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THE EFFECTS OF OVERFILLED POLYETHYLENE TUBE INTRAOSSEOUS IMPLAN--ETC(U)
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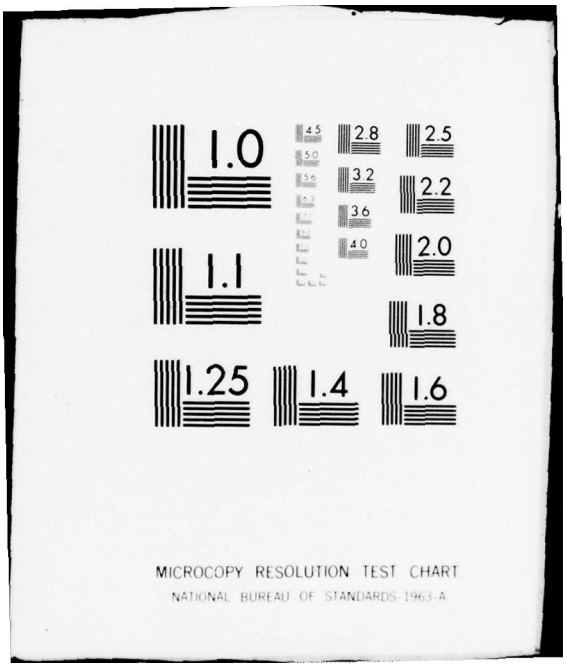
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Polyethylene tubes obturated flush at one end and overfilled 2 mm at the opposite end with unset Grossman's cement and gutta percha were implanted in rat tibias. Microscopic evaluation of the specimens revealed (1) the gutta percha, Grossman's sealer, and the polyethylene tubing are well tolerated by rat intraosseous tissue; and (2) a canal overextended 2 mm beyond the apex with Grossman's cement and gutta percha produces an early mild to moderate inflammatory response which resolves by the 30 day interval. The overfillings did not significantly

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THE EFFECTS OF OVERFILLED POLYETHYLENE TUBE INTRAOSSEOUS IMPLANTS IN RATS

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ABSTRACT

Polyethylene tubes obturated flush at one end and overfilled 2 mm at the opposite end with unset Grossman's cement and gutta percha were implanted in rat tibias. Microscopic evaluation of the specimens revealed that (1) the gutta percha, Grossman's sealer, and the polyethylene tubing are well tolerated by rat intraosseous tissue; (2) a canal overextended 2 mm beyond the apex with Grossman's cement and gutta percha produces an early mild-to-moderate inflammatory response which resolves by the 30 day interval. The overfillings did not significantly compromise the healing of rat intraosseous tissue.

In endodontic treatment the obliterating materials may terminate short of the apex, flush with it, or extend into the periapical tissues. In the latter instance these are usually referred to as overfillings. Since these materials remain permanently embedded, a knowledge of the biocompatibility of the materials used in obliterating root canals is essential for successful endodontic treatment.

Overfilling, whether intentional or accidental, occurs frequently during endodontic therapy. The ideal termination of the obliterating material is at the cemento-dentinal junction.[1] Kuttler's studies[2] have shown that the cemento-dentinal junction is .5 mm to .75 mm from the apical foramen. Also, numerous studies have demonstrated that the main apical foramen rarely coincides with the anatomical apex.[3,4] Thus, although the obliterating material may appear to terminate at the roentgenographic apex, the canal is probably overfilled and the material is extruded into the apical tissues. In teeth with areas of apical rarefaction resorption has usually altered the normal morphology, and increases the difficulty in terminating the filling material at the cemento-dentinal junction.[5]

The apical termination of the root canal materials in relationship to endodontic success has been noted in the literature. In the Washington Study[6] 3.85% of the failures were attributed to overfillings. Schilder,[7] however, has maintained that overfilling does not prejudice the outcome of a case provided that complete obliteration of the root canal space has been accomplished.

Seltzer and Bender reported that teeth overfilled were less successful (70.6%) compared with those teeth which were underfilled (87.2%) or those teeth which were filled flush with the roentgenographic apex (86.8%).[8,9] However, in a study of endodontic failures by Seltzer and associates,[10] of the failures evaluated, only 14% were due to overfilling as opposed to 29% in the underfilled groups and 31% in the groups filled flush with the apex.

Seltzer and colleagues,[11,12] studied the effects of overfilling versus underfilling in the teeth of monkeys and humans and concluded the optimal tissue repair occurred when the obliterating material was confined within the canal. Repair was delayed when canals had been overfilled. The inflammatory response was more severe and persisted for longer periods of time. There was also a tendency toward proliferation of cell rests of Mallassez.

