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ARMY INSTITUTE OF DENTAL RESEARCH  
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TUBE INTRAOSSEOUS IMPLANTS IN RATS

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ABSTRACT

Polyethylene tubes obturated flush at one end and 1 mm short at the opposite end with gutta percha and Grossman's cement as the cementing media were implanted in rat tibias. The gutta percha, the set Grossman's cement and the polyethylene implant were well tolerated by the rat intra-osseous tissues. There was no significant inflammatory response at either the flush end or the short end of the polyethylene implant.

A primary factor in planning successful endodontic therapy is a knowledge of the reaction of apical tissues to foreign substances used in obliterating the root canals, and is essential in planning endodontic treatment. Since these materials remain permanently embedded, it is obvious that the methods which incite the least reaction are to be preferred as obliterating agents.

In 1931, Rickert and Dixon<sup>1</sup> presented a paper entitled "The Controlling of Root Surgery", that has had a profound influence on endodontics and in particular the procedure and management of root canal obliteration. They reported evidence of inflammation around open ends of implanted hollow steel and platinum tubes but not around solid implants of non-porous materials. This led to the conclusion by Coolidge<sup>2</sup> in 1933, that tissue fluids may enter the empty space of unfilled or incompletely filled canals and break down into irritating substances which diffuse into the periapical tissue and promote an inflammatory response. Coolidge, Kalnins and followers<sup>3</sup> postulated that organic matter might pool in the dead space of a hollow tube, such as an unfilled or underfilled root canal and become infected. This theory has been an influential factor in the concept that root canals should be filled to the anatomical apex and completely obliterate the space so that no unfilled portion of a root canal could serve as a reservoir for accumulated tissue fluid or inflammatory exudate and, thus, delay or prevent periapical repair.

More recent studies have cast doubt on this postulate. Selye<sup>4</sup>, Torneck<sup>5</sup>, and Davis<sup>6</sup> studying glass, polyethylene tubing and unfilled dog canine root canals respectively, found little or no inflammation around the open ends of the tubes. They reported that in some cases

fibrous tissue bridges grew into or through the tubes. Torneck<sup>5</sup> found the ability of the tissue to bridge through the tubes appeared dependent on the diameter and lengths of the tubes and on their being open at both ends. In the teflon tube implant study Goldman and Pearson<sup>7</sup> found that, although tissue fluid did enter the hollow tubes, the exchange of fluid was free and was not associated with inflammation around the open ends of the tubes. However all previous implant studies were conducted in soft tissue and it cannot be assumed that comparable results would be achieved in periapical tissue.

Hodosh, Povar and Shklar<sup>8</sup> demonstrated that holes drilled in plastic teeth, which were then implanted in fresh sockets did not produce inflammation around the open ends and in many cases these holes became filled with fibrous tissue or bone. Davis, Joseph and Bucher<sup>6</sup> working with under filled dog teeth noted that with adequate apical opening the canals became filled with the tissue that predominated in the area, either cementum, bone or fibrous tissue. If the only access to the unfilled space was via the original small opening of the apical foramina, little or no viable tissue filled the space. From these studies it is becoming apparent that hollow tubes in living tissue need not be accompanied by inflammation or tissue destruction, but may be associated with physiological repair instead.

Seltzer and his colleagues<sup>9</sup> have pointed out that fillings that appear flush with the apex, radiographically are usually overfilled when viewed in microscopic sections and that overfilling produces an inflammatory reaction, whereas underfilling produces generally excellent

repair. Seltzer and associates<sup>10,11</sup> studied the effects of endodontic procedures on periapical tissue in monkeys and in humans and concluded that optimum results in terms of tissue repair were obtained when root canals were instrumented and filled short of the apices of the teeth following vital pulp extirpation.

