The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats

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The problem of maintaining the vitality of a clinically exposed dental pulp by various conservative procedures to encourage the development of dentinal bridging has been the subject of numerous investigations.[1-4] The unpredictability of results obtained from these procedures, however, has been the source of great consternation to most operative clinicians. In those instances of successful management of pulpal exposures, a considerable part of the success has been attributed to the extraordinary degree of pulpal tissue resistance.[5] Reasons given for the failure in formation of a hard-tissue pulpal seal have included age of the patient, degree of surgical trauma, excessive sealing pressures, improper choice of medication, bacterial infection, and a low threshold of host resistance.

Because of the frequency of adverse results obtained after the pulp-capping treatment of acute or chronically infected dental pulps, regardless of all the other conditions present, these particular cases are no longer considered for this type of procedure. The microorganisms in these acute and chronically inflamed pulpal exposures have therefore been thought to be the most significant cause of failures in attempts at dentinal bridging. To test conclusively the influence of viable microorganisms on the fate of a surgically exposed dental pulp, a study utilizing germ-free animals was undertaken.

MATERIALS AND METHODS

Thirty-six 7-week-old inbred Fisher rats, divided equally as to sex, were used for this experiment. The group consisted of twenty-one germ-free animals

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and fifteen conventional control animals. The germ-free rats were maintained in a Reynier germ-free system unit. Both the germ-free and the conventional animals were fed, ad libitum, identical diets of pellet-form autoclaved Purina 5010 laboratory chow and distilled water.

The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (4 mg. per 100 grams of body weight). A carbide round bur (size ½), mounted in a jeweler's spindle-topped hand mandrel, was used to drill a hole through the occlusal thickness of enamel and dentine of the maxillary right first molar in order to expose the pulp tissue. An attempt was made to limit the exposure entry of the bur to the coronal portion of the tooth (Fig. 1, *A* and *B*).* In many instances, however, the crown was unintentionally penetrated laterally or apically (Fig. 1, *C*). No attempt was made to restore or seal the exposures; therefore, food, debris, and contaminating microorganisms (in the conventional animals) could become impacted into the pulpal tissues. The animals were killed at intervals of from 1 to 42 days postoperatively. A jaw block of the maxillary right quadrant was removed and fixed in formalin. The tissues were serially cut in a mesiodistal plane at a thickness of 6 microns and stained with hematoxylin and eosin, Masson's trichrome, Giemsa, and Brown and Brenn stains.

RESULTS

Conventional control animals

Serial sections from a total of fifteen conventional animals were studied microscopically. The pulp was exposed on the occlusal surfaces of all the first molars, and perforations through to the proximal, lingual, or apical surface occurred to varying degrees in eight of the specimens.

All the prepared occlusal cavitations were packed with food and debris. Specimens taken on the eighth experimental day showed vital pulp tissue remaining only in the apical half of the roots, the remaining coronal portions of the pulp being necrotic and purulent (Fig. 2, *A*). Colonies of microorganisms were usually seen (Fig. 2, *B*).

Without exception, the first molar sections from all the specimens obtained after the eighth experimental day showed complete pulpal necrosis with chronic inflammatory tissue and abscess formations in the apical areas (Fig. 3). A few specimens exhibited microorganisms in the soft tissues beyond the apical foramina (Fig. 4). One specimen revealed an embedded hair shaft that had passed through the pulp canal and extended into the periapical area (Fig. 5, *A*). Abscesses were also found at accessory foramina (Fig. 5, *B*). In one specimen an epithelial lining had begun to form within an apical granuloma. Numerous foam cells similar to those found in dental granulomas of man were also found (Fig. 6).

In no instance did any of the injured pulpal tissues show evidence of repair. Especially lacking were matrix formation and attempted dentinal bridging.

When unintentional perforations occurred, the impaction of food and debris into the periodontal tissues resulted in an inflammatory response which initiated

*All illustrations show microscopic sections stained with hematoxylin and eosin unless otherwise designated.

