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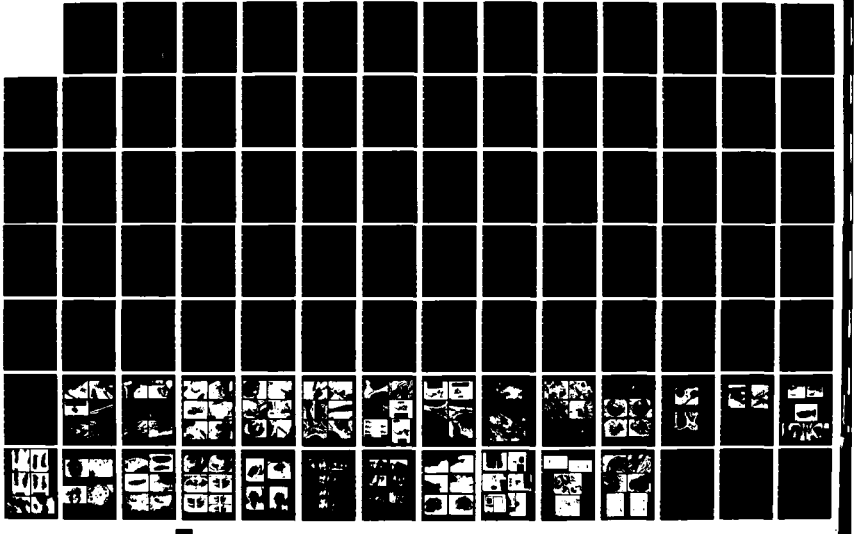

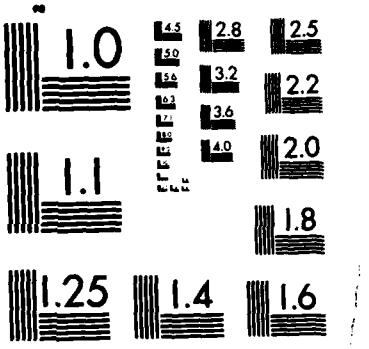

FINAL TECHNICAL REPORT

ONR Contract N00014-82-K-0305

REVASCULARIZATION IN MAXILLOFACIAL BONE HEALING

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REVASCULARIZATION IN MAXILLOFACIAL

BONE HEALING

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Callus formation in fracture healing is initiated by cell proliferation and capillary sprouting from the periosteum, bone marrow and surrounding connective tissues. The purpose of this investigation was to elucidate factors crucial to revascularization, the key event in fracture or bone defect repair. Their delineation could modify current treatment modalities to encourage bone revascularization during

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the reversible ischemic phase of injury; this would result in enhanced healing and faster recovery. Experimental bony defects were produced in monkeys, rabbits and rats. The principal model was a nonhealing defect drilled through the relatively avascular ramus of the mandible of the rat. Infiltration with fibrovascular tissues, revascularization, and subsequent ossification of the defects was assessed after implantation with plaster of Paris alone, demineralized bone, and ceramic hydroxylapatite particles with various binders such as bovine serum albumin, collagen or plaster of Paris. Revascularization was also studied in specimens from humans where ceramic hydroxylapatite had been used clinically for mandibular ridge augmentation and in specimens from monkeys where it has been used experimentally with plaster of Paris to achieve more adequate ridge augmentation than can be achieved when hydroxylapatite is wetted with saline. The revascularization and repair of mandibular defects implanted with ceramic hydroxylapatite is clearly promoted by a nonimmunologic foreign body granuloma which aids incorporation of the implant particles. The use of plaster of Paris not only aids implant formation and containment of the hydroxylapatite particles but contributes to the angiogenic and osteogenic effects of the implants. Hyperbaric oxygen also markedly enhances osteogenesis and probably revascularization by the implants.

Plaster appears to be osteogenic and over a period of months, many of the particles close to the host bone become incorporated into new cancellous bone.

These studies in our laboratory have shown that plaster