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REVIEW

Endodontic Microbiology. I. Etiology

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The role of bacteria in the etiology of endodontic lesions has been intensively studied. Recent work has elucidated specific mechanisms by which bacterial components such as endotoxin and other cell wall components can contribute to the inflammatory processes. In addition, developments in anaerobic microbiology have facilitated accurate and reproducible identification of endodontic pathogens. This article reviews and correlates clinical and laboratory research bearing on this important topic.

Microbial infection of the dental pulp, often the result of dental caries, is the most common prelude to endodontic treatment. Successful endodontic therapy depends upon reduction or elimination of these microorganisms. Failures in endodontic therapy may be due to the persistence of infection. Therefore, the endodontist should be knowledgeable about the specific bacterial species involved in pulpal and periapical pathosis and the mechanisms whereby they perpetuate these inflammatory lesions. Such information facilitates rational treatment directed toward microbial elimination.

Bacteria are major deterrents to the repair of exposed pulpal tissues. In their classical study utilizing germ-free rats, Kakehashi et al. (1) demonstrated that exposed pulpal tissue became necrotic in the presence of bacteria with the development of chronic inflammation and, eventually, periapical granulomas. On the other hand, in a germ-free environment the response of exposed pulpal tissue was characterized by minimal inflammation and the formation of dentinal bridges.

Any microorganism in the oral cavity, nasopharynx, or gastrointestinal tract can infect a root canal. The results of past studies have generally agreed that the microorganisms most commonly isolated from infected root canals were the α-hemolytic streptococci. γ-Streptococci and enterococci were commonly found whereas *Staphylococcus aureus* and β-hemolytic streptococci were isolated less frequently (2–5). There were occasional reports of new types and smaller forms, such as mycoplasma (6). However, attempts at culturing other agents, such as viruses, from root canals or periapical tissues have been uniformly unsuccessful (7, 8).

For the most part, these previous studies utilized aerobic incubation which favored facultatives, such as streptococci, and ignored other species, such as Bacteroides, which are anaerobes. Thus, it became axiomatic that streptococci were the most prevalent bacteria in infected pulps. However, these organisms by themselves could not totally account for the histopathology of pulpal and periapical lesions nor could they fulfill Koch's postulates.

Many studies on the microbiology of root canal infections were flawed. Many specimens were improperly taken and thus contaminated. Frequently, bacteriological media, which were incapable of supporting the growth of significant organisms, were used. More important, the use of anaerobic transport and culturing methods was ignored. In addition, poor taxonomic classifications often rendered the findings meaningless.

In recent years, considerable interest has been generated in the role of anaerobic microorganisms in causing inflammation and pain. Anaerobes in mixed root canal infections may be responsible for the inhibition of neutrophil chemotaxis and phagocytosis, interference with antibiotic sensitivity (9, 10), and the production of enzymes and endotoxins (11, 12) resulting in the persistence of painful periapical lesions (13).

Today, no serious investigation of endodontic infection can be undertaken without special attention to anaerobic culturing. Meticulous care must be taken to prevent contact of the specimen with atmospheric oxygen. The canal orifice must be flushed with nitrogen gas during sampling. The emergence of anaerobic bacteria as important endodontic pathogens has somewhat paralleled their importance in oral microbiology and periodontal disease (14). The association of anaerobic or microaerophilic microorganisms, such as black-pigmented Bacteroides, fusobacteria, actinobacilli, and capnocytophaga, with periodontal disease has been definitively established (15, 16).

Anaerobic infections occur when there is a compromised blood supply or antecedent infection by aerobic bacteria, both of which produce a milieu with a low oxygen-reduction potential (Eh). Anaerobes are in the normal flora and thus become secondary invaders, spreading beyond the confines of the pulp and periapex. They are not usually primary pathogens. Infected sites tend to harbor a complex flora consisting of both aerobic and anaerobic components (17). Brown and Rudolph (2) studied the bacteria isolated from traumatized teeth which, clinically, appeared to be unexposed to the saliva. Anaerobic and aerobic culturing, as well as dark-field and phase contrast monitoring for spirochetes, was performed. At

that time, techniques for limiting exposure to atmospheric oxygen during culturing had not been perfected nor had the best medium been formulated for optimal growth of anaerobes. Thus, anaerobes accounted for only 24% of isolates, surpassed by facultatives (51%) which were mostly streptococci.

The importance of anaerobic isolation and culturing was underscored by Berg and Nord (18). They found that 50% of the bacteria isolated from pulps when anaerobic procedures were used did not grow when only aerobic incubation was used. Their study also demonstrated that the proper procedure for maintenance of anaerobiosis was the use of nitrogen gas flow over the pulp orifice when the samples were taken.