Muruzabal and associates,[13] in a study of overfillings in rat molars concluded that the material protruding beyond the apex acts as a foreign body and the reaction of the tissues is dependent on the inherent physico-chemical properties of those materials. They noted that when the protruding material formed a consistent mass, not soluble in the body fluids, the surrounding tissue tended to encapsulate it.

Gutierrez and colleagues[14] investigated overfillings in dentin implants in the subcutaneous tissues of rabbits. A delay in healing was noted around the overfilled implants, but healing was generally not compromised. Gutta percha in contact with the tissues and exudate was disintegrated and later removed by macrophages. This corresponded with

the clinical phenomenon noted in periapical regions of human teeth.

Erasquin and Muruzabal,[15,16] tested various root canal sealing agents in molars of rats with overfilling of the canals. The most favorable tissue response occurred in specimens with fillings short of the apex. All of the root canal cements tested in cases of overfilling showed a tendency to be resorbed. When the cements were in contact with the alveolar surface, necrosis and resporption occurred.

Thus, there appears to be contrasting opinions concerning the biocompatibility of filling materials which terminate beyond the apical foramen. Also, it appears questionable whether the overfilling per se contributes to a greater incidence of failure.

Friend and Browne[17] reported that in the studies undertaken on the reaction of living tissue to foreign materials placed in the root canals of experimental animals, the conditions under which the tests were conducted introduced too many variables into the experimental system and thus made the interpretation of the results difficult. Langeland and associates,[18] employed polyethylene tube implants in their study and concluded that many confounding variables could be eliminated using polyethylene tubes as a vehicle in which various materials could be implanted. They were well tolerated by the tissues and simulated the prepared portion of the root apex. Polyethylene tube implants have been used by many investigators to study the biologic response to endodontic materials.[19-22]

To date no studies have been conducted to determine the histological effects on bone of an implant of polyehtylene tubing filled flush at one

end and overfilled at the other end with gutta percha as the solid core and Grossman's cement as the cementing media. Since this obliterating system is in wide spread use in endodontic treatment, and overfillings frequently occur, it is important to know the histologic effects of this technique on bone tissue. Therefore, the purpose of this study was to investigate the histologic effect that an overfilled polyethylene tube implant had on rat osseous tissue.

MATERIALS AND METHODS

In conducting this research, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Science - National Research Council.

Forty-eight white, male, 250 to 300 gram Walter Reed strain rats were used for this study. Eight rats were used for each interval of 4, 7, 14, 30, 60, and 90 days. Time intervals less than four days were omitted because the initial trauma of the surgical procedure would have precluded the discrimination of an acute response to the materials, even in the presence of the control. The remaining intervals permitted an objective discrimination of the inflammatory response and repair. Polyethylene tubing, 4 mm long x 0.7 mm internal diameter with 0.46 mm thick walls, was filled flush at one end and overfilled by 2 mm with gutta percha and Grossman's cement at the opposite end. For the convenience of the experimentors, the implants were prepared in advance.

Prior to implantation, the exposed portions of the gutta percha were coated with a thin mix of freshly mixed Grossman's cement to simulate the unset cement present in clinical overfillings. An unfilled piece of hollow polyethylene tubing 4 mm long was placed in the adjacent tibia for control.

The animals were anesthetized with sodium pentobarbital and the area of the tibia shaved. The tissues overlaying the tibia were incised to bone and the periosteum was reflected by blunt dissection. The bony socket for the insertion of the polyethylene tubing was prepared with a 703 fissure bur to a depth sufficient to accomodate the tubing within the confines of the tibia. During the surgical procedure, the area was irrigated with sterile isotonic saline during preparation of the tube slots in the tibia to prevent over-heating and bone necrosis. The tube inserts were sterilized with ethylene oxide gas; placed parallel to the long axis of the tibia; and the incision was closed with number four gut, spaced 2 mm apart.

The animals were sacrificed at 4, 7, 14, 30, 60, and 90 days with .3 cc of 2% sodium pentobarbital injected interperitoneally and the implant sites were removed by gross dissection and placed in a 10% buffered formalin solution. The specimens were prepared for histological examination. The tissue samples were oriented in the paraffin so that longitudinal sections were cut at 6 micra and tissue slides stained with hematoxylin and eosin for histological analysis.

The histological evaluation employed a scaled* assessment of the amount of acute and chronic inflammation around both ends of each implant for each time period measured. The degree of inflammation for each implant was determined by the degree of vascularity, fibrosis, necrosis, osteolytic activity, and osseous sequestration at each end of the implant. The results were analyzed for statistical significance using the Chi-squared technique.

