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Alloplastic Implant in Primates: Calscorbate-Culture Medium 199 - a Sequential Histopathologic Study

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ALLOPLASTIC IMPLANT IN PRIMATES: CALSCORBATE-CULTURE MEDIUM 199 - A SEQUENTIAL HISTOPATHOLOGIC STUDY

by

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DEDICATION

To my father and mother, Wil and Lois Eastman, for their love, encouragement, guidance and confidence in me. Without the great sacrifices they have made, these last six years would never have been possible.

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VITA

Lindsay B. Eastman was born on August 31, 1952 in Nassau, Bahamas. He is the first son of Wilbert and Lois Eastman, Jr.

He grew up in Park Ridge, Illinois, and went to Maine South High School. He graduated in May, 1970, and went on to study at Purdue University, West Lafayette, Indiana, for four years.

He entered Loyola University College of Dentistry in September, 1974, and graduated with his D.D.S. Degree in May, 1978. While he was in his senior year at Loyola, he taught Anatomy at Triton Junior College. In September, 1978, he entered the Periodontics Graduate Residency at Loyola University and graduated in May, 1980.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Replacement of the loss of supporting structures of teeth has been the major obstacle in the treatment of moderate to advanced periodontal disease. A number of therapeutic procedures were investigated involving treatment of bone loss by osseous recontouring -- osteoplasty and ostectomy (1). These techniques, although presently utilized because of their predictability, for the most part involve the additional loss of bone.

Replacement of lost bone, rather than further reduction of bone, to achieve a physiologic osseous and gingival architecture, would be the most desirable aim of periodontal therapy involving loss of supporting structure. Yet, replacement techniques lack the degree of predictability and often are not practical in a daily clinical situation.

To attempt replacement of alveolar bone lost by disease, a number of materials were investigated for osseous grafting. Autografts were utilized with some success (2,3,4); but often the disadvantages of additional surgical trauma, treatment and postoperative morbidity limit their value as a therapeutic modality.

Autogenous hematopoietic bone marrow has demonstrated success within limits (5). However, availability of sufficient quantities has posted problems using only the host as a source of bone graft material. Studies using allograft bone have indicated risked biologic accepta-

bility and the possible hazard of immunological incompatability, disease transmission, or other postoperative problems (6).

An early attempt by Beube and Silvers (7) utilizing devitalized heterogenous sheep bone powder in surgically created defects claimed benefit in healing possibly by the availability of calcium.

Peltier (8) described the work of Dressman (1892) in the Trendelenberg Clinic at Bonn, Germany as the first reported use of plaster of paris to fill defects in bone.

Bahn (9) noted biologic acceptability utilizing sterilized plaster of paris as an alloplast graft. Alderman (10) reported bone fill utilizing sterile plaster of paris to fill intrabony defects. But Schaffer and App (11) found no evidence of bone fill six months following the implantation of plaster in intrabony defects.

Stallard and Hiatt (12) implanted bone, cementum and dentin chips into surgically created intraosseous defects in dogs and desribed the implants serving as niduses for bone formation.

Henefer et al, (13) described acceptability of implanted acrylic amide sponges into created alveolar defects.

Pedersen (14) utilized a porous woven ceramic implant to fill surgically created extraction sockets noting complete bone fill within the pores.

Levin et al, (15) utilized a tricalcium phosphate ceramic in dogs, reporting excellent tissue tolerance and regeneration of lost periodontium.

Thus autografts and substitutes for autografts were attempted.

These included, in part, Boplant, (16) boiled cow bone powder, (17) plaster of Paris, (18) sclera, (19) and ceramics. (20)

Previously researched materials utilizing Calscorbate (21) and Culture Medium 199 (22) as alloplast materials reported biologic acceptability and an obtainable readily available source. A more ideal consistency alloplast material was suggested by combining a 50/50 mixture of Calscorbate with Culture Medium 199. (23) The purpose of this investigation is to examine histologically the sequential healing phenomenon of surgically created two-walled osseous defects in the Rhesus monkey using a 50/50 mixture of Calscorbate powder,* and Culture Medium 199** as an alloplast implant material.

* Calscorbate, Cole Pharmacal Co., Inc.
** Culture Medium 199, Cat. No. E-12, Grand Island Biological Co. Grand Island, New York

CHAPTER II

LITERATURE REVIEW

Bone regeneration as a reparative modality in the treatment of periodontal disease is a desired aim of periodontal therapy. To date no completely predictable method of bone replacement exists.

Alveolar bone throughout life is in the process of bone formation, maintainance, and remodeling. In the normal condition the factors or environment must be such to provide maintainance. In the pathological state of periodontal disease, bone resorption predominates resulting in bone loss. (24)

Normal alveolar bone formation is related to membrane ossification - bone formation directly, without a cartilagenous matrix. Also, remodeling of every bone occurs by membrane (intramembranous) ossification. (25) The concept of an osteoid in the formation of bone apposition was introduced by Pommer (1885). (26)

Recent studies (Frank, 1978) (27) have shown that the feature of membranous ossification in utero and the differentiation of bone trabeculae in adults are similar. A monolayer of osteoblasts line up along a noncalcified osteoid matrix adjacent to calcified bone and then undergo differentiation. Frank 1978, demonstrated that the transformation of an osteoblast into an osteocyte embedded in its lacuna is the result of cellular activity. After the osteoblasts synthesize an osteoid matrix, the matrix undergoes mineralization leaving osteoblasts

immediately adjacent to newly mineralized bone. The osteoid matrix is secreted by the second line of osteoblasts and completely surrounds the first line of osteoblasts. The thickness of the collagenous matrix progressively increases as the second line of osteoblasts withdraws from the first, leaving the first line surrounded on one side by bone and on the other by osteoid (termed osteoid osteocytes by Dudley and Spiro, 1961). (28) Thus, an organic matrix is first formed followed by mineralization of the osteoid around the osteoid osteocytes.

The most widely accepted theory regarding the initial mechanism of calcification is still the nucleation or epitactic theory (Glimcher and Krane, 1968). (29) Mineralization consists first of a local deposition of amorphous calcium phosphate followed by its transformation into minute apatite crystals which grow to mature size.

Howell 1971, (30) still reports of the unconfirmed biochemical and biophysical consitions required for the seeding of calcium phosphate in the organic matrix. Howell stated that collagen may act as an organic nucleator or epitactic agent for the formation of the first nuclei of hydroxyapatite. Collagen plays a major role in inorganic nucleation. Calcium may be transferred directly to osteoid and bone by diffusion through the intercellular spaces of medullary fibroblasts and osteoblasts. (31)

Bone resorption exceeds bone regeneration in advanced periodontal disease. The bone resorption is primarily under the control of large multinucleated cells - the osteoclasts. (32) The osteoclasts may also be a mononuclear cell. (33) The osteoclast, a multinucleated

cell with phagocytic vacuoles, is capable of catabolic activity. (34) Osteoclast resorption consists initially of apatite crystals, thereby exposing the collagen fibrils with typical periodic cross-striations. Secondly, collagen destruction occurs by lysing the fibrils adjacent to the extracellular spaces by the osteoclast. (35) Osteoblasts are probably involved in bone remodeling. Pluripotential mesenchymal cells can be either bone forming or bone resorbing.

Urist (36) termed the "bone induction principle" stating that a tissue substrate may exert a physio-chemical effect upon competent mesenchymal cells stimulating differentiation into osteoblasts capable of osteogenesis and further induction.

Osteoblasts may arise from pre-existing osteoblasts, (37) from the endosteal lining of marrow cavities, (38) from perivascular undifferentiated mesenchymal cells, (39) or a combination of the above.

A certain number of substances such as hormones, vitamins and others, either stimulate or inhibit osteoclastic activity. (40) Hormones and vitamins influence differentiation of osteoblasts from precursor cells. Osteoblasts are induced to differentiate by parathormone, while calcitonin does the opposite. (41) Hydrocortisone promotes either resorption of bone or its deposition, depending upon the dose. (42) Vitamin C depresses resorption of bone and may help differentiation of osteoblasts as it enhances osteogenesis. (43)

Other factors are of concern in the repair phenomenon. Ascorbic acid is instrumental in the formation and maintainance of collagen in healing wounds. (44) The hydroxylation of proline to hydroxyproline

and lysine to hydroxylysine in the amino acid incorporation into the collagen molecule involves the presence of ascorbic acid. (45) Jeffrey 1966, (46) suggested that ascorbic acid exerts a direct action on collagen synthesis by stimulating the hydroxylation of peptide-bound proline.

The necessity of Vitamin C is demonstrated by deficiency situations. Wolbach (1926), (47) in a classic article, demonstrated the lack of collagen formation in dentin, bone, and wound healing in ascorbic deficiency states; and the prompt appearance of collagen development after administration of ascorbic acid.

It has been demonstrated that ascorbic acid is a prerequisite for the maintainance of a collagen pool in tissues (Ostergaard and Loe, 1975). (48) Ascorbic acid is required for normal wound healing. (49) It was suggested that a megadose of ascorbic acid therapy may be of value during the acute phase of healing, but its value is less apparent in long term healing (Kramer et al, 1979). (50) A citrate-soluble collagen fraction accelerated bone mineral hydroxyapatite formation and deposition - demonstrated both in vitro, (51) and in vivo. (52) A citrate extract of ox bone treated with pepsin, placed in surgically created periodontal pockets in dogs demonstrated rapid proliferation of repair cells and accelerated healing in connective tissue, bone, and cementum, also yielding greater amounts of new bone formation. (53)

Calcium has been demonstrated as necessary for the calcification of newly forming bone and cementum. (54) When calcium ascorbate was

injected subcutaneously into rats the amount of bone healing was significantly greater than that of animals injected with calcium gluconate suggesting that the availability of calcium may be increased in the presence of ascorbate. (55) The increase in calcium turnover in bone in the presence of ascorbic acid may possible be through increased metabolic activity of bone cells. (56)

Calcification is believed to occur either from a high enough local concentration of calcium and phosphate somehow causing precipitation; and/or that some organic component - collagen in the case of bone - may have properties mimicking those of apatite crystals. (57) Extracellular fluid is supersaturated and metastable in relation to calcium and phosphate. An organic component may act as a seed crystal starting calcification.

Beube, (58) demonstrated that the boiled cow bone powder used in a graft, may supplement the calcium concentration available from the circulatory system. The presence of implanted bone chips in resorbing calcified tissues may be a factor in the differentiation of osteoblasts. (59)

Wadkins et al, in 1974, (60) demonstrated that the exposure of ordinarily noncalcifying matrices in vivo to an unphysiologically high concentration of calcium can produce a matrix which initiated bone mineral formation. The exchange of calcium at the bone surface probably takes place in the surface layer 1 - 4 microns thick. (61)

Various investigations utilized mineralized materials as a calcium source to stimulate osteogenesis, including "Anorganic bone,"

(Toto et al), (62) and plaster of paris (Randentz and Collings). (63)

Mineralization was shown to be more dependent upon calcium than on phosphate ion concentration, and calcium is the first ion found. (64)

Vitamin D may exert a local role in calcification, by maintaining the calcium and phosphate concentration of plasma at supersaturated levels in regard to bone mineral which in turn is necessary for normal calcification in bone, and in maintaining calcification. (65) Vitamin D may facilitate the transport of calcium from bone fluid to the bone cell. (66)

Another vitamin important for the growth and maintainance of bone is Vitamin A, expecially in bone remodeling. (67) Na_2HPO_4 and $CaCl_2$ have osteogenic induction properties when placed intramuscularly. (68) Glucose and sucrose supported bone growth and maintainance in tissue culture medium. (69)

A good demonstration of essential and supportive substances acting in tissue maintainance, is noted by tissue culture experiments in bone growth and maintainance, and in infusion intravenous solutions utilized on patients.

The intravenous nutrition utilized for patients introduce needed elements directly into the blood stream and has been demonstrated to support growth in a child and restore weight loss in an adult. (70) This support is, of course, systemic. Local tissue support is well demonstrated in tissue culture experiments in vitro. The in vivo use of tissue culture medium has shown cellular stimulated growth in mice (Toto, 1968). (71)

Classic reference in regard to tissue culture medias is Paul, 1959. (72) He demonstrated that a medium must possess certain properties and be in balance for cellular support. Sustained cellular support requires more than a balanced salt solution. Normally those factors present in whole serum should be present. In addition to a balanced salt solution, all essential amino acids, oxygen, vitamins and serum protein should be present in addition to the other amino acids, co-enzymes, nucleosides and vitamins. (73)

The presence of an agent capable of inducing bone growth is illustrated by the classic research of Goldhaber. Goldhaber (1961) (74) demonstrated in vivo the concept of an osteogenic induction factor. Implanted material on one side of a millipore diffusion chamber elicited growth of new bone on the host side of the filter.

Composition of bone's organic matrix (about 22% by weight) includes its main component of collagen (18.5% by weight) predominantly of Type I; some of Type III collagen; and 3.5% by weight of a phosphoprotein rich in serine, glycoproteins, proteoglycans, lipid and citrate. (75) Water makes up about 8% by weight of bone; and the inorganic part consisting of some amorphous calcium phosphate and numerous, tiny hydroxapatite crystals. The biochemical composition of mineralized human bone and cementum is similar (Cole and Eastoe, 1977) (76)

In the literature it has been shown that materials like gel foam with cementum and dentin, (77) and plaster of paris, (78) may have a scaffolding role to bone formation but do not stimulate new bone formation. Ceramics that are biodegradable (79) also may act as

a scaffold but do not demonstrate osteogenesis capabilities. Sclera grafts as those by Klingsberg (80) demonstrate possible enhancing capacity of the repair of intrabony lesions but have the potential hazard of being a foreign protein capable of possible delayed sensitivity reactions. Thus, ideally it would be desirable to find a well-tolerated material that may act as a scaffold and/or stimulate osteogenesis and new bone formation.