Several studies have been carried out in connective tissue<sup>12-14</sup>. The studies which have been conducted in bony tissue and teeth seem to verify the conclusions of the soft tissue studies,<sup>15-18</sup> but do not conclusively support the notion that canals must be completely obliterated for successful treatment<sup>19-27</sup>. In addition, to date, no studies have been conducted to determine the histological effects on bone of an implant of polyethylene tubing filled short at one end and flush at the other end with gutta percha as the solid core and Grossman's cement as the cementing media. Since this filling system and the technique of filling a root canal short of the radiographic apex has become more popular in endodontic treatment, it would be of importance to know the histologic effect of this technique on bone tissue. Therefore the purpose of this study will be to investigate the validity of the hollow tube theory and the histological effect that a partially filled polyethylene tube implant has on rat osseous tissue.

## METHODS AND MATERIALS

Forty, white, male Walter Reed strain rats of 250 to 300 grams of body weight were used for this study. Eight rats were used for each time interval of 3, 7, 30, 60, and 90 days. Polyethylene tubing, 4.0mm long x 0.7 mm internal diameter with 0.46 mm thick walls, was filled flush at one end and underfilled by one mm at the opposite end with gutta percha and Grossman's cement. An unfilled piece of hollow polyethylene tubing, 4.0 mm long was placed in the other tibia as a control.

The animals were anesthetized with sodium pentobarbital and the area over both tibias was shaved. The tissue overlying the tibia was incised down to bone and the periosteum was reflected by blunt dissection. A bony socket for the insertion of the polyethylene tubing was made with a 703 fissured bur deep enough to accommodate the tubing within the confines of the tibia. The tube inserts were sterilized with ethylene oxide gas, placed parallel to the long axis of the tibia, and the incision was closed with 0000 gut sutures spaced 2.0 mm apart.

The animals were sacrificed at 3, 7, 30, 60 and 90 days with 0.3cc of 2% sodium pentobarbital injected intraperitoneally. The implant sites were removed by gross dissection and placed in a 10% buffered formalin solution. The specimens were sent to the histological laboratory to be processed and double imbedded in paraffin. The tissue samples were oriented in the paraffin blocks so that longitudinal sections could be cut at 6.0  $\mu$  thickness and the tissue slides stained with Harris'

hematoxylin and eosin for histological analysis.

The histological analysis was determined by a scaled evaluation of the amount of acute and chronic inflammation around both ends of each implant for each time period measured as mild, moderate and severe. The degree of inflammation for each implant was determined by the degree of vascularity, fibrosis, necrosis, osteolytic activity, osseous sequestration and round cell infiltrates at each end of the implant. Evidence of bone ingrowth into the unfilled portion of the polyethylene tube was also determined. The results were analyzed for statistical significance using the chi-square technique.

## RESULTS

Microscopic examination of the hollow and gutta percha filled polyethylene tubes at 3, 7, 30, 60, and 90 postoperative days revealed the following:

### 3 Day Gutta Percha

The implant specimens were enveloped by extravasated blood, a fibrin clot and bone sequestra. These occupied the surgical site filling completely the short end of the tube and forming a stalklike invagination (Fig.1). An early attempt was apparent to organize the clot by the infiltration of fibroblasts, collagen fibers and vascular channels around the implants and up into the short end (Fig. 2). Seven specimens showed moderate inflammation and one showed a severe response. All inflammation was confined to the tissues at the end of the tube and characterized by an infiltrate of polymorphonuclear leukocytes, erythrocytes

and few lymphocytes with no evidence of necrosis. There was evidence of resorption and early remodelling of the cortical bone plate, marrow trabeculae and bone sequestra by multinucleated giant cells in the implant site of all specimens. Both the cortical bone adjacent to the implant site and the marrow tissue at either end of the implant appeared healthy and viable. Course granules of Grossman's cement were noted in the gutta percha spaces of four specimens.