* 0 = no inflammation; 1 = mild; 2 = moderate; 3 = severe inflammation

RESULTS

Microscopic examination of the experimental implants (overfilled polyethylene tubes) and the control implants (empty polyethylene tubes) at 4, 7, 14, 30, 60, and 90 post-operative days revealed the following:

4 Day Experimental Group

Examination of the histologic specimens showed the implants enveloped by extravasated fluid with a fibrin blood clot and bone sequestra. There was an early attempt at organization of the clot through an infiltration of fibroblasts and collagen fibers around the implants. Numerous segments of devitalized bone undergoing osteoclastic activity were present. The cortical bone adjacent to the implant site as well as the marrow tissue at either end of the implant appeared healthy and vital. Multinucleated cells resembling megakaryocytes were frequently observed. Gross quantities of Grossman's cement and gutta percha were observed at the overfilled end of the tubes and Grossman's cement was noted in the adjacent marrow spaces (Fig. 1). There were several foci of

moderate to severe inflammation at both ends of the implant, adjacent to the gutta percha and the excess Grossman's cement. The inflammation was characterized by an infiltrate of polymorphonuclear leukocytes and lymphocytes with no evidence of tissue necrosis. The inflammatory reaction, however, was not universal. Five of the specimens exhibited a moderate to severe reaction, limited to the tube ends, while three of the specimens showed a mild response.

4 Day Control Group

There was a well-defined outline of the hollow polyethylene tube, enveloped by extravasated fluid, a fibrin clot, and bone sequestra (Fig. 2). The fluid and nuclear debris extended through the entire length of the tube while the bone sequestra were limited to the open third of the tubes. Clot organization was apparent by the infiltration of fibroblasts and collagen fibers around the surgical site and extending a short distance into the tube end. The bone sequestra showed evidence of osteoclastic activity although the surrounding cortical bone appeared healthy and vital. The inflammatory response of this group was characterized by a mixed infiltrate similar to the experimental group. Two of the specimens demonstrated a moderate response while the remaining six showed a mild response.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations falling in the extremes, adjacent categories were combined and the data were arrayed in a 2 x 2 contingency table and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 2.28$, $df = 1$, p = n.s.) revealed

a non-significant x^2, indicating that there was no difference in the amount of inflammation between the amount observed in the experimental and the control groups.

7 Day Experimental Group

Observations of the 7 day experimental specimens revealed that the well-organized fibrin clot was being replaced with fibrous connective tissue. There appeared to be an attempt to wall off the entire implant through the proliferation of fibroblasts and collagen fibers (Fig. 4). Along the borders of the implant, the fibrous connective tissue had formed into a continuous band varying from 5 - 10 cell layers thick. Osseous debris was observed throughout the surgical site and was undergoing resorption; there was evidence of matrix formation and new bone trabeculae. Foci of inflammatory cells, primarily lymphocytes and polymorphonuclear leukocytes, were observed at either end of the implants, particularly around the gutta percha (Fig. 4). The excess Grossman's sealer appeared to invoke a response but this response was not universal or consistent. Overall, the inflammatory response was mild in four specimens and moderate to severe in four specimens.

7 Day Control Group

As in the 7 day experimental group the hollow tube controls revealed complete organization of the fibrin clot with replacement by fibrous connective tissue. The connective tissue surrounded the entire implant and had progressed from each end of the tube towards the center, but the center of the tube was filled with a fibrin clot and nuclear debris

(Fig. 5). Bone sequestra were apparent throughout the surgical site and undergoing resorption by osteoclasts. There was new bone formation at the tube ends, as evidenced by matrix formation and new bone trabeculae. The inflammatory reaction was mild in all of the specimens and characterized by lymphocytes and polymorphonuclear leukocytes.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations falling in the extremes, adjacent categories were combined and the data were arrayed in a 2 x 2 contingency table and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 5.34$, $df = 1$, $p < .05$) revealed a statistically significant difference between the two groups, thus indicating that the experimental group showed more inflammation.

14 Day Experimental Group

The implants were further walled off by fibrous connective tissue with a layered arrangement, 5 - 10 cells thick along the sides of the tube. A thicker band of fibrous connective tissue encapsulated the ends of the tube (Fig. 6). There was evidence of new bone formation and most of the bone sequestra appeared to have been resorbed or had served as matrix for new bone formation. Several areas of mild to moderate inflammatory foci were present adjacent to the gutta percha. The Grossman's cement appeared to be well tolerated and portions of excess cement in the marrow spaces did not invoke an overt inflammatory response. Four of the specimens revealed a mild inflammatory response and three showed a moderate response. All of the responses occurred at the ends of the tube and were characterized by an infiltrate of lymphocytes with

a few polymorphonuclear leukocytes.