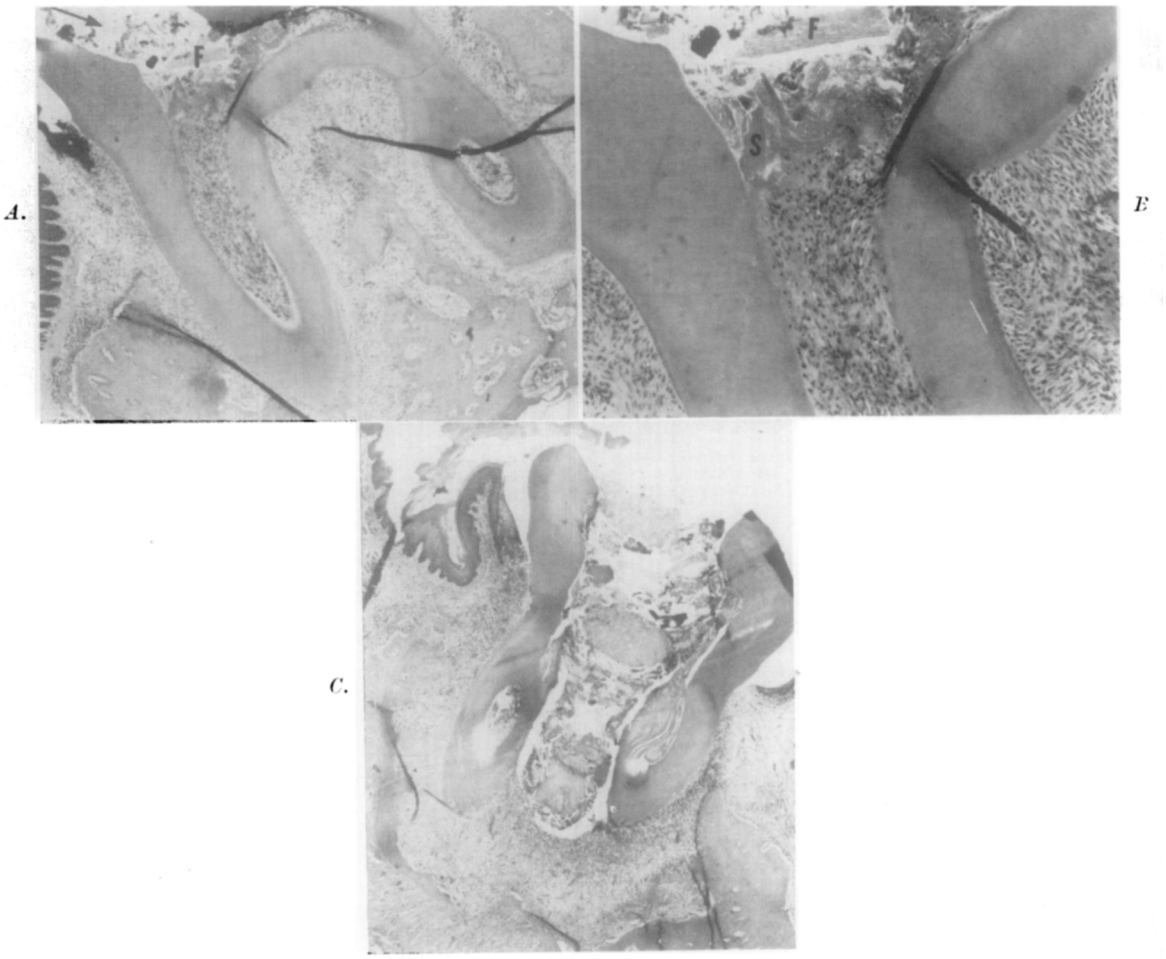


Fig. 1. A, Specimen D63-250-7-15, GFP44, No. 1, 7 days postoperative. Germ-free specimen showing intentional exposure (arrow) of first molar. Food debris *(F)* has been forced into exposure and even into pulp tissue. (Magnification, ×40; reduced ¼.)