### 3 Day Hollow Polyethylene Control

As in the 3 day gutta percha specimen there was a well-defined outline of the hollow tube with the infiltration of extravasated blood, fibrin clot and bone sequestra that extended through the entire length of the hollow tubes (Fig. 3). In most specimens, the bony sequestra had been partially pushed up from the ends of the tube toward the center but never more than a quarter of the distance at each end. Clot organization was apparent by the infiltration of fibroblasts, collagen fibers and vascular channels around the surgical site and extending a short distance into the ends of the tubes. Moderate inflammation in all specimens with no appearance of necrosis was observed. The inflammatory response was characterized by a mixed infiltrate similar to the gutta percha specimens, with similar osseous proliferation and remodelling patterns.

### 7 Day Gutta Percha

Histologically, the areas around the implants appeared identical to those at 3 days except that the fibrin clot had become completely

organized and replaced with fibrous connective tissue, collagen fibers, numerous vascular channels, immature bony trabeculae lined by large plump osteoblasts, and some ingrowth of marrow tissue from the extremities of the surgical site (Fig. 4). There was some indication of osseous debris scattered around the surgical site. This occurred notably in the short end where it was packed against the gutta percha and was undergoing continuous osteoclastic resorption (Fig. 5). Lying between the implanted tube and bony trabeculae that formed along the borders of the implants was a fibrous band of tissue varying from 5 - 14 cell layers thick adjacent to the gutta percha and 1 - 4 cells thick along the polyethylene tubing. Contiguous to this fibrous capsule a border of immature bony trabeculae was observed forming along all sides of the implant and up into the short end. In two specimens there was evidence of an overzealous osseous proliferation at the ends of the implants possibly representing a reaction by the animal to the surgical procedure. At seven days mild inflammation was still present in all specimens but only at the ends of the tube implants. This consisted of a mixed infiltrate of predominantly lymphocytes and a few polymorphonuclear leukocytes. Both the cortical bone of the tibia and the marrow spaces around the implants appeared normal.

#### 7 Day Hollow

Similar changes were noted in the hollow polyethylene tubes as were observed in the 7 day gutta percha implants. The fibrin clot around the implant was replaced with fibrous connective tissue, vascular channels, and a fibrous band which separated the border of immature trabeculae from

from the polyethylene tube. Bony sequestra were scattered throughout the surgical site and undergoing active osteoclastic resorption. These same changes had progressed into the hollow space from each terminal end of the tube but did not extend to the center where there was still a small portion of the clot remaining. The maturation of the fibrous connective tissue, fibrous band or capsule and bony trabeculae decreased progressively from the ends of the implants toward the center. The inflammatory reaction matched the 7 day gutta percha sample and was mild. It was limited to the tube ends and characterized by lymphocytes and a few polymorphonuclear leukocytes.

#### 30 Day Gutta Percha

A well-defined thin fibrous band encapsulating the entire implant was seen in specimens of the 7 day group. The implant was covered by a border of mature lamellated bone with encapsulated osteocytes and complete haversian systems. The width of the fibrous band ranged from 1 - 3 cells along the polyethylene tubing to 4 - 20 cells along the gutta percha (Fig. 6 and 7). The bands lining the short end of the tubes did not appear to be as well organized or dense as those of the flush-ends on most specimens. By this period the connective tissue had been totally replaced by bone and bone marrow which lined the implant and filled the surgical wound (Fig. 8). There was no indication of bone sequestra, necrosis, round cell infiltrate or other signs of inflammation around or within the implanted tubes.

### 30 Days Hollow

The observations of the 30 day controls were analogous to those seen in the 30 days gutta percha implants. The fibrin clot, bone sequestra and fibrous connective tissue in the lumen of the tube had been completely supplanted by bone and bone marrow. In addition a band of fibrous tissue separated the polyethylene tube from the bony border which lined the lumen of the tube. The maturation of the bone marrow within the tubes interior varied from least mature at the center to the most mature at the ends (Fig.9). The 30 day specimens were completely free of inflammation.

### 60 Day Gutta Percha

Except for continuing maturation of the bone at the flush and short end of the gutta percha borders the observations were essentially identical to those observed at 30 days. (Fig. 10 and 11). A fibrous band 5-14 cells thick was present and separating the gutta percha from the mature bone border. At 60 days there was no evidence of inflammation.