14 Day Control Group

The entire implant was surrounded by mature fibrous connective tissue which, on the periphery of the tube, was 5 - 10 cell layers thick. The lumen of the tube was almost entirely penetrated by connective tissue fibers and only a small portion of fibrin clot remained (Fig. 7). There was evidence of new bone formation with few areas of devitalized sequestra present. A mild inflammatory response was observed in four of the specimens; three were free of any inflammation.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations falling in the extremes, adjacent categories were combined and the data were arrayed in a 2 x 2 contingency table and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 3.83$, df = 1, p = n.s.) revealed a nonsignificant x^2, indicating no differences between the groups in the amount of inflammation.

30 Day Experimental Group

The implant was bordered by a fibrous connective tissue capsule which along the length of the tube was 4 - 10 cell layers thick. The fibrous capsule was much thicker at the tube ends and was thickest at the overfilled portion. Cement was extruded into the marrow spaces and appeared to be invoking a mild inflammatory response. There was also a mild inflammatory response in the connective tissue capsule adjacent to the gutta percha in some specimens (Fig. 8). The inflammatory cells were of the chronic series. A fluid layer of undetermined origin was noted between

the gutta percha and the capsule. Six samples exhibited mild inflammation and two showed moderate response.

30 Day Control Group

The fibrin clot, bone sequestra, and fibrous connective tissue in the center of the tube had been replaced by bone marrow and bone (Fig. 9). A thin band of fibrous tissue 1 - 4 cell layers thick separated the tubes from the adjacent bone that lined the lumen and periphery of the tubes. The maturation of the bone marrow was least mature at the center of the tubes. There was no inflammation present in the 30 day controls.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations falling in the extremes, adjacent categories were combined and the data were arrayed in a 2 x 2 contingency tables and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 2.28$, df = 1, p = n.s.) revealed a nonsignificant x^2, indicating no difference in the amount of inflammation observed between the groups.

60 Day Experimental Group

There was continued maturation of the connective tissue capsules at either end of the tube with the implant being completely walled off. The capsule was thickest, 15 - 25 cell layers thick, at the overfilled portion of the tube (Fig. 10,11). A thin band of fibrous connective tissue 1 - 5 cell layers thick separated the tubes from the adjacent bone. Some specimens showed inflammatory cells in the capsule adjacent to the gutta percha; portions of Grossman's sealer were found in the adjacent marrow spaces and were well tolerated. Three of the specimens

were free of inflammation and three were invoking a mild response. Two of the specimens showed a moderate inflammation at the overfilled portion of the implant.

60 Day Control Group

The 60 day control implants were similar to the 30 day control implants except for continued maturation of the bone lining the lumen of the tube and continued maturation of the marrow elements (Fig. 12). A thin band of fibrous connective tissue 1 - 4 cells thick separated the bone from the implant. As in the 30 day controls, there was no inflammation present.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations in the extremes, adjacent categories were combined and the data were arrayed in a 2 x 2 contingency table and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 2.28$, $df = 1$, p = n.s.) revealed a nonsignificant x^2, indicating no differences between the groups.

90 Day Experimental Group

The 90 day experimental implants were similar to the 60 day group. The implants were entirely walled off by mature fibrous connective tissue which was thickest at the overfilled portion, 10 - 20 cell layers thick (Fig. 13). Most of the specimens were free of inflammation. Three specimens revealed a mild response at the overfilled portion.

90 Day Control Group

The 90 day controls were very similar to the 60 day controls. Some of the tube lumens were filled with mature bone and exhibited few marrow elements (Fig. 14). No inflammation was noted.

The amount of inflammation was assessed in all samples. Because of the small number of evaluations falling in the extremes, adjacent categories were combined and the data was arrayed in a 2 x 2 contingency table and subjected to a Chi-squared analysis. The results of the analysis ($x^2 = 0.00$, $df = 1$, $p = n.s.$) revealed a nonsignificant x^2, indicating no differences between the two groups in the amount of inflammation noted.

DISCUSSION

The histologic responses to the hollow polyethylene controls in this study were similar to those reported by previous investigators who used polyethylene tubes.[17,19,20,21,22,23] The response was characterized by an initial mild to moderate inflammatory reaction, probably the result of the surgical trauma, which appeared resolved in specimens at the 30 day interval. Healing was complete and each implant was bordered by a thin capsule of fibrous connective tissue, 1 - 4 cell layers thick. Mature cancellous bone and marrow elements filled the tube lumens and was also separated from the implant by a thin band of fibrous tissue. It was the opinion of the authors that the response of the controls had established a favorable criteria with which to compare the experimental implants. Differences noted in the experimentals would therefore be due to the implanted root filling materials and their overextension, rather than the polyethylene tubes and surgical trauma.