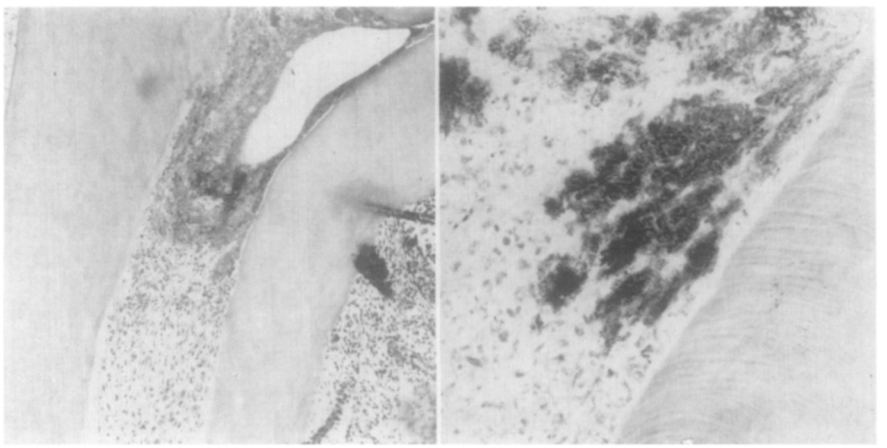
B, Higher magnification of specimen shown in *A*. Food debris *(F)* and dentinal spicules *(S)* can be seen within pulp chamber. (Magnification, ×100; reduced ¼.)

C, Specimen D63-253-7-6, GFC44, No. 2, 8 days postoperative. Conventional specimen with perforation penetrating through coronal pulp chamber into interradicular area. Debris is on a level with root apices. (Magnification, ×35; reduced ¼.)

the proliferation of epithelial rests of Malassez. These proliferating areas of epithelium coalesced to line the embedded substances, thus forming periodontal lesions and pockets. Peripheral sections of such lesions would reveal apical migration of the epithelium. Similar periodontal lesions also occurred in the germ-free animals (Fig. 7, *A* and *B*).

Germ-free experimental animals

Of the twenty-one germ-free animals, eighteen survived the operative procedures. Despite the pulpal exposures and even the frequent gross perforations of the first molars, no completely devitalized pulp was observed in any of the



A. *B.*

Fig. 2. *A*, Specimen D63-252-5-15, GFC44, No. 1, 8 days postoperative. Conventional specimen with pulp exposed and lingual root visualized. Note junction of necrotic and vital tissues in lingual root canal. Acute inflammatory cells can be observed infiltrating necrotic and vital tissues. (Magnification, ×100; reduced ¼.)

B, Adjacent section of specimen shown in *A*, stained by Giemsa technique to reveal colonies of microorganisms in necrotic tissue. (Magnification, ×450; reduced ¼.)

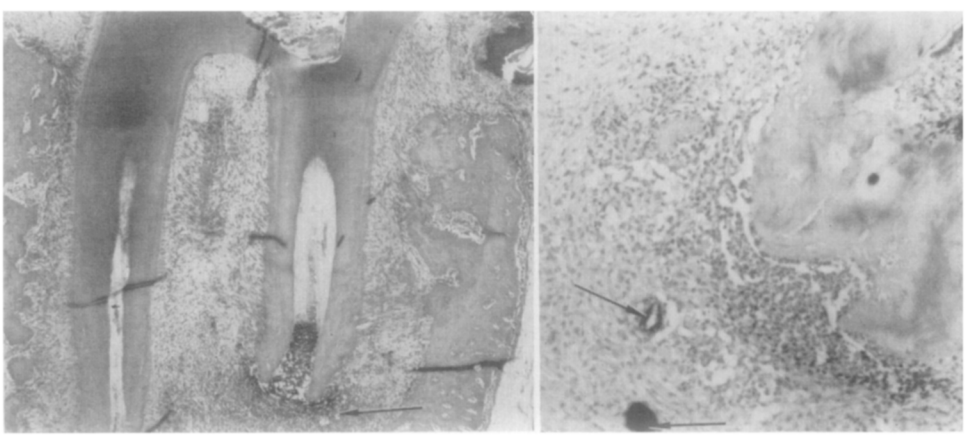


Fig. 3. *Fig. 4.*

Fig. 3. Specimen D63-266-5-2, GFC44, No. 5, 14 days postoperative. Conventional specimen demonstrating complete pulpal necrosis in both roots with apical abscess formations (arrow). (Magnification, ×40; reduced ¼.)

Fig. 4. Specimen D63-285-5-15, GFC44, No. 17, 41 days postoperative. Conventional specimen stained by Brown and Brenn technique exhibiting two colonies of microorganisms in soft tissues beyond apical foramen (arrows). (Magnification, ×440; reduced ¼.)

germ-free animals. The pulpal inflammation resulting from the exposure was minimal in every specimen. Not a single apical abscess was found.

