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SCHOOL OF DENTISTRY
Department of Oral Surgery

Final Progress Report

# BONE INDUCTION WITH LYOPHILIZED BONE AND ADDITIVES

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## CHAMBER DESIGN

The technical problems of handling the standard millipore chamber without distortion of its contents have originally produced a rather inefficient model. In addition, the success of bone induction with this vehicle and bone marrow has been minimal. In search of a more efficient vehicle chamber, a single circular sheet of millipore paper was contoured by first folding it in half. The paper had been moistened to prevent cracking. About one half of the semicircular peripheral edge was glued together and compressed with a hemostat along the edge. This created a pocket for placement of material. The small paper pocket was then sterilized, the material to be studied was placed inside, and the remaining edge glued, folded, and compressed.

## HYPOTHESIS

The studies of Urist and his co-workers have concluded the lyophilized bone in an extraskeletal environment is a reliable model for the study of the bone induction phenomenon. In order to use the millipore chamber as a vehicle to study this phenomenon, it was decided to use lyophilized bone with its supply of intact protein as the inducing agent in a series of experiments.

## METHODS AND MATERIALS

In a pilot study, the mouse was used as the laboratory animal. The lyophilizing process was the same as described by Urist. Five variables were investigated: the millipore chamber in an empty state, lyophilized bone alone inside the chamber, lyophilized bone and bone marrow, lyophilized bone and fresh cortical bone, and the lyophilized bone and fresh striated muscle combinations inside the chambers.

The bone marrow was obtained from mouse femora as previously described. The fresh bone was obtained by stripping the periosteum and using small fragments of cortical bone. Muscle was obtained at the dissection site and cut into small multiple pieces. The donor material was all allogeneic. The chambers were placed singly in a host mouse through a dorsal mid-line incision at uniform depth.

All five groups were sacrificed at 2, 4, 6, 8, and 10 weeks. It was found that three of the early chambers leaked, as noted by the presence of capillaries seen inside the chamber. The chamber construction was then modi-

fied by doubling the compressed edge over and placing the serrated edge of the hemostat perpendicular to the original sealing serrations. All specimens were placed in formalin for fixation. The control specimens and the chamber containing lyophilized bone and marrow were not decalcified; the remaining two specimens were decalcified. The material was blocked in paraffin and cut at 10 μ and stained with H & E Lesson's Alcian Blue. The undecalcified material in addition was stained for calcium with Von Kossa's stain. A full set of 10-week specimens were available for study.

## RESULTS

The chamber containing no material had no cellular infiltrate as would be noted if there were a leak at the compressed and glued junction. A thin fibrous capsule was seen outside the chamber with flattened inactive fibroblasts as the major component (Figure 1). The chamber containing lyophilized bone alone was identical except for the content of original lyophilized bone (Figure 2).

The chamber containing lyophilzed bone and fresh bone at 10 weeks showed a rather large amount of new bone formation covering about two-thirds of the surface of the inside of the chamber (Figure 3). In areas, this new bone appeared in continuity with the lyophilized bone and fresh bone (Figure 4). There was a loose cellular component interspersed, which had a fibro-myxomatous character. Under higher magnification, the new bone had a lamellated appearance undergoing active osteogenesis (Figure 5). In some cases, there were osteocytes with vacuolated cytoplasm which took on the appearance of chondrocytes but no actual cartilage matrix was seen (Figure 6). For the most part, the new bone was in intimate relationship to the surface of the inside of the filter chamber. In these areas, there was characteristic basophilic staining of the filter as noted by Heiple and Goldhaber (Figure 7). In selected areas, but certainly not uniform, bone induction was seen (Figure 7). This was characteristized not only by the presence histologically of identifiable bone, but by the polarization of the adjacent cells, with an increase of cytoplasm-nucleus ratio. There was the same increase of basophilic staining within the filter on the outside surface of the chamber. In some areas, this was intense (Figure 8). In areas where there was not any histologically identifiable bone, the cells along the outside surface of the filter maintained a more deeply staining active appearing nucleus. The cytoplasmic material was more abundant and more eosinophic in character. The cells appeared polarized.

On the inside of the chamber where new bone appeared in contact with lyophilized bone and fresh bone, the junction appeared appositional and although the contact was intimate, there did not appear to be penetration into the lyophilized bone or activation of the fresh bone (Figure 9).

The chamber containing lyophilized bone and bone marrow had many similarities. There was a large amount of new bone formation intimate with the inner surface of the chamber (Figures 10 and 11). Von Kossa's stain showed this was obvious calcification. At the junction with lyophilized bone, the same apparent appositional relationship was noted (Figure 12). Similarly the calcified matrix extending into the inner surface of the filter was readily evident. On the outside surface, no histologic evidence of induced bone was noted but the characteristic polarization of these cells as seen with lyophilized bone and fresh bone was quite evident (Figure 13). In addition, calcification within the filter wall on its outside surface was noted in numerous areas (Figure 14). It appears as if the collagen fibers which penetrated into the outside surface of the filter had become calcified before there was histologic evidence of induced bone formation. Further studies with sequential evaluation for both earlier or longer periods of time are in order to determine when this calcification starts and the time lag before histologic evident bone induction begins.