When compared to the control specimens the experimental implants appeared to evoke a slightly greater inflammatory response, but this difference was statistically significant only at the 7 day interval. All of the implants were well tolerated and most of the inflammatory

response had subsided by 60 days. An increased fibrous tissue response was noted adjacent to the ends of the tubes with the greatest reaction occurring around the overfilling. It was not possible to determine if this fibrous band resulted from the stimulating irritation of the implant or from an overt endosteal tissue reaction representing a normal response to vital bone formation. The thickness of this capsule was not used as a criterion for grading the inflammation since the capsule did not appear to interfere with adjacent tissue healing.

The tissue reactions to the gutta percha observed in the present study were similar to those reported by previous investigators.[24,25] The gutta percha appeared to elicit an initial inflammatory response which later subsided. It is possible that particle size and surface texture may be an important factor in determining the kind of response which occurs. Larger particles were well tolerated; smaller particles and those with uneven appearing surfaces were characterized by greater inflammatory responses. These observations would seem to merit further investigation.

The response of the tissues to the unset Grossman's sealer in this study agrees with the findings of Curson and Kirk.[26] The unset sealer was found to evoke an inflammatory response which after a period of time was well tolerated. The statistically significant difference in the inflammation between the 7 day experimental group and the 7 day control group may have been the result of the irritating properties of the unset sealer. However, upon setting the sealer appeared to be

well tolerated - an observation which agrees with those of previous investigations of set Grossman's sealer.[17,27] The reaction of the sealer and gutta percha combination at the flush end of the implant agrees with the results of Wenger and associates,[23] who studied underfillings in polyethylene tubes.

Prior to extrapolating clinical correlations from the results of this study, the limitations of this model for studying the effects of overfillings and reactions to root canal filling materials must be delineated. The implants were placed into surgical wounds made in healthy tissues. One can only speculate what effects these procedures would have in areas of apical pathology. Also, the implants were all sterile and completely obturated, eliminating treatment variables such as cleansing and shaping the root canal system, sterilization of the root system, and obturation of the prepared canals. Finally, this model does not duplicate the effects of physiological tooth migration or the effects of masticatory forces on the overfilled portion of a root apex. However, the elimination of the above mentioned variables may also permit us to extend out observations by attempting to resolve some of the controversy surrounding this issue.

The results of this study would indicate that the apical terminus of a root canal filling beyond the apical foramen would not impair healing. This disagrees with the studies of Seltzer and associates,[11,12] who, in their observations of overfills in humans and monkeys, noted impaired healing. Also, Erausquin and Muruzabal,[13,15,16] in their studies of overfillings in rat molars, likewise found the overfillings

to seriously impair healing. In light of the disagreement, one must consider the possibility that variables such as incomplete obturation and incomplete cleaning and shaping of the teeth used in those studies contributed to their observed failures. The results of this study do agree, however, with Gutierrez and associates[14] who, in their study of overfillings, noted that although there was an initial delay in healing, overall healing time was not compromised.

The results of this study are also in agreement with the observations of Schilder.[7] Overfillings, per se, do not induce failure, but extension of an underfilling or incomplete obturation may contribute to failure. The importance of cleaning and shaping the complete root canal system and its subsequent obturation with a suitable filling material is of greatest importance in endodontic therapy. Should overfilling of the root canal system occur, it may not be necessary to surgically remove the excess if the root canal system was correctly prepared and obturated.

SUMMARY

Forty-eight white male Walter Reed rats were used to study the osseous tissue inflammatory response to polyethylene tubing filled 2 mm long on one end and flush at the other end with unset Grossman's cement and gutta percha. One of these tubes was placed in one of the animal's tibia while the other tibia received a hollow polyethylene tube as a control. The animals were sacrificed in six groups of eight each at 4, 7, 14, 30, 60, and 90 days following tube insertion. Histologic examination of the specimens revealed the following:

1. The gutta percha, Grossman's sealer, and polyethylene tubing were well tolerated by rat intraosseous tissue.
2. A statistically significant difference in the amount of inflammation was noted in the 7 day experimental group as compared with the controls. This difference was thought to be the result of the irritating properties of unset Grossman's sealer.
3. The overfillings per se did not significantly compromise the healing of rat intraosseous tissue.