THE GROWTH OF MICROORGANISMS IN SPECIFIC MEDIUM

The type of culture medium used influences the results of root canal cultures. Appropriate medium, which specifically supports organisms commonly found in infected root canals, should be utilized. Dextrose broth, brain-heart infusion broth, trypticase dextrose broth, and serum dextrose broth have been used for endodontic culturing. However, all of these media are not suitable for the growth of anaerobic microorganisms. The addition of small amounts of agar to some of these media permits the growth of some microaerophilic species and the addition of serum, blood, or ascitic fluid facilitates the growth of others (19). However, in any mixed flora, some microorganisms grow while the growth of others is suppressed.

Leavitt et al. (3) cultured for anaerobes using trypticase soy broth supplemented with 0.1% agar. Forty-five percent of their isolates were α-hemolytic streptococci; 33% were anaerobes, such as anaerobic streptococci and Veillonella. The presence of staphylococci in 54% of the samples was suggestive of skin contamination. However, agar incorporation in the trypticase broth as the sole means to produce anaerobic conditions is unreliable for culturing of some pulpal pathogens. Even the use of medium containing reducing agents, such as thioglycollate, does not guarantee growth of strict anaerobes. In teeth with necrotic pulps, Griffee et al. (20) found that 94% were infected when the contents were grown in prereduced thioglycollate medium while only 41% were positive using trypticase soy broth with agar.

The limitations of thioglycollate as the only medium for the isolation of anaerobes has been underscored in studies of bacteria from wounds and abscesses in hospital laboratories. With thioglycollate only 2 to 3% of the specimens yielded anaerobes. The use of anaerobic jars increased isolation to 9%. More rigorous techniques, such as the use of transport tubes, prereduced media, and anaerobic chambers, increased the proportion of anaerobes to almost 40% (21). Fulghum et al. (22) compared the isolation of pulpal pathogens by the use of the Virginia Polytechnic Institute (VPI) method (23) with that of thioglycollate. Although 18 of 24 pulps (75%) produced growth on VPI prereduced media, only 8 (33%) were positive for growth on thioglycollate. In earlier studies, Crawford et al. (5) failed to identify any bacteroides from 57 teeth when they depended on thioglycollate for anaerobic isolation. Engström and Frostell (24) cultured single-rooted teeth with necrotic pulps using thioglycollate broth. Although 25 of 36 teeth (72%) were infected with bacteria that could be seen microscopically, only 21 (58%) had visible growth in broth cultures. They did find an association between the clinical detection of a gangrenous odor and bacterial isolation; 17 of 18 teeth that manifested an odor yielded positive growth.

When more attention has been paid to anaerobic isolation, the number of such isolates have increased. In a study by Wittgow and Sabiston (25), 80% of 40 teeth were positive for growth. All but one was infected with obligate anaerobes, mainly Gram-negative rods. Kendell et al. (26) used the VPI system to isolate and identify anaerobes from teeth with vital and nonvital pulps. Both necrotic and vital pulps yielded 80% positive cultures; however, anaerobes could only be isolated from the nonvital cases. Gram-positive rods (Actinomyces and propionibacteria) and Gram-negative rods (Bacteroides and fusobacteria) were isolated most frequently. However, sensitivity to oxygen is not the only factor which limits isolation of anaerobes from root canals (27). Anaerobes can survive exposure to air for up to 2 h if the microorganisms are processed in medium supplemented with blood. Catalase in blood serves to break down hydrogen peroxide which mediates the oxygen toxicity for anaerobes.

Byström and Sundqvist (28) cultured 30 teeth with necrotic pulps and isolated bacteria from all. Eighty percent of the isolates were anaerobic. Most canals yielded more than one microbe, the median number being four. The most frequent isolates were anaerobes such as fusobacteria, eubacteria, peptostreptococci, and Bacteroides. Zavistoski et al. (29) attempted to quantitate the number of microorganisms in necrotic pulps. The mean number of bacteria was approximately 10^8 per g, which is similar to bacterial concentrations at other infected anatomical sites. Sixty-three percent of all bacteria in necrotic pulps were anaerobes, such as Bacteroides, lactobacillus, *Propionibacterium acnes*, and Veillonella. A summary of the studies of the microbiology of root canal infections appears in Table 1.

SPIROCHETES

Brown and Rudolph (2) found spirochetes by dark-field and phase contrast microscopy in 14% of their pulp samples but most could not be cultured. These spirochetes were subsequently studied and identified by Hampp (30) as small treponemes, the most anaerobic of oral bacteria (31). In a later study, Kantz and Henry (32) failed to observe or culture any spirochetes from 24 pulpless teeth. The presence or absence of oral spirochetes may be an aid in differentiating between endodontic and periodontic abscesses. Dark-field examination of exudates from 15 patients showed that periodontal abscesses were heavily populated with spirochetes (30 to 60% of observed microorganisms), whereas endodontic abscesses had few or none (less than 10%) (33).