Dentinal bridging was already evident at 14 days, with prominent quantities of matrix formation (Fig. 8, *A* and *B*). The older specimens presented matrix formation completely bridging or sealing the exposure. In every instance, the pulp tissue remained vital beneath the newly formed reparative dentinal bridge. The bridging occurred at any angle, regardless of the angle or severity of the exposure (Fig. 9, *A* and *B*). As postoperative time intervals increased, a gradual

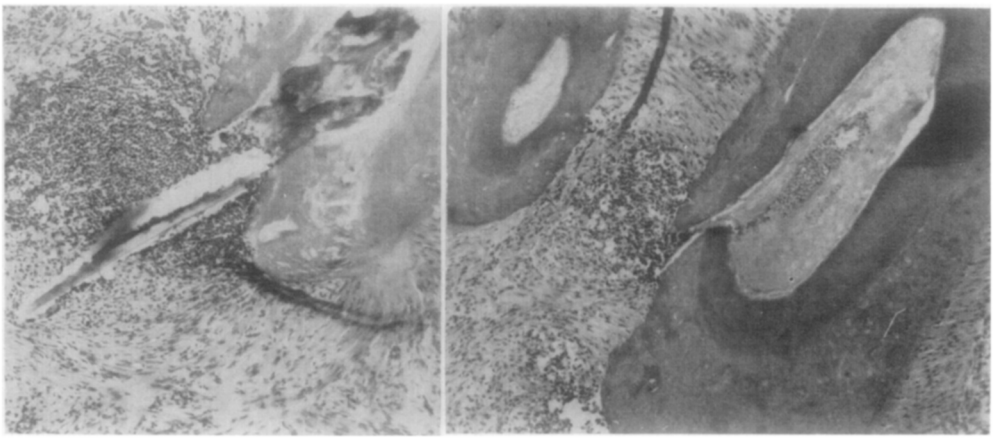


Fig. 5. A, Specimen D63-286-6-11, GFC44, No. 18, 41 days postoperative. Conventional specimen with hair shaft protruding from apical foramen and surrounded by leukocytes. Masses of microorganisms can be seen in root canal. (Magnification, ×100; reduced ¼.)

B, Specimen D63-286-2-28, GFC44, No. 20, 41 days postoperative. Conventional specimen with necrotic pulp connecting with abscess in periodontal membrane by way of accessory canal. (Magnification, ×100; reduced ¼.)

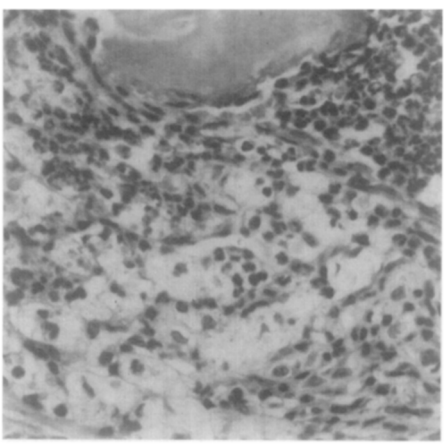
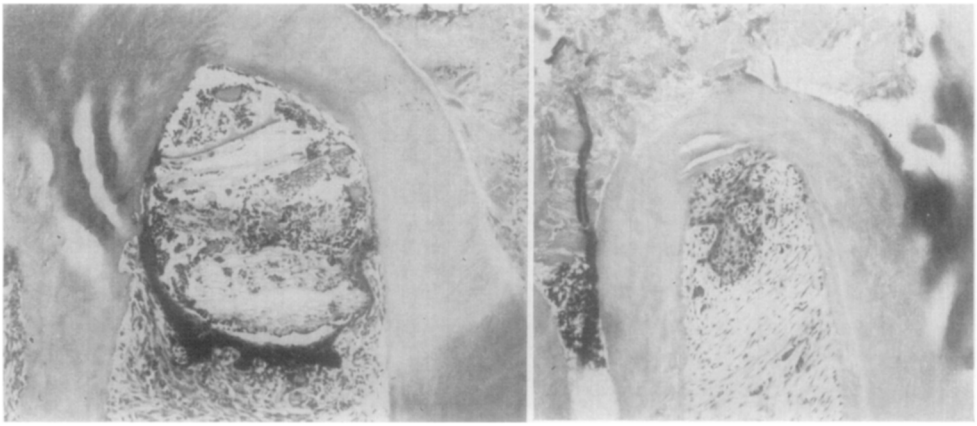


Fig. 6. Specimen D63-286-14-5, GFC44, No. 18, 41 days postoperative. Conventional specimen with apical lesion demonstrating typical foam cells so frequently seen in human dental granulomas. (Magnification, ×440; reduced ¼.)

constrictive obliteration of the pulp chamber with new matrix, containing many cellular inclusions, was observed (Fig. 10, *A*). The oldest postoperative specimens demonstrated matrix deposition filling the entire coronal chamber and root canals. Sometimes even accessory canals in the apical root area showed constriction by matrix formation in response to the occlusal exposure (Fig. 10, *B*).