### 60 Days Polyethylene Control

The 60 day implants appeared identical to the 30 day samples except for continued maturation of the fibrous band and development of the bone and marrow elements through the center of the tubes in the latter. As in the preceding time period an inflammatory infiltrate was absent.

### 90 Day Gutta Percha and 90 Day Polyethylene Control

All implants at 90 days appeared similar to those at 60 days. (Fig. 12, 13).

The amount of inflammation in all samples was assessed, the data

anged in a 2 x 2 contingency table and subjected to chi-squared analysis. The results of the analysis revealed a nonsignificant  $\chi^2$  ( $\chi^2=0$ ,  $df=1$ ,  $p=N.S.$ ), indicating no differences in the amount of inflammation between the experimental and the control groups.

#### DISCUSSION

The inflammatory responses to the gutta percha and polyethylene tubing were similar at the sacrifice periods of 3, 7, 30 and 90 days. The responses ranged from a moderate inflammation with round cell infiltrates, fibrin clot and bone sequestra at 3 days to no inflammation at 30 days with new bone and marrow development. The response to the polyethylene tubing and gutta percha implants was similar. It progressed from a moderate reaction and terminated with an absence of inflammation and complete healing. The authors feel that the initial exsanguination of erythrocytes, fibrin clot formation, sequestered bone and round cell infiltrate was primarily attributed to the surgical procedure.

The absence of a severe reaction and the relatively short duration of inflammation indicated that the implanted materials were well tolerated by the tissues and therefore contributed little to the inflammatory response. These results agree with previous studies which show polyethylene tubing and gutta percha to be well-tolerated in vivo.<sup>5, 28, 29, 30</sup> Curson and Kirk<sup>31</sup> demonstrated that set Grossman's cement is slightly irritating in vivo; however, our study revealed no irritation.

Previous bone and connective tissue implant experiments have used the thickness of the fibrous capsule surrounding the implant, the capsule cell

arranged in a 2 x 2 contingency table and subjected to chi-squared analysis. The results of the analysis revealed a nonsignificant  $\chi^2$  ( $\chi^2=0$ ,  $df=1$ ,  $p=N.S.$ ), indicating no differences in the amount of inflammation between the experimental and the control groups.

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Previous bone and connective tissue implant experiments have used the thickness of the fibrous capsule surrounding the implant, the capsule cell

density and the direct contact of the implant with bone as criteria to measure the inflammatory response.<sup>15, 16, 32.</sup> In this study a fibrous capsule or band was observed enveloping all the implants and separating them from newly formed fibrous connective tissue and bone. This band ranged from 1 - 4 cells thick along the border of the polyethylene tubing to 4 - 20 cells thick at the flush and short ends of the implants. Organization and cell density were more apparent at the flush-end than at the short-end. The distinction could not be clearly made whether this fibrous band resulted from the stimulating irritation of the implant or as an over-zealous endosteal tissue reaction representing a normal response to vital bone formation. Previous investigators have favored the former explanation. However, because of our inability to interpret the origin of this fibrous capsule, it was decided not to use it as a criteria for evaluating the degree of inflammation since the capsule did not appear to interfere with healing of the adjacent tissue. Some histological sections were observed with a bone-implant interface. This was artifactual and not representative of the actual situation.

Torneck<sup>5</sup> found that hollow polyethylene tubes implanted in rats connective tissue were well tolerated and caused no inflammation at the implant ends. He also noted that the fibrous connective tissue proliferated throughout the tube length if the tubes were open at both ends. Similar results within bone were found in the present study with the additional finding that viable osseous tissue extended in all cases through the length of the hollow tubes.

