Ultrastructural Observations on Bacterial Invasion in Cementum and Radicular Dentin of Periodontally Diseased Human Teeth*

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IN THIS STUDY THE BACTERIAL INVASION in root cementum and radicular dentin of periodontally diseased, caries-free human teeth was examined. In addition, structural changes in these tissues, which could be related to the bacterial invasion, were reported. Twenty-one caries-free human teeth with extensive periodontal attachment loss were studied by light and scanning electron microscopy. At the base of the gingival pocket, bacteria were found in the spaces between remnants of Sharpey's fibers and their point of insertion in the cementum. In teeth that had been scaled and root planed, most of the root cementum had been removed. Bacterial invasion was found in the remaining root cementum. The invasion seemed to start as a localized process, often involving only one bacterium. In other areas bacteria were present in lacunar defects in the cementum. These lacunae extended into the radicular dentin. In 11 teeth bacteria had invaded the dentinal tubules. Most bacteria were located in the outer 300 μm of the dentinal tubules, although occasionally they were found in deeper parts. In two of the nontreated teeth, bacteria were detected on the pulpal wall. No correlation was found between the presence of bacterial invasion and the absence of radicular cementum. No bacteria were found in the portion of the root located apically to the epithelial attachment. These data are in agreement with our results from cultural studies of the bacterial flora in these structures. It was also demonstrated that in spite of meticulous scaling and root planing and personal oral hygiene, bacterial plaque remained present on radicular surfaces. Both the invaded dentinal tubules and the lacunae could act as bacterial reservoirs from which recolonization of treated root surfaces occurs. From these reservoirs bacteria could also induce pulpal pathoses. Since these bacterial reservoirs are not eliminated by conventional mechanical periodontal treatment, it seems appropriate to combine mechanical periodontal therapy with the use of chemotherapeutic agents.

In periodontal disease the root surface is exposed to the subgingival environment and microflora. The exposure to crevicular fluid, as well as to enzymes and metabolites produced by subgingival plaque bacteria, induces physicochemical and structural alterations of the radicular cementum.[1-14] Since periodontal disease is an intermittent process, i.e., phases of active tissue breakdown and remission alternate with periods of inactivity,[15,16] the intimate contact of the exposed root surface with the subgingival bacterial flora is maintained for a prolonged period of time.

The exposed radicular cementum is a thin, often discontinuous barrier between the underlying dentin and the oral environment.[17] During scaling and root planing, most of the cementum is removed[18] and the radicular dentin exposed. It has been suggested that most of the exposed radicular surface is covered with a discontinuous, partially mineralized cuticle derived from the gingival inflammatory exudate.[19] However, little information is available on the structural changes in radicular dentin of periodontally diseased teeth.[20,21]

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Using an anaerobic technique we have been able to isolate and culture significant numbers of viable bacteria from the radicular dentin and pulp tissue of periodontally diseased human teeth.[22,23] The composition of the invading bacterial flora indicates that these bacteria are derived from the subgingival microflora.[24]

The purpose of this investigation was to study structural and ultrastructural changes observed in the radicular cementum and dentin of periodontally diseased human teeth. Particular interest was given to the observation of bacterial invasion in the radicular cementum and dentin.

MATERIALS AND METHODS

Twenty-one extracted human teeth affected by periodontal disease were used in this study. All teeth had connective tissue attachment loss of two-thirds or more of the roots. Eight of them had connective tissue attachment loss reaching the apex. Molar teeth had Class III furcation involvement.[25] None of these 21 teeth had any clinically detectable carious lesion.

Six teeth were extracted before any periodontal treatment was started. The remaining 15 teeth were extracted after the initial hygienic treatment phase. This consisted of supragingival and subgingival scaling and root planing using ultrasonic and hand instruments, oral hygiene instructions and monthly prophylaxis sessions. The latter teeth had been *in situ* for at least four months after the start of the hygienic treatment phase. During the extraction procedure, all necessary precautions were taken to avoid damages to the exposed interdental radicular surfaces.

Immediately after extraction, adherent blood and saliva were removed by abundant rinsing with a physiologic saline solution. Fixation was performed in a 2.5% sodiumcacodylate buffered (0.1 M) glutaraldehyde solution (pH = 7.4). After dehydration in ascending ethanol, the teeth were freeze-fractured longitudinally.[26] One-half was processed for light microscopy, the other half for scanning electron microscopy.

Specimens for light microscopy were rehydrated and demineralized in a citric acid-sodium citrate solution (250 ml citric acid, 100 g sodium citrate, 750 ml distilled water). Completion of demineralization was determined radiographically. Using routine techniques, these specimens were embedded in Paraplast Plus,* oriented on a Leitz microtome,† and 5- to 6-μm thick longitudinal mesiodistal sections obtained. Hematoxylin-eosin and Brown and Brenn[27] stained sections were examined and representative photomicrographs taken on an Ultraphot II light microscope.‡

Specimens for scanning electron microscopy were critical point dried in a Polaron E3000 critical point drying apparatus.§ They were mounted on stubs in such a way that the radicular surface as well as the freeze-fractured surface could be examined. Mounted specimens were sputter coated with 15 nm gold in a Polaron E5000 diode sputtering apparatus and examined in a JEOL JSM-U3 ‖ or AMRAY 1000-B¶ scanning electron microscope, operated at an accelerating voltage of 5 to 15 kV.

RESULTS

Light Microscopic Observations

Both the supragingival and subgingival radicular surfaces from untreated teeth and from treated teeth (Fig. 1) were covered with bacterial plaque. However, the plaque layer on the non-treated teeth was considerably thicker and its composition more complex.