The chamber containing lyophilized bone and muscle contained viable muscle at the end of the 10-week period (Figure 15). No new bone formation was seen within the chamber although unfortunately there appeared to be an artifactual component to this chamber. The cells along the outside surface of the chamber had the characteristic plumping of nucleus with marked evidence of and increase in nucleus-cytoplasm ratio (Figure 16). The cytoplasm was again eosinophilic in character. These cells appeared polarized. On this a priori basis, it was felt that histologic bone induction was imminent.

## DISCUSSION

The value of an isolation chamber in studying the histogenesis model of bone induction appears rewarding. The design of this smaller chamber allows for a technically accecptable preparation of material to be studied. It appears from the work of Urist that an acid insoluble macromolecular protein complex found in lyophilized bone is one of the essential factors in bone induction. Isolation of the lyophilized bone within a millipore chamber establishes a model by which further study of this complex phenomenon can be evalulated. The interaction between the lyophilized bone and the additives does not appear to be on a cellular basis alone and certainly the polarization of cells and the bone induction seen outside the chamber is not on a cell contact basis. Urist calls this induction principle the Bone Induction Principle (BIP). With bone marrow, it can be understood that the primitive recitular cells could respond to BIP stimulus to differentiate to osteoblasts and form bone. The modulative capability of the osteocyte could respond similarly. Urist has shown that mesenchymal cell of striated muscle also contains this capability for modulation. This study tends to corroborate these theories. In addition, it seems that new bone formation within the chamber is probably necessary for bone induction to occur outside the chamber.

The electron microscope work of Heiple has shown that calcification does penetrate into the pores of the filter for about one-fifth of the filter's width and that collagen fibers do penetrate another fifth, but the central fifth of the chamber wall is void of either collagen of calcification. The reverse is true as complete penetration through the filter is made. This microscopic evidence would be in accord with the staining characteristics of the filter when new bone and bone induction is seen on either side of the filter material. Interestingly, in the specimen with lyophilized bone and bone marrow, no histologic evidence of bone formation from induction is seen and yet calcification is seen within the filter at the outside fifth with the outline of calcified collagen fibers noted in this study. Urist also has shown that the responding cell outside of the chamber is the perivascular fibroblast which differentiates to an osteoblast. Apparently the collagen matrix is laid down in the filter first and calcified prior to calcification outside of the chamber. However, the communicator material that instigates this process is still unknown. It is felt that the cellulose acetate filter of the chamber may be a mechanism to isolate the protein complex which may be passing through the pores and give a better understanding of the BIP or communicating material.

## PERSONNEL RECEIVING CONTRACT SUPPORT

Investigators

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Gilbert S. Small, D.D.S.

Graduate Student

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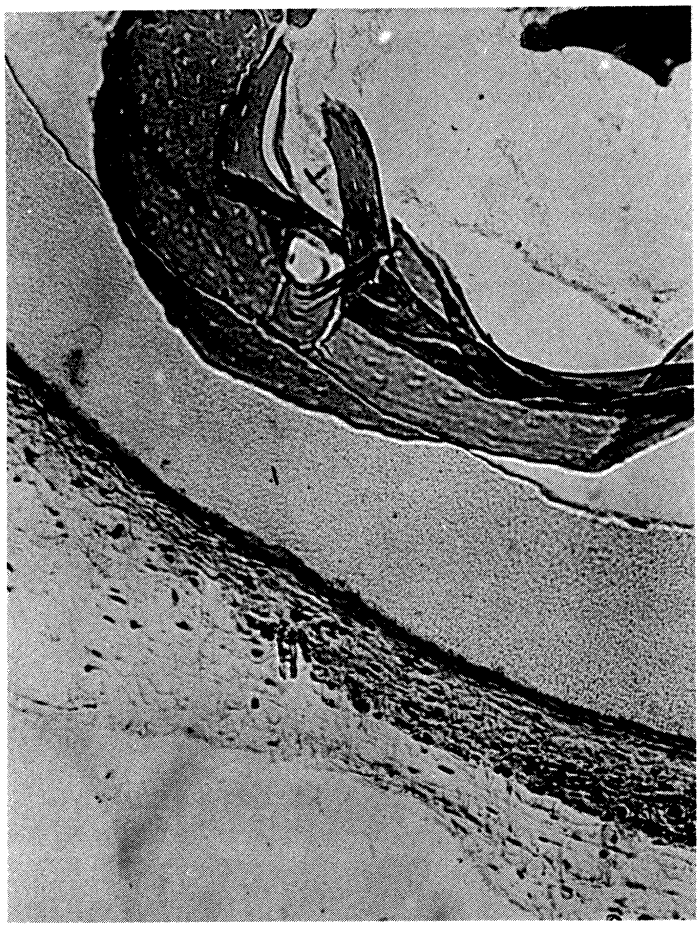