Human reports have demonstrated that healing of the periapical tissue

after root canal obliteration in teeth without areas of radiolucencies is enhanced when the filling material is placed short of the apex instead of flush or beyond the apex.<sup>10,11</sup> Our results in animals disagree with these findings. We found similar reactions in implants that were either filled flush or filled short at the apical end. These implants were well tolerated, did not interfere with healing and were sealed by bone and bone marrow. These findings indicate that both ways of obturating a canal with gutta percha, either short or flush with the apex, will promote normal healing. However, Kesel<sup>34</sup> has demonstrated that when a canal is filled flush with the radiographic apex, it is in reality likely to be overfilled since the apical foramen is seldom located at the radiographic apex. In addition, other investigators have shown that root canal fillings placed flush with the apex create a mechanical pressure on the periapical tissue causing tissue necrosis and healing delay.<sup>11,35</sup> The fact that apical conditions are affected by various physical and physiological factors not encountered in this in vivo study suggest that a root canal should be obturated short of the apex to promote optimal healing. However, more definitive studies are required before this issue can be resolved.

The lack of inflammation or the absence of the halo effect around the hollow tube or either implants agrees with the studies of Torneck<sup>5</sup>, but not with Rickert and Dixon's "Hollow Tube Theory". In no instance in the present study was it observed that an unfilled area promoted inflammation through the accumulation and percolation of tissue fluids into the hollow space.

In the present study it was observed that all hollow spaces were

initially completely filled with exsanguinated blood and sequestered bone. These were subsequently replaced by granulation tissue, fibrous connective tissue and finally osseous tissue. This sequence of events corresponds very closely with Osby's<sup>36</sup> theory of apical healing by the formation of a blood clot. Erasquin and Muruzabal<sup>37</sup> observed similar findings of blood clot healing at the apex of rat molars.

It has been assumed that the apical diameter of the root canal can influence the infiltration of blood, the formation of a clot and eventual obliteration of the hollow space with the bone. Torneck<sup>5</sup> has shown that the diameter of the polyethylene tube does affect the infiltration of fibrous connective tissue into hollow tubes. Further research should be conducted to determine whether the diameter of the implant will affect the formation of a blood clot and eventual osseous repair.

A thin film of set Grossman's cement was used to seal the gutta percha into the polyethylene tube. This condition does not represent the actual clinical situation since freshly mixed cements would normally be placed in the canal prior to filling and could be in contact with the periapical tissue. Previous experiments on the irritability of unset Grossman's cement have noted variable results ranging from mild to severe inflammation.<sup>31,35,38,39</sup> Had freshly mixed Grossman's cement been utilized with the gutta percha a change might have been evident in the inflammatory reaction to the implants.

#### SUMMARY

Forty male white Walter Reed rats were used to study the tissue

inflammatory response to polyethylene tubing filled 1 mm short of the tube at one end and flush at the other end with Grossman's cement and gutta percha. The tibiae of each of the animals were implanted with either a hollow polyethylene tube control or a polyethylene tube filled with gutta percha. The animals were sacrificed in five groups of eight each at 3, 7, 30, 60 and 90 days following tube insertion. The results revealed that: (1) the gutta percha, set Grossman's cement and polyethylene tubing are non-irritating and are well tolerated by rat intra-osseous bony tissue. (2) a polyethylene tube implant filled 1 mm short of the orifice with set Grossman's cement and gutta percha elicited little or no inflammation. There was no evidence of interference with the healing of the surrounding apical tissue. Within 30 days the involved hollow area became filled and sealed with osseous tissue.

