Clinical, radiographic, and histological study of endodontically treated retained roots to preserve alveolar bone

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Endodontically treated, submerged roots in two Macaca speciosa monkeys were studied clinically, radiographically, and histologically. The roots were successfully covered by soft tissue, except in two areas. In several sites, radiographic evidence of bone formation was observed; this was confirmed by histologic examination. Bone formation coronal to the submerged roots was not a predictable occurrence. Even though epithelium and inflammation commonly occurred over the amputation sites, their presence did not seem to affect bone formation.

The alveolar ridge resorbs after tooth extraction whether or not it supports a prosthesis. This can be observed clinically, radiographically, and histologically,[1-5] and can cause loss of facial contour, lack of stability, and ill-fitting dentures. Concern about the alveolar bone has stimulated numerous researchers to study materials for impressions and prosthetic techniques that would result in prostheses that could minimize alveolar bone loss. This concern has prompted others to study materials and surgical methods that could be used to restore or increase the height and width of the ridge to provide more favorable support.[6-10]

Only a few studies have tested the hypothesis that root retention, of either vital or pulpless teeth, improves denture stability and function.[11-15] In separate studies in dogs and humans, Björn,[16] and Björn, Hollender, and Lindhe[17] demonstrated regeneration of alveolar bone around endodontically treated teeth that were covered by a surgical flap. Howell[18] used a clinically successful procedure for retaining endodontically treated roots that were amputated at the level of the alveolar bone and covered by a full-thickness flap for improved denture support. Similar procedures without pulpal extirpation, by Poe, Hillenbrand, and Johnson,[19] and Whitaker and Shankle[20] in dogs and monkeys, have been reported as successful. Histologically, when the amputated roots were maintained in an aseptic environment and completely closed by a mucoperiosteal flap, the pulps remained vital with the cut ends demonstrating calcification. Another study in primates, by Johnson and associates[21] consisted of vital amputation 3 mm below the original alveolar crest. Pulp vitality and bridging with osteocementum occurred, but epithelial proliferation and root resorption, without new bone formation, were noticed.

The purpose of this study was to determine whether new bone will form coronal to retained endodontically treated roots. Through this investigation, it was hoped to formulate a practical method of preserving alveolar bone.

MATERIALS AND METHODS

Two *Macaca speciosa* monkeys were used. The experimental sites included the right mandibular central and lateral incisors, second premolar, and first molar. Before each experimental procedure, the animals were tranquilized by an intramuscular injection of 5 mg of phencyclidine hydrochloride.* Profound anesthesia was then obtained by an intravenous injection of sodium pentobarbital† in the dose of 22 mg/kg body weight.

Routine preoperative radiographs were made of the experimental sites. A custom-made film holder and a target-distance device were constructed for each monkey to maintain the same position and angulation during radiographic procedures. Contralateral teeth were extracted to serve as a control and to permit observation of differences in bone loss.

The four mandibular teeth in each monkey were treated with conventional endodontic procedures. An aseptic technique with a rubber dam was used. The first session consisted of access opening, pulp extirpation, and cleansing and shaping of the root canals to within 0.5 to 1 mm of the radiographic apex. During the second session, the canals were filled with the use of a Premier master gutta-percha cone with lateral condensation of accessory cones, and Kerr Tubli-seal. Care was exercised to avoid overfilling. A sealant of zinc oxide and eugenol‡ was placed in the coronal aspect of the root.

The endodontically treated roots were then amputated below the crest of the alveolar bone. In each area, a labial mucoperiosteal flap was repositioned with vertical relaxing incisions. Using a high-speed, small-barrel, diamond bur with water spray, the crown was removed to the level of the alveolar bone. Next, the coronal aspect of the root segment was amputated approximately 2 to 3 mm below the alveolar bone, maintaining the interseptal radicular bone but rounding sharp edges. At the same time, contralateral teeth were extracted as atraumatically as possible in an effort to preserve alveolar bone. Relaxing incisions through the periosteum at the depth of the flap were made to facilitate coronal repositioning and approximation of the labial flaps to the lingual tissue. The crevicular epithelium was removed, and an effort was made to provide tight closure of the mucosal layers with no. 3-0 silk sutures. After the first surgical procedure in the anterior segment, it was necessary to make a vestibular relaxing incision before the flap was reflected because of the extreme muscular pull of the mentalis muscle. This allowed better tissue adaptation with less muscle tension.