In the group of teeth that received initial hygienic treatment, the supragingival areas were covered with a thin layer of plaque, consisting of 10 to 20 layers of mainly cocci and coccoid bacteria. The subgingival plaque layer was four to eight times thicker and comprised a dense layer of filaments and cocci in contact with the root surface, and a less dense peripheral layer. The composition of the latter layer was morphologically more complex and consisted of cocci, coccoid bacteria, rods, filaments and spirochetes. The majority of the bacteria had cell walls that stained as those of typically gram-negative bacteria.

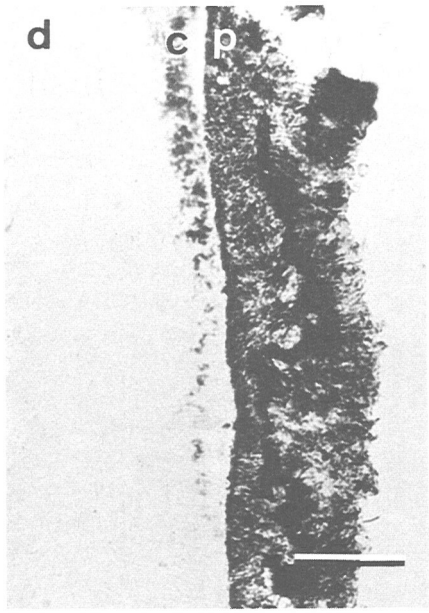

Figure 1. *The radicular surface of a tooth that received initial hygienic treatment is covered with a thick layer of subgingival bacterial plaque* (p). *In the radicular cementum* (c) *and in the underlying dentin,* (d) *positively staining areas are present, indicating the presence of invading bacteria (Brown and Brenn staining). Bar = 50 μm.*

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‖ JEOL, Tokyo, Japan.
¶ AMRAY, Bedford, MA.

Radicular cementum, in which deepest part granular structures could be observed, was present on most tooth surfaces from both groups. However, in the initial hygienic treatment group, in numerous areas, especially supragingivally, all root cementum had been removed and the radicular dentin exposed. In the subgingival area, lacunar defects were observed in the root cementum. These lacunae often extended into the radicular dentin.

In Brown and Brenn stained sections, bacteria were detected inside the dentin. These bacteria were located in the lumina of the dentinal tubules (Fig. 2). In the treatment group the numbers of invading bacteria and the depth of invasion were greater in areas where the root cementum was absent, although it was not always possible to follow the dentinal tubules from the surface to the area where the bacteria were observed. The majority of invading bacteria was present in the outer third of the radicular dentin. Occasionally, however, bacteria were observed in deeper parts of the dentin.

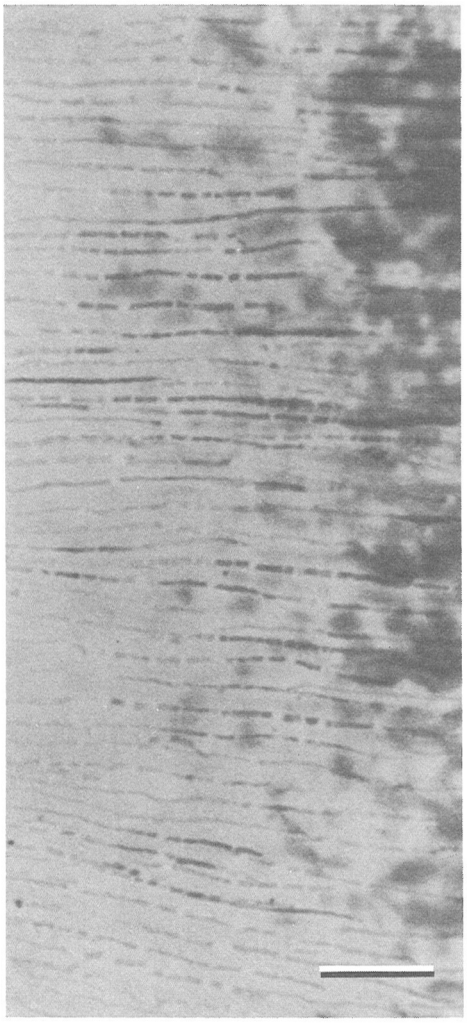

Figure 2. *Bacterial invasion in the radicular dentin of a tooth that received initial hygienic treatment. The bacteria appear to follow the course of the dentinal tubules. The cementum was completely removed. (Brown and Brenn staining). Bar = 50 μm.*

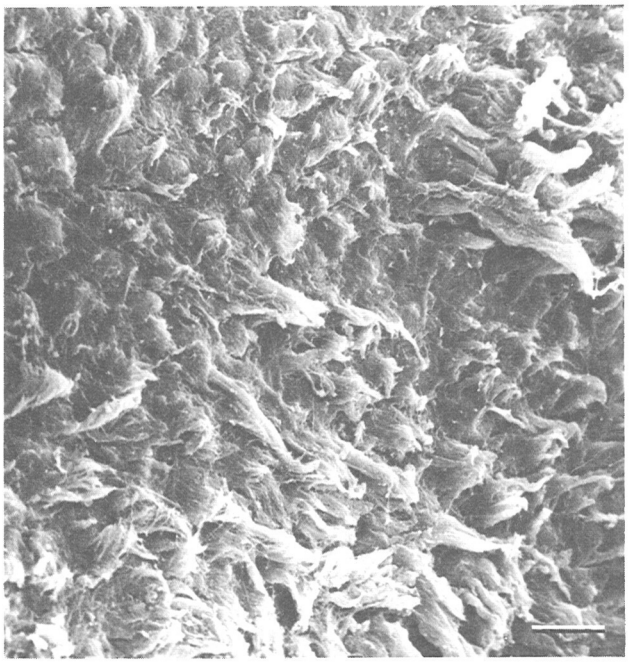

Figure 3. *The surface of the radicular area located apically of the epithelial attachment, consisting of normal cementum, with dome-like structures and remnants of torn Sharpey's fibers attached to the cementum. Bar = 10 μm.*