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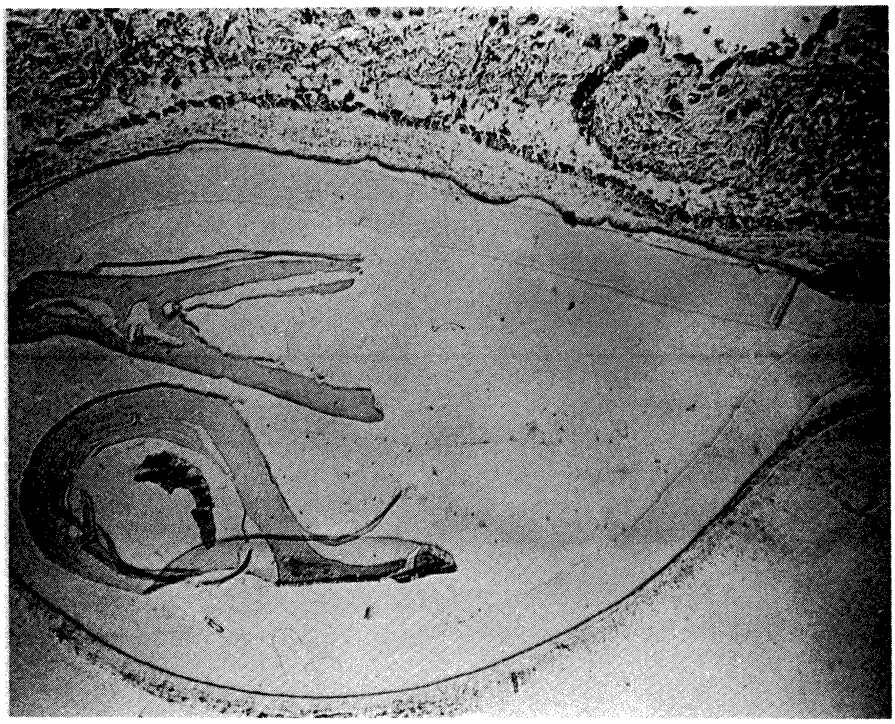

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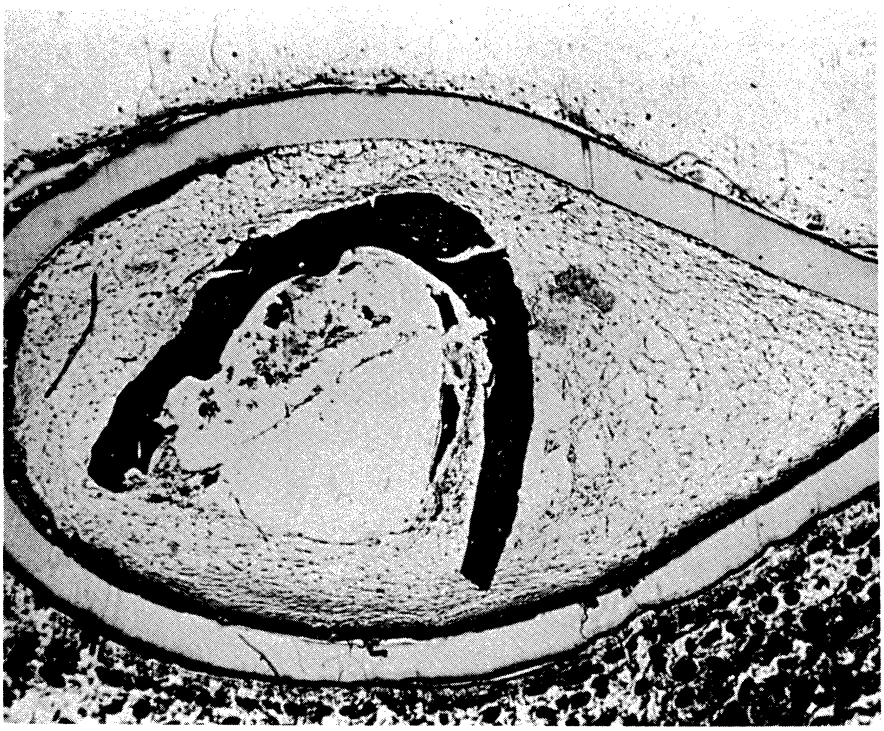