## ILLUSTRATIONS

- Figure 1 Surgical sequestered bone and blood clot occupies the unfilled end of the 3 day polyethylene tube (P) and gutta percha implant (G) (20x). There is a moderate inflammatory reaction at both ends of the implant.
- Figure 2 A stalk-like invagination of bone sequestra (B) and blood clot (C) is formed in the unfilled end of the 3 day implant (75x) with a moderate inflammatory infiltrate present.
- Figure 3 An outline of the 3 day hollow polyethylene implant (P) is formed by the surgical clot (C) and sequestered bone (B) with a moderate inflammatory infiltrate at both ends (20x).
- Figure 4 In the 7 day gutta percha (G) implant, the fibrin clot and bone sequestra is being replaced by immature bony trabeculae (BT), connective tissue and vascular channels. There is mild inflammatory infiltrate at both ends of the implant.
- Figure 5 The unfilled portion of the 7 day gutta percha and polyethylene implant is occupied by remnants of the surgical clot, bony sequestra (B) new bony trabeculae (BT), connective tissue (CT), vascular channels, (VC) bone marrow (BM), and mild inflammatory infiltrate (75x).
- Figure 6 At 30 days the polyethylene tube and gutta percha implant is encapsulated by fibrous tissue and lamellated bone that extends along all surfaces of the implant (20x). In the center of the implant there is evidence of Grossman's sealer (S) that was retained during slide preparation.

There is no evidence of inflammation either end of the implant at 30 days.

Figure 7 At the flush end of the polyethylene tube and gutta percha, a layer of fibrous tissue (F), lamellated bone (L) and bone marrow (BM) has developed adjacent to the implant after 30 days (80x).

Figure 8 After 30 days, the unfilled portion of the polyethylene tube and gutta percha implant was similar to the flush end, except that the fibrous band layer was only 1 to 3 cell layers thick (75x). Fibrous band (F), lamellated bone (L), and bone marrow (BM).

Figure 9 The blood clot and connective tissue in the center of the 30 day hollow polyethylene tube implant has been replaced by lamellated bone (L) and bone marrow (BM) with no evidence of inflammation (16x).

Figure 10 There is continued osseous proliferation into the unfilled end of the polyethylene and gutta percha implant at 60 days (20x) with no evidence of inflammation.

Figure 11 The unfilled end of the gutta percha implant at 60 days (80x) shows continued proliferation of lamellated bone (L), bone marrow (BM), and a thin fibrous tissue lining (F).

Figure 12 The 90 days polyethylene tube and gutta percha implant shows a mature lamellated bone (L) and bone marrow (BM) at both ends of the implant (20x). There is no evidence of inflammation.

Figure 13 An overview of hollow polyethylene tube of 90 days shows a lamellated bone layer, (L) and bone marrow (BM) that fills the center of the implant with no evidence of inflammation (20x).