Both Calscorbate (Appendix I) and Culture Media (Appendix II), as previously mentioned, appeared to be well tolerated by the host when used as an alloplastic graft material. The combination of the calscorbate powder with an equal portion of culture media may produce a favorable consistency material with desirable nutritive or supportive capabilities in the repair phenomenon of healing bone. Thus, the study of the use of equal portions of Calscorbate powder and Culture Medium 199 were employed as alloplastic graft materials into surgically created defects of the alveolar bone of Rhesus monkeys.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Design

In this study, the experimental models utilized were two adult female rhesus monkeys (Macaca mulatta). The experiment covered a one-hundred and seven day span with the first twenty-one days being utilized as a quarantine and tuberculosis inoculation period. This period allowed the monkeys to acclimate to the Loyola animal care facility and the establishment of a definite parameter of health. Throughout the experimental period, the monkeys physical parameters were maintained as was recorded during their quarantine, and they appeared to be in good health during the remaining eighty-six days of experimental period.

Upon examination of the oral cavity, the animals demonstrated slight marginal gingivitis with various amounts of material alba, plaque and calculus. The gingiva was firm in consistency and pink in color. The sulcus depths were examined and found to be within acceptable limits. (Figure 1)

The posterior teeth of the maxillary and mandibular quadrants were utilized as experimental sites. Therefore, four maxillary and four mandibular quadrants were included in the study. Three, two-walled (consisting of a lingual and distal wall of bone with a mesial wall of cementum and/or dentin) surgically created osseous defects were created. Defects made distal to the second premolars were used as the control area, while the created defects distal to the first premolar

and the first molar were the experimental sites used for the implant mixture consisting of equal parts of Calscorbate and Culture Medium. The control site preparation and correction (by surgical curettage without the placement of the implant mixture) was performed at the same time as the preparation and correction of the analgous implanted defects within the same quadrant.

The one-hundred and seven day schedule of the experiment was planned to allow for a twenty-one day quarantine and an eighty-six day experimental period. This allowed the two-walled osseous defects to be created thirty days before the initial implantation. The predetermined time schedule was designed so as to allow the sacrifice of the control and the Calscorbate-Culture Medium implants on 0, 3, 7, 14, 21, 28, 42 and 56 days, postoperatively. Each of the time sequences allowed for a control defect and for a defect to be corrected by the mixture of Calscorbate-Culture Medium.

Twenty to twenty-five minutes prior to the surgical procedures, the appropriate monkey was given an intramuscular injection of 14mg Sernylan* for sedation. When more surgical time was anticipated, an additional dose of 5mg Sernylan was given intramuscularly as needed for sedation. Prior to beginning the surgical procedure, a local anesthetic of xylocaine 2%** with 1:100,000 epinephrine was injected into the area of surgical intervention.

Throughout the course of each surgical procedure, strict conditions of asepsis were maintained both for the operator as well as for

* Bio-Ceutic Labs
** Astra Pharmaceutical Products, Inc., Worchester, Mass.

the monkey's protection. Any and all pertinent clinical observations were recorded throughout the experimental period.

B. General Preparation

Full buccal mucoperiosteal flaps from the distal of the canine to the distal of the second molar were utilized for both the creation and the correction (30 days later) of the defects. An intrasulcular incision (scalloped to allow for better primary closure) was carefully designed with a #15 C Bard-Parker blade. Using a small periosteal elevator (#7 wax spatula), the flaps were meticulously reflected so as to insure that the tissue would not be perforated.(Figure 2) Tissue tags on the inner surface of the flap were carefully removed with a LaGrange tissue scissors. Gracey curettes were used to remove all chronic inflammatory tissue and to thoroughly root plane the exposed root surfaces.

C. Preparation of the Defects

The interproximal osseous septae distal to the first premolar, second premolar and first molar were the areas chosen for the creation of the osseous defects. These areas were chosen not only because they were easily accessible and plainly visible, but also because they offered a more than sufficient amount of bone to accommodate the creation of the two-walled defects. These experimental sites also offer protection postoperatively due to the interproximal contact areas between adjacent teeth, and due to the protection of the anatomical convexities of the crowns. The defects created distal to the first premolar and the first molar were to receive the Calscorbate-Culture Medium implant, while the osseous defect created distal to the

second premolar was designated to be the control site in the experiment.

Two-walled, surgically created, osseous defects were made in the selected sites. A #557 straight fissure bur was used in a slow speed belt-driven dental handpiece to remove 5 mm of alveolar crest bone along the distal root surfaces of the selected teeth. A width and depth of 3 mm was carefully maintained with the use of a callibrated periodontometer.* The resulting osseous defects consisted of a distal and lingual wall of bone, a mesial wall of cementum and/or dentin, and no buccal wall. Isotonic sterile saline was used (during the creation of the osseous defects) to flood the surgical site to avoid temperature increase which would damage the bone. (Figure 3)

D. Introduction of Chronic Irritants

A sterile, wooden toothpick approximately measured at 6 mm in length was then introduced into each osseous defect to function as a chronic irritant. The excess length afforded a wedging effect for retention of the toothpick. The toothpick so placed acted as a route of direct communication from the oral cavity to the defect, thereby allowing the ingression of bacteria and their associated toxins. This step was performed in an attempt to stimulate a chronic defect in human periodontitis. (Figure 4)

After proper positioning and wedging of the chronic irritant, the full mucoperiosteal buccal flap was repositioned and adapted as close as possible to its original position with interrupted, vertical, mattress, interproximal 5-0 Ethaflex** sutures which were tied on the

* Hu-Freidy - Michigan Probe
** Ethicon - Johnson and Johnson

lingual surface. This suturing technique provided additional stabilization of the flap as well as the chronic irritant.

E. Post Operative Care

The monkey received a prophylactic injection of an antibiotic prior to the initiation of any surgical procedure. A lcc intramuscular injection of 300,000 units of Flo-cillin* (Benzathine Penicillin G and Procaine Penicillin G) was given. This amount of Flocillin was enough to give prophylactic antibiotic coverage for 72 hours. Post operative instructions specified that the animals receive no food for the first 24 hours, a soft diet for the following 2 days, and thereafter a normal diet.

F. Removal of Irritants

On the seventh day following the surgical creation of the osseous defects the animal was sedated, as before with Sernylan, and the sutures and wooden irritants were removed. At this time, a clinical impression of chronically inflammed gingiva was noted.

G. Procurement of Implant Material

A carefully measured 50/50 mixture of Calscorbate** powder (the tablets were crushed until a fine, even consistency powder was obtained) and Medium 199*** was mixed with triple distilled water until a thick, pasty consistency was obtained. This Calscorbate/Culture Medium paste without further modifications was utilized as the implant material.

*Bristol Meyers
**Cole Pharmacal Company, Inc.
***Grand Island Biological Co., Grand Island, New York

H. Correction of the Defects

Exactly 30 days postoperative to the surgical creation of the two-walled osseous defects, the surgical sites were reopened, and the osseous defects were exposed for curettage or addition to the implant mixture (control vs. experimental site respectively). To expose the sites, the same surgical procedures were performed, as outlined previously. All chronic inflammatory tissue was carefully removed from the 30-day, two-walled defects, and the root surfaces were again thoroughly planed. (Figure 5)

The control defect distal to the second premolar was carefully debrided and irrigated with isotonic, sterile saline - to ensure that the control defect was free of any material.

The Calscorbate/Medium 199 paste was placed in the debrided defects distal to the first premolar and the first molar, being careful to keep the implant mixture within the confines of the two-walled defect. The implant mixture was placed without difficulties, and as evidenced by Figure 6, hemorrhage in the areas was easily controlled.

At this point, the control and experimental sites were then recovered by the full mucoperiosteal buccal flap which was repositioned and held in place by the interproximal suturing technique as was previously described. As before, the same postoperative antibiotic and diet instructions were given.

On the seventh day after the surgical correction of the defects, the monkey was again sedated and the sutures removed. The monkey was then maintained until the next scheduled procedure or until the final sacrifice.

I. Collection of Specimens

On the day of the scheduled sacrifice, the animals were sedated as previously described and were then sacrificed by giving them an intra-cardial injection of a lethal dose of Beuthanasia-D.*

A Stryker saw was used to obtain the block sections. The obtained specimens were washed with distilled water and placed in pre-labeled jars containing formalin for fixation. The volume of formalin was 20 times that of the specimen.

J. Preparation for Histologic Examination

After two weeks in fixation, the specimens were decalcified in a 50/50 mixture of formic acid and sodium citrate. After 5 weeks in the decalcifying solution, the sections were trimmed after which radiographs were taken (60 kvp at 0.6 sec.) at weekly intervals for another 6 weeks until it was determined that a proper degree of decalcification had been reached. At this point, the sections were embedded in parafin, sectioned at 5 microns in a transverse buccolingual plane, and finally stained with hematoxylin and eosin. After a complete light microscopic examination of all slides, a representative histologic section was selected for a detailed histologic analysis.

* Burns-Biotec Lab

CHAPTER IV

FINDINGS

A. Clinical Observations

At the beginning of the experiment, the clinical preoperative examination of both monkeys revealed a chronic generalized marginal gingivitis (Figure 1). Both the papillary and marginal gingiva were boggy, edematous, and discretely magenta in color. The attached gingiva was adequate, firm in consistency, and pink in color. Slight hemorrhage was ellicited upon probing. Pocket depths ranged from one to three millimeters.

One week following the surgical creation of the two-walled osseous defects and subsequent placement of the implanted irritants, the gingiva displayed a marked increase in inflammation. The marginal and papillary gingiva were erythematous, boggy in consistency, and displayed hemorrhaging upon both pressure and probing. Areas of granulation tissue were also noted - especially near the irritants.

Thirty days following the surgical creation of the osseous defects, a marked decrease in the amount of gingival inflammation was noted as compared to areas at seven days following the creation of the osseous defect. The thirty day post-defect gingival inflammation was greater than that initially observed before beginning the experiment.

Upon re-entry thirty days following creation of the defects, both bone resorption and apposition was detected in the walls of the

defect. There evidently was additional bone resorption noted on the mesial and distal aspects of the buccal cortical plate thereby presenting a larger V-shaped defect than that produced by surgical intervention. The detected bone apposition was noted to have occurred mainly in the area of the lingual wall of the defect.

One week following surgical correction of the defects and with addition of the implant, the marginal and papillary gingiva were erythematous, edematous and boggy. Upon examination on the fourteenth post-operative day, there was a marked decrease in gingival inflammation. By the twenty-first day, the areas appeared clinically normal.

B. Histological Observations

1. Control

a. Epithelium

0 Day

Prior to sacrifice, the area was surgically flapped and curetted. This procedure also was followed in all of the control defects from 3 - 56 days. The gingiva was covered by normal keratinized stratified squamous epithelium; however, there was no attachment to the tooth.

3 Day

There was no evidence of a mucosal flap over the area of the defect. However, keratinized stratified squamous epithelium was observed over the non-defect area of the mucosa. The sulcular epithelium exhibited intercellular edema and a fibrinopurulent exudate.

7 Day

The reflected flap appeared partly attached to the tooth surface, and the actual implant site was not well defined. The mucosa contains keratinized stratified squamous epithelium, and only slight inflammation was seen in the sulcular epithelium.

14 Day

The reflected flap was not closely adapted to the bony defect site. The mucosa was covered by keratinized stratified squamous epithelium demonstrating short, broad rete pegs.

21 Day

There was an apparent flap reattachment. The mucosa was covered by slightly keratinized stratified squamous epithelium. The nonkeratinized sulcular epithelium showed localized edema, inflammation, and cellular disorientation.

28 Day

There was a definite epithelial attachment with the junctional epithelium. It extended from the sulcus apically to cementum where the epithelium appeared artifactually detached. The sulcular epithelium and adjacent tissue showed polymorphonuclear leukocytic infiltration. The mucosa was covered by keratinized stratified squamous epithelium.

42 Day

The mucosa was covered by keratinized stratified squamous epithelium. The sulcus was covered by normal nonkeratinized epithelium. However, the sulcus epithelium showed infiltration by monocytic cells and a few polymorphonuclear leukocytes, indicating a low-grade gingivitis.

56 Day

Keratinized stratified squamous epithelium covers the mucosa and a nonkeratinized epithelium of normal width lined the sulcus.

b. Connective tissue

0 Day

Within the fibrous connective tissue, dilated blood vessels, extravasated blood and residual muscle bundles were noted. Near the detached flap, while no inflammatory reaction was noted, there were red blood cell extravasation, dilated blood vessels, and vascular stasis.

3 Day

There was an acute inflammatory reaction predominated by polymorphonuclear leukocytes within the loosely arranged connective tissue fibers. There also were sequestrated bone spicules interspersed within the connective tissue and defect area, probably as a result of re-entry and curettment of the area.

7 Day

The collagenous fibers appeared homogeneous and contained large sinusoidal dilated blood vessels, both deep and superficial to the defect. Polymorphonuclear leukocytes were seen throughout the tissue, and there was evidence of fibroblastic repair as fibroblast-like cells with plump, well-developed nucleoli were seen. It appeared that inflammation and reparative granulation tissue were simultaneously present.

14 Day

The reparative granulation tissue appeared similar to that

seen at 7 days, as it showed dilated capillaries and numerous fibroblast-like cells.

21 Day

There was an apparent notch in the cementum due to instrumentation. Between the cementum and the superficial portion of the flap was evidence of loosely arranged dense collagenous fibers. Adjacent to the cementum the surface of the tooth showed irregularities probably due to the original intervention. This defect was covered by cemental repair. Deeper, there was dense connective tissue with extravasated blood and a few bundles of striated muscle.

28 Day

The lamina propria contained polymorphonuclear leukocytes. Within the defect site both mature as well as immature collagen fibers were noted. The cemental defect was seen apical to the junctional epithelium extension and showed cemental repair. Restoration of the periodontal ligament was also apparent.

42 Day

Adjacent to the tooth was a normal periodontal ligament. Also, there was evidence of cementum formation. The periodontium at this site has repaired.

56 Day

A completely restored periodontal ligament was seen. The lamina propria showed mature collagen with some slight inflammation.

c. Alveolar bone and defect

0 Day

There was a widening of the adjacent marrow spaces and

osteoclastic resorption of the alveolar bone proper adjacent to the periodontal ligament. Some small spicules of residual curetted bone were noted among the fibrin clot debris.