Scanning Electron Microscopic Observations

The radicular cementum in the area located apically of the epithelial attachment displayed the typical dome-like surface. Remnants of Sharpey's fibers, which had been torn during the extraction, were inserted in the cementum (Fig. 3). In some cases, minute bone fragments that had accompanied the tooth during the extraction were present. In these specimens, intact Sharpey's fibers could be observed from their insertion in the alveolar bone to their insertion in the radicular cementum (Fig. 4). Bacteria were never observed on this part of the radicular cementum, nor were any bacteria present between the Sharpey's fibers attached to this part of the radicular surface.

The subgingival bacterial plaque covering the radicular surfaces was a complex flora consisting of cocci, coccoid cells, short and long rods, filamentous and fusiform bacteria, and spirochetes (Fig. 5). Microcolonies could be recognized as small clusters of morphologically identical bacteria (Fig. 6). In addition, combinations of different morphological types of bacteria could be seen (Fig. 7), and intercellular junctions were apparent. Older plaque could be distinguished through the presence of crystalline or amorphous mineral components between the bacterial cells (Fig. 8), indicating the presence of subgingival calculus.

On longitudinally fractured specimens, in the area close to the bottom of the pocket, remnants of Sharpey's fibers inserting into the cementum were present (Fig. 9). A dense layer of subgingival plaque covered this surface. Bacteria filled the spaces between the Sharpey's

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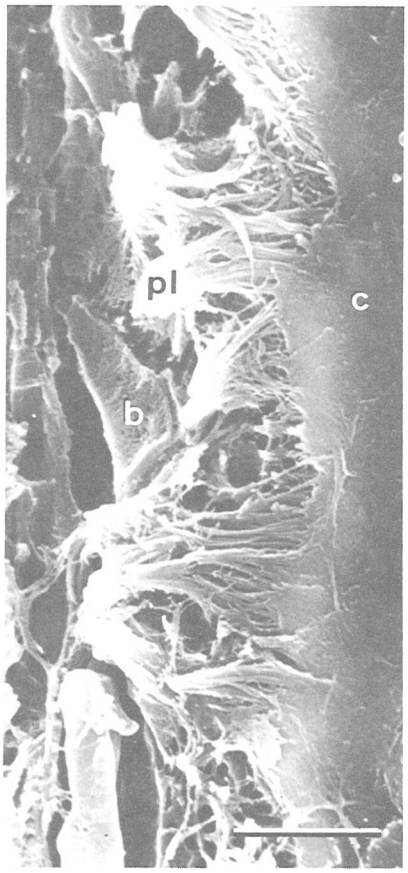

Figure 4. *Longitudinal fracture through the periodontal ligament in the area located apically of the epithelial attachment. The collagen fibers of the periodontal ligament* (pl) *connect the root cementum* (c) *with the cribriform plate of the alveolar bone* (b). *Bar = 5 μm.*

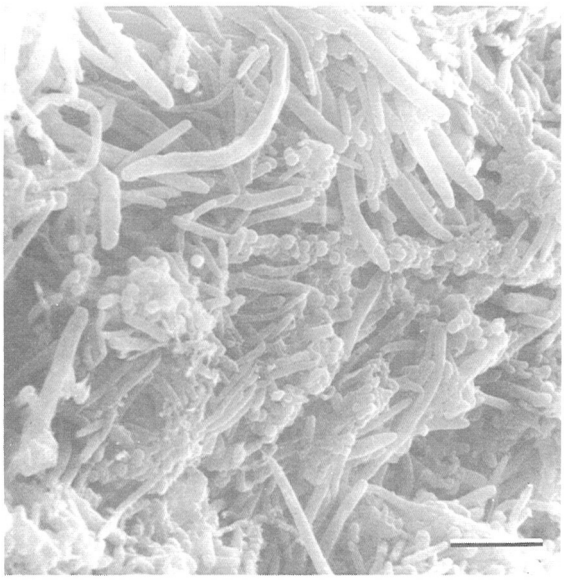

Figure 5. *The complex subgingival microflora, consisting of cocci, coccoid cells, short and long rods, filamentous and fusiform bacteria and spirochetes. Bar = 5 μm.*

fibers and the structures in the cementum where these fibers inserted.

More coronally, these fibrous structures were absent, and the cementum had a more uniformly mineralized

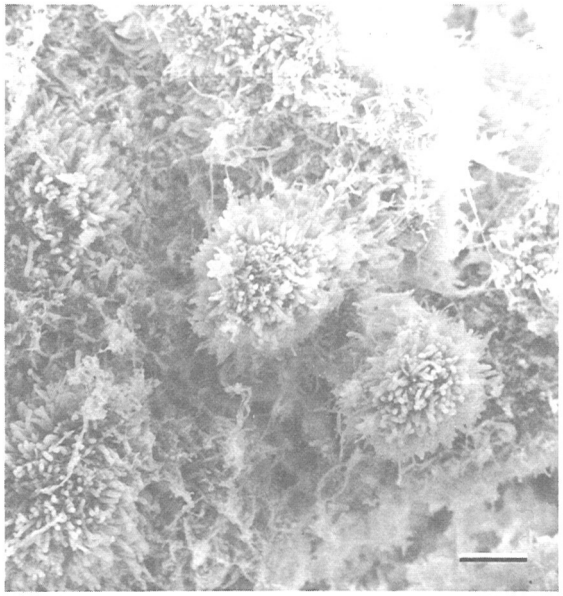

Figure 6. *Microcolonies of subgingival bacteria attached to the subgingival root surface. Bar = 10 μm.*

