 (Zone B). The corresponding plasma cell/lymphocyte ratios were 15/1 (Zone A) and 5/1 (Zone B). A closer analysis of the data from the individual biopsies revealed that in six out of seventeen samples the volume ratio (Zones A + B)

between plasma cells and lymphocytes was 10/1. In only one lesion analyzed, lymphocytes were more numerous and occupied a larger volume than plasma cells (lymphocytes: $N_v = 38.5$ percent, $V_v 17.4$ percent; plasma cells; $N_v = 19.9$ percent, $V_v 15.2$ percent). In this particular specimen neutrophils occupied a comparatively large portion of the ICT volume ($N_v = 22$ percent, $V_v = 15$ percent).

The peripheral part of the lesions (Zone C) was characterized by the high density of collagen structures (Table I). Thus, 80.8 percent (S.E. = 5.9 percent); V_v) of the volume of Zone C was occupied by collagen, whereas residual tissues and vascular structures occupied only 5.8 percent (S.E. = 6.6 percent; V_v) and 2.6 percent (S.E. = 3.1 percent; V_v), respectively. The cells were dominated by fibroblasts (92.5 percent, S.E. = 4.1 percent; N_v) with monocytes/macrophages (1.5 percent, S.E. = 0.4 percent; N_v), lymphocytes (1.1 percent, S.E. = 0.2 percent; N_v), and mast cells (2.1 percent, S.E. = 0.7 percent; N_v) being represented in low numbers.

Fig. 3 describes graphically the numerical and the volumetrical densities of various tissue components in Zones A, B, and C. It is important to emphasize that the distinction between Zones A and B was often difficult to make, whereas Zone C was easily distinguishable from Zones A and B. The histograms in Fig. 3 show that the fibroblast and collagen densities gradually increased from Zone A to Zone C, while all other cell types decreased in number and volume.

DISCUSSION

The present investigation revealed that all periapical biopsy material examined contained an inflammatory cell infiltrate which was dominated by plasma cells and lymphocytes. As a rule, few polymorphonuclear leukocytes were observed within the lesions. In fourteen out of seventeen specimens the infiltrate was separated from the surrounding alveolar bone by a dense, noninfiltrated, connective tissue. In only two biopsy specimens could inflammatory cells be found in marrow spaces of the adjacent bone. This, together with the fact that at least 2 years had elapsed since endodontic treatment was performed, suggests that the biopsy material examined in the present study represented inflammatory periapical lesions of long standing.

By means of a morphometric analysis, the lesions were characterized by the relative occurrence of different cells and tissue constituents. Measurements were performed at three different locations at various distances from the tooth apex and in two levels within the tissue sample to provide a basis for a

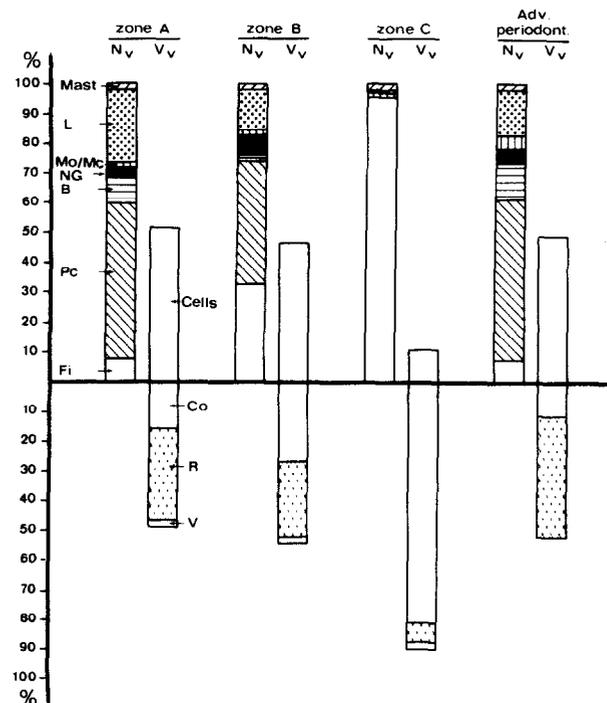


Fig. 3. Comparison of tissue composition of advanced marginal periodontitis lesions (figures from Lindhe, Liljenberg, and Listgarten²⁵) and the periapical lesions of the present study. N_v percent = average numerical density and V_v percent average volumetric density of infiltrated connective tissue (ICT). *Zone A* = close to the apex; *Zone B* = intermediate area; *Zone C* = peripheral area (see Fig. 1). *Mast*, mast cells; *L*, lymphocytes; *Mo/Mc*, monocytes and macrophages; *NG*, neutrophilic granulocytes; *B*, blast cells; *Pc*, plasma cells; *Fi*, fibroblasts; *Cells*, total volume of inflammatory cells; *Co*, collagen fibrils; *R*, residual tissue; *V*, vessels.