3 Day

Widened marrow spaces were seen in this tangential section. Spicules of necrotic bone were found interspersed within the alveolar bone defect. The defect was filled with a fibrinopurulent exudate, loosely arranged fibrous connective tissue, and polymorphonuclear leukocytes. Osteoblastic activity was noted within the adjacent marrow and on the surface of the alveolar bone defect.

7 Day

An intact alveolar bone proper was seen near the tooth surface as the actual defect was not well defined in this section. The bone seen in this section was covered by a thick layer of periosteal cells. There was also osteoclastic resorption with widened spaces within the bone marrow.

14 Day

This section appeared to depict the base of the defect area, which showed granulation tissue on the surface of the bone. There was osteoblastic bone apposition present in areas where osteoclastic resorption had already occurred. The surface of the defect showed new bone formation. Also viewed was regenerating marrow with dilated capillaries, fibroblasts and osteoblastic activity. Therefore, there was apparent new bone formation in both the marrow spaces as well as on the bone surface.

21 Day

The alveolar bone crest showed new bone formation. Apically, it was noted that new osteophytic bone had formed which probably was a reaction from previous flap intervention.

28 Day

At the level of the alveolar crest, there was evidence of recent bone formation with osteoblastic activity. This extended from the tooth surface to the crest and to the buccal surface where active osteoblastic activity was seen. Previous bone resorption at the site was noted by reversal lines. There was repair on the crest, buccal and periodontal ligament side of the bone.

42 Day

The cortical bone showed osteophytic bone indicative of repair which was covered by a moderately thick layer of periosteum with osteoblasts. There was also bone repair along the alveolar bone proper.

56 Day

The defect site showed a fragmented artifactual defect. The crest displayed osteophytic bone formation and a moderately cellular marrow with active osteoblasts. The communication between the new bone, with a lining of osteoblasts, and the periodontal ligament had been restored.

2. Calscorbate-Medium 199 Implant

a. Epithelium

0 Day

Prior to sacrifice, the area was surgically flapped and

curetted. A 50/50 mixture of Calscorbate-Medium 199 was implanted into the osseous defect. The reflected mucosal flap was made up of keratinized stratified squamous epithelium.

3 Day

The mucosa was covered by keratinized stratified squamous epithelium. The epithelium was supported by a weak, edematous attachment with the underlying dense fibrous connective tissue; due in part to the existence of a fibrinopurulent exudate.

7 Day

The reflected flap contained keratinized stratified squamous epithelium with some fragments of bone undergoing osteoclastic resorption. Many of the fragments showed empty lacunae indicative of necrotic material. Also seen were a few polymorphonuclear leukocytes and extravasated red blood cells.

14 Day

The keratinized stratified squamous epithelium covering the surface of the implant site was very thin. The epithelium was apparently regenerating and contained a few polymorphonuclear leukocytes.

21 Day

This tangential section showed keratinized stratified squamous epithelium and an intraepithelial infiltration with ploymorphonuclear leukocytes. It appeared that there was a normal epithelial attachment.

28 Day

This section showed normal keratinized stratified squamous epithelium and a normal gingival sulcus. Deeper, there was some

intercellular edema with polymorphonuclear leukocytic infiltration. There was a long junctional epithelial attachment.

42 Day

Both the keratinized stratified squamous epithelium and the epithelial attachment appeared normal.

56 Day

The mucosa was covered by a normal keratinized stratified squamous epithelium with a normal gingival sulcus with an accompanying epithelial attachment. There appeared to be polymorphonuclear leukocytic infiltration within the sulcus.

b. Connective tissue

0 Day

The connective tissue showed edema with some infiltrated polymorphonuclear leukocytes. The periodontal ligament was intact. Extravasated blood was present between the tooth and flap.

3 Day

The dense fibrous connective tissue displayed increased vascularity with an acute inflammatory response. Although this was a poor representative section, the activity appeared to be similar to that occurring in the 3 day control.

7 Day

The periodontal ligament was intact, and was in contact with the alveolar bone proper. The reflected flap contained some dilated blood vessels and substantial proliferating fibroblasts surrounding the dilated capillaries indicative of a repairative process. Also seen were a few polymorphonuclear leukocytes and extrav-

asated red blood cells. There appeared to be a split thickness separation - separating the connective tissue through the papilla down to the underlying alveolar bone.

14 Day

The connective tissue covered the alveolar crest and formed a contact with cementum. There was evidence of cementum resorption and cementogenic repair. In this specimen, the periodontal ligament fibers run parallel to the root surface.

21 Day

This tangential section showed a lamina propria containing collagenous fibers, fibroblasts and dilated capillaries. There was active fibrogenesis occurring. Normal periodontal ligament fibers were seen extending from the alveolar bone proper to the cementum.

28 Day

In this section, the instrumentation defect in the root showed secondary dentin and new cementum formation. Active osteogenesis was seen in the alveolar bone proper as were well orientated intervening periodontal ligament fiber bundles. There was evidence of repair of the periodontal ligament.

42 Day

Evidence of cementogenesis on the resorbed tooth surface was seen. Beneath the epithelium was an inflammatory process with plasma cells, monocytes and a few polymorphonuclear leukocytes within fibrous connective tissue.

56 Day

The instrumentation defect seen in the root had been repaired by the apposition of cementum. Well oriented periodontal ligament fibers insert into the periodontal ligament and adjacent bone. There was a dense fibrous connective tissue periosteum covering the cortical buccal bone.

c. Alveolar bone and defect

0 Day

The implant site was bordered by well trabeculated bone and contained blood, fragments of dentin and a yellow-black or blue-grey pigmented material which represents Calscorbate-Medium 199 granules. Calscorbate may be described as an amorphous, yellow-black pigment in Hematoxylin and Eosin. Culture Medium 199 seems to stain blue-grey with Hematoxylin and Eosin.

3 Day

The implant site base in this section was composed of fibrous connective tissue totally surrounded by polymorphonuclear leukocytes. On the surface was a poorly defined blue-grey material believed to be Culture Medium 199. Widened marrow spaces with osteoclastic activity were seen adjacent to the implant site. There was also new bone formation possibly related to the previous injury.

7 Day

Adjacent to the implant site, there was a proliferation of periosteal cells, differentiating osteoclasts and resorption of small bone fragments as a result of curettment. There was a repair

process with remodeling resorption in the adjacent bone. The implant site was not seen.

14 Day

The alveolar crest showed evidence of surface osteoclastic resorption, but no osteoclasts are seen. The surrounding marrow showed cellularity with many capillaries. There was also active osteoblastic activity with osteophytic bone formation on the surface of "old" bone (wall of defect), thought to be indicative of a repair process somewhere slightly below the implant site.

21 Day

This tangential section is a poor representation of the implant site. Surface osteoclastic resorption was seen on the alveolar crest.

28 Day

The alveolar crest showed evidence of osteoblastic activity with subsequent new bone formation. This new alveolar bone communicates directly by fibrous connective tissue bundles to the root surface restoring the periodontal ligament. Extending from the new bone apically to the mature bone, one can see active osteogenesis on the alveolar bone proper as well. There was evidence of repair of the periodontal ligament with active osteogenesis.

42 Day

A dense fibroperiosteum was seen covering the buccal cortical bony surface. Osteophytic bone was present. Because of the widened marrow spaces with surface resorption as well as apposition, it

can be presumed that since the operative procedure was performed 42 days ago, there must have been severe trauma with resultant damage (i.e. remnants of osteoclastic activity). The base of the implant site however was repaired with a new layer of bone on its surface.

56 Day

There was evidence of new bone formation on the buccal, crestal and periodontal ligament aspects of the alveolar bone. There was active osteoblastic activity on the surface of the alveolar bone. The implant site was repaired and covered bucally by an active periosteum. The amount of new bone formation appeared similar to that seen in the control.

CHAPTER V

DISCUSSION

This study utilized apparently clinically healthy adult Rhesus monkeys. No deficiency states or abnormal conditions were clinically noted. Thus, the healing of a mucosal and bony wound would be that ordinarily expected in health.

In exposure of the alveolar bone in this experiment for both the creation of the defect and the placement of the alloplasts, a reverse bevel full thickness mucoperiosteal flap was made. Regarding the normal healing phenomenon of reverse bevel periodontal flaps in monkeys, the most detailed description of healing is presented by Caffesse et al, 1968. (81) Within the first twenty-four hours, a thick band of polymorphonuclear cells, accompanied by a superficial necrosis of the surgical surfaces of the flap and over uncovered interproximal bone occurs. Only a minimal inflammatory response is seen where the flap is placed in close approximation to bone. However, after forty-eight hours, the implant site shows a slight proliferation of epithelial cells and the connective tissue is noted which produces with fibrosis. The inflammatory reaction extends into the marrow spaces adjacent to the wound area. Three to eight days after the surgery, fibroblastic and angioblastic activity is noted originating from the periodontal membrane surface, marrow spaces and the margin of the flap. At fourteen days after surgery, a new epithelial attachment develops on the surface of the cementum of the adjacent teeth, and new bone forms

on the surface of necrotic bone. Ideally, when the flap had been adapted very close to the bone, then healing appears to be complete within 21 days. However, if the flap is poorly adapted to the bone then healing may be delayed in the flap up to 72 days. Thus, the sequence of healing of the flap procedures appeared dependent upon the degree of flap adaptation to the tooth and bone. With close flap adaptation to the tooth, the epithelium grows apically on the connective tissue surface of the flap and a new epithelial attachment forms at seven days. If the flap does not make contact with the tooth, then the epithelium continues to proliferate. This is similar to a gingivectomy wound which shows much more inflammation as the wound granulates. When the bone is actually left exposed, then the implant site spaces become filled with blood between the flap and the bone. This leads to marked delayed healing, continued inflammation and more bone resorption.

Staffileno et al (1962), (82) reported healing of gingival wounds in dogs. Of particular interest is the fact that the underlying bone responds to wound injury of the overlying soft tissue. As early as 2 days post-operatively, there is evidence of resorption of the internal surfaces of the vestibular alveolar plate. This was not accompanied by osteitis. Resorption of bone reached a maximum at six days; thereafter, bone resorption gradually gave way to osteogenesis, which leads to restoration of lost bone.

Linghorne and O'Connell (83) found that a new periodontal ligament will form adjacent to newly formed bone in healing after excision of the periodontal membrane and alveolar bone margin. The periodontal

membrane regenerates where there is a restoration of alveolar bone; but the epithelium covers appreciable areas of the root surface to which connective tissue was attached, pre-operatively.

Healing studies of free osseous autografts have demonstrated that bone grafts are resorbed and replaced by new bone formation. (84) The autograft is first surrounded by a blood clot, then it becomes partially resorbed. New bone is deposited on the autograft and new cementum on the exposed dentin. An active remodeling of the graft with new supporting bone being deposited has been observed up to eight months post-operatively. (85) Plaster of Paris was demonstrated to act as a non-irritating scaffold but did not stimulate new bone formation. (86)(87) Delayed sensitivity reaction to foreign proteins are always a possible complication in allografts. (88)

This experiment demonstrated no unusual variability in the response of the epithelium between the control and experimental tissues other than the expected inflammation as seen in the normal gingiva or that which results from the surgical procedure.

The connective tissue is generally similar, both in the control and experimental tissues. Fibrous deposition is not unusual in either. Amorphous, small, dark, pigmented particles seen in the experimental connective tissue, but not seen in the control probably represents remnants of graft material.

Osseous tissue of the alveolar process in which surgical defects are produced, demonstrates osteogenesis both in the control and experimental animals, as well as areas of osteoclastic activity.

Quantitation of bone repair between the experimental and control alveolar bone is not specifically possible by the experimental design used in this study. However, the qualitative bone repair appears similarily equal in both the experimental and the control.

Specifically, the findings present the fact that the Calscorbate-Medium 199 implant is well accepted by the host tissue alveolar bone. There is no evidence of rejection associated with the graft and no delayed sensitivity noted after the 56 day period of this study.

The possible benefits of the Calscorbate-Medium 199 implant may be in providing an environment rich in nutrients, supportive repair of bone by osteogenesis. The implant may possibly induce osteogenesis by the induction principle suggested by Urist (89).

The graft may also contribute to favorable conditions by limitation of the size of the blood clot within a bony defect and may thus hasten repair. It is likewise possible that the clot is retained and supplied with essential supportive nutrients protecting the vitality of the underlying connective tissue and bone.

The graft may indeed first occupy space in bone, and after being actively resorbed, stimulate bone repair. This was demonstrated well by Klingsberg's sclera grafts (90). If, indeed, the dark particulate areas found in the experimental specimens were graft material, then they in part may be occupying space though not to the same quantitative degree as that suggested in Klingsberg's work.

There may be an involvement of one or more of these reactions or others occurring in the healing process involving favorable allo-

plasts. Selection of an alloplast with components supportive in the healing sequence seems reasonable. An unphysiologically high concentration of calcium has been demonstrated by Wadkins et al, (91) capable of producing an in vivo matrix which catalyzes mineral formation. The rate of bone healing was increased with calcium ascorbate (92). These and other previously discussed important properties of both calcium and ascorbate in the alloplast may play a favorable role in osseous regeneration.

The culture media in the alloplast material may provide essential nutrients similar to that provided by whole serum. A synthetic mixture of certain essential amino acids, vitamins, serum protein and minerals can provide survival of mammalian cells for long periods of time. And when incorporated in the alloplast, graft material may influence the defect environment in a favorable manner by providing nutrients for bone regeneration.

The graft material did not interfere with the usual formation of the periodontal ligament or with the formation of cementum. Therefore, while contributing nutrients to the repair process, it does not produce any delay in the healing of bony defects.

The experimental design of the defects attempted to simulate chronic defects created in chronic periodontitis. But it must be remembered this is only a simulation and probably does not duplicate the exact situation of the disease state. Also, animal studies cannot be directly correlated to the human disease situation. Also, creation of defects such as three walled defects may actually be better to test

graft potentiation.