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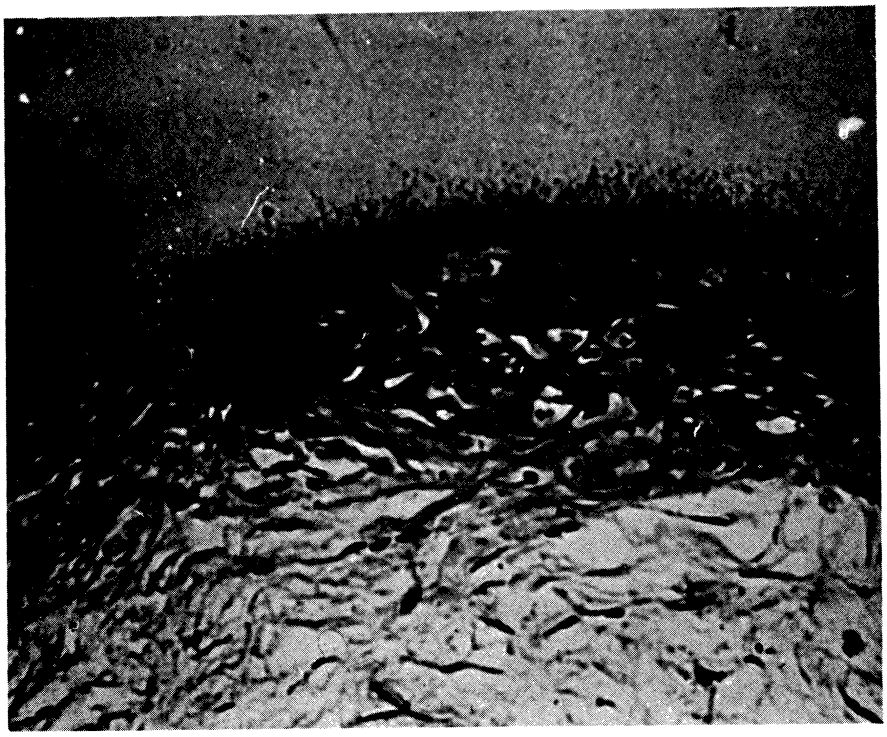

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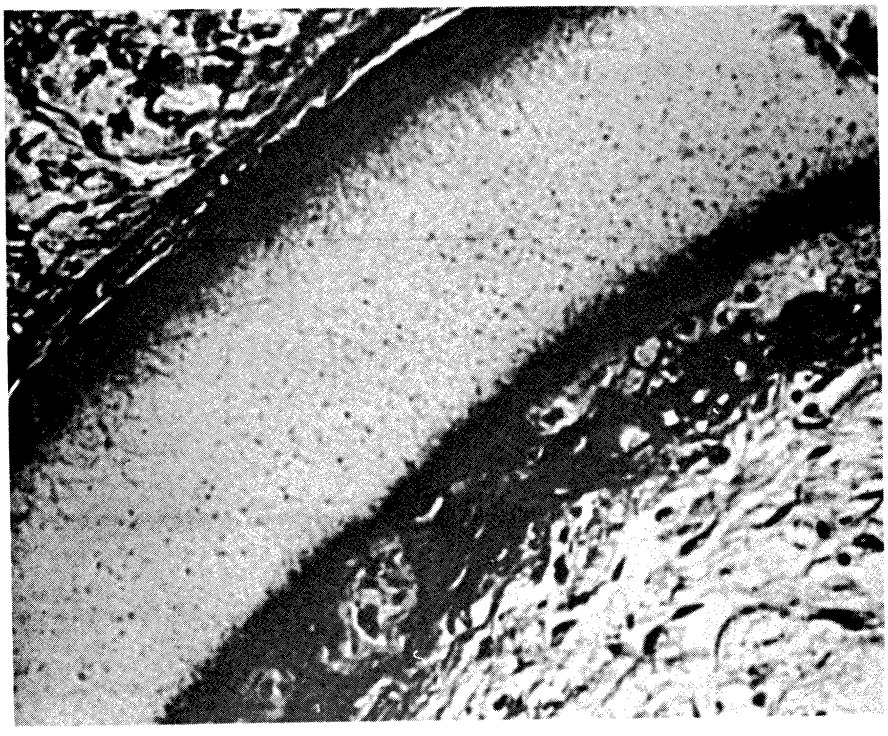