Clinical and radiographic examinations were conducted at 6-, 12-, and 20-week intervals with the use of photographs and intraoral and extraoral radiographs. To label newly formed bone, a Procion dye§ was administered intraperitoneally to each monkey in doses of 100 mg/kg body weight a week before they were killed.

After five months, the monkeys were killed by a lethal dose of sodium pentobarbital administered intravenously. Block sections were fixed in 10% Formalin, decalcified in 5% formic acid, and embedded in paraffin. Serial sections 7μ thick were prepared and stained with hematoxylin and eosin (H & E) for microscopic examination. Selected unstained sections were examined under fluorescent microscopy for Procion labeling.

Two additional monkeys were treated in the manner described; they will be used for a study of one- and two-year durations, the results of which will be reported at a later date.

RESULTS

Clinical

The two monkeys showed essentially normal and uncomplicated healing. The clinically edentulous anterior segments in both had normal attached and unattached alveolar mucosa and an adequate vestibule. With the exception of a slightly narrower, buccolingual ridge over the extraction site (Fig 1, top), it could not be discerned if the site covered a true edentulous area or the retained roots. The only site of inflammation occurred in both monkeys after five months at the extreme distobuccal aspect of the right posterior segment that enclosed retained root tips (Fig 1, bottom). Except for these two erythematous areas, coverage by soft tissue appeared quite successful clinically.

Radiographic

Throughout the radiographic observation period, the normal resorptive process of the alveolus that occurs after any surgical flap procedure continued to a certain point.

Monkey no. 1 had a faster rate of alveolar crest resorption in the anterior retained root segment than did monkey no. 2. At five months, or

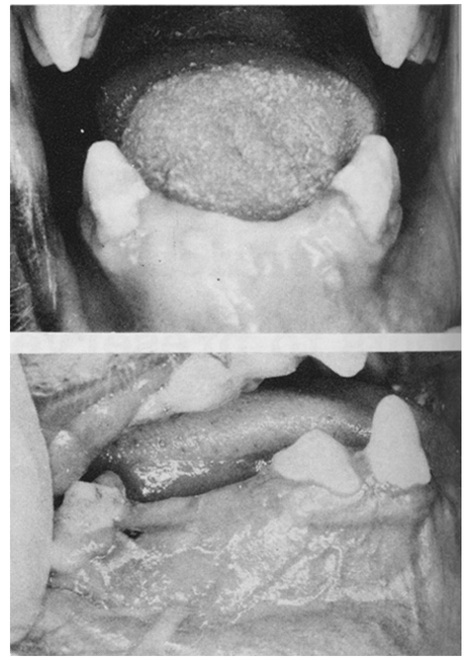
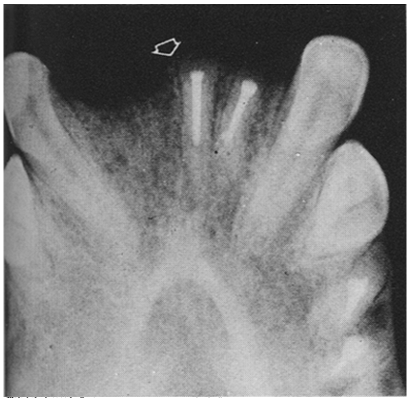


Fig 1—Top: Anterior segment of monkey no. 1, 12 weeks after surgery. Notice normal attached and unattached alveolar mucosa and adequate vestibule. Bottom: Five-month postoperative view of ridge with retained roots of monkey no. 2 illustrating broad normal ridge. A 2-mm area at distobuccal aspect appears erythematous and slightly hemorrhagic.

probably sooner, this process had ceased or slowed to an undiscernible amount. Overall, the resorption continued to a point even with or below the coronal level of the retained roots. The only area in the anterior segment that showed any semblance of bone formation was coronal to the right mandibular central incisor of monkey no. 2 (Fig 2, top left).