FLORA OF OPEN AND CLOSED CANALS

A difference exists between the flora of infected root canals which have been open to the oral fluids for some time and the flora isolated from freshly opened canals (2, 5, 34, 35). The types of microorganisms from the canals which are open to the oral cavity vary widely and correspond generally to the oral flora; there is less variation in the microorganisms from

TABLE 1. Microbiology of pulpal infections

Study	Anaerobes as % of Total Isolates	Prominent Isolates
Möller, 1966 (121)	73	Fusobacteria Anaerobic streptococci
Kantz and Henry, 1974 (32)	27.2	Bacteroides Actinomyces
Berg and Nord, 1973 (18)	73	Lactobacilli Corynebacteria Eubacteria Peptococci Bacteroides
Wittgow and Sabiston, 1975 (25)	84	Eubacteria Bacteroides
Sundqvist, 1976 (71)	94	Eubacteria Bacteroides Propionibacteria Fusobacteria
Kendell et al., 1976 (26)	NS*	Propionibacteria Bacteroides Eubacteria
Zavistoski, 1980 (29)	63	Bacteroides Lactobacilli Peptococci
Byström and Sundqvist, 1983 (28)	88	Peptostreptococci Fusobacteria Bacteroides

* NS, not specified.

closed canals. All open root canals from teeth with necrotic pulps have yielded positive cultures, whereas in teeth with closed root canals and necrotic pulps approximately one-half gave positive cultures (36, 37). Teeth with periapical radiolucent areas have a higher incidence of infected root canals than teeth without such areas (37, 38).

In closed canals, the bacterial flora is predominantly anaerobic (12, 25, 29, 32). The isolates from closed root canals are restricted in their growth and there is a tendency for anaerobic strains to increase in number and proportions to other types. Möller et al. (39) found that, in experimentally infected monkey root canals, some microorganisms, including facultative anaerobes, tended to disappear with the passage of time, possibly due to a change in the oxygen environment and disturbance of the interbacterial nutritional relationships.

PHLOGISTIC COMPONENTS OF BACTERIA

Lipopolysaccharides (Endotoxin)

The cell walls of Gram-negative bacteria, such as black-pigmented Bacteroides or fusobacteria, contain endotoxin. These are heat-stable molecules containing polysaccharides and phospholipids. This bacterial component is also referred to as lipopolysaccharide (LPS) (40). LPS is a major virulence factor; it exerts a plethora of biological effects which result in the amplification of inflammatory reactions. Endotoxins are nonspecific weak antigens which are poorly neutralized by antibodies. They are capable of activating the complement cascade without antibody. This activation can be accomplished by way of the classical pathway (via C'1) or through the alternate or properdin pathway (via C'3) (41). Complement activation results in activation of the kinin (42) and coagulation (43) cascades.

The administration of endotoxin in vivo results in degranulation of mast cells with the release of histamine and heparin (44). Endotoxin also causes the release of collagenase (45) and cachectin, a hormone known as tumor necrosis factor, from macrophages (46). In small doses, endotoxin induces fever. The pyrogenicity of endotoxin is largely due to its ability to stimulate the release of interleukin 1 from macrophages. Interleukin 1, formerly referred to as endogenous pyrogen, acts on the hypothalamus, the body's thermoregulator (47). Endotoxin also enhances neutrophil-mediated injury by promoting adherence to endothelial cells and potentiating the release of oxygen radicals and proteases, leading to tissue injury (48). Endotoxin is a nonspecific stimulator of B lymphocytes (B cell mitogen) (49, 50). In periodontal disease, LPS inhibits the attachment of gingival cells to cementum (51, 52).

Endotoxin in Root Canals

Many of the chronic periapical lesions are associated with the presence of anaerobic microorganisms, alone or in combination with other species (53). Even small amounts of endotoxin are capable of inducing a periapical inflammatory response (11). The presence of endotoxin in necrotic pulps has been documented by Schein and Shilder (54) and Dahlén and Bergenholtz (12). Both studies measured endotoxin by use of the limulus lysate test which depends upon the gelation of the lysate of the amebocytes of the horseshoe crab by picogram quantities of endotoxin. The test is sensitive and does not yield false-positive reactions with other bacterial toxins (55). Schein and Schilder found higher levels of endotoxin in the root canals of pulpless teeth with radiolucencies than in canals of teeth with vital pulps but without radiolucencies. Dahlén and Bergenholtz found a correlation between the limulus titer and the number of Gram-negative bacteria which could be isolated from infected pulps.