In cases in which the pulpal exposure resulted in severance of a root, the pulp of the severed root retained its vitality (Fig. 11). When perforations permitted the embedding of food and debris deep into the periodontal tissues, the resulting inflammatory response caused resorption of the roots even though the



A. *B.*

Fig. 7. A, Specimen 630631-1-5, GFP44, No. 4, 14 days postoperative. Germ-free specimen in which perforation through floor of the chamber has permitted impaction of food and debris into interradicular area and has stimulated the proliferation of epithelium. (Magnification, ×100; reduced ¼.)

B, Specimen 630643-11-8, GFC44, No. 10, 28 days postoperative. Conventional specimen in which perforation through floor of pulp chamber has stimulated proliferation of epithelium

root pulp remained vital (Fig. 12). As the root resorbed, new bony matrix was deposited to compensate for the reduction in root size.

DISCUSSION

The over-all limited success in the treatment of pulpal exposures by pulp-capping procedures has narrowed the selection of teeth to be considered for this therapy to the adolescent dentition.[5] Success in this age group has been attributed primarily to the presence of an abundant pulpal blood supply due to incomplete root formation.[6] On the other hand, retrospective analysis of the failures obtained in pulp-capping procedures has been fraught with empiricism. Many of the suggested factors in these failures should be critically re-examined. It may be of interest to note that in a given person, regardless of age or state of health, two similar teeth with paralleling histories of tooth insult, clinical symptoms, and treatment may run postoperative courses ranging to opposite extremes, from complete success to pulpitis followed by necrosis and periapical abscess formation. It is possible that, in spite of all the obvious similarities between these two teeth, the clinician in no way is enlightened as to the chance history of degenerative pulpal changes in the tooth that failed. For the clinician, however, there does exist the onus of a possible unsuspected bacterial infection due to the pathologic condition present or to the required manipulative procedures. In laboratory animals, the classic experiments by Boling and Robinson[7] showed that the integrity of an unexposed dental pulp can be maintained after severe injury only in the absence of a bacterial infection.

It should be pointed out that in the present experiments the conditions were deliberately chosen to compare the effects on pulp healing of opposite extremes of the microbiologic environment, namely, complete abscence of all bacteria versus a grossly contaminated environment. Second, no attempts were made to influence the course of the reparative processes by application of any therapeutic measures. Under these conditions, the results indicate the major significance of the role of