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FIGURE 1



FIGURE 2



FIGURE 3

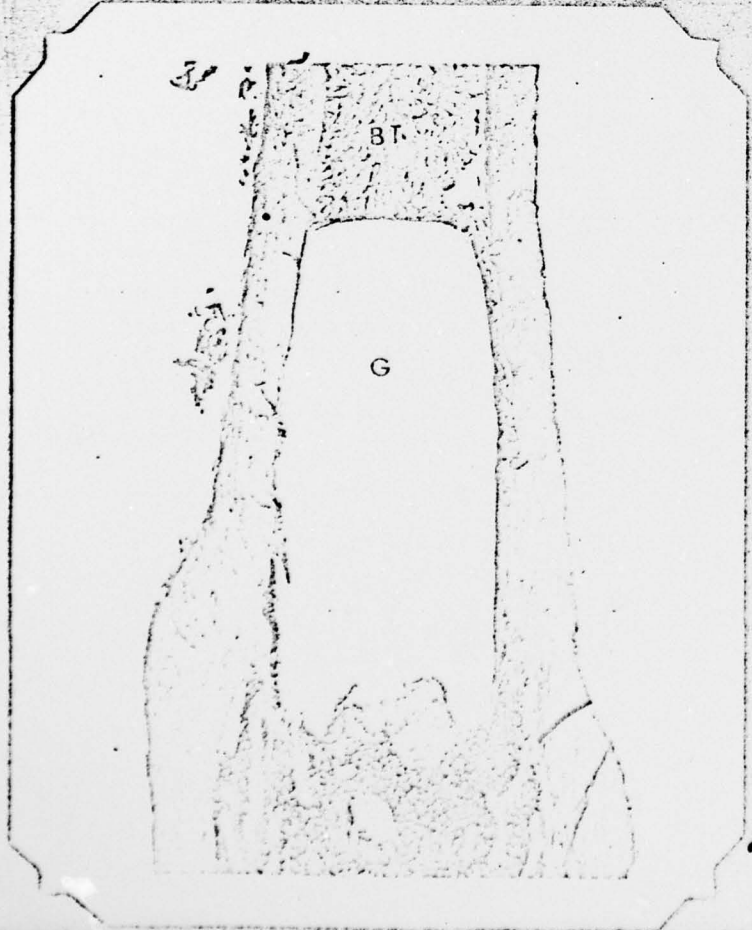


FIGURE 4



FIGURE 5



FIGURE 6

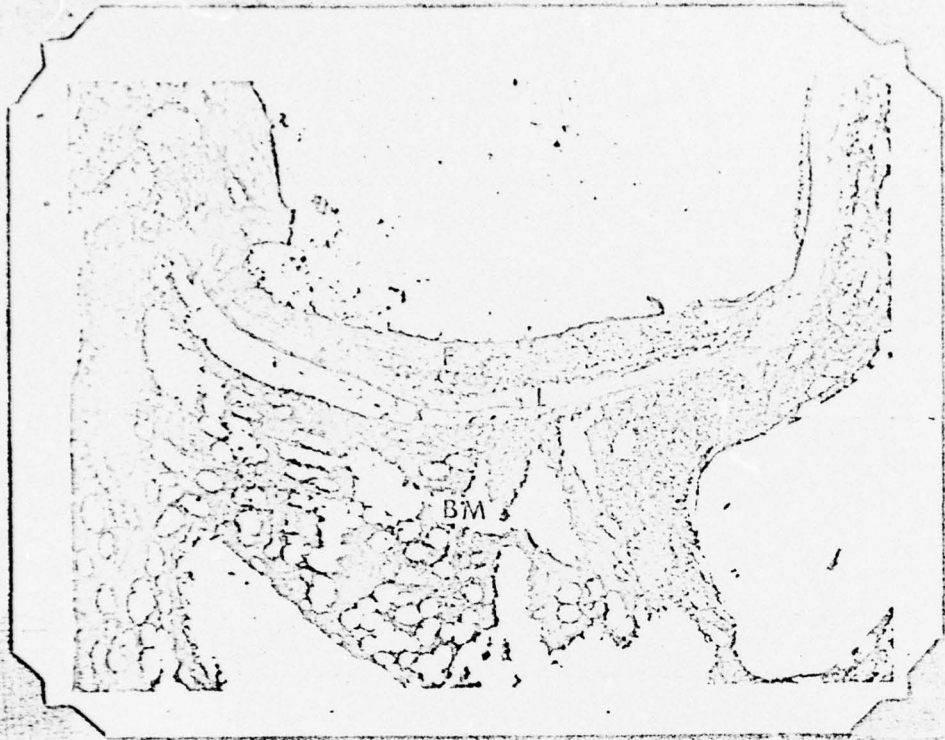