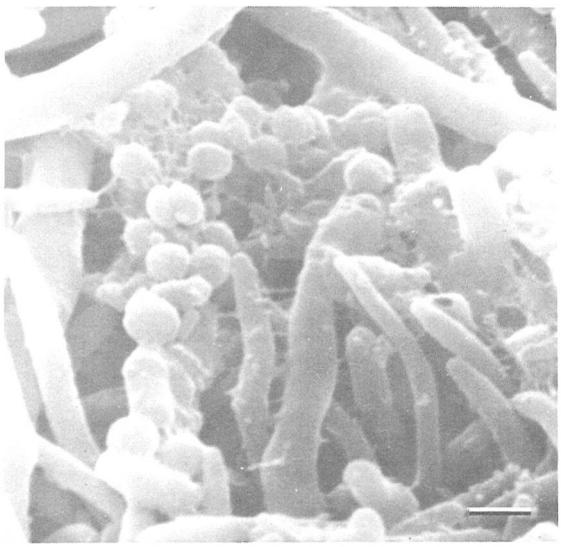

Figure 7. *Intercellular junctions between subgingival plaque bacteria. Bar = 1 μm.*

appearance. Bacterial invasion into the root cementum was frequently found (Fig. 10). This invasion started as a very localized process, often involving only one single microorganism (Figs. 10a and 10b). No particular morphological type of bacteria appeared to be more frequently involved.

In this part of the root, multiple lacunar defects filled with bacterial plaque were found (Fig. 11a and 11b). In all specimens, areas were present where several of these lacunae had become confluent, thus forming multilocular defects (Fig. 12). Most of these lacunae were completely colonized by subgingival plaque bacteria. However, in some lacunae the bacterial population was less dense, which allowed for the observation of the bottom of the lacunae. This bottom extended into the radicular dentin, as could be ascertained from the presence of

the orifices of dentinal tubules (Fig. 13). The surface of the bottom of the lacunae was rough, with numerous single bacteria present between globular and granular components of the exposed intertubular dentin. In many of the tubular orifices, bacteria invading the dentinal tubules were seen (Fig. 14).

On longitudinally fractured surfaces, invading bacteria were found in the dentinal tubules (Figs. 15 and 16). Bacteria were present in the dentin in three of the six untreated teeth and in eight of the 15 teeth of the initial hygienic treatment group. In the zone where the attachment loss had occurred, multiple dentinal tubules in the outer third of the radicular dentin contained cocci, short rods and filamentous bacteria. However, most bacteria were present in the outer 300 μm of the dentinal tubules. Occasionally, bacteria were found in deeper parts of the tubular lumen. In two nontreated teeth, bacteria were detected on the predentinal surface

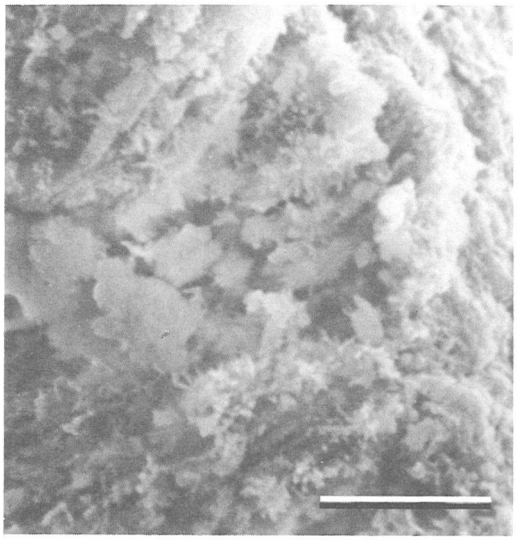

Figure 8. *Subgingival calculus with distinct mineralized substances between the bacteria. Bar = 5 μm.*

Figure 9. *Longitudinal fracture of the subgingival portion of the root near the bottom of the pocket. Remnants of Sharpey's fibers* (s) *insert into the cementum* (c). *Subgingival plaque bacteria* (b) *cover the root surface and penetrate into the spaces where the Sharpey's fibers insert into the cementum. Bar = 5 μm.*