Experimentally, endotoxin has been demonstrated to induce periapical inflammatory lesions and bone destruction in laboratory animals (56, 57). Dwyer and Torabinejad (56) found that when endotoxin was inoculated into the pulp chambers of cat canines, large apical radiolucent areas were induced after 2 wk. Root canals inoculated with either saline or detoxified endotoxin failed to elicit this reaction. The inflammatory exudate consisted primarily of neutrophils with fewer numbers of lymphocytes and macrophages as well as osteoclasts associated with bone resorption. Pitts et al. (57) obtained similar results when Salmonella endotoxin was placed into the root canals of dog molars and premolars. The induced periapical inflammatory lesions were heavily populated with neutrophils; periapical bone was resorbed. Dahlén et al. (58) sealed LPS from an oral fusobacterium into the root canals of monkey molars and premolars. Six of 27 treated teeth developed periapical destruction after 3 months. Histologically, there was chronic periapical inflammation with bone resorption and histochemical evidence of increased enzyme activity by bone cells. The ability of LPS to produce inflammatory lesions in monkey pulps at shorter time intervals was demonstrated by Warfvinge et al. (59). They prepared class V

cavities in teeth so that less than 1 mm of dentin remained over the pulp. A paste composed of lyophilized LPS from the oral organisms, *Bacteroides oralis* or *Veillonella parvula* mixed with saline, was placed on the floors of the cavities and sealed. Histological examinations were made after 8- and 72-h periods. Control applications of bovine serum albumin resulted in only mild neutrophilic infiltration. However, in the pulps of the cavities sealed with LPS, leukocytic infiltrates increased in intensity from 8 to 72 h.

However, not all investigators agree that endotoxins constitute a major factor in the initiation or maintenance of chronic inflammation and the sequelae of such inflammation. Repeated application of endotoxin can induce immunologically nonspecific tolerance as measured by passive hemagglutination and lymphoblast assays (46). This enhanced nonspecific resistance can be produced by Gram-negative and some Gram-positive organisms as well as by viruses. In endotoxin-tolerant rats, alveolar bone resorption is significantly reduced (60). In an experimental animal model, Wesselink et al. (61) used the local Shwartzman reaction to assess the effect of *Escherichia coli* endotoxin on the connective tissue of rats. They found that tissue reactions to implanted endotoxin-filled polyethylene tubes were no different than those surrounding unfilled control tubes after challenge with a provocative intravenous injection of endotoxin.

Although endotoxin is generally inhibitory to cells, variations in the endotoxin preparations and differences in susceptibility of target cells might account for the divergent observations. For example, endotoxin has been shown to have a stimulatory effect on human and bovine fibroblasts derived from pulpal tissues. Pinero et al. (62) found that *E. coli* endotoxin at levels of 5 or 125 μg per ml stimulated both human and bovine dental pulp cell utilization of [^{3}H]thymidine, implying increased production of DNA, increased cell division, and increased glycosaminoglycan production. At higher concentrations (625 μg per ml), collagen production was increased in the human pulp and bovine cell lines showed increased sulfate utilization. These responses resemble those of fibroblasts during wound healing. Thus, infections associated with low concentrations of endotoxin may actually stimulate repair mechanisms.

ENDOTOXIN AND PAIN

It would appear as if the endotoxins elaborated from infected root canals may contribute to increasing vasoactive and neurotransmitter substances at the nerve endings of inflamed periapical lesions. *Bacteroides melaninogenicus* produces enzymes which are collagenolytic (63) and fibrinolytic (64). Its endotoxin is capable of activating Hageman factor which leads to the production of bradykinin, a potent pain mediator. Bradykinin production is also enhanced when human leukocytes are exposed to endotoxin (65).

Considerable evidence has accumulated supporting the fact that bacterial endotoxins possess neurotoxic properties (66–68). Parnas et al. (69) have indicated that bacterial endotoxin acts on presynaptic nerve terminals, causing them, in response to an applied stimulus, to release an increased amount of neurotransmitter.

In teeth with infected root canals and periapical lesions, the relationship of bacterial endotoxins with pain has been demonstrated by Schein and Schilder (54) and by Schonfeld et al. (70); more endotoxin was found in the periapical areas of painful teeth than in those of asymptomatic teeth. Sundqvist (71) used the VPI method in his classical work on the bacteriology of pulps rendered nonvital by impact injury. This study was thorough in isolating, characterizing, and speciating the isolates. He found that, of 32 tooth samples, only those with periapical radiolucencies yielded bacterial growth. Eighteen of 19 teeth with radiographically detectable periapical lesions contained bacteria; over 90% were anaerobes. A greater number of different bacterial strains was isolated from patients with painful symptoms. There was a significant association between the clinical symptoms and the presence of *B. melaninogenicus*. This anaerobic Gram-negative rod was isolated only from symptomatic teeth and was absent from those that were asymptomatic. In declining order of frequency, the isolates were fusobacteria, Bacteroides, eubacteria, peptococci, peptostreptococci, and campylobacter. No facultative streptococci were isolated.