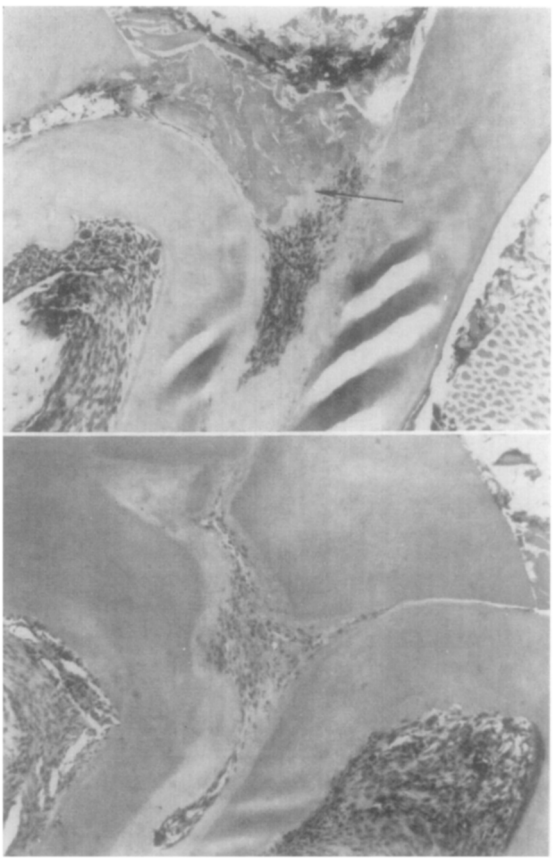


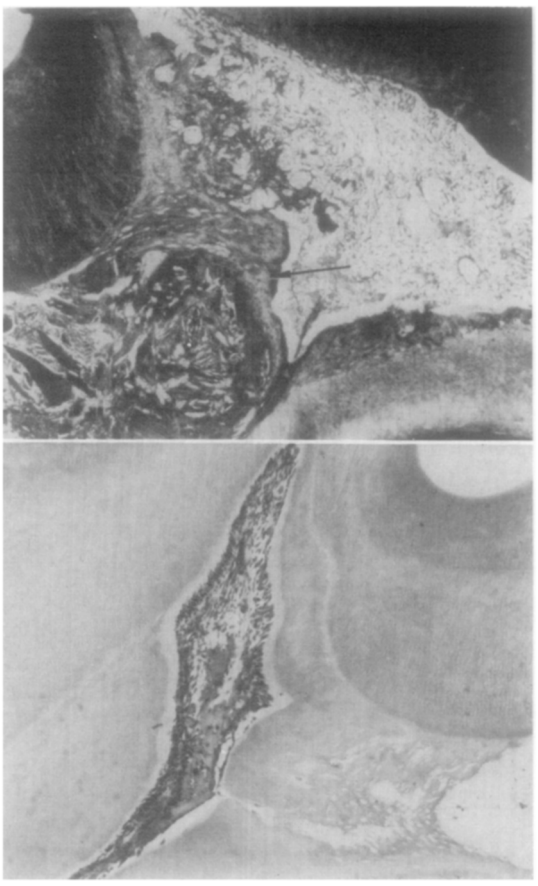
Fig. 8. A, Specimen 630630-4-2, GFP44, No. 3, 14 days postoperative. Germ-free specimen with food and debris in occlusal exposure. Nuclear detail of surviving pulpal tissue can be observed beneath bridge consisting of dentinal fragments united by new matrix (arrow). (Magnification, ×100; reduced ¼.)

B, Another section of germ-free specimen shown in *A*, showing matrix formation filling pulp chamber. The stimulus causes formation of matrix on all surfaces. A plug of fractured dentine can be seen contributing to formation of the bridge. (Magnification, ×100; reduced ¼.)

microorganisms in the repair of the exposed and severely damaged pulp. In spite of severe surgical trauma complicated by impaction of food and debris, the germ-free animals exhibited a recovery and reparative response leading to dentinal bridging. In contrast, in the conventional animals under similar conditions, the situation deteriorated progressively from an initial severe pulpal inflammation to complete necrosis. Since it is not possible to say which of the many microbial species involved were most responsible for the observed findings, it must be concluded that present techniques for the clinical management of exposed pulps must be critically reviewed to include maximal measures for the eradication of all bacteria from the site.

SUMMARY

The purpose of this study was to observe the pathologic changes resulting from untreated experimental pulp exposures in germ-free rats as compared with



A.

B.

Fig. 9. A, Specimen 630631-7-3, Masson, GFP44, No. 4, 14 days postoperative. In germ-free specimen, despite large perforation in addition to pulp exposure, perpendicular bridging is apparent (arrow). Matrix production is also occurring on adjacent surfaces. (Magnification, ×100; reduced ¼.)

B, Specimen 630636-10-4, GFP44, No. 9, 32 days postoperative. In germ-free specimen, note completion of bridge with vital noninflamed pulp tissue remaining. Bridging can occur at any angle. (Magnification, ×100; reduced ¼.)

conventional rats with a normally complex microflora. The pulp tissues of these rats were exposed by drilling through the occlusal surface of the maxillary right first molar with a carbide round bur mounted in a jeweler's spindle-topped hand mandrel. After varying postoperative time intervals (1 to 42 days), the animals were killed and the appropriate tissues were serially sectioned.

By the eighth day, vital pulp tissue remained only in the apical half of the roots in the conventional animals. Complete pulpal necrosis with granulomas and abscess formation occurred in all older specimens. Evidence of repair was uniformly lacking.

In contrast, no devitalized pulps, apical granulomas, or abscesses were found in the germ-free animals. Dentinal bridging began at 14 days and by 21 and 28 days was complete, regardless of the angle or severity of the exposure.