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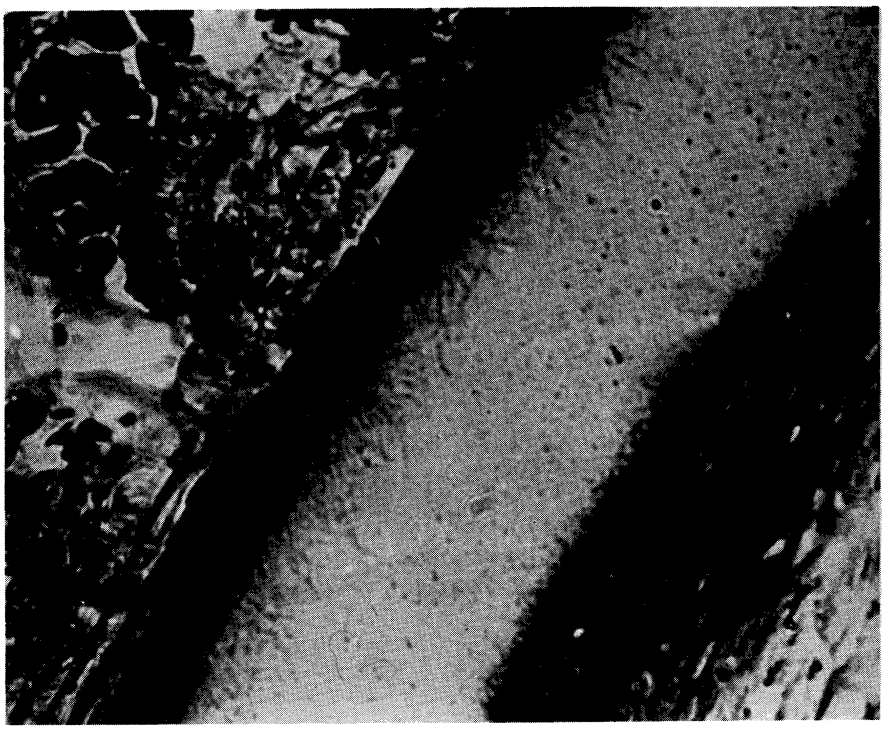

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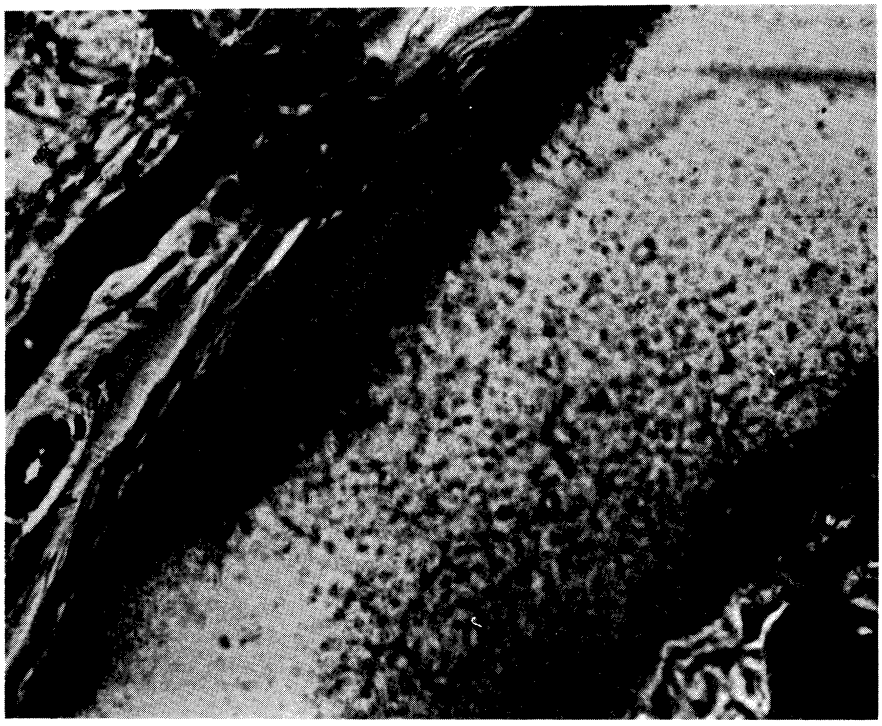

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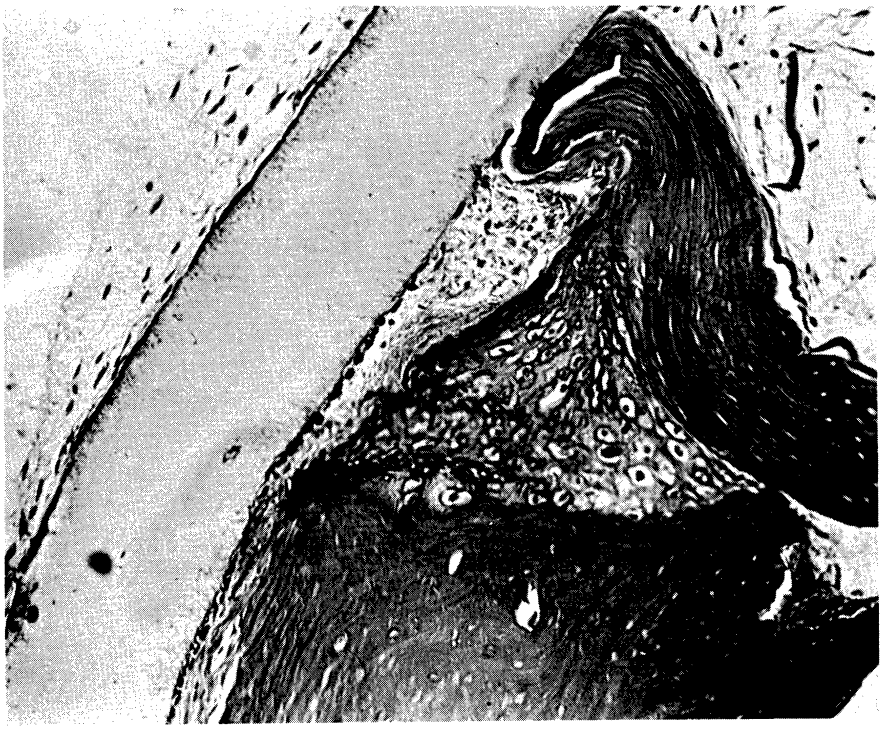