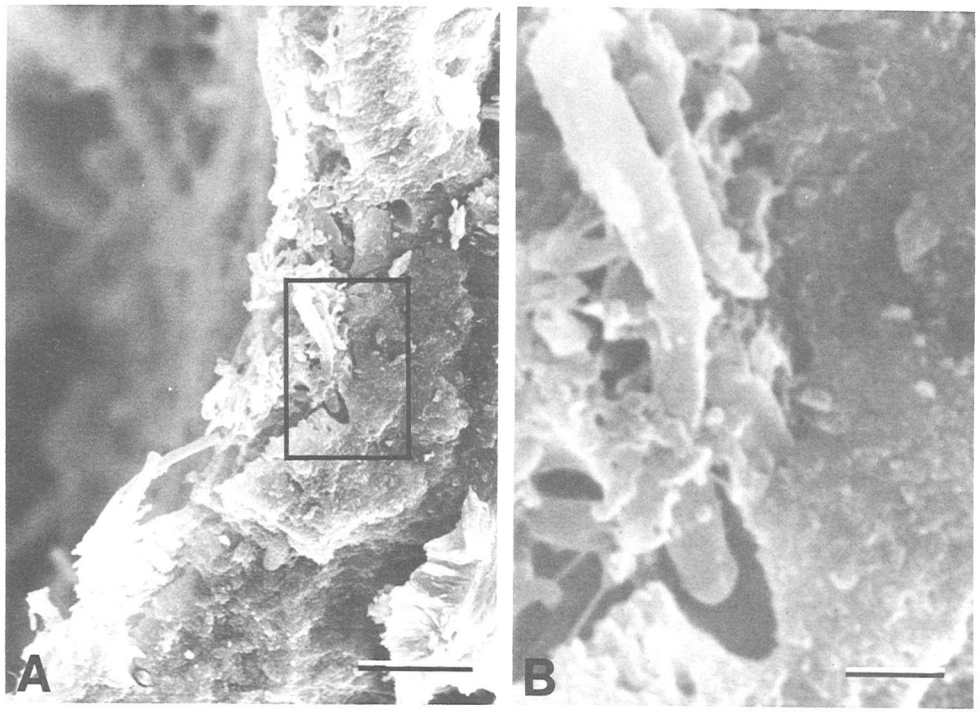

Figure 10. A. *Longitudinally fractured root in the subgingival area. A filamentous organism in a localized defect in the cementum surface. Bar = 5 μm.* **B.** *Detail of boxed area in Figure 10A. Bar = 1 μm.*

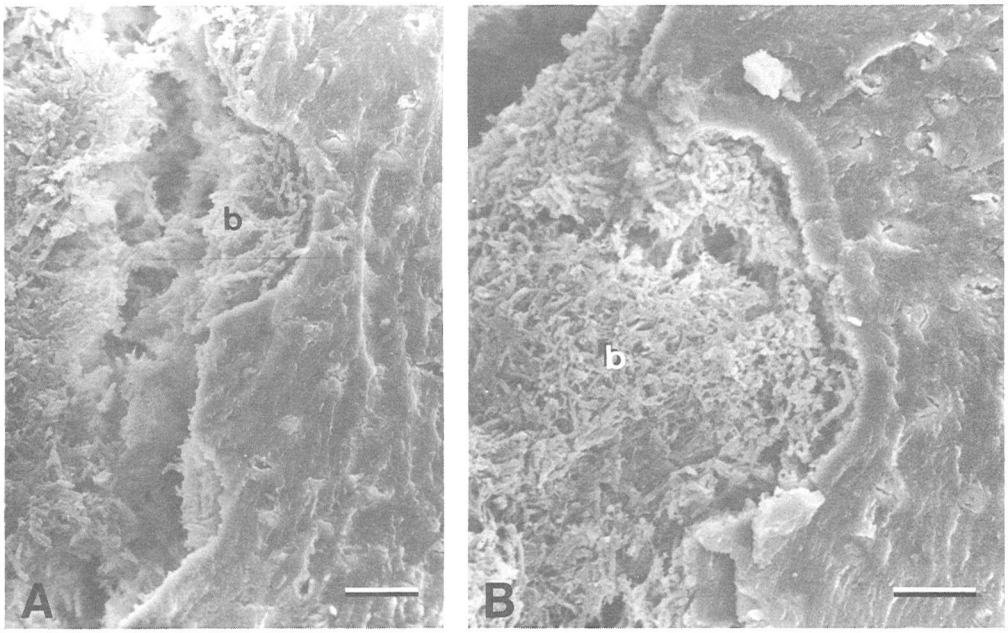

Figure 11. A. *Longitudinally fractured root surface of a tooth that was treated by scaling and root planing. Lacunar defects contain subgingival plaque bacteria* (b). *Bar = 10 μm.* **B.** *Longitudinal fracture through a lacunar defect in the radicular surface. Bacterial plaque* (b) *completely filling the lacuna. Bar = 10 μm.*

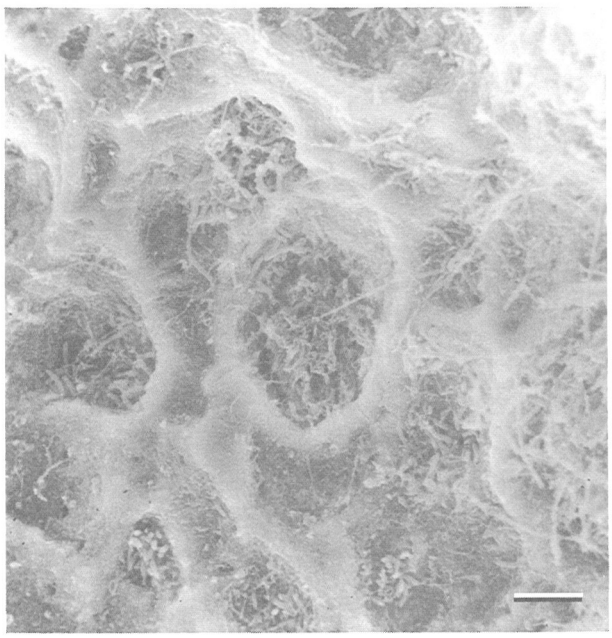

Figure 12. *Multilocular defects developed in the subgingival root cementum when individual lacunar defects became confluent. Abundant masses of bacterial plaque are present in most lacunae. Bar = 10 μm.*