The sequela of bone resorption preceding bone formation was also evident in the posterior retained segments. This process had reached its peak at six weeks and diminished or ceased at 12 weeks, with no further destruction evident at the time the monkeys were killed. In monkey no. 1, bone formation was observed coronal to the distal root of the second premolar and mesial root of the first



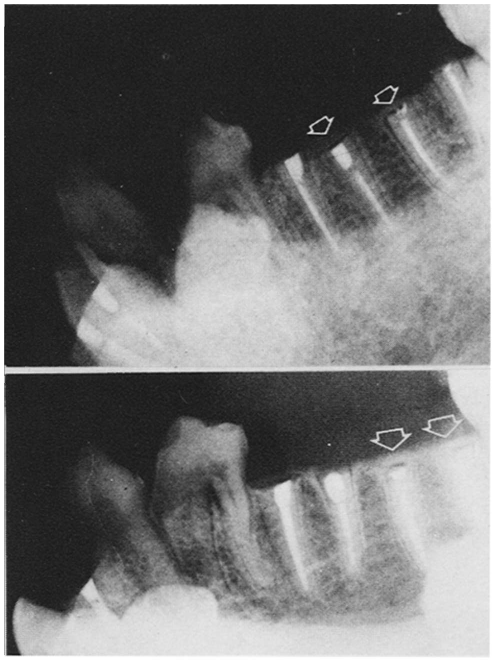


Fig 2—Top, left: Anterior segment of monkey no. 2 after five months. There is semblance of bone formation coronal to right central incisor (arrow). Top, right: Posterior segment in monkey no. 1 after five months showing no further bone destruction. Bone formation is apparent coronal to distal root of second premolar and mesial root of first molar (arrows). Bottom, right: Posterior segment in monkey no. 2. Notice bone formation coronal to mesial and distal roots of first molar (arrows).

molar (Fig 2, top right). Similar results were noticed coronal to the mesial and distal roots of the first molar of monkey no. 2 (Fig 2, bottom right). Bone formation was apparent in one of four anterior and four of eight posterior retained root tips.

Histologic Observations

Microscopic examination of the amputated roots of both monkeys was made at five months. The results are summarized in the Table.

• *Central and Lateral Incisors, Monkey no. 1.* The retained roots were successfully covered by soft tissue; there was no communication with the oral environment. The alveolar ridge was covered with intact, stratified squamous epithelium supported by connective tissue. Overlying the amputated root surface was a thin band of stratified squamous epithelium (Fig 3, top), adjacent to which were scattered a few inflammatory cells (Fig 3, bottom). There was no evidence of bone formation over the amputated roots of either incisor.

• *Central and Lateral Incisors, Monkey no. 2.* The findings were generally similar to those of the central and lateral incisors of monkey no. 1. However, areas of new bone formation over the central incisor of monkey no. 2 (Fig 4) were evident, as was osteocementum over the amputated dentin surface of the central incisor.

• *Premolar and Molar Areas, Monkey no. 1.* All roots were successfully covered by soft tissue, except for a minute area of communication between the distal root of the first molar and the oral environment (Fig 5). Except for the mesial root of the molar, all roots were covered with a band of stratified squamous epithelium. This epithelium was continuous with the overlying oral epithelium over the distal root of the first molar, which communicated with the oral environment (Fig 6). Inflammation over the amputation sites ranged from mild to moderate, except over the site of communication, where it was severe. Osteocementum was evident over the amputated dentin surface of the