The association of *B. melaninogenicus* and clinical symptoms was partially confirmed by Griffee et al. (72) who studied 33 teeth with nonvital pulps. *Bacteroides melaninogenicus* was isolated from 12 teeth; its presence was related to pain, foul odor, and the formation of sinus tracts. However, no relationship was established between the presence of the organism and the presence of periapical rarefaction.

The emphasis on the significance of Gram-negative anaerobes in the production of pain and swelling does not negate the fact that Gram-positive bacteria may also be involved in root canal infections and flare-ups. It appears as if a myriad of microorganisms are associated with infectious exacerbations.

CLINICAL CLUES OF ANAEROBIC INFECTIONS

Anaerobic infections usually produce tissue necrosis with abscess formation. Clinically, the patient develops a swelling and experiences pain and fever. The purulent discharge has a foul smell caused by bacterial metabolites such as ammonia, indoles, urea, and amino acids. All of these have been shown to induce the firing of pulp neurons (73, 74). Failure to recover pathogens from aerobic cultures and failure to respond to antibiotics which are effective against aerobes should lead to the suspicion that the root canal is the seat of an anaerobic infection. The common anaerobes have typical morphological appearances when Gram stained. Thus, the findings from Gram staining the exudate may suggest the presence of anaerobic infection.

GRAM-POSITIVE CELL WALL COMPONENTS

The cell walls of Gram-positive bacteria, such as streptococci and Actinomyces, are also capable of influencing inflammatory reactions. The cell wall components include peptidoglycans and lipoteichoic acids. Peptidoglycan, which accounts for 40% of the mass of the cell, is made up of glycan chains (*N*-acetylglucosamine and *N*-acetyl muramic acid) with peptide cross-bridges. Together they form a large, net-like structure which lends rigidity to the bacterial cell (75). Peptidogly-

cans are arthritogenic in laboratory animals, particularly rats (76) and mice (77). The granulomatous reactions that are induced by peptidoglycans have been extensively studied as a model for human rheumatoid arthritis. These reactions are dependent on thymus-derived lymphocytes and are characterized by the presence of numerous macrophages. The former produce lymphokines, such as osteoclast-activating factor and fibroblast-activating factor, as well as prostaglandins. All of these mediators may play important roles in the pathogenesis of periapical lesions (78, 79). Peptidoglycan also activates complement (80) and acts as a polyclonal stimulator of B lymphocytes (81).

Another class of compounds are the lipoteichoic acids which are covalently linked to peptidoglycans. They are composed of polymers of glycerol phosphate with a glycolipid moiety at one end. Both of these bacterial components are powerful biological response modifiers. Because of their glycolipids, lipoteichoic acids have the ability to bind to cell membranes, particularly of lymphocytes and macrophages. This association results in cell activation (82). Lipoteichoic acids also induce bone resorption in tissue culture (83). The complement cascade is also activated by this bacterial component (84).

Experimentally, Gram-positive bacteria have been shown to induce pulpal and periapical inflammation. Whole cells or sonicates of *Streptococcus mutans* elicited pulpal necrosis and periapical bone resorption 6 months after injection into the pulps of cats (85). However, when compared with other oral bacteria, *S. mutans* was unable to induce periapical inflammation in monoinfected gnotobiotic rats. Korzen et al. (86) compared pulpal and periapical responses after pulp exposure and instrumentation in gnotobiotic and conventional rats. The most severe inflammatory reactions at these locations were seen in conventional animals. Their gnotobiotic counterparts, which were monoinfected with *S. mutans*, developed pulpal inflammation which did not spread to the periapical tissues. However, Rosengran and Winblad (87), utilizing a proteolytic mutant of *S. mutans*, induced periapical bone destruction and abscess formation in conventional rats. Bergenholtz and Warfvinge (88) tested the ability of sonicated Gram-positive organisms to elicit pulpal inflammation in monkey teeth. Class V cavities were prepared and Actinomyces and streptococcal sonicates were placed into the cavities and sealed. Eight hours later, histological examination revealed that streptococcal preparations caused only slight inflammatory responses in the pulp; the placement of Actinomyces produced a severe neutrophilic infiltrate. The role of complement in this reaction was also studied. Activation of complement by either LPS or lipoteichoic acid results in release of C'3 which is chemotactic for neutrophils. Cobra venom factor, which specifically inhibits C'3, was shown to have no effect on the pulpal inflammation evoked by Actinomyces. This finding would indicate that Gram-positive bacteria might induce pulpal inflammation by secreting chemotactic factors independent of complement activation. Such activity has been shown for *P. acnes*, a prominent anaerobic pulpal isolate which nonspecifically stimulates the mononuclear phagocytic system (89) and releases low molecular weight chemotactic factors (90).