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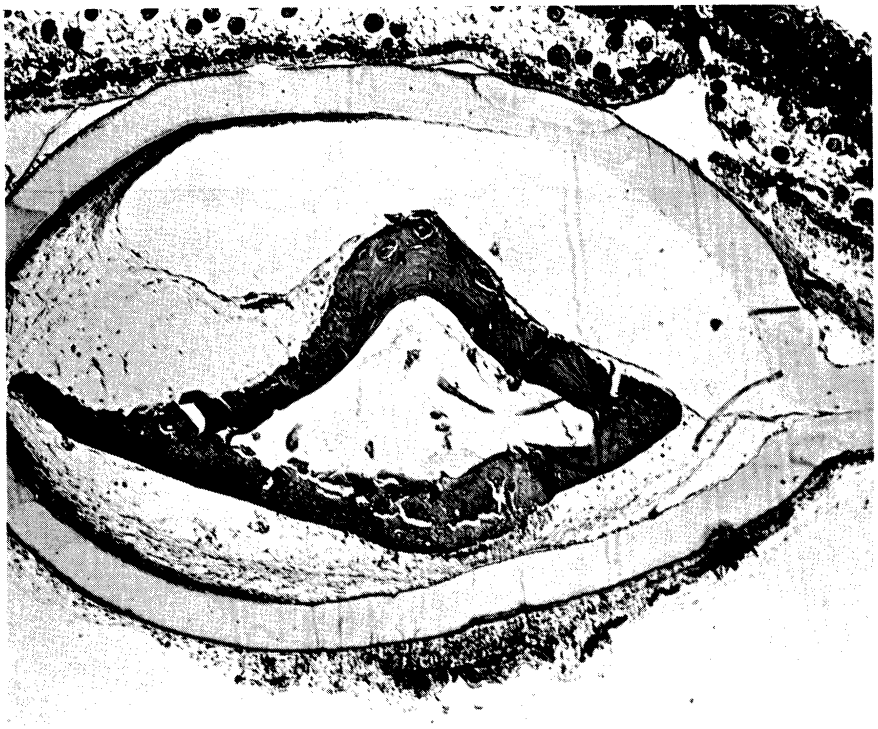

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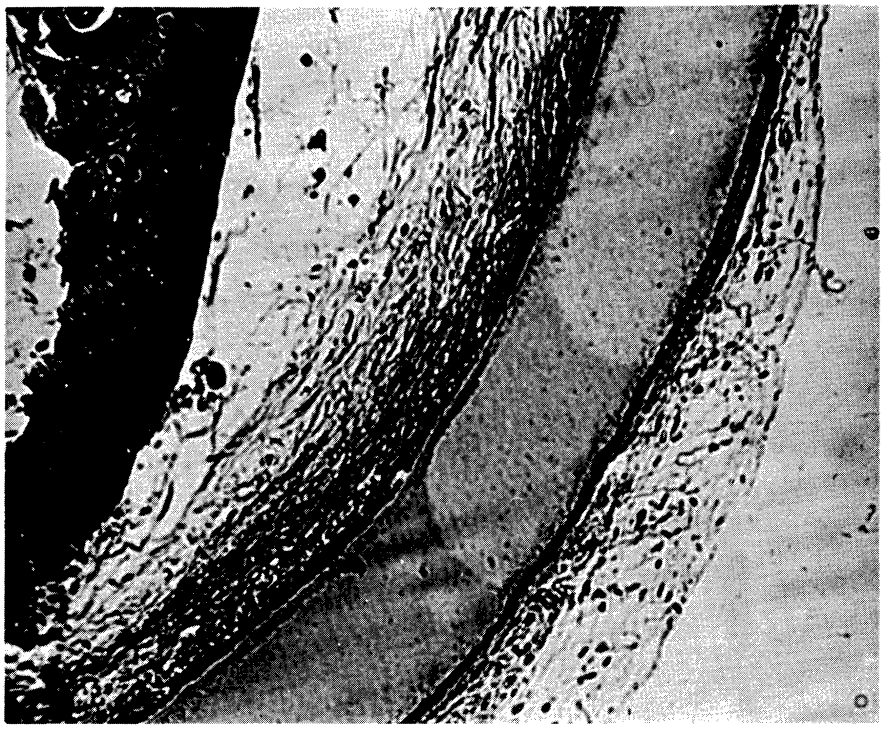