three-dimensional analysis of the specimen. Morphometric techniques for quantitative evaluation of tissue morphology have been used frequently in studies of gingival and periodontal disease^{20, 24, 25} and also recently to characterize the cell composition of periapical lesions.⁹ From the comparatively low variances of the various assessments (Table I), it can be seen that the lesions investigated showed a fairly homogeneous tissue profile. Thus, areas close to the apical foramen (Zones A and B) contained large numbers of plasma cells, lymphocytes, and blast cells, whereas more peripheral areas (Zone C) were almost devoid of such cells but were dominated by collagen structures and fibroblasts. In fact, a further analysis of the data clearly demonstrated (Fig. 3) that with increasing distance from the apex, there was a decreasing number of plasma cells, lymphocytes, and blast cells, while the volume occupied by

fibroblasts and collagen increased. The numerical density parameters describing the composition of the cellular infiltrate are at variance with the findings reported by Stern and associates⁹. While Stern and associates⁹ claimed that macrophages were the most predominating cell type in periapical granulomas and cysts, plasma cells and lymphocytes dominated the lesions of the present material. This discrepancy may be explained by the fact that the present study used electron microscopy to identify the various cells, whereas Stern and associates⁹ made their observations in paraffin-embedded tissue sections in the light microscope.

When comparing the cellular content of Zones A and B with that characterizing the advanced lesion of marginal periodontitis,²⁵ obvious similarities between the two lesions can be observed. In both lesions the cell population is dominated by plasma cells, lymphocytes, and blast cells; macrophages, neutrophils and mast cells are present in low numbers (Fig. 3). This similarity in the composition of the cellular infiltrates of the advanced marginal and the periapical lesions is interesting and may represent a similar tissue reaction to the same causative agents—in periodontal disease to microorganisms in plaque on the tooth surface and in periapical disease to microorganisms within the root canal system. Lindhe, Liljenberg, and Listgarten²⁵ also demonstrated how different stages of destructive periodontal disease were associated with a varying composition of the inflammatory cell infiltrate. They furthermore suggested that different periodontal lesions vary with different microbial populations in the plaque. As the advanced plaque-associated periodontal lesion and the periapical lesions studied in the present report have been found to have many morphologic features in common, there are reasons to suggest that both lesions are associated with a similar microbiota.

Several investigations have shown that gram-negative anaerobic bacteria usually predominate the microflora not only of infected root canals^{26,27} but also the subgingival plaque of advanced periodontal disease.²⁸ Although the causative agents of inflammatory periapical processes are most likely of infectious origin, we cannot rule out the possibility that the lesions studied in the present investigation were—at least partly—mediated by chemical irritation produced by the filling material of the endodontically treated canals. It has been suggested that materials used to medicate and fill root canals may release noxious agents that could either act as haptens or alter host tissue to become antigenic.²⁹ The inflammatory cells of the lesions examined may thus have accumulated in response to bacteria and

their products as well as to noxious agents and denaturated host tissue. However, it should be remembered that the root canal of the resected root tip frequently housed microorganisms of different morphologic forms, thus supporting the hypothesis of a major role for bacteria in producing and maintaining the lesions observed.

Microorganisms not only were confined to the root canal of the teeth examined but also were in a few cases, found at the outer root surface. This confirms the observation that root canal bacteria occasionally may invade the periapical tissues.³⁰

Both plasma cells and lymphocytes have important functions in the immune response of the human body. Plasma cells are immunoglobulin-producing cells. In periapical lesions they have been shown to produce large amounts of IgG but less of IgA, IgM, and IgE.^{8,13-15} No attempt, however, has been made to study the specificity of the antibodies produced in periapical granuloma. It is reasonable to assume that they are related to antigens released into the periapical tissues. However, according to Brandzaeg and Tolo,³¹ the chances of finding immunocytes in chronic inflammatory lesions forming antibodies against a specific antigen are small. In studies of human gingival biopsies and experimental chronic inflammatory processes, they demonstrated that the great majority of plasma cells seemingly produces antibodies to a great number of different antigens, many of which were irrelevant for the lesion (altered IgG, tissue antigens etc.).

The neutrophils of the apical lesions studied were almost invariably found within strands of epithelial cells, leaving the connective tissue part almost devoid of this cell type. This confirms the observation of Hill⁴ who, in a histologic study of human dental granulomas, found that proliferating epithelium was associated with neutrophil infiltration. A similar finding has been made in studies on the development of marginal periodontitis, where large numbers of neutrophils are found within the junctional and pocket epithelium of the gingiva,^{22,32} whereas comparatively few such cells occur in the gingival connective tissue. The reason neutrophils are found more often within the epithelial tissue component of both apical lesions and marginal periodontitis lesions is not properly understood at present.

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