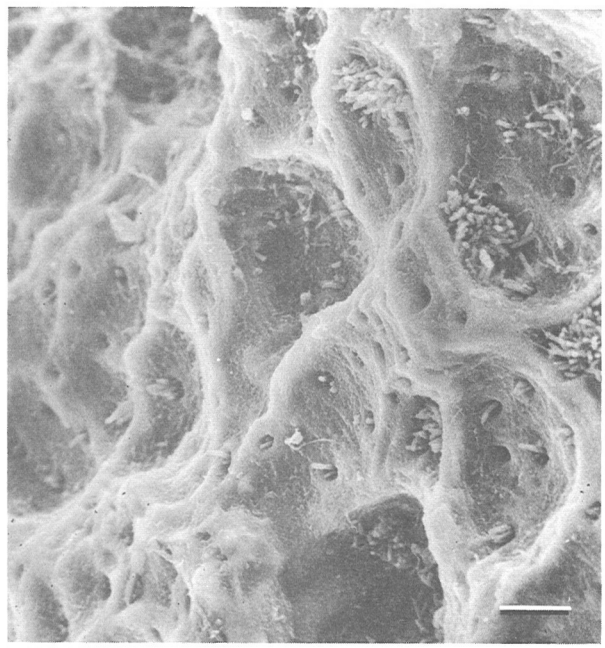

Figure 13. *The bottom of most resorption lacunae extend into the radicular dentin. The orifices of the dentinal tubules are easily discernible. Bacteria are present on the bottom of the lacuna and several dentinal tubules contain invading bacteria. Bar = 10 μm.*

of the pulpal dentin wall. Bacterial invasion did not occur in all dentinal tubules, as many empty tubules, even immediately adjacent to invaded tubules, were present. As far as could be ascertained from the specimens in which longitudinally fractured tubules could be followed from the radicular surface to the area where the invading bacteria were present, no correlation existed between presence of bacterial invasion in the dentinal tubules and presence or absence of remaining radicular cementum. Bacteria were confined to the tubular lumen and in none of the specimens were bacteria found in the intertubular dentin. No bacteria were found in the portion of the root located apically to the epithelial attachment and where intact Sharpey's fibers connected the cementum to the alveolar bone.

The ultrastructure of the dentin did not display any major differences from the ultrastructure of normal radicular dentin. Odontoblast processes were found

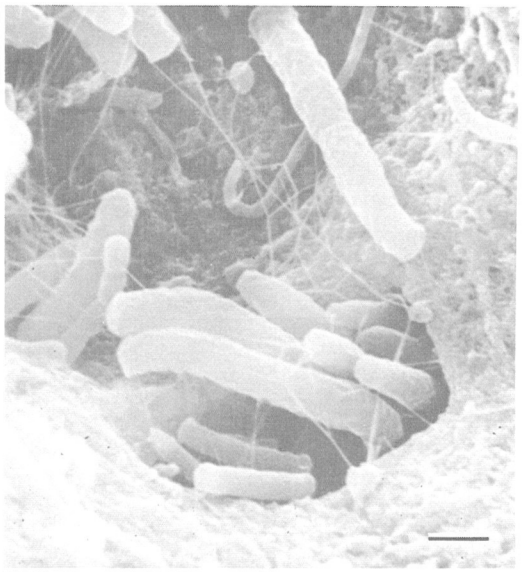

Figure 14. *Filamentous bacteria invading the dentinal tubules at their orifices in the bottom of a resorption lacuna. Bar = 1 μm.*

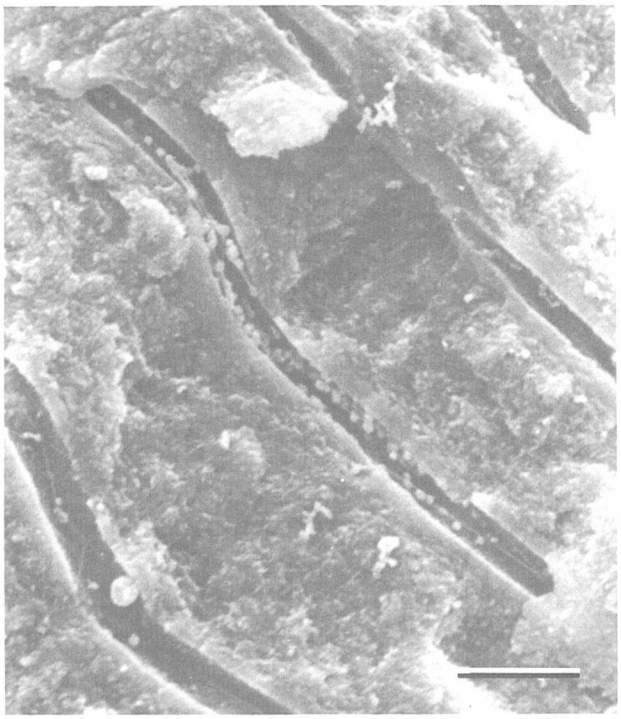

Figure 15. *Longitudinally fractured dentinal tubules in the radicular dentin area corresponding to the exposed subgingival root surface. Bacteria are present in the dentinal tubules. Bar = 5 μm.*

only in the inner third of the dentin. Occasionally, mineral inclusions were observed in the lumen of a single dentinal tubule.

DISCUSSION

This light and scanning electron microscopic study has demonstrated bacterial invasion in the radicular cementum and radicular dentin of periodontally diseased caries-free human teeth. These findings are in agreement with our results from studies in which bacteria were cultured from the dental pulp and radicular dentin of these teeth.[22–24] The terminology "*invasion*" is appropriate since bacteria are moving into the host tissues with displacement and destruction of the tissue components,[28] in this case Sharpey's fibers, radicular cementum and odontoblast processes.