These results, even in the face of gross food impactions, indicate that the

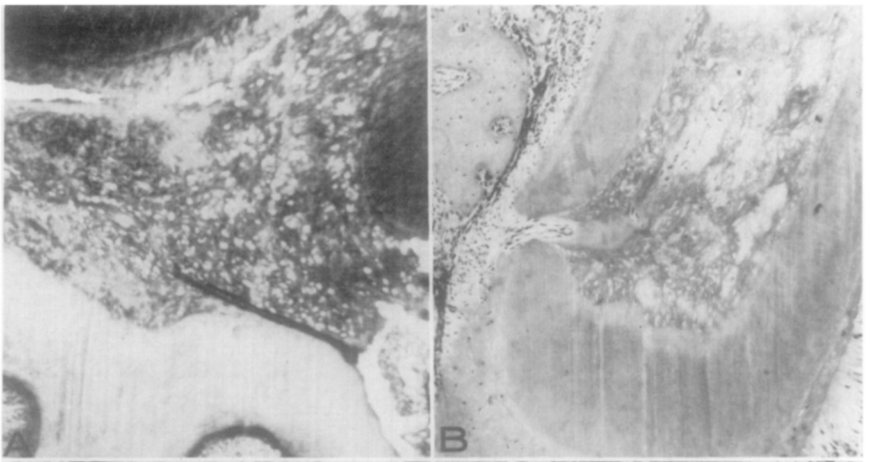


Fig. 10.

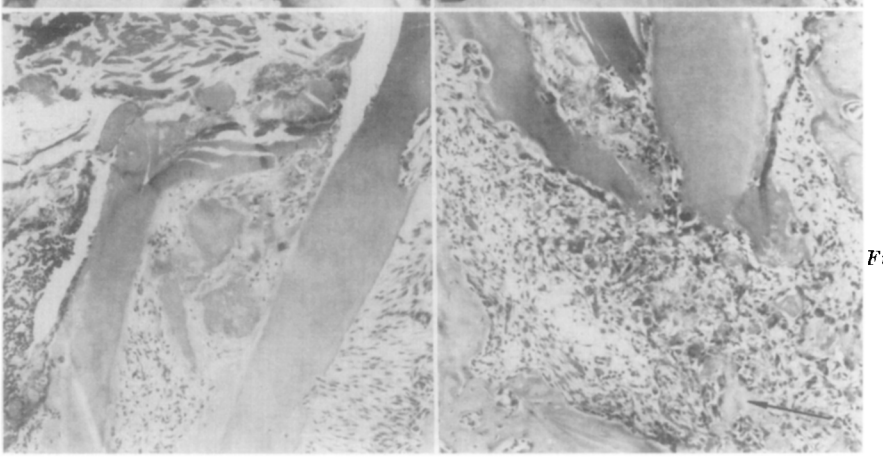


Fig. 11. *Fig. 12.*

Fig. 10. A, Specimen 630638-12-3 Masson, GFP44, No. 11, 32 days postoperative. In germ-free specimen, note obliteration of coronal pulp chamber by matrix formation and number of lacunae from cellular inclusions within matrix. (Magnification, ×100; reduced ¼.)

B, Specimen 630635-19-6, GFP44, No. 8, 32 days postoperative. In germ-free specimen, gradual obliteration of pulp chamber and root canals has occurred. Here obliteration of root canal is evident, leading to constriction of an accessory canal. Note many cellular inclusions. (Magnification, ×100; reduced ¼.)

Fig. 11. Specimen 630632-8-4, GFP44, No. 5, 14 days postoperative. Germ-free specimen exhibiting severance of root. Nevertheless, remaining pulp tissue in severed root remains vital. Note dentinal fragments being coated with new matrix. (Magnification, ×100; reduced ¼.)

Fig. 12. Specimen D63-251-11-11, GFP44, No. 2, 7 days postoperative. Germ-free specimen in which perforation has occurred, permitting food and debris to be channeled into deeper periodontal tissues. Resulting inflammation has led to root resorption, even though pulp in root canal remains vital. As root is resorbed, new alveolar bone is being deposited (arrow) to compensate for decrease in root size. (Magnification, ×100; reduced ¼.)

presence or absence of a microbial flora is the major determinant in the healing of exposed rodent pulps.

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