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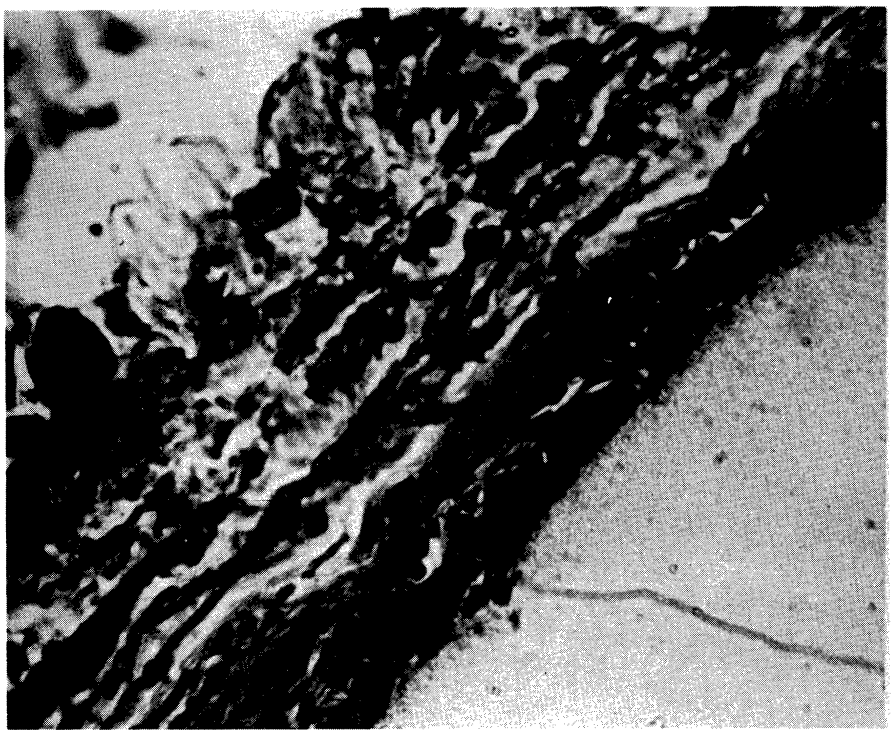

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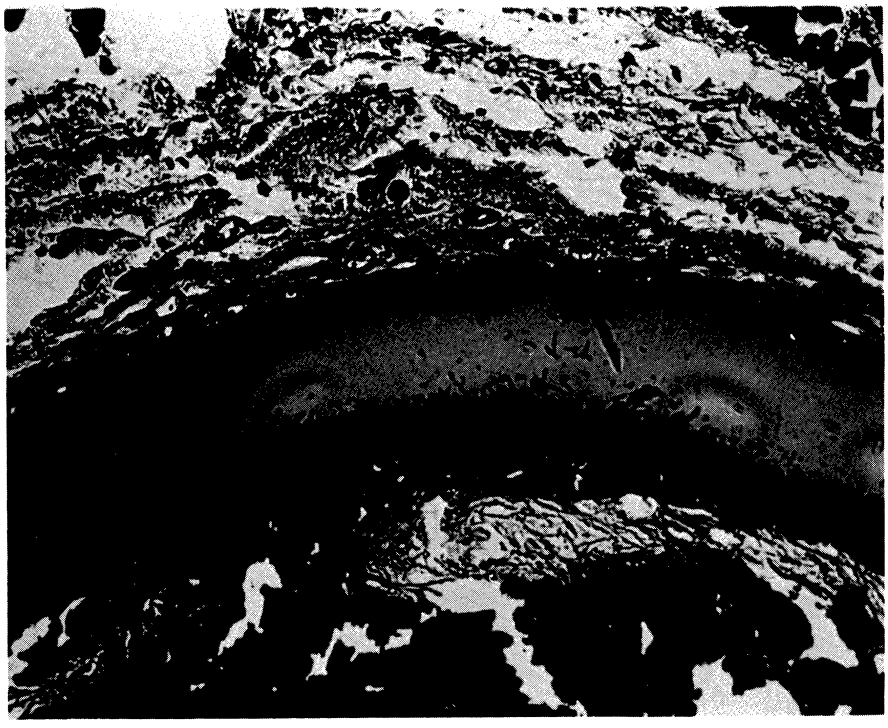