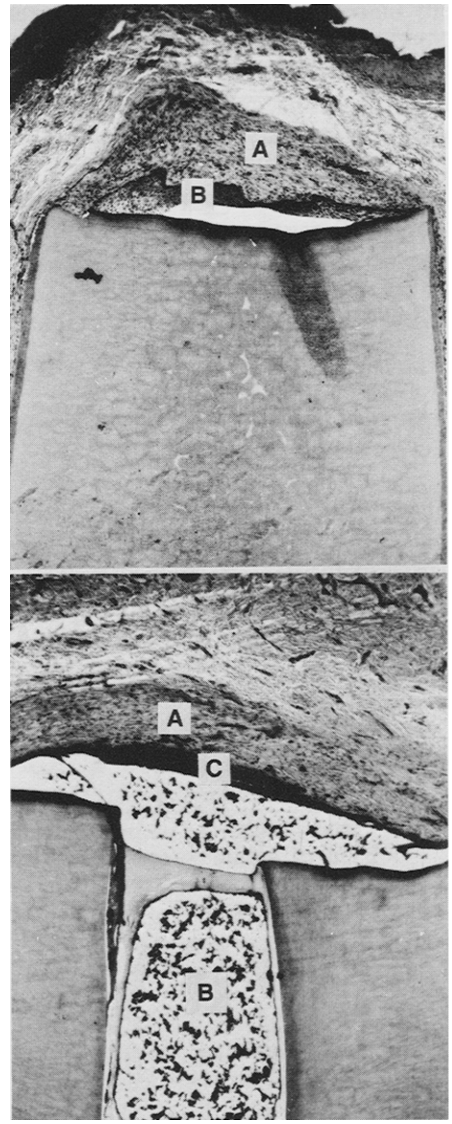


Fig 3—Top: Stratified squamous epithelium overlying amputation site of monkey no. 1. Connective tissue (A), stratified squamous epithelium (B) {H & E, orig mag X35}. Bottom: Scattered inflammatory cells overlying amputation site of monkey no 1. Inflammatory cells (A), (B), stratified squamous epithelium (C) {H & E, orig mag X35}.

distal root of the second premolar and the mesial root of the first molar. These retained roots were completely covered by new bone formation (Fig

Table • Microscopic findings of amputated roots at five months.

Segment no.	Coverage: Bone and soft tissue	Coverage: Soft tissue	Inflammation*: Site of amputation	Inflammation*: Periapical	Inflammation*: Lateral	Overlying amputation site: Epithelium	Overlying amputation site: Osteocementum
Monkey no. 1							
Central	—	+	Mild	—	—	+	—
Lateral	—	+	Mild	—	+	+	—
Premolar mesial root	+†	+	Moderate	+	—	+	—
Premolar distal root	+	+	Mild	—	—	+	+
Molar mesial root	+	+	Mild	—	—	—	+
Molar distal root	—	—‡	Severe at site of communication	—	—	+	—
Monkey no. 2							
Central	+†	+	Mild	—	—	+	+
Lateral	—	+	Mild	+	—	+	—
Premolar mesial root	+†	+	Moderate	—	—	+	—
Premolar distal root	—	+	Moderate	—	+	+	—
Molar mesial root	+	+	None	—	—	+	+
Molar distal root	+	—‡	Severe at site of communication	—	—	+	—

Note: *Mild—Scattering of inflammatory cells or a thin band of inflammatory cells adjacent to amputation site; Moderate—Dense band of inflammatory cells adjacent to amputation site; Severe—Heavy diffuse inflammatory infiltration.
†Slight bone formation over the amputation site.
‡Except for a small area of communication to the oral environment, there was soft tissue coverage.

7). The mesial root of the premolar was partially covered by new bone, but there was no evidence of bone formation over the distal root of the first molar.

• *Premolar and Molar Areas, Monkey no. 2.* Soft tissue coverage, including communication with the oral environment in the same area, was similar to that described in monkey no. 1. Occurrence of stratified squamous epithelium and mild to moderate inflammation over the amputation sites followed the same pattern as in monkey no. 1. Osteocementum was observed only over the mesial root of the first molar. New bone formation confirmed by Procion labeling partially covered the mesial root of the premolar and completely covered both molar roots.