* * * * * * * *

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

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In conducting research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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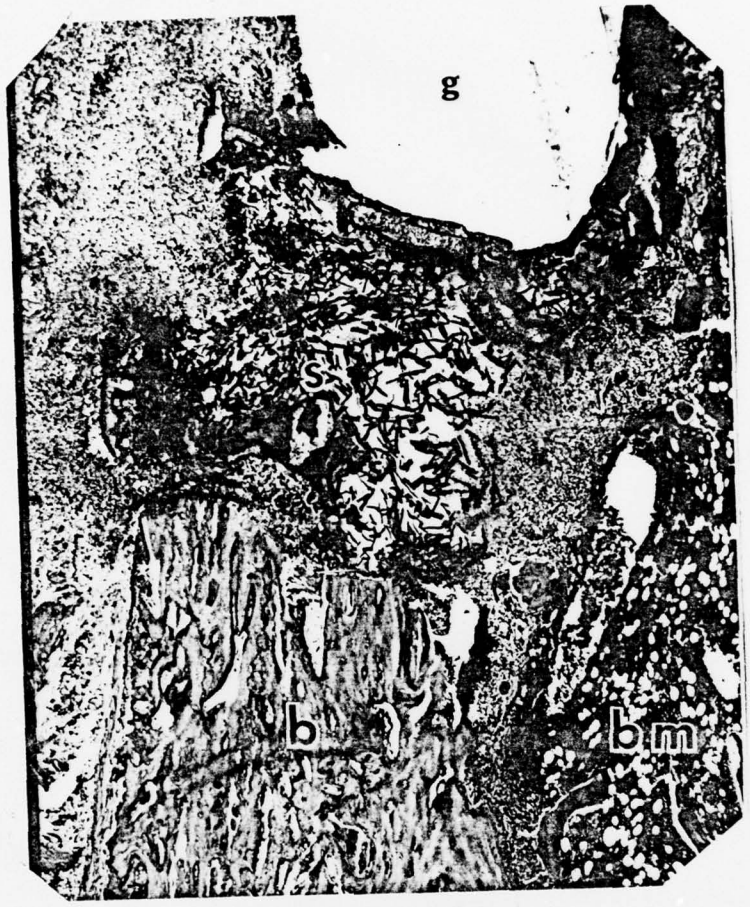

Figure 1 Overfilled portion of 4-day experimental gutta percha implant (g) illustrating excess Grossman's sealer (s) in bone marrow spaces (bm) and moderate inflammatory reaction to the gutta percha and excess sealer. Cortical bone of rat tibia (b). (40X)

Figure 2 An outline of the 4-day hollow control implant (p) is formed by the surgical clot (c) and sequestered bone with a mild inflammatory response at both ends. Cortical bone of rat tibia (b). (16X)

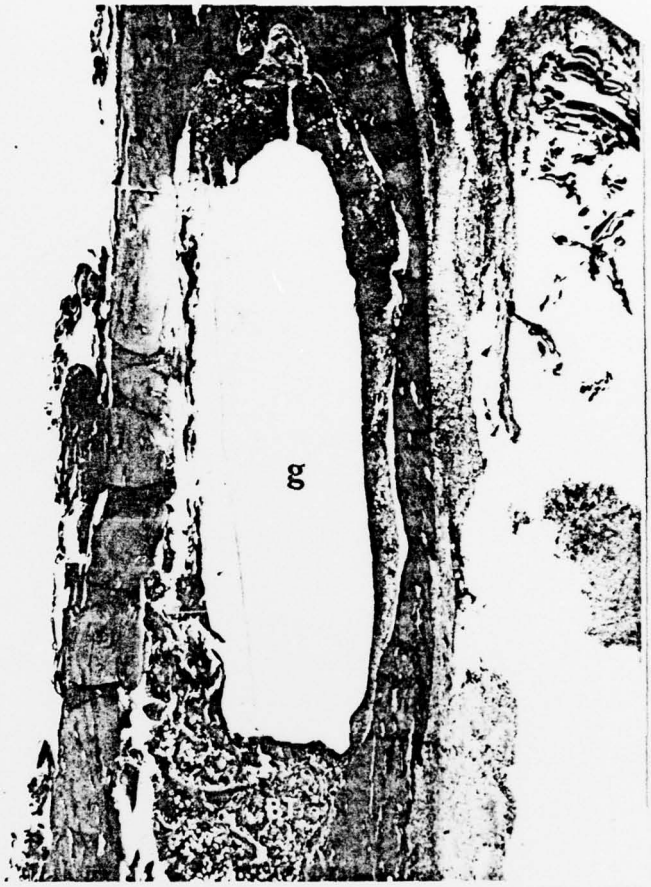

Figure 3 7-day experimental gutta percha implant (g) illustrating proliferation of fibroblasts in an attempt to wall off the implant. Moderate inflammatory reaction around overfilled portion of the implant. Note new trabeculae (BT) at tube end. (16X)