The media in the form of a thick paste facilitates its implantation in bony defects. However, calscorbate powder alone also has ease of placement into the defect. The presence of protein in the graft mixture may have the added potential complication of a long term delayed sensitivity reaction which may not be as likely with the calscorbate mixture alone.

Thus, from the results obtained in this study compared to the independant studies of L. Jenkins (93) and P. Haupers (94), no increased benefit can be established by the use of a mixture of calscorbate and culture media over the use of pure calscorbate or culture media alone.

CHAPTER VI

CONCLUSIONS

1. The two-walled osseous defects corrected by an equal mixture of Calscorbate-Culture Medium 199 were accepted by the host site and favorable osteogenesis did occur.

2. The experimental defects created probably do not duplicate osseous defects caused by chronic periodontal disease.

3. The graft may possibly act by creating an environment rich in nutrients supportive of the growth of adjacent bone and osteogenic cells, by inhibiting the size of the blood clot, and/or by acting as a "space occupier" allowing for a greater number of undifferentiated mesenchymal cells and host induction of osteogenesis.

4. Qualitative bone repair in the experimental defect appeared to parallel that seen in the control.

5. The combination of Calscorbate with Culture Medium 199 may provide an abundant calcium source, influence bone collagen, and provide an environment rich in essential nutrients.

6. An experiment to test quantitatively the regeneration of bone utilizing Calscorbate alone is recommended.

CHAPTER VII

SUMMARY

The purpose of this study was to histologically examine the sequential healing pattern of mechanically induced two-wall periodontal osseous defects in Rhesus monkeys using an equal mixture of Calscorbate-Medium 199 as an alloplastic implant material.

In this investigation, two adult, female Rhesus monkeys were utilized as control and experimental models from which 0 to 56 day biopsy specimens were taken. Both control and experimental graft specimens were taken at 0, 3, 7, 14, 21, 28, 42 and 56 days post-operatively.

While control defects were treated only by re-entry and subsequent surgical curettment, the experimental defect consisted of implanting a 50/50 mixture of commercially available Calscorbate and Medium 199 incorporated into a thick paste (using distilled water).

Histologic sections from each specimen were carefully prepared and stained with Hematoxylin and Eosin, after which a light microscopic evaluation and description of the sequential healing events was recorded, and thereafter the results were discussed.

It was found that the implanted Calscorbate-Medium 199 mixture was compatible with osteogenesis and was well accepted by the host site. By experimental design, no quantitative comparison can be made between controls and experimentals.

CHAPTER VIII

ILLUSTRATIONS

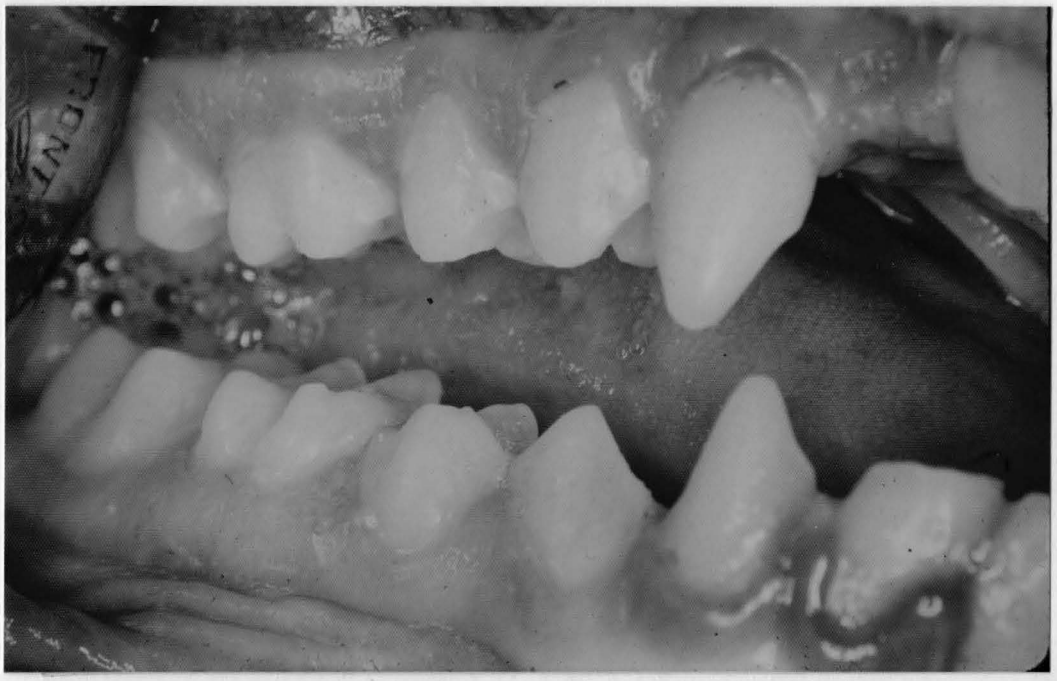

Figure 1: The preoperative appearance of experimental area.

Note the slight papillary and marginal gingivitis.

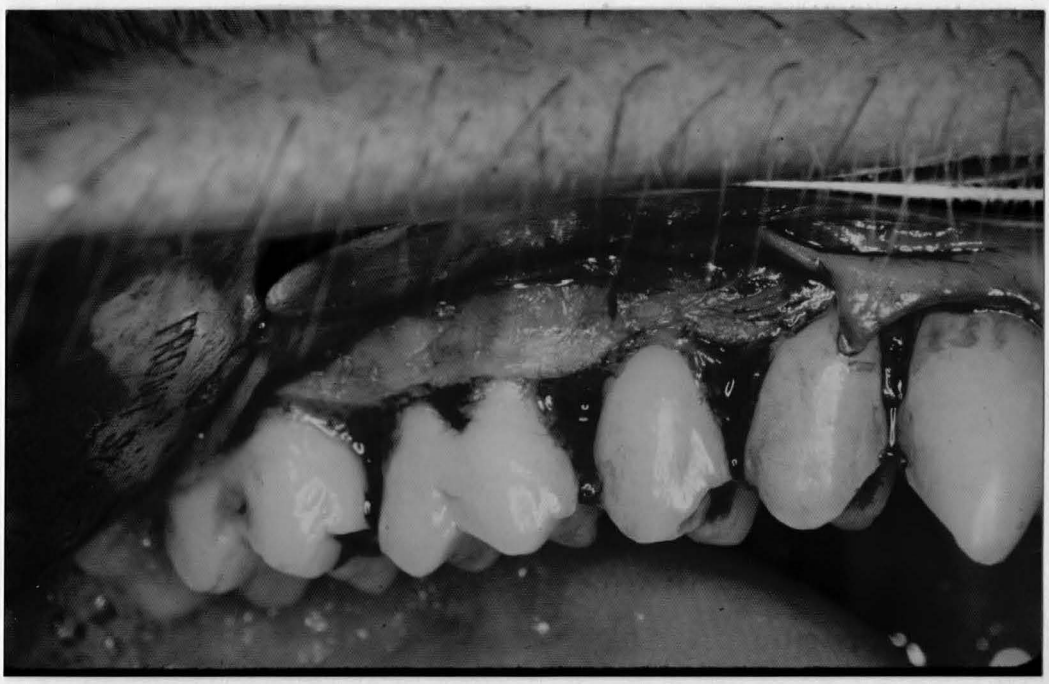

Figure 2: Full, buccal mucoperiosteal flap from the distal of the canine to the distal of the second molar.

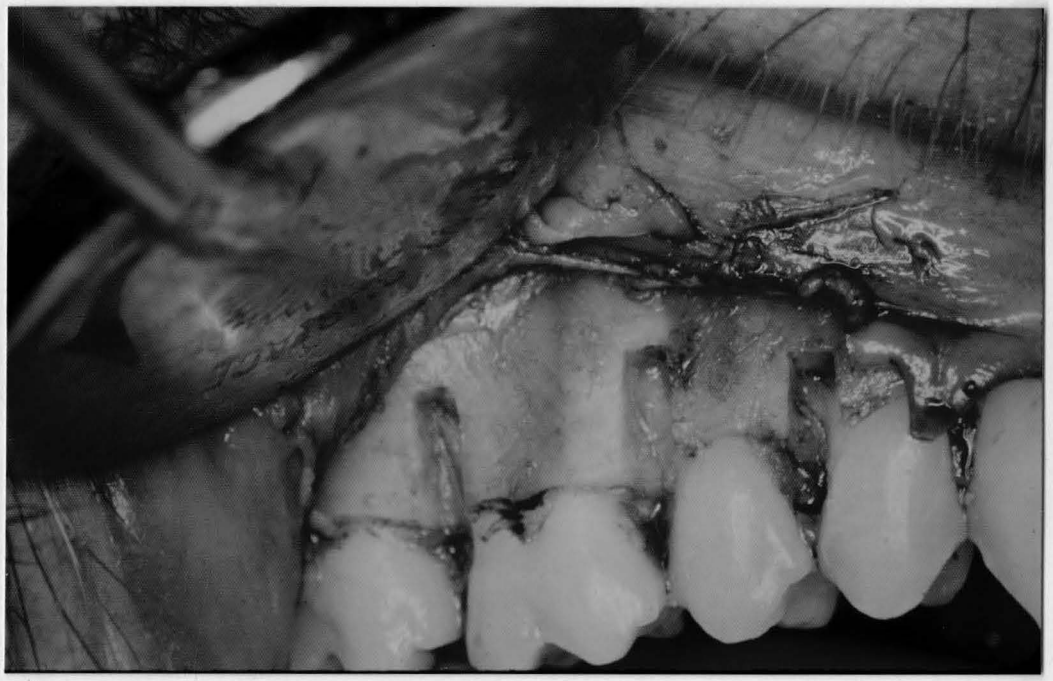

Figure 3: Experimentally created two-walled osseous defects distal to the first premolar (implant), second premolar (control) and first molar (implant).

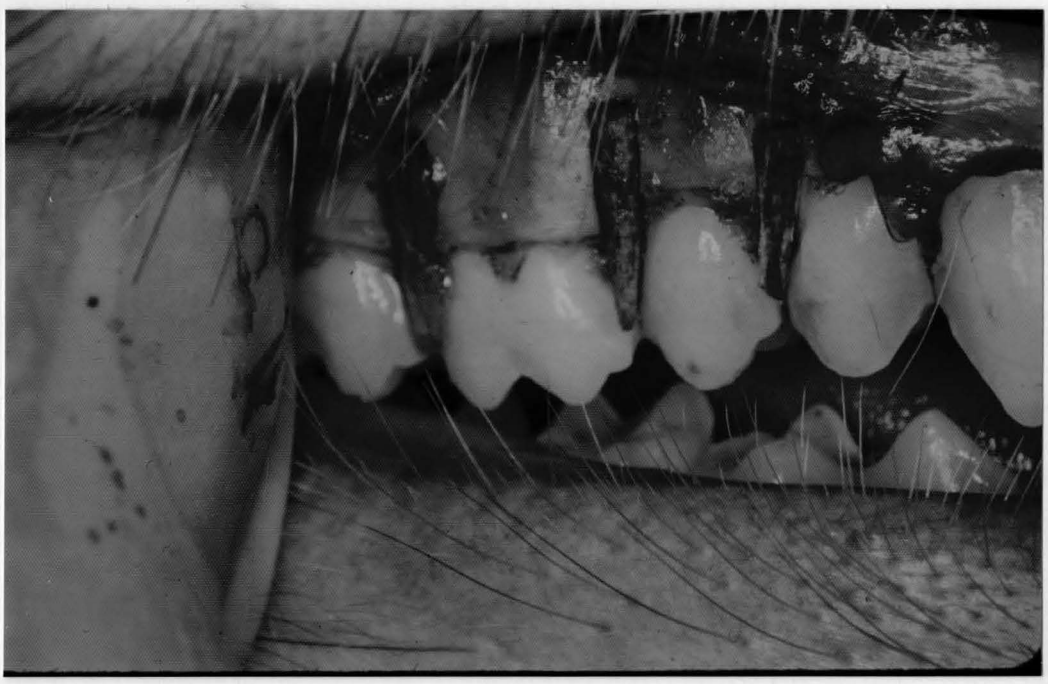

Figure 4: Introduction to the chronic irritant into each osseous defect.

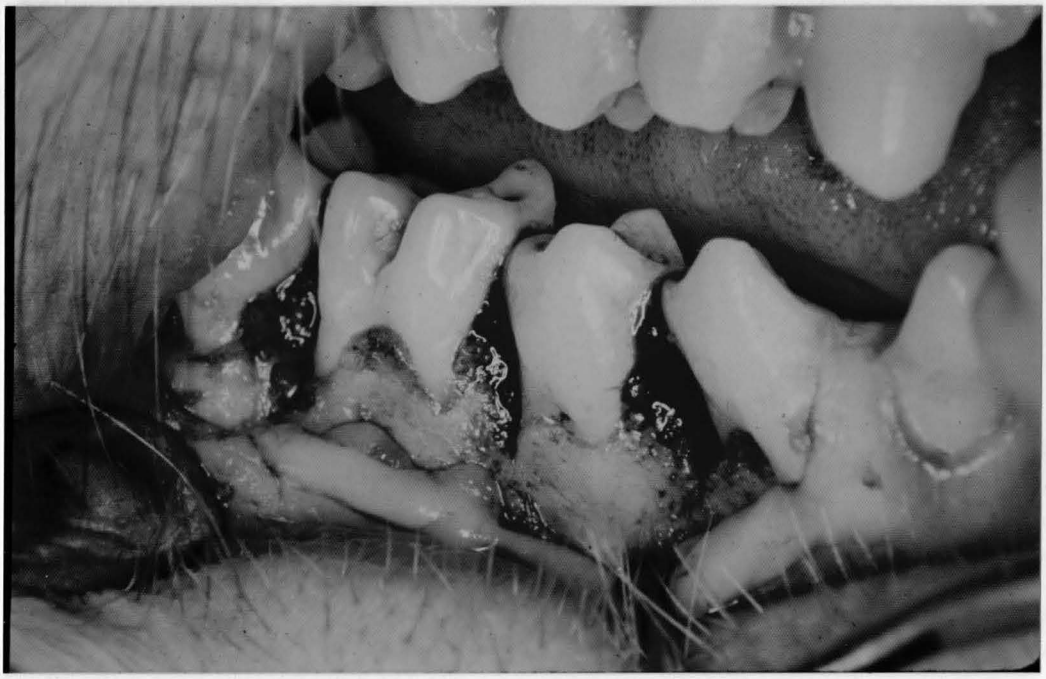

Figure 5: Thirty day postoperative appearance of the two-walled osseous defects.