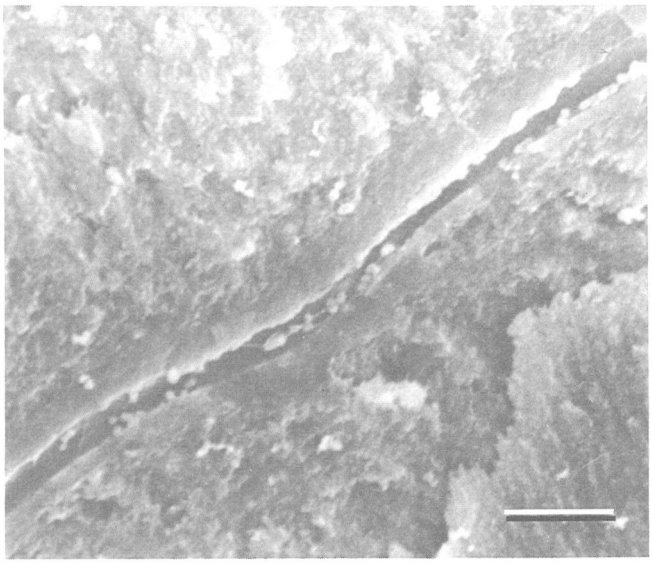

Figure 16. *Groups of cocci and short rods in the lumen of a longitudinally fractured dentinal tubule. Bar = 5 μm.*

Bacterial invasion into the radicular cementum of periodontally diseased teeth has been described.[8,13,17,29–32] In the studies reported by Hartzell[30] and Daly et al.,[13] bacteria were present in the outer half of the cementum. Using Humberstone's Gram staining method, Daly et al.[13] found single bacteria or small groups of gram-positive and gram-negative bacteria to a depth of 12 μm below the plaque-covered cementum surface. Others[17,29,31] found bacteria throughout the entire cementum, including the deeper layers. Within a seven-day experimental period, Fine and Greene[33] observed bacterial penetration into cementum strips inserted in gingival pockets of chronic adult periodontitis patients.

In the present study, bacterial invasion of the cementum exposed to the subgingival environment associated with periodontal disease has been described. It appears from the present material that where Sharpey's fibers are attached into the cementum, these areas were used by the bacteria during the invasion of the cementum. This supports the hypothesis proposed by Hart.[29] However, several investigators have been unable to detect holes or spaces where Sharpey's fibers once inserted into the cementum.[34,35] In the present study, these spaces were only found in the most apically located parts of the exposed cementum. In the more coronally located areas they were not detected, as they probably had been removed during scaling and root planing. Likewise, the scaling and root planing performed on the teeth studied by Jones et al.[34] and Pameijer et al.[35]

would have been responsible for the absence of these spaces in their material.

However, in the more coronally, but still subgingivally located areas of the radicular cementum, bacterial invasion also occurred. Bacterial enzymes and acid metabolites might be present locally in concentrations sufficiently high to induce localized damages to the cementum and to a partially mineralized cuticle which eventually covers the exposed root surface.[19] The net result of this would be that bacterial invasion into the deeper cementum layers can occur. It is interesting to note that collagen, which is the predominant organic constituent of cementum, is only attacked by collagenase. Although this enzyme is produced by *Bacteroides gingivalis*,[36–39] *Actinobacillus actinomycetemcomitans*,[38] *Bacillus cereus*,[40,41] spirochete strains[42,43] and *Capnocytophaga* species,[44] all isolated from periodontally diseased sites in humans, the majority of plaque bacteria lacks this specific enzyme. However, collagen is easily denatured by bacterial acid metabolites and, in its denatured state, can be further broken down by almost any aspecific proteolytic enzyme, which most subgingival bacteria produce in sufficient quantities.[45]

The presence of lacunar defects has been described previously as a common feature in the root surfaces of periodontally diseased human teeth.[8,46–49] The origin of these lacunae could not be determined in this study. They might have been caused by the action of plaque bacteria, as was suggested by some small defects in which single bacteria were present. On the other hand, Schroeder and Rateitschak-Plüss[49] have proposed a hypothesis for the origin of these lacunae without the involvement of bacterial acids. External resorption lacunae are formed in the radicular surface of periodontally healthy caries-free teeth under a variety of physiological conditions, and their formation is enhanced by the presence of orthodontic or traumatic forces. Similarly, these lacunae are formed under conditions of acute and chronic inflammation of the marginal or apical periodontal tissues. Usually, these lacunae are filled with osteocementum after cessation of the resorptive process. However, if marginal periodontal inflammation is accompanied by the apical migration of the subgingival plaque, with the concomitant breakdown of the interface between the tooth and the periodontal tissues, the resorption lacunae are exposed to the subgingival environment without further possibility of repair. Subsequently, these lacunae are colonized by subgingival plaque bacteria. In some areas (Fig. 12) the morphology of the lacunar defects corresponded to the description of those in the material from Schroeder and Rateitschak-Plüss[49] and, therefore, tends to support their hypothesis. However, in other areas, the lacunar defects were obviously induced by the action of the bacteria present in the defect (Figs. 10a and 10b).

The radicular resorption lacunae, which harbor subgingival plaque bacteria and subgingival calculus, are poorly accessible for instrumentation. In a study on 84 extracted human teeth, which had been meticulously scaled and root planed, Waerhaug[47] found that these lacunae were only partially eliminated. Particularly, lacunae close to the bottom of the pocket were hard to eliminate completely. Bacteria left behind in these lacunae were responsible for the recolonization of treated areas, thus resulting in recurrence of periodontal disease.

In the present study invading bacteria were not only found in the radicular cementum, but also in the dentinal tubules of periodontally diseased caries-free human teeth. Invasion was observed in the portion of the root where the attachment loss had occurred. Most of the invading bacteria were present in the outer third of the radicular dentin. Few investigators have studied, by electron microscopy, the presence of invading bacteria in the radicular dentin of periodontally diseased teeth. However, this phenomenon has been documented in a small number of light microscopic studies in humans.[20,21] Kopczyk and Conroy[20] performed scaling and root planing followed by a period of up to four and a half months before teeth were extracted and Gram-stained paraffin sections were examined. In one of the 16 teeth, they found bacteria in the dentinal tubules from which the orifices had been exposed to the oral environment during therapeutic procedures. Bacteria were present in the external half of the radicular dentin. Langeland et al.[21] found bacterial invasion in the dentinal tubules in five of 60 extracted periodontally diseased teeth. Again, bacteria were observed in the outer half of the dentin only. In all five teeth, a superficially demineralized root surface was present.