It is apparent that bacterial cell wall components are important instigators of pulpal and periapical inflammation. These reactions are summarized in Fig. 1.

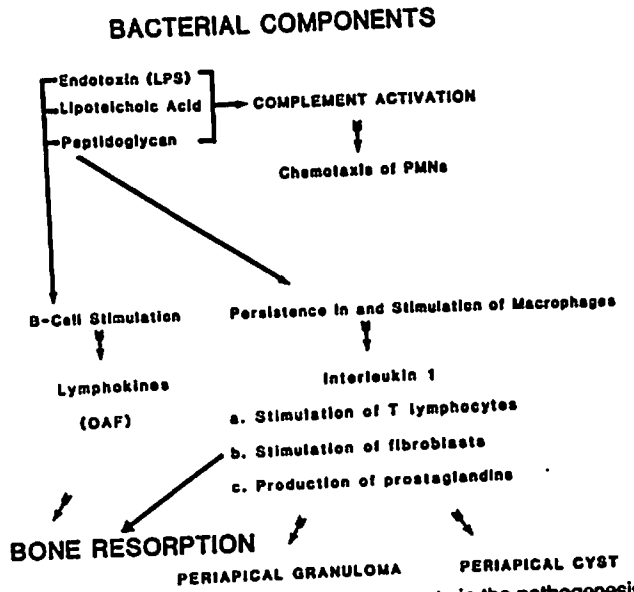

FIG 1. The role of bacteria and their components in the pathogenesis of pulpal and periapical inflammatory lesions.

PERIAPICAL INFECTIONS

Vital pulp tissue appears to act as a buffer against periapical inflammation. Periapical destruction rarely occurs when vital pulp elements are present (86, 91). However, when a periapical lesion is present, there have been differing views regarding the bacterial status of lesions. In the past, two major studies, which utilized similar protocols, emerged with completely irreconcilable results. Hedman (92) placed a cannula into the root canal, through which a culture wire was then passed to sample the periapical area. Even though a culture medium which could not support anaerobic growth was used, bacteria were found in 68% of 82 periapical lesions. The organisms were mainly streptococci and staphylococci. Ten years later, Shindell (93) used a similar, but modified, technique and found growth in less than 5% of 62 lesions. In light of newer techniques for culturing oral microorganisms, it is now generally agreed that most periapical lesions harbor bacteria.

The significant role of oral bacteria in the induction of periapical inflammation has been confirmed experimentally. Möller et al. (94) exposed pulpal tissues of monkey teeth to the oral environment for 7 days. The teeth were then sealed and examined 6 months later. All of the exposed teeth became infected with microorganisms such as α-hemolytic streptococci, enterococci, coliforms, and anaerobes such as Bacteroides, eubacteria, propionibacteria, and peptostreptococci. Ninety percent of the teeth developed radiographic periapical lesions. In a subsequent study, Fabricius et al. (95) found that the ratio of anaerobes to aerobes increased with the passage of time following the closure of the exposed pulps. The ratio increased from 3.9 at 90 days to 11.3 at 1,060 days. *Bacteroides melaninogenicus* was found in higher percentages in the apical regions. Bacteroides has also been shown to be capable of inducing abscesses in a variety of other experimental models, either in pure culture or in conjunction with facultative organisms (96–98).

Tronstad et al. (99) studied six patients who had asympto-

matic lesions which were refractory to nonsurgical endodontic treatment. After gaining access to the periapical lesions by an aseptic surgical technique, soft tissue and scrapings from the surface of the root tips were cultured for aerobes and anaerobes. *Propionibacterium acnes* as well as *Bacteroides gingivalis* and *endodontalis* were isolated.

BACTEROIDES

The surface components of Bacteroides species have been implicated in the etiology and pathogenesis of periodontitis, pregnancy gingivitis, acute necrotizing ulcerative gingivitis, and pulp and periapical inflammation. These surface components play a role in adherence, colonization, and subsequent tissue destruction. The surface components which are involved in attachment or act as virulence factors are fimbriae, capsules, and LPS in the form of membranous vesicles (100). Fimbriae are seen as peritrichous, fibrillar structures in the electron microscope (101). Although unable to induce abscesses by themselves, the presence of these organisms in mixed infections appears to be significant in the production of purulent inflammation and pain in pulpless teeth (9, 71, 72, 102, 103).