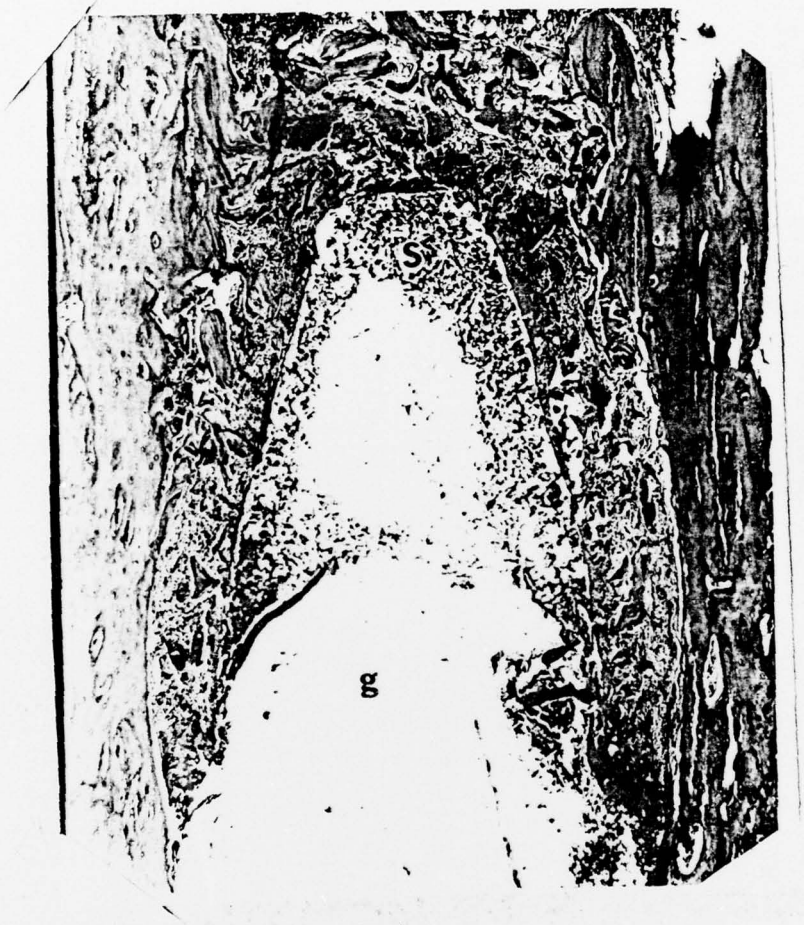

Figure 4 Overfilled portion of 7-day experimental gutta percha implant (g). Excess sealer (s) is evoking moderate inflammatory response. Boney trabeculae (BT) developing unimpeded at over extended tube end. (40X)

Figure 5 7-day hollow control implant (p). Proliferation of fibroblasts and connective tissue into tube lumen, fibrin clot (c) and nuclear debris remain at center of tube. (40X)

Figure 6 14-day experimental implant (58X). A thick band of fibrous connective tissue (f) encapsulates the end of the implant. Inflammatory response is mild to sealer (s). (58X)

Figure 7 14-day hollow control implant (p). The lumen of the tube is filled with connective tissue elements and inflammatory response is minimal. (48X)

Figure 8 30-day experimental gutta percha implant (g). Fibrous connective tissue capsule (f) is regularly arranged at tube end. Inflammatory cells are concentrated at tube end. (64X)

Figure 9 The blood clot and connective tissue in the center of the 30-day control tube (p) have been replaced by marrow elements (bm). Note mature lamellated bone (L) lining tube lumen. (16X)