FIGURE 7



FIGURE 8

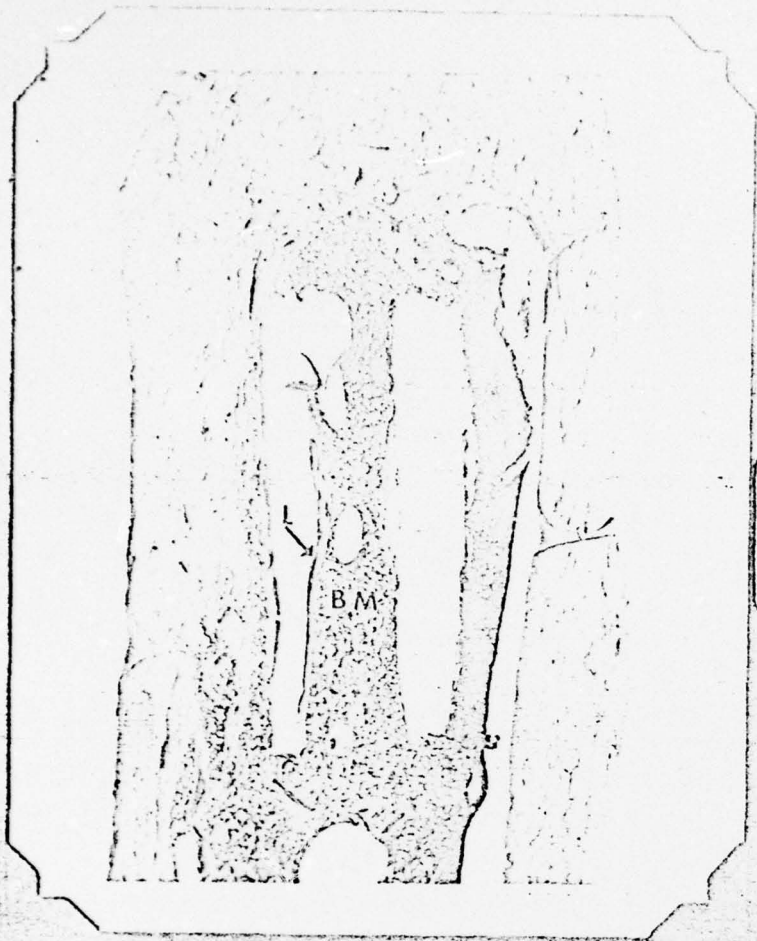


FIGURE 9



FIGURE 10



FIGURE 11

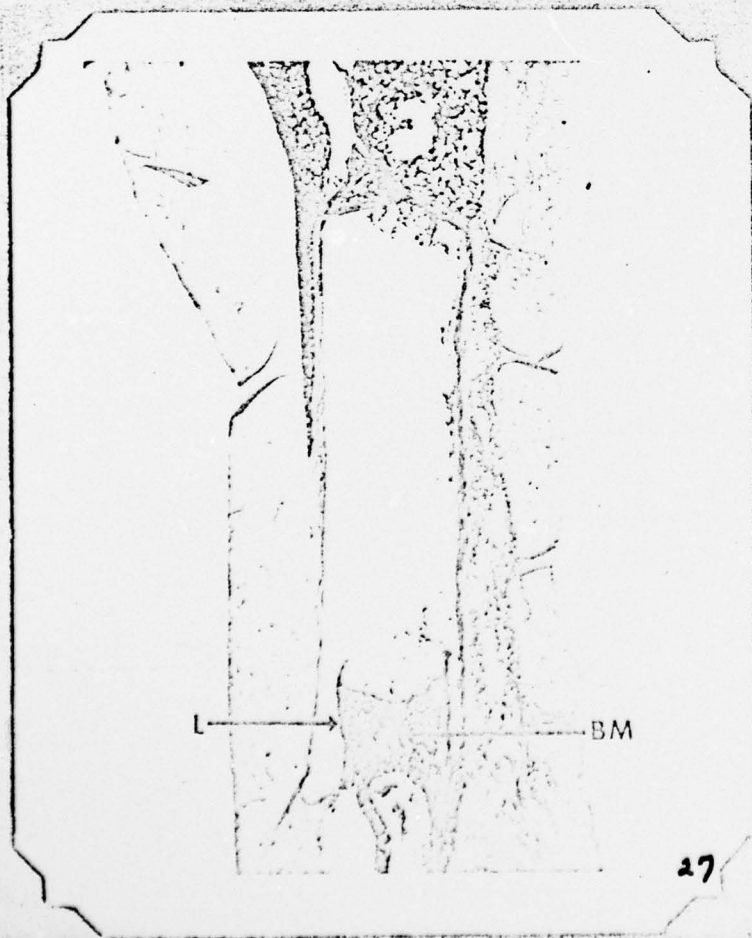


FIGURE 12



FIGURE 13