Numerous studies have implicated endotoxin-producing species, such as *B. melaninogenicus* and *Bacteroides asaccharolyticus*, as inflammation and pain inducers (71). Sundqvist et al. (9) found that combinations of bacteria, which included strains of *B. melaninogenicus* or *B. asaccharolyticus*, produced transmissible infections with purulence when inoculated subcutaneously in guinea pigs. Thus, bacterial synergism is of major importance in maintaining Bacteroides infections.

CLASSIFICATION OF BACTEROIDES SPECIES

Two strains of *B. melaninogenicus*, isolated by Sundqvist, were studied in more detail by van Steenbergen et al. (104). They were found to be sufficiently different, by DNA homology and enzyme tests, to warrant a new species designation of *B. endodontalis*. The specificity of this organism for the root canal was demonstrated by van Winkelhoff et al. (105). They cultured 28 odontogenic abscesses divided into three categories based on tissue of origin, namely, endodontal, periodontal, and pericoronal. Twenty-six (93%) contained Bacteroides, which constituted 10 to 50% of the cultivable flora. *Bacteroides endodontalis* could only be isolated from endodontic infections, along with *B. intermedius* and *B. gingivalis*. *Bacteroides endodontalis* differs from its periodontal counterpart, *B. gingivalis*, by being more oxygen sensitive and less proteolytic. Other investigators have found and characterized previously undescribed pulp isolates which were initially thought to be Bacteroides but were found to differ significantly both structurally and biochemically (106). Subsequently, Haapasolo et al. (107) placed these organisms into another genus, Mitsuokella, with the species designation dentalis. Ongoing research has indicated that Bacteroides encompass a heterogeneous group of bacteria. A large number of species has replaced the single species of *B. melaninogenicus*. Included under the taxonomy of *B. melaninogenicus* are several subspecies including the saccharolytic species melaninogenicus and intermedius and the asaccharolytic subspecies asaccharolyticus. The Bacteroides subspecies melaninogenicus group currently includes three species, *B. melaninogenicus*, *B. loescheii* and *B. denticola*. The Bacteroides subspecies, intermedius, contains two species, *B. corporis* and *B. intermedius*. At the present time, *Bacteroides asaccharolyticus* is subclassified into *B. asaccharolyticus*, *B. gingivalis*, and *B. endodontalis* (Table 2).

Twelve strains of *B. endodontalis* have been isolated from the root canals of teeth with periapical lesions and from oral submucous abscesses (105, 106). In another study (108) it was found that 26 of 28 odontogenic abscesses were associated with black-pigmented Bacteroides. The most frequently isolated species (19) was *B. intermedius*. Nine periapical abscesses contained *B. endodontalis*. *Bacteroides gingivalis* was found in 2 of 17 periapical abscesses. As interest in Bacteroides has increased, newer species, such as *B. capillus*, with a Ruthenium red staining external cell wall layer (S-layer) outside the outer membrane, are being detected in human periapical lesions (109). Final classifications have yet to be determined.

SPREAD OF ENDODONTIC INFECTION

The endodontist is often called upon to treat dental abscesses which have evolved from pulpal and periapical infections. These fluctuant swellings may also originate from the infected periodontium. The origin is often difficult to discern. Although these infections are usually limited by anatomical barriers to the orofacial areas, complications such as mediastinal, intracranial, and retropharyngeal abscesses may result. Early diagnosis and treatment by the endodontist is imperative to prevent such complications.

Sabiston and Gold (110) and Sabiston et al. (111) used the VPI system to study the bacterial flora of 65 oral abscesses. Seven specimens yielded no growth. One to 12 different bacteria were isolated from 58 samples, with an average of 3.8. Obligate anaerobes comprised 66% of the total bacteria. Prominent isolates were fusobacteria, Bacteroides, peptostreptococci, and Actinomyces. Chow et al. (112) studied 31 patients with various pyogenic orofacial odontogenic infections. Obligate anaerobes were cultured from 94% of the patients, while aerobes were recovered from 55%. Bacteroides, peptostreptococci, and streptococci were the most prevalent pathogens. In most cases, only streptococci would have been isolated if anaerobic culture techniques were not used. Kannangara et al. (113) studied 61 cases of oral infections; the majority suffered from posttraumatic dental infections. In seven cases, the cultures were negative. Forty-five of the remaining 54 (83%) had anaerobic organisms, whereas 59%

TABLE 2. Current classification of *B. melaninogenicus* species

Subspecies saccharolyticus	Subspecies asaccharolyticus
B. melaninogenicus	*B. asaccharolyticus*
B. melaninogenicus	*B. gingivalis*
B. loeschei	*B. endodontalis*
B. denticola	
B. intermedius	
B. corporis	
B. intermedius	

contained aerobic Gram-positive cocci. The average number of isolates per sample was 2.6. One unusual finding, which was probably due to the large number of posttraumatic cases, was the isolation of *Bacteroides fragilis* from 18 patients. This microorganism is usually associated with abdominal infections and is not ordinarily found in the oral cavity. These findings have not been duplicated by others.