DISCUSSION

Soft tissue coverage was found over all roots except over the distal root of the molars in each monkey, where minute areas of communication with the oral environment were microscopically evident. A mild to moderate inflammatory infiltrate was evident adjacent to most amputation sites. There was no constant contamination with the oral flora or irritation caused by gutta-percha, as suggested by Whitaker and Shankle.[20] The inflammation was probably due to irritation from the zinc oxide and eugenol used as a coronal sealant and from the Tubli-seal, which was used with the gutta-percha in the canal obturation.

No correlation was apparent between the amount of inflammatory infiltrate overlying the amputation site and the new bone formation. In one case, microscopic communication with the oral environment did not preclude the possibility of bone formation. Also, the level of amputation did not seem to affect new bone formation or soft tissue coverage.

Even though stratified squamous epithelium over the amputation site was a common occurrence, this epithelium did not seem to interfere with new bone formation. Three possible sources of epithelium overlying the amputation sites are suggested: down-

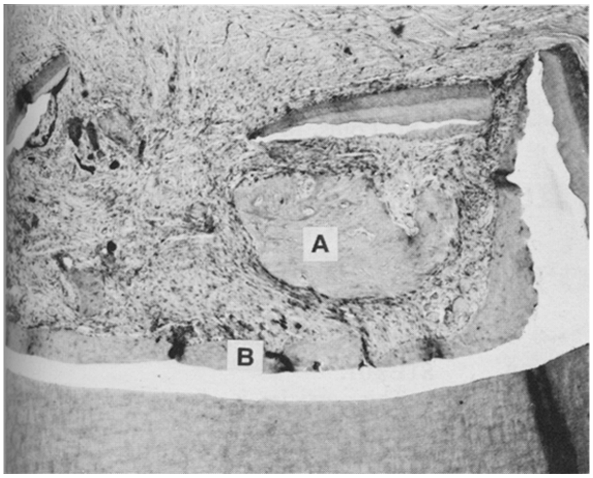


Fig 4—Bone formation over amputation site of central incisor of monkey no. 2. Island of new bone (A), osteocementum (B) {H & E, orig mag X100}.

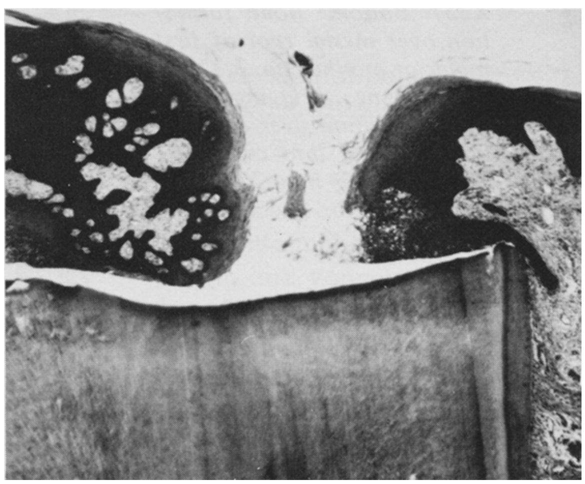


Fig 5—Distal root of first molar of monkey no. 1. Notice communication with oral environment {H & E, orig mag X35}.

growth of the surface epithelium, the cell rests of Malassez that are present in significant number in the cervical third of the root, and implantation of crevicular epithelium into the underlying connective tissue at the time of surgery. As suggested by Howell,[18] and Poe, Hillenbrand, and Johnson,[19] a special effort was made in this study to remove the crevicular epithelium. Complete removal might not have been accomplished, however. At this point, the band of epithelium adjacent to the amputated dentin did not seem to be interfering with healing or bone formation.

Osteocementum, which was found in a few instances over the amputated dentin surface, seemed to be confluent with the normal root cementum. Its presence was not a prerequisite to bone formation or soft tissue coverage.