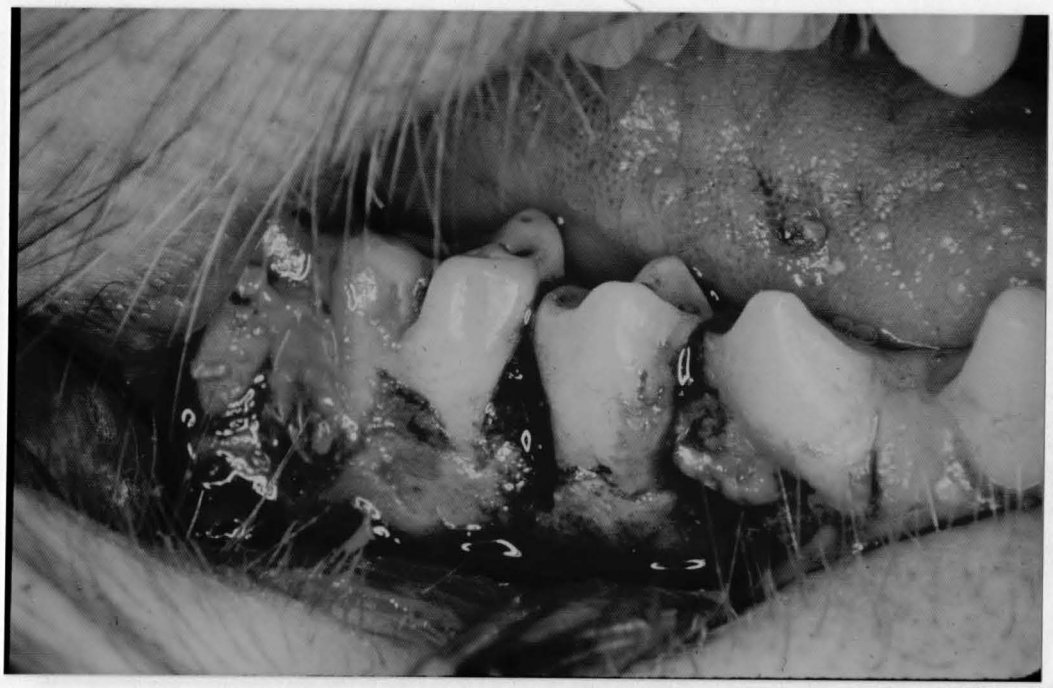

Figure 6: Placement of the Calscorbate-Medium 199 implant in the defect areas distal to both the first premolar and first molar with the control area distal to the second premolar.

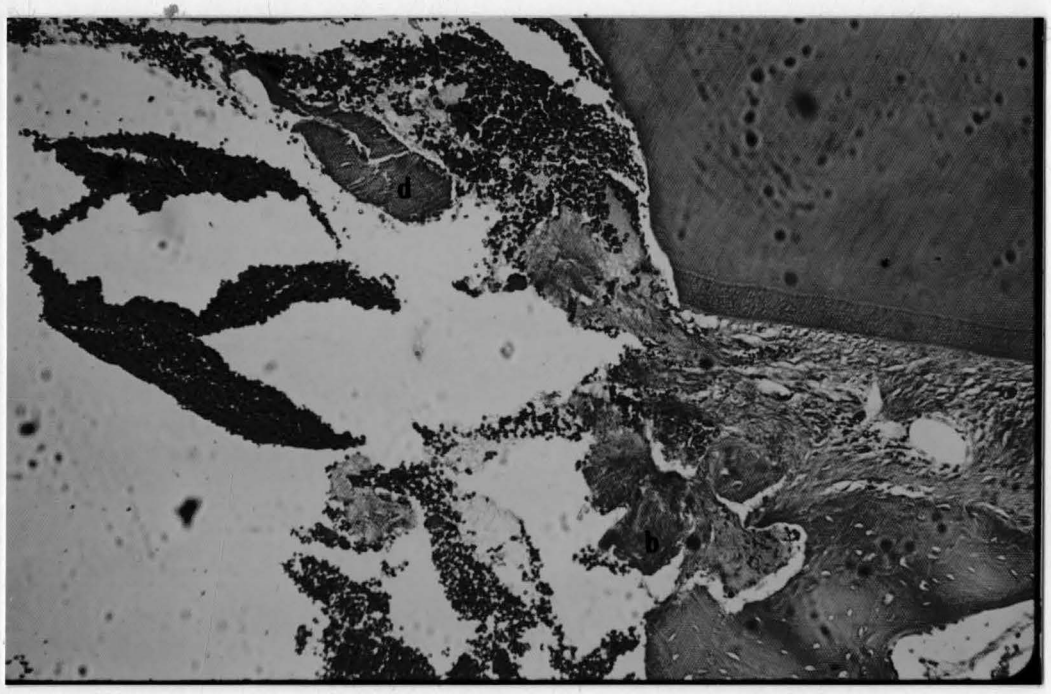

Figure 7: 0 day flapped control, 10X. Surgically created defect with fibrin clot debris showing pieces of dentin (d) and spicules of bone (b).

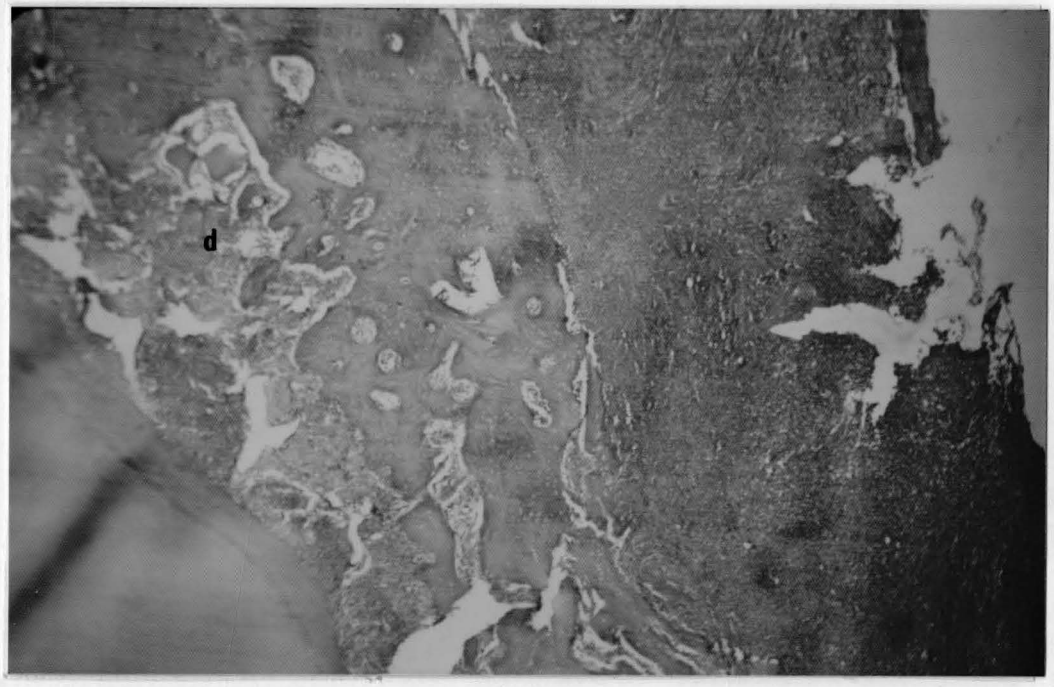

Figure 8: 3 day control, 4X. Tangential section showing the defect area (d), within mature bone.

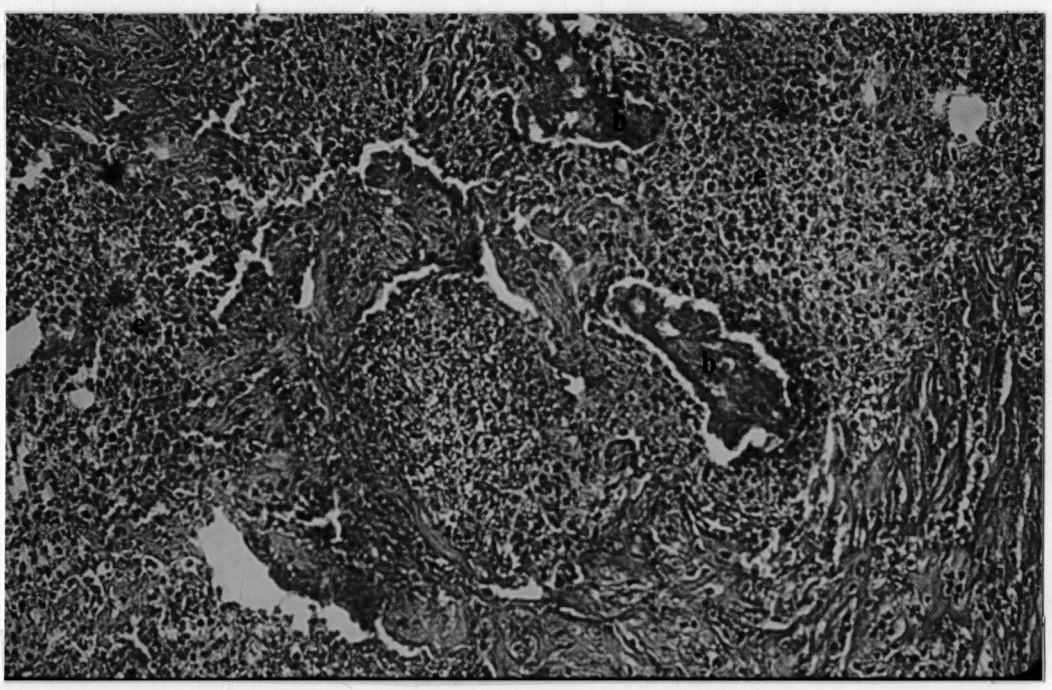

Figure 9: 3 day control, 20X. The defect area is filled with a fibrinopurulent exudate (e), and spicules of bone (b).

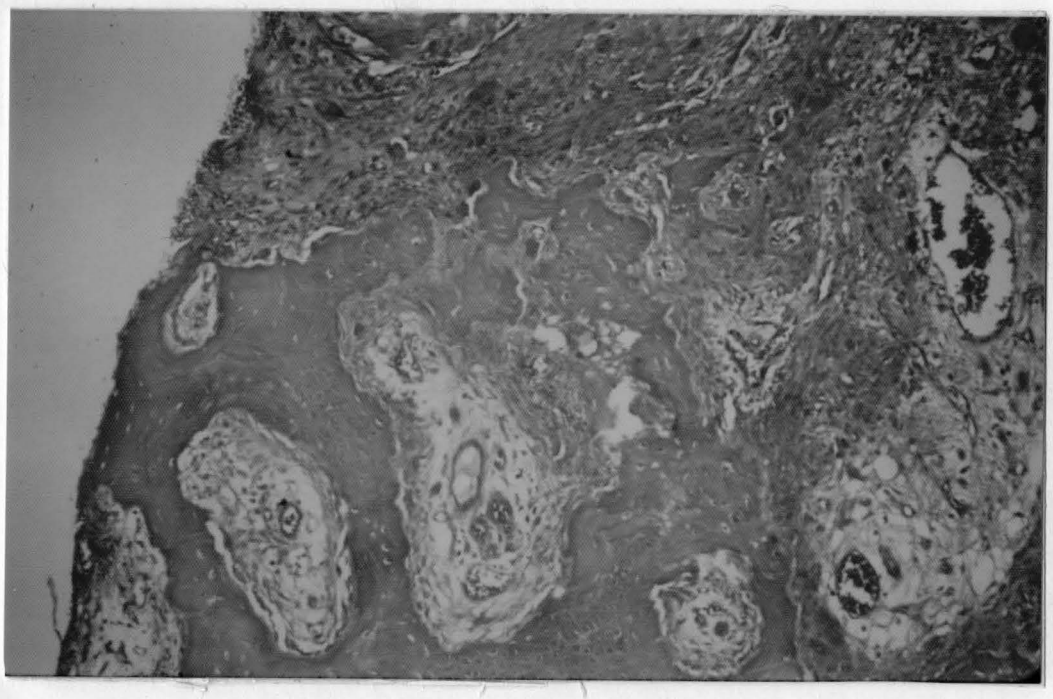

Figure 10: 7 day control, 10X. Mature bone with large marrow spaces. Repairative granulation tissue covers the crest of the bone.

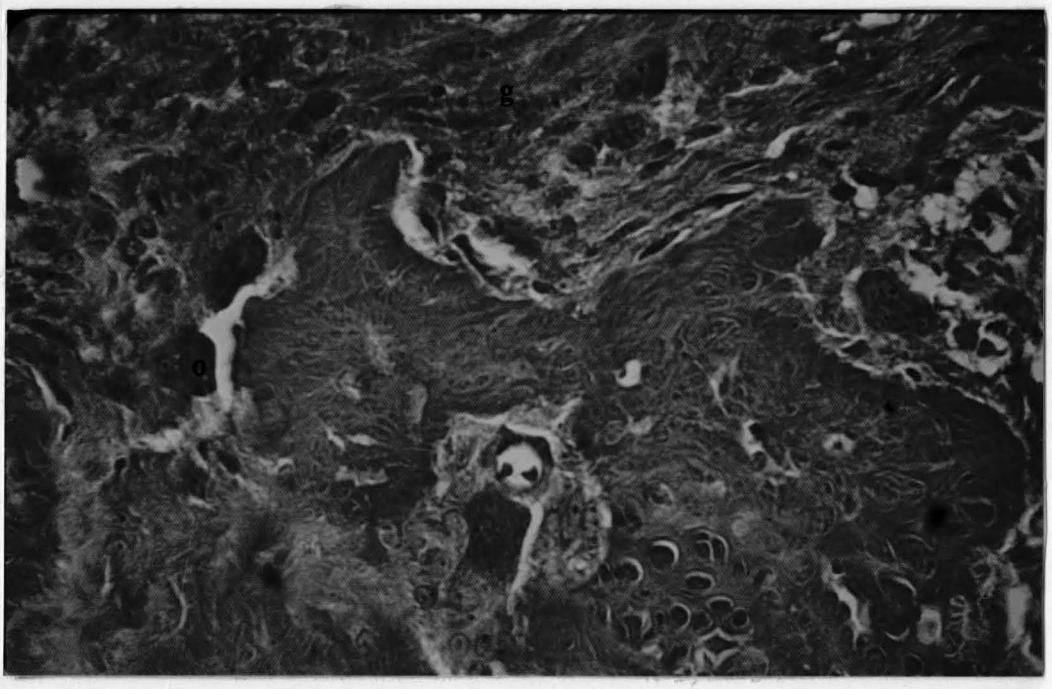

Figure 11: 7 day control, 45X. Osteoclastic resorption (o) within Howship's lacunae. Note the overlying repairative granulation tissue (g).