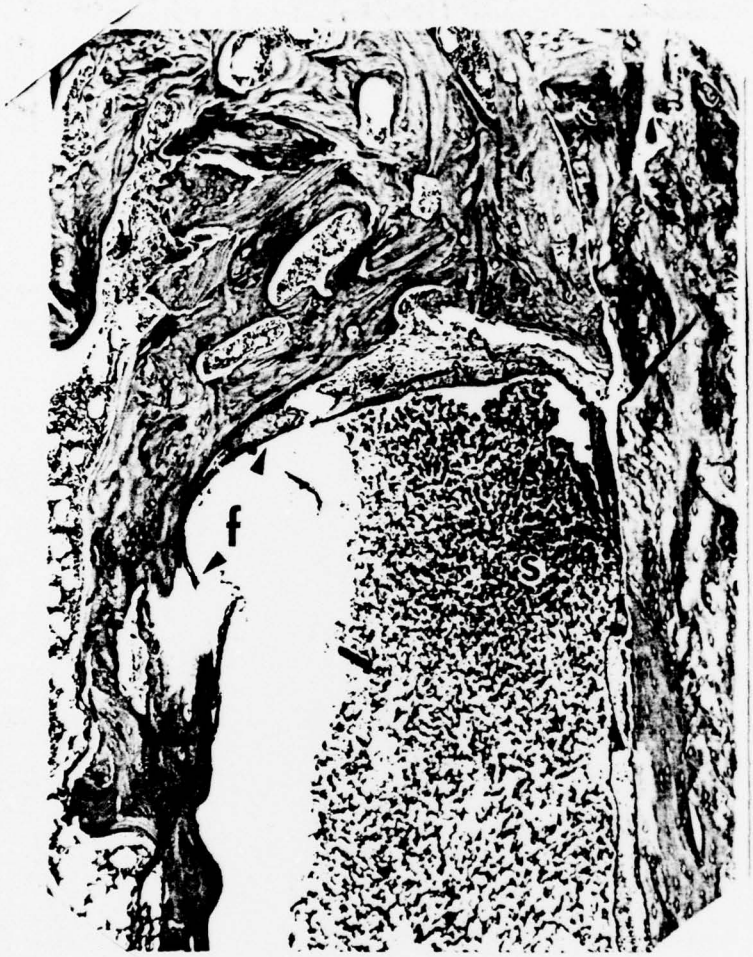

Figure 10 Overextended portion of 60-day experimental implant & sealer (s) illustrating fibrous connective tissue capsule (f) and mild inflammatory response. (64X)

Figure 11 Flush end of 60-day experimental gutta percha implant (g) illustrates fibrous connective tissue capsule (f) and mild inflammatory response. (64X)

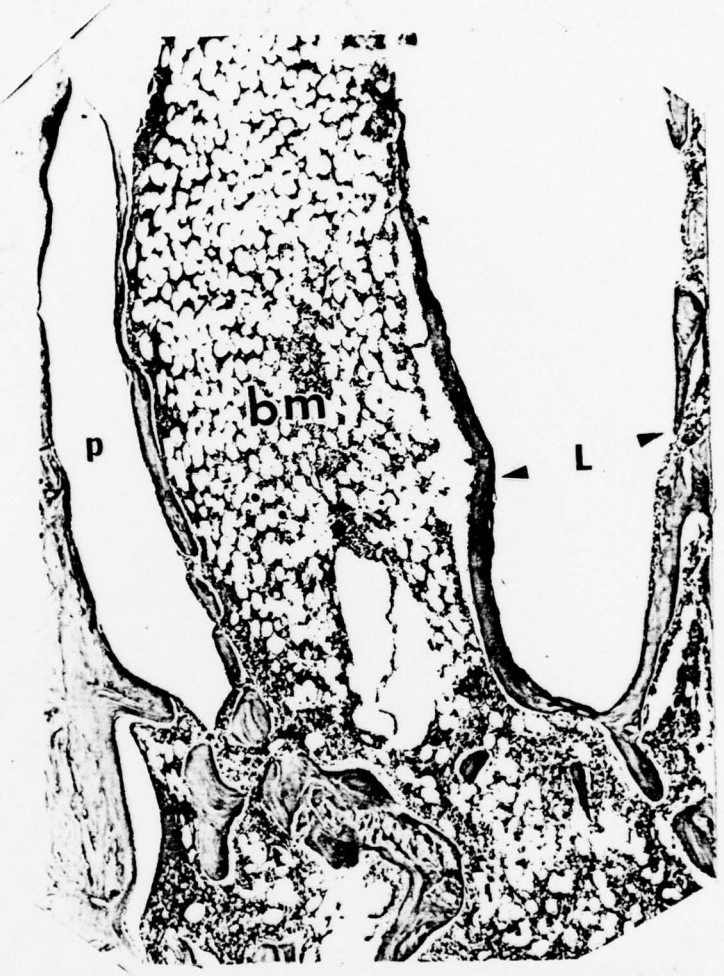

Figure 12 Lamellated bone (L) lines the periphery of the tube (p) in the 60-day control. Mature marrow (bm) elements and lamellated bone (L) occupy the tube lumen. (52X)

Figure 13 90-day experimental gutta percha (g). Surrounding bone and marrow elements well organized. Fibrous connective tissue capsule (f) 10 - 20 cell layers thick at end of tube free of inflammation. (64X)

Figure 14 90-day hollow control. Mature bone (b) with few marrow elements occupy lumen of tube (p). Fibrous capsule (f) surrounds implant. (40X)