In this study, a positive correlation existed between the clinical observation of an erythematous area and the histological picture representing communication with the oral environment. Therefore, the success rate of soft tissue coverage can be determined quite accurately by clinical examination. Furthermore, bone formation with complete coverage of the amputation site can be determined quite accurately through radiographic examination. As expected, a positive correlation existed between bone formation observed radiographically and bone formation depicted histologically, even though radiographic evidence of bone formation lagged behind the microscopic appearance of new bone.

Seven of the 12 retained root segments were covered either partially or completely with new bone, giving a rate of success for coverage by bone of about 58%. However, formation of new bone over the amputated root segments did not seem to be predictable. Neither the level of amputation nor the presence or absence of inflammation or epithelium over the amputation site seemed to correlate with new bone formation.

Future studies in humans should be conducted for evaluation of the usefulness of this procedure. Howell[18] has been successful in constructing dentures over endodontically treated, retained roots amputated to the alveolar crest. A study using both vital and endodontically treated roots should be done to determine if bone formation coronal to the amputated roots is necessary to either act as a cushion or to retard the extrusion of the roots.

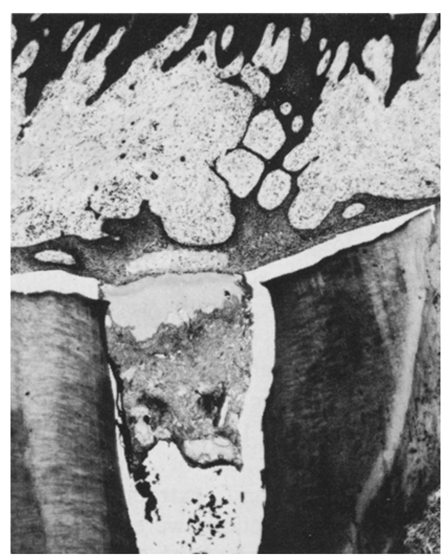


Fig 6—Epithelial continuity of oral epithelium and epithelium overlying amputation site of distal root of monkey no. 1 {H & E, orig mag X35}.

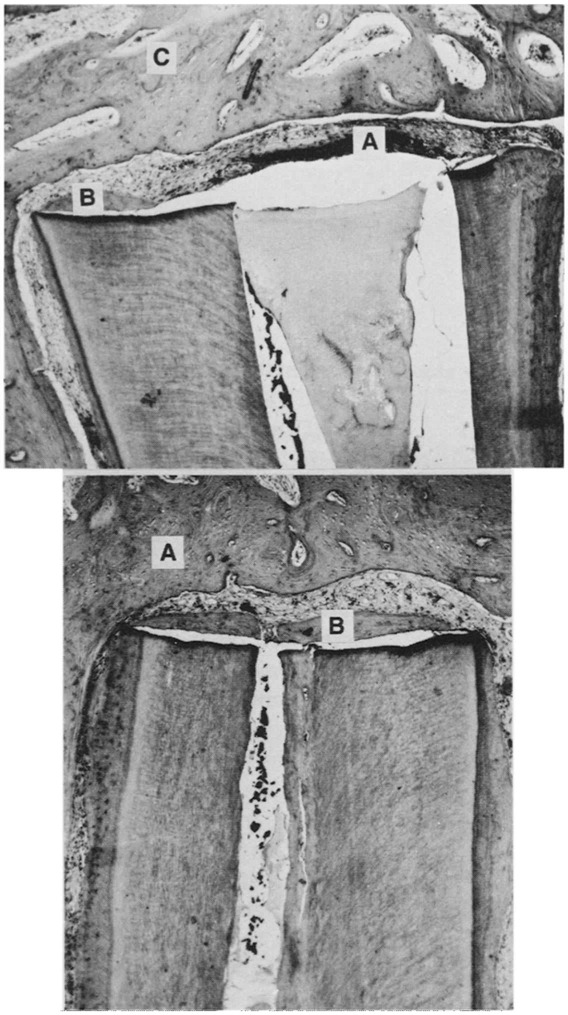


Fig 7—Top: Bone formation over distal root of premolar of monkey no. 1. Inflammatory cells (A), osteocementum (B), complete bone bridging (C) {H & E, orig mag X35}. Bottom: Bone formation over mesial root of first molar of monkey no. 1. Complete bone bridging (A), osteocementum over amputation site and into canal (B) {H & E, orig mag X35}.