Aderhold et al. (114) found that prior to anaerobic culturing, 29% of oral abscesses sampled produced no bacterial growth. Coincidentally, they found that, of 50 infections which were cultured both aerobically and anaerobically, 28% yielded only anaerobes, such as Bacteroides and fusobacteria. *Bacteroides fragilis* was not isolated from any of these cases which were confined to endogenous dental infections. In another study of 50 patients, Labriola et al. (115) found that 86% contained at least one anaerobe. Bacteroides species, including melaninogenicus, oralis, and ruminicola as well as peptostreptococci, fusobacteria, and peptococci, were isolated. Forty-two percent of the bacteria were penicillin resistant. A similar spectrum of anaerobes was detected by von Konow et al. (116). Bacteria were isolated from 55 of 57 patients with various periapical and periodontal infections. *Bacteroides ruminicola*, *Fusobacterium nucleatum*, and *Streptococcus intermedius* were isolated most frequently. These authors also recommended surgical drainage and use of penicillin as preferred treatments. The microbiota of periapical abscesses in 12 children aged 5 to 16 yr was investigated by Brook et al. (117). Anaerobes were isolated from all specimens. Of the 53 anaerobes, 20 were Bacteroides with *B. melaninogenicus* the most common. β-Lactamase (penicillinase) production was noted in one-third of the organisms.

Two recent studies demonstrate the variability in the bacterial flora of periapical abscesses. Both studied 10 patients and used the latest cultivation techniques. One group (118) isolated 25 strains of bacteria. Of these, anaerobes were isolated from 80% of the lesions. Six abscesses harbored only two species and none contained more than four. Strict anaerobes comprised 48% of the isolates; facultative streptococci represented 40%. Fusobacteria and *Streptococcus mitis* were found as co-infectants in 6 of the 10 abscesses. No spirochetes or motile rods were seen. The second group (119) cultured anaerobes from 9 of 10 abscesses of endodontic origin. In six of these, the flora was exclusively anaerobic. Gram-negative anaerobic rods, mostly fusobacteria, accounted for 37% of the total. One-quarter of the isolates were anaerobic cocci, mostly peptostreptococci. This study confirmed the predominant role of anaerobic bacteria in periapical abscesses; Bacteroides and fusobacteria were the most frequent pathogens. Facultative streptococci accounted for only 21%.

Bacterial isolates were quantitated from 50 abscesses by Lewis et al. (120). They confined their studies to periapical abscesses which extended through the alveolar bone and produced intraoral swellings, but had no communication with the oral cavity. Seventy-four percent of all isolates were strict anaerobes. Peptococcus, *B. oralis*, *B. gingivalis*, and *B. melaninogenicus* were most frequently identified. The most common facultative microorganism was *Streptococcus milleri*, a microaerophilic, CO_2-dependent species. The investigators reasoned that facultatives such as streptococci were the first microbes present in periapical areas. These bacteria were isolated early in the clinical course of infection and prepared the environment for the anaerobes which appeared after 2 to 3 days. Table 3 lists the studies of periapical infections and the percentage of anaerobic infections.

Table 3. Microbiology of periapical and other orofacial abscesses

Study	Anaerobes as % of Total Isolates	Prominent Isolates
Sabiston et al., 1976 (110)	65.9	Bacteroides Fusobacteria
Chow et al., 1978 (112)	69	Bacteroides Peptostreptococci Streptococci
Aderhold et al., 1981 (114)	72	Bacteroides Fusobacteria
Brook et al., 1981 (117)	90	Peptococci Peptostreptococci Bacteroides Fusobacteria
von Konow et al., 1981 (116)	72	Bacteroides *S. milleri* Fusobacteria
Oguntebi et al., 1982 (118)	48	Fusobacteria *S. mitis*
Labriola et al., 1983 (114)	86	Bacteroides Fusobacteria Peptococci
Williams et al., 1983 (119)	70	Fusobacteria Bacteroides Peptostreptococci
van Winkelhoff et al., 1985 (108)	93	Bacteroides (used selective techniques)
Lewis et al., 1986 (120)	74	Peptococci Bacteroides *S. milleri*

CONCLUSION

In recent years, knowledge of the role of microorganisms in endodontic infections has reached a high level of sophistication. The development of anaerobic culturing techniques as well as elucidation of the role of bacterial cell wall components, such as endotoxin and lipoteichoic acid, in inflammatory processes have added to our understanding. How this new knowledge impacts on treatment modalities is discussed in a subsequent paper.

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