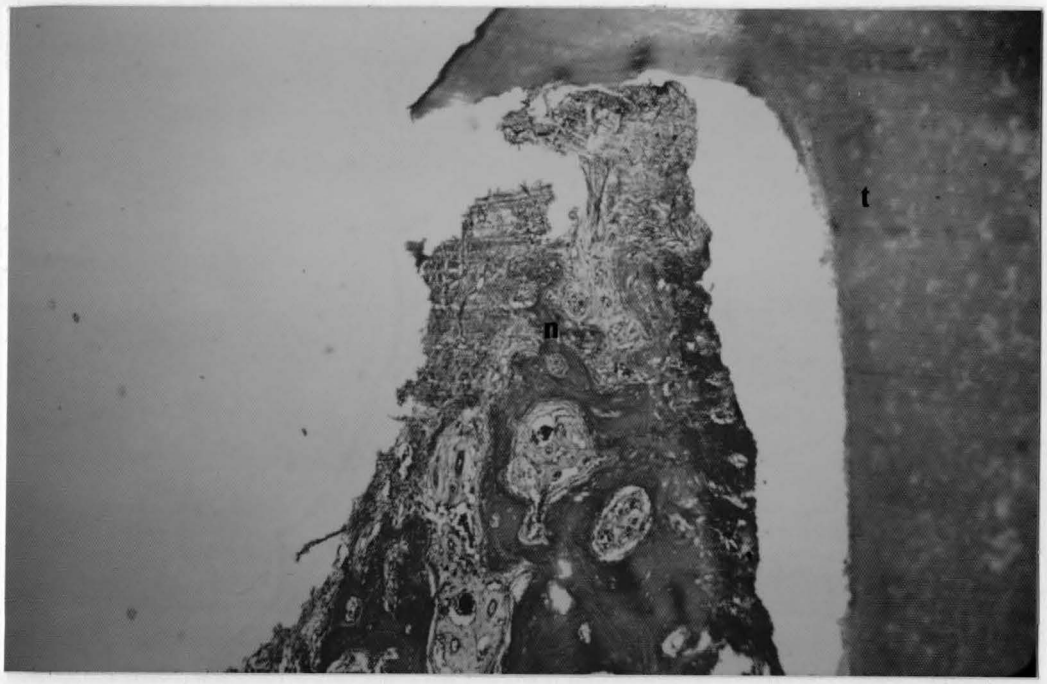

Figure 12: 14 day control, 4X. Apparent defect within the tooth (t). New bone formation (n).

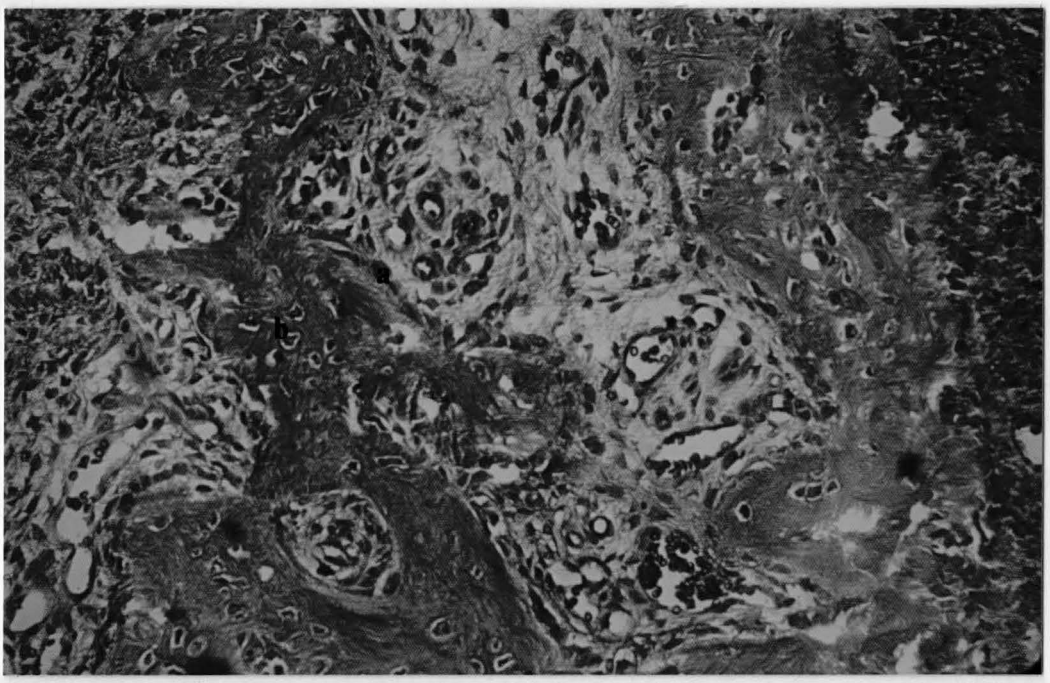

Figure 13: 14 day control, 20X. Osteoblastic activity (a) surrounding osteophytic bone (b).

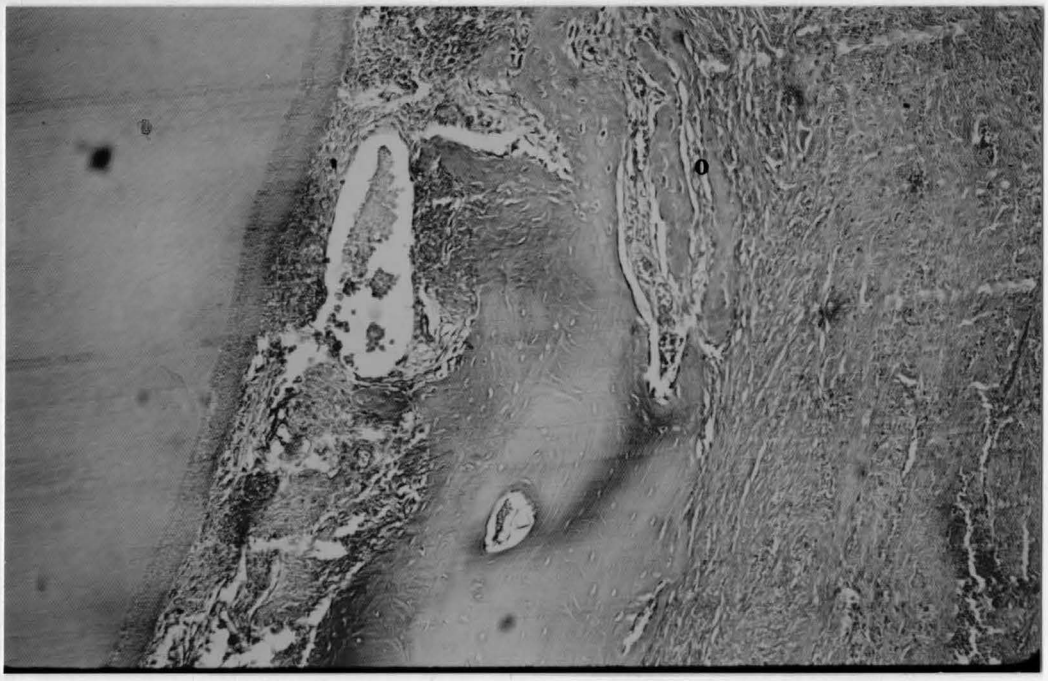

Figure 14: 21 day control, 10X. Osteoblastic activity (o) adjacent to more mature bone.

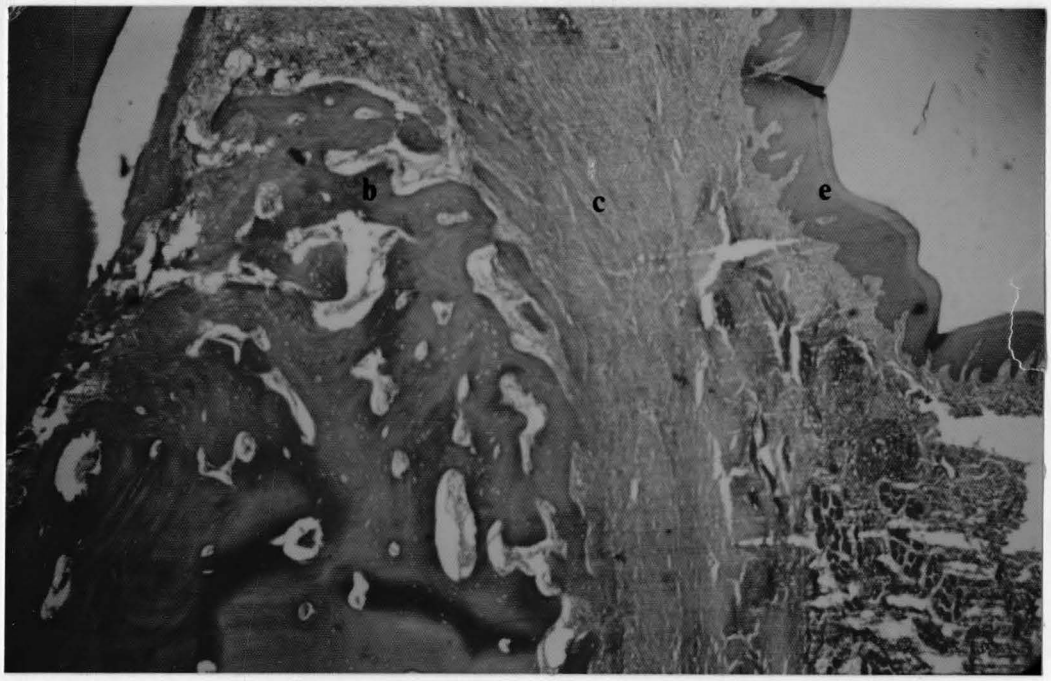

Figure 15: 28 day control, 4X. Keratinized stratified squamous epithelium (e), maturing fibrous connective (c) and osteophytic bone (b).

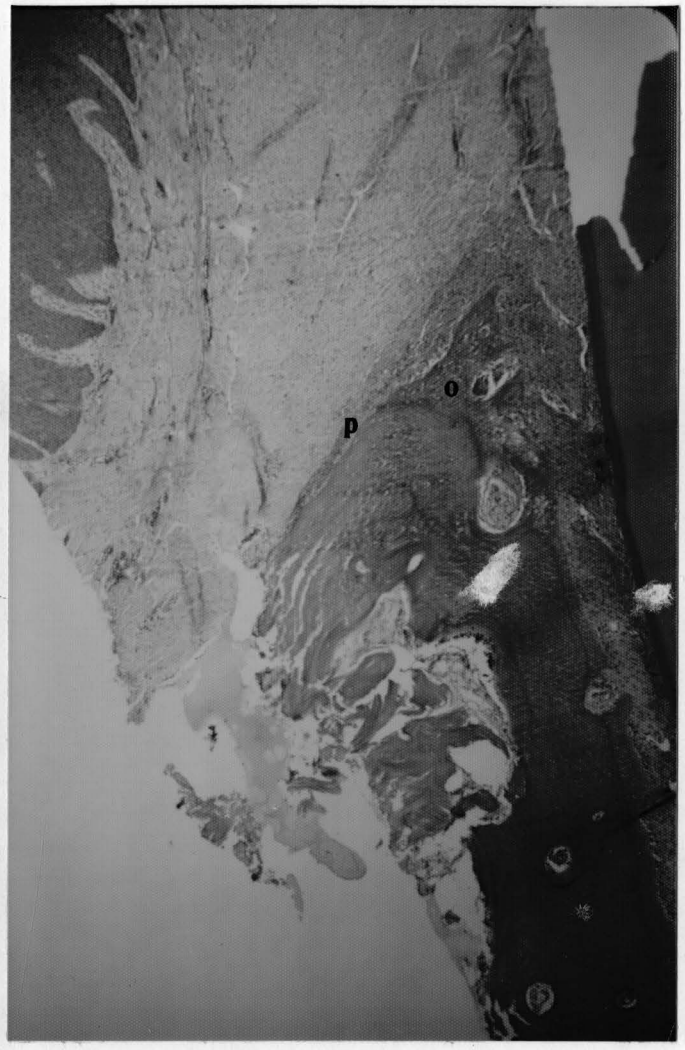

Figure 16: 42 day control, 4X. Moderately thick layer of periosteum (p) covering osteophytic bone (o).