The presence of bacteria in the dentinal tubules of the radicular dentin has also been described in gnotobiotic rats and hamsters.[50–52] In a study of the pulpal response to citric acid in cats,[53] bacterial invasion was found in the dentinal tubules of citric acid treated teeth. The depth of the bacterial invasion increased with longer observation periods (up to 83 days). This suggested that the bacteria were indeed able to actively invade the radicular dentin.

In *in vitro* experiments it has been demonstrated that bacterial invasion in dentinal tubules occurred when pure cultures of *Streptococcus mutans*,[54] *Actinomyces naeslundii*[55] and *Capnocytophaga gingivalis*[56] as well as bacterial plaque from periodontal lesions[57] were grown on human dentin. In these studies, it was demonstrated that the presence of motile bacteria in the flora resulted in bacterial invasion to greater depths.[58]

Compared with the few existing reports, the occurrence of invading bacteria in the radicular dentin in the present study is relatively high. This might be attributed to the fact that in scanning electron microscopy the detection of bacteria does not depend on a staining technique and that the resolution is far superior to that of light microscopy. The numbers of teeth containing

bacteria were even higher (87%) when cultural techniques were used.[22,23] This is due to a higher sensitivity of the cultural technique as compared with microscopic techniques. From the cultural studies it is known that bacterial concentrations averaged 16,000 colony forming units per mg of dentin in the outer dentin layer in which the highest bacterial concentrations were found. Based on an approximate density of 2 mg/mm^3 for dentin, it would take roughly 100 serial 5-μ thick sections and nine high-power fields to be examined in each one of these sections in order to count the bacteria present in 1 mg of dentin.

Several authors have claimed that radicular cementum that has been exposed to the oral environment contains substantial amounts of bacterial endotoxin and that this endotoxin is capable of interfering with the healing of the periodontal tissue after treatment through its cytotoxic effect.[59,60] From experiments with treated roots of periodontally diseased teeth in which the endotoxin levels were measured,[61–63] or in which the growth of different cell types was studied,[64,65] the complete removal of all orally exposed cementum was advocated as a key to successful periodontal therapy.

However, Nakib et al.[66] reported that commercially available *E. coli* endotoxin loosely adhered to the root cementum of either periodontally diseased or healthy teeth, without penetrating into the cementum. Daly et al.[67] suggested that endotoxin from the cell wall of gram-negative bacteria left behind after scaling and root planing might be the major source of the *Limulus* amebocyte lysate assay reactivity obtained from orally exposed root surfaces. Several studies[68–70] have demonstrated that in the presence of a variety of compounds, such as ribonucleic acids, ribonuclease, gram-positive cell wall fractions, peptidoglycan, thrombin and thromboplastin, a positive *Limulus* amebocyte lysate assay was obtained. On this basis the specificity of this assay for endotoxin has been questioned. Based on our present findings, we suggest that the *Limulus* amebocyte lysate reactivity that was found by several investigators in orally exposed root surfaces of periodontally diseased teeth might be derived from components of whole gram-positive and gram-negative bacteria present in the root cementum and radicular dentin. This hypothesis is further supported by our cultural data, which demonstrated the presence of viable bacteria in both tissues of periodontally diseased teeth.[22–24] This means that a variety of toxic products from invading bacteria might induce inflammatory responses in the periodontal tissues and in the dental pulp.

In clinical studies, the presence of pulpal pathosis in combination with periodontal disease has been well documented.[21,71–75] However, histologic studies[76,77] failed to find correlations between the presence or severity of periodontal disease and morphologic changes in the pulp tissue. In monkeys, inflammatory reactions and secondary dentin formation were observed[78] in dental pulps of teeth exposed to experimentally induced periodontal disease, followed by a single episode of scaling and root planing. Although clinical and histologic studies gave conflicting or at least inconclusive results, a number of arguments have been presented that support the induction of pulpal reactions by subgingival periodontopathic bacteria. When freshly cut dentin of monkeys was exposed to either human dental plaque extract,[79] or components of cultured dental plaque bacteria,[80] these substances induced an acute inflammatory pulpal reaction within eight to 32 hours. When sonicates of pure cultures of *A. actinomycetemcomitans* and *A. viscosus* were applied to freshly cut dentin in monkeys, polymorphonuclear leukocyte infiltration of the pulp tissue below the cavity was consistently found.[81]

Bacteria present in the dentin of the root surfaces that were exposed by periodontal disease will not be eliminated by a meticulous scaling and root planing unless substantial portions of dentin are removed. Since this is impossible for obvious reasons, the bacteria left behind in the dentin can recolonize the treated root surfaces. Thus, these bacteria will interfere with the initial healing process and could cause recurrence of the periodontal disease. This recolonization of treated root surfaces might be particularly harmful in cases where therapy aims at the creation of new connective tissue attachment by retardation of apical migration of the epithelium and in which a high percentage of failures has been reported.[82–86]

Since mechanical debridement alone is unable to remove bacteria present in the radicular dentin, it appears logical that chemical means of eliminating them are needed. Antimicrobial agents that are applied topically, such as chlorhexidine, fluoride or tetracyclines, and systemic antibiotics, such as metronidazole or tetracyclines, probably could be used effectively. Although numerous studies have shown the positive effects of combining antimicrobials with the mechanical scaling and root planing, no studies are available to document the effect of these agents on the invading bacterial population. The question remains whether bactericidal concentrations of these compounds can be obtained in radicular dentin.

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