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine whether new bone would form coronal to retained endodontically treated roots. Two *M speciosa* monkeys were used. The right mandibular central and lateral incisors, second premolar, and first molar were treated with conventional endodontic procedures. Contralateral teeth were extracted to serve as a control and to permit observation of differences in bone loss. With the aid of surgical flaps and high speed burs, the endodontically treated teeth were amputated coronally and reduced 2 to 3 mm below the alveolar crest. Clinical and radiographic examinations were conducted at 6-, 12-, and 20-week intervals with the use of photographs and intraoral and extraoral radiographs. Block sections were fixed, decalcified, and embedded in paraffin. Serial sections 7μ thick were prepared and stained with H & E for microscopic examination. Selected unstained sections were examined under fluorescent microscopy for Procion labeling.

Clinically, coverage by soft tissue seemed to be quite successful, except in two areas of communication with the oral environment. This correlated with the microscopic appearance showing direct communication and downgrowth of surface epithelium to the amputation sites. Radiographic evidence of bone formation was observed and then confirmed through histological examination. As expected, the radiographic appearance of bone lagged behind actual bone formation depicted microscopically. Even though epithelium and inflammation commonly occurred over the amputation sites, their presence did not seem to affect bone formation. Osteocementum occasionally formed over the cut dentinal surface.

It was concluded that bone will form coronal to amputated endodontically treated roots; however, this is not a predictable occurrence.

*Sernylan, Bio-Ceutic Laboratories, Inc., St. Louis, Mo.
†Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.
‡Merck & Co., Rahway, NJ.
§Procion Brilliant Red H-8BS, I.C.I., Organics, Inc., Providence, RI.

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Antibiotic sensitivities of enterococci isolated from treated root canals

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Enterococci that persisted in debrided, medicated root canals were tested by the Kirby-Bauer procedure for sensitivity to various antibiotics. The 50 strains tested were uniformly sensitive to ampicillin and vancomycin. More than 90% were also sensitive to erythromycin. Varying degrees of sensitivity and resistance were noted to bacitracin, cephaloridine, cephalothin, chloramphenicol, gentamicin, and tetracycline. All organisms were either partly or wholly resistant to clindamycin; penicillin; streptomycin; and sulfadiazine, sulfamerazine, and sulfamethazine (triple sulfas).

Practitioners in the endodontic clinics at the University of Pennsylvania routinely culture root canals before closing the tooth. During a period of 18 months from January 1974 to June 1975, positive cultures taken from debrided, medicated root canals were screened for the presence of enterococci. Of the cultures examined, 44% (69 of 156) contained *Streptococcus faecalis* or one of its varieties, *S faecalis* ss *zymogenes* or *S faecalis* ss *liquefaciens*. Since antibiotic sensitivities of microorganisms change with time, and since resistance can be transmitted rapidly by plasmids[1-3] among microorganisms, including *S faecalis,* we thought it was necessary to assess the current status of the enterococci that persisted in the treated root canals with a variety of antibiotics. Patterns of sensitivity and resistance to antibiotics used clinically by endodontists and to antibiotics used for enterococcal infections at sites other than the oral cavity[4-6] were determined.

Materials and Methods

Debrided, medicated root canals were cultured using sterile paper points and tubes of semisolid (0.1% agar) brain-heart infusion medium (BHI).* Tubes in which there was obvious growth after incubation were subcultured by streaking them on azide-containing agar (Streptosel),† which was incubated anaerobically for 48 hours. This procedure is selective for the growth of gram-positive, facultative or oxygen-indifferent organisms, and against the growth of gram-negative and obligately aerobic organisms.

Isolated colonies were subcultured into fresh BHI broth tubes for use as inoculum for diagnostic media. A portion of the BHI broth cultures was