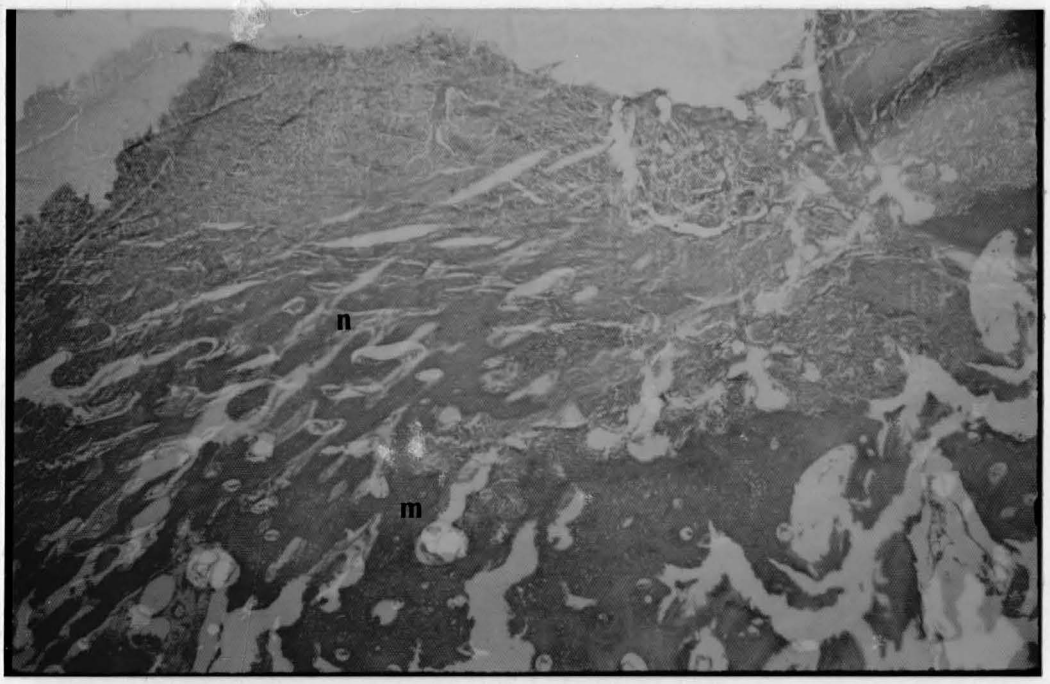

Figure 17: 56 day control, 4X. New bone (n) is seen covering more mature bone (m).

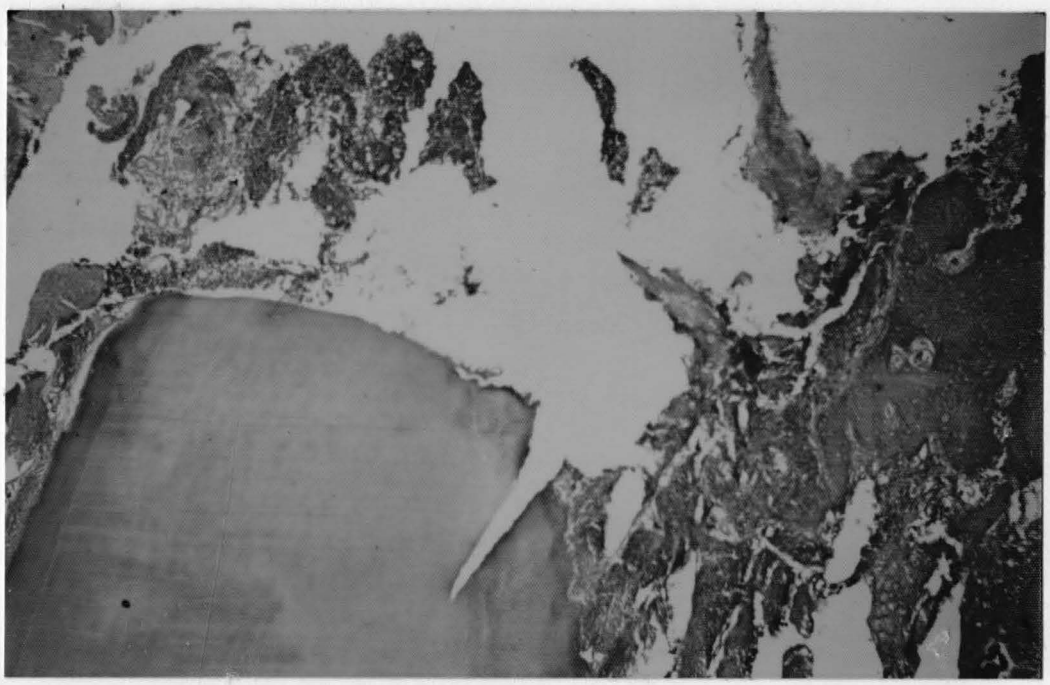

Figure 18: 0 day Calscorbate-Medium 199 implant, 4X. Surgically created defect with red blood cells, bone spicules and connective tissue.

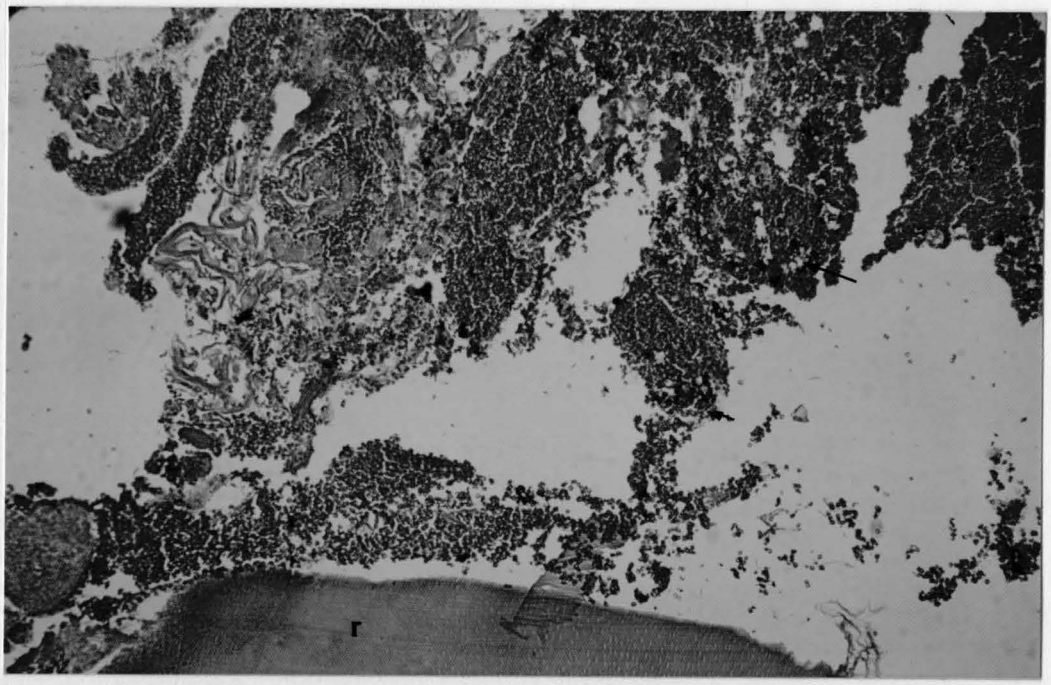

Figure 19: 0 day Calscorbate-Medium 199 implant, 10X. Adjacent to the root surface (r), note the yellow-black calscorbate implant material (arrow) within the red blood cells.

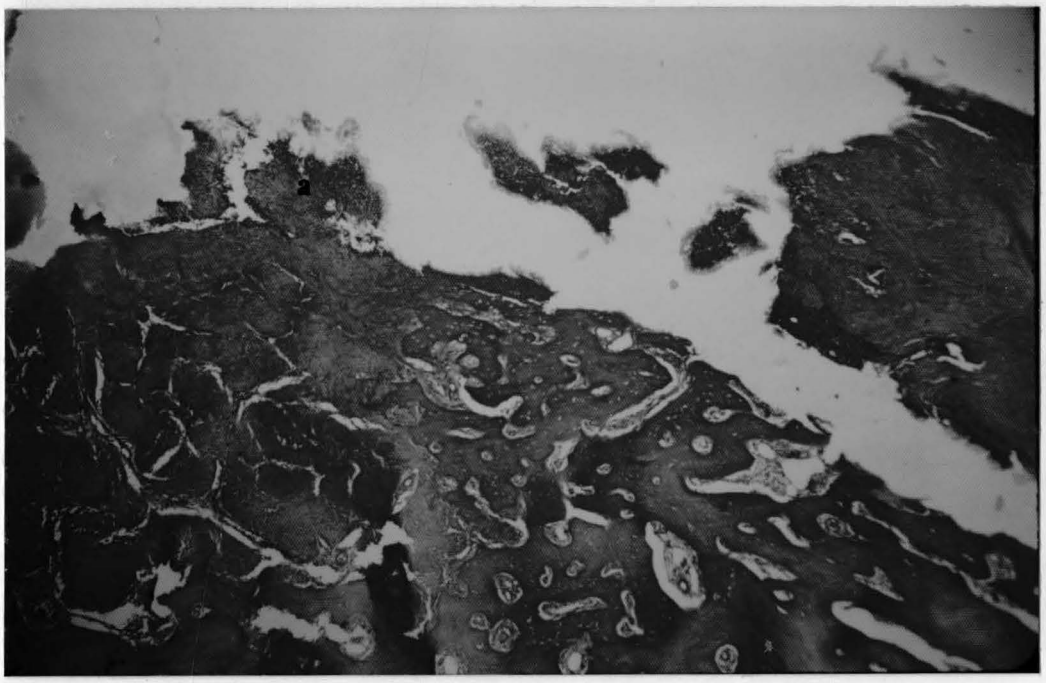

Figure 20: 3 day Calscorbate-Medium 199 implant, 4X. Acute inflammatory reaction (a) occurring on the surface of the connective tissue and bone.

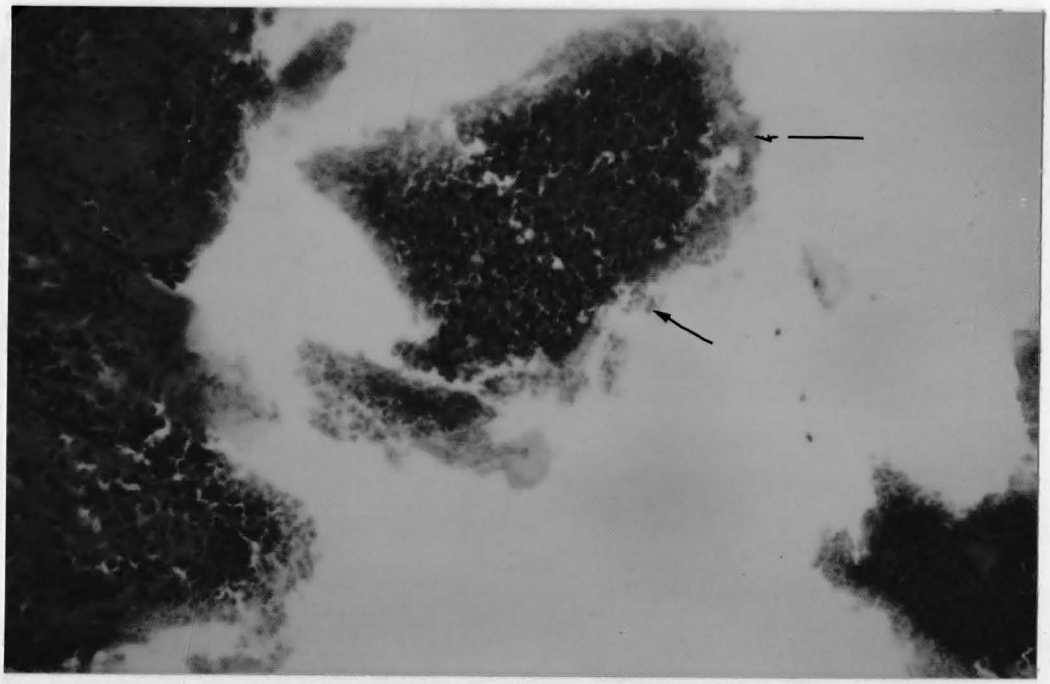

Figure 21: 3 day Calscorbate-Medium 199 implant, 20X. Poorly defined blue-grey material (arrow), probable Medium 199 implant, surrounding dense fibrinopurulent exudate.

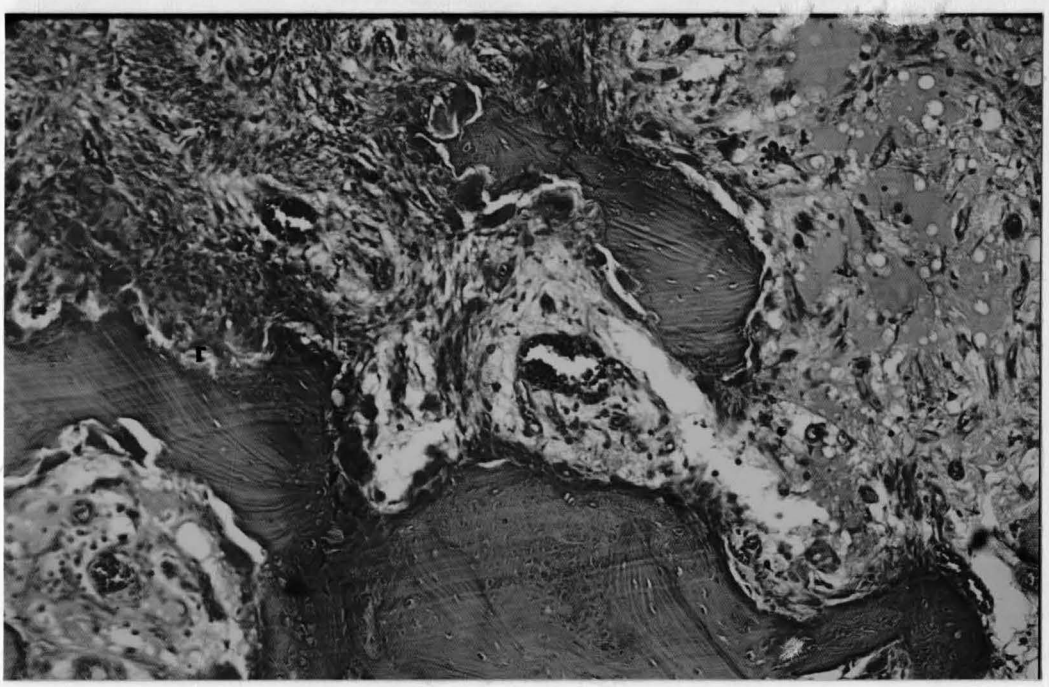

Figure 22: 7 day Calscorbate-Medium 199 implant, 20X. Extensive osteoclastic resorption (r) is seen within the defect area.