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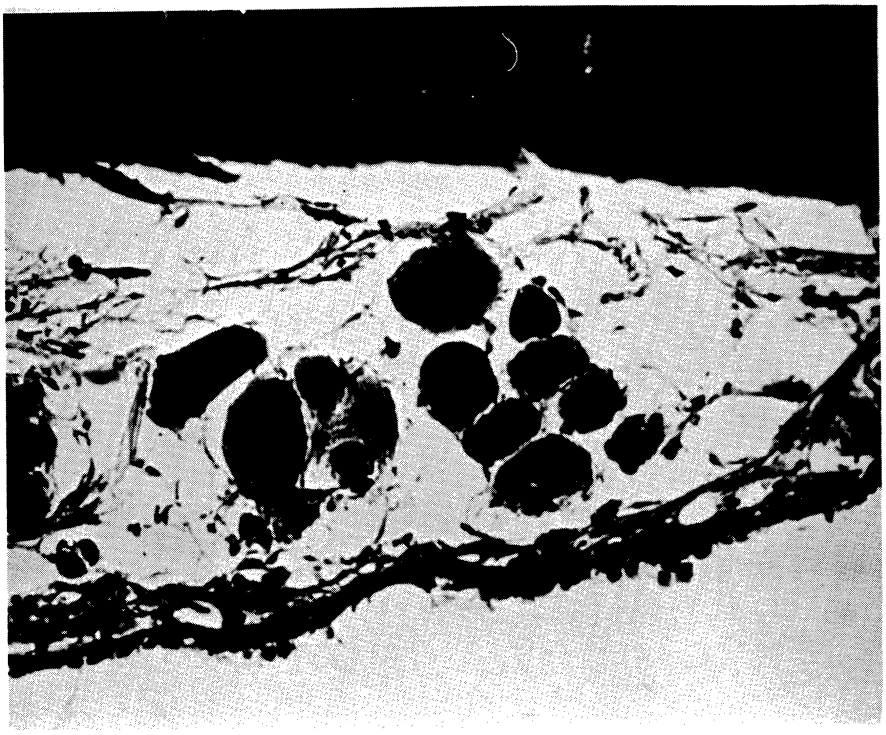

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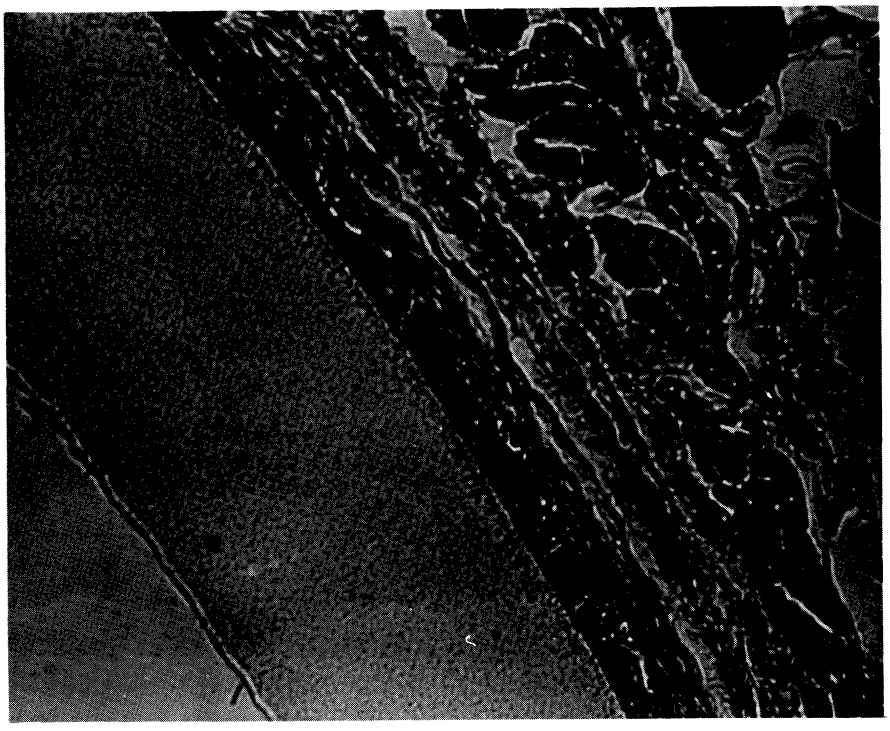

Figure 16

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13. ABSTRACT


Lyophilized bone has bone induction capability when placed alone in soft tissue sites. If placed in the isolation of a millipore chamber it has none. Selective additions as shown do produce bone induction. The millipore chamber therefore presents an isolation vehicle for studying the bone induction phenomenon. The penetration of a bone induction communicating substance has been demonstrated.


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