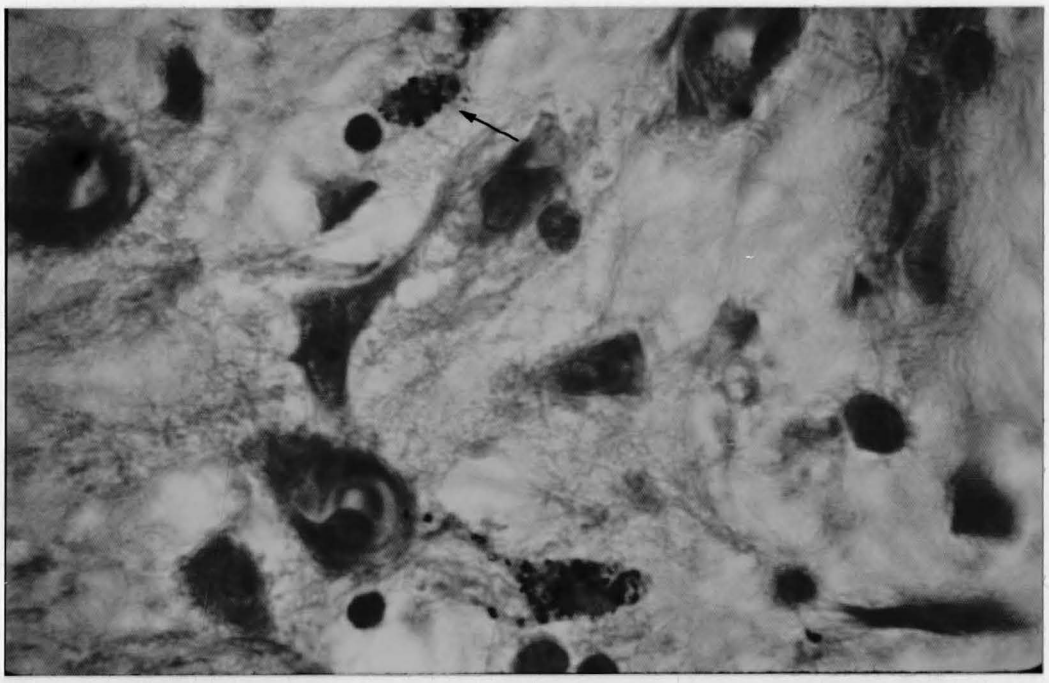

Figure 23: 7 day Calscorbate-Medium 199 implant, oil immersion. Probable implant material being engulfed by macrophages (arrow). Young capillaries and proliferating fibroblasts are seen.

Figure 24: 14 day Calscorbate-Medium 199 implant, 4X. Keratinized stratified squamous epithelium (e). Active osteogenesis (a) adjacent to mature bone.

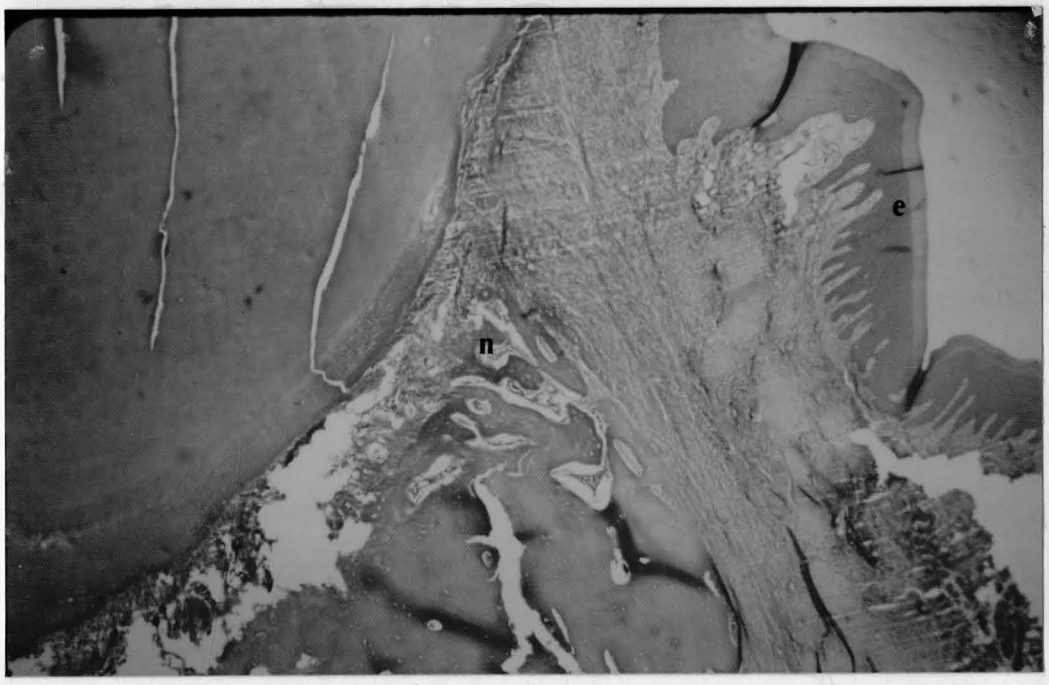

Figure 25: 28 day Calscorbate-Medium 199 implant, 4X. Keratinized stratified squamous epithelium (e) overlying maturing fibrous connective tissue. New bone formation (n) in the defect area.

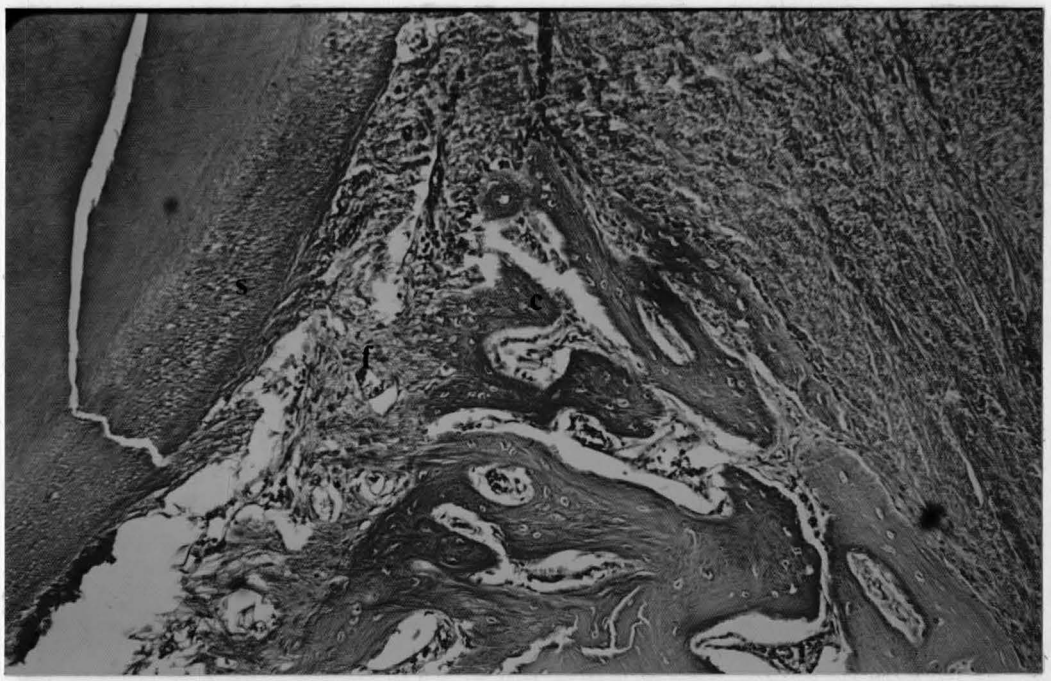

Figure 26: 28 day Calscorbate-Medium 199 implnat, 10X. Periodontal ligament fibers (f) and newly formed osteophytic crestal bone (c). Secondary dentin is evident (s).

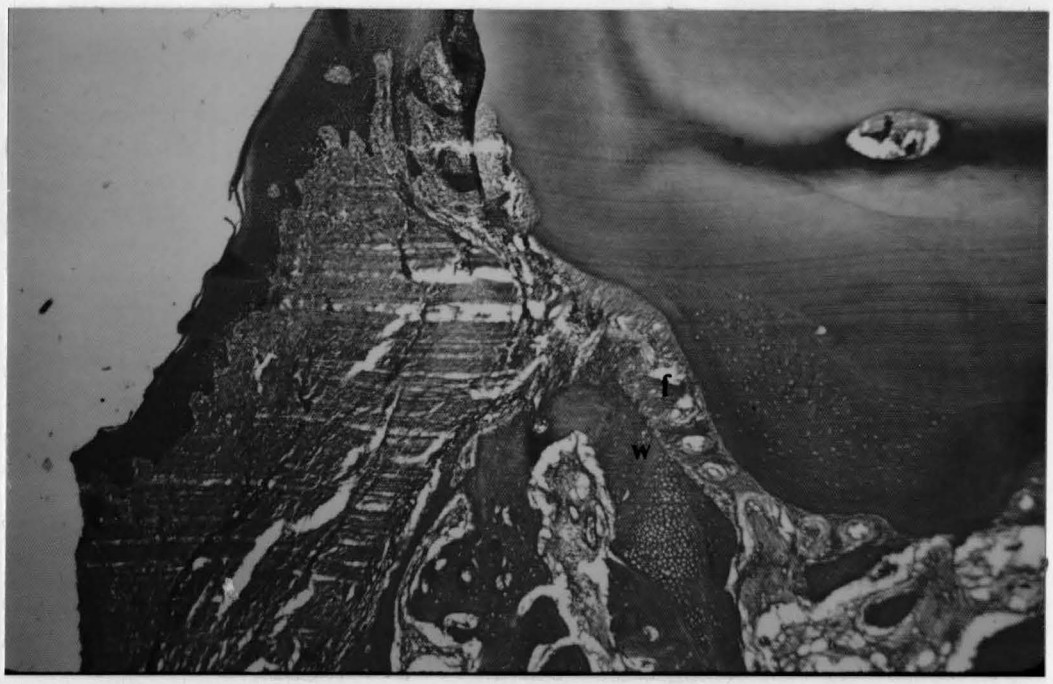

Figure 27: 42 day Calscorbate-Medium 199 implant, 4X. Periodontal fiber arrangement (f) adjacent to woven bone (w). A fibroperiosteum is seen covering the alveolar bone.

Figure 28: 56 day Calscorbate-Medium 199 implant, 4X. Keratinized stratified squamous epithelium supported by residual muscle fiber bundles (m). Osteophytic bone with continuing osteoblastic repair (r) is seen.

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APPENDIX I

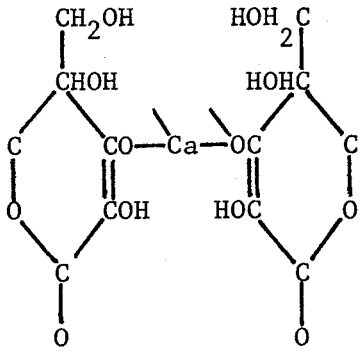
Calscorbate*

A. Each Calscorbate tablet contains:

- Calcium Ascorbate (100 mg.)
- Dibasic Calcium Phosphate (Hydrous) (1.08 gm)
- Vitamin D_2 (Calciferol) (50 U.S.P. Units)

B. Calscorbate chemically:

- Two moles Ascorbic Acid
- One mole Calcium
- $(C_6H_7O_6)_2$ Ca



(Calcium Ascorbate)

* Cole Pharmacal Company, St. Louis, Mo.

APPENDIX II

MEDIUM No. 199

L-Arginine	70.0	Riboflavin	.01
L-Histidine	20.0	Pyridoxine	.025
L-Lysine	70.0	Pyridoxal	.025
L-Tryosine	40.0	Niacin	.025
DL-Tryptophan	20.0	Neacinamide	.025
DL-Phenylalanine	50.0	Pantothenate	.01
L-Crystine	20.0	Biotin	.01
DL-Methionine	30.0	Folic Acid	.01
DL-Serine	50.0	Choline	.5
DL-Threonine	60.0	Inositol	.05
DL-Leucine	120.0	p-Aminobenzoic acid	.05
DL-Isoleucine	40.0	Vitamin A	.1
DL-Valine	50.0	Calciferol (D)	.1
DL-Glutamic acid	150.0	Menadione (K)	.1
DL-Aspartic acid	60.0	a-Tocopherol phosphate (E)	.01
DL-Alinine	50.0	Ascorbic acid	.05
L-Proline	40.0	Glutathione	.05
L-Hydroxyproline	10.0	Cholesterol	.2
Glycine	50.0	Tween 80 (oleic acid)	20.0
Cysteine	.1	Sodium acetate	50.0
Adenine	10.0	L-Glutamine	100.0
Guanine	.3	Adenosine triphosphate	10.0
Xanthine	.3	Adenylic acid	.2
Hypoxanthine	.3	Ferric nitrate	.1
Thymine	.3	Ribose	.5
Uracil	.3	Deoxyribose	.5
Thiamin	.01		

This medium also contains a balanced salt solution (Hanks). The above values are milligrams per 1000 ml.

HANKS SALT SOLUTION
(Grams per litre)

NaCl	8.0	Na_2HPO_4-$2H_2O$	.06
KCl	.4	KH_2PO_4	.06
$CaCl_2$	.14		
$MgSO_4$-$7H_2O$	.1	Glucose	1.0
$MgCl_2$-$6H_2O$	.1	Phenol Red	.02

Taken from: Paul, J.: Cell and Tissue Culture. Livingstone Ltd., London, 1959. pp. 68 & 74.

APPENDIX III

Graft Terminology

Former	Present	Definition
Autograft	Autograft	Same Individual
Isograft	Synograft	Identical Twins
Homograft	Allograft	Within Same Species
Heterograft	Xenograft	Different Species
	Alloplast	Bone Substitute

APPROVAL SHEET

This thesis, submitted by Lindsay B. Eastman, D.D.S., has been read and approved by the following committee:

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The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

4-14-80
Date

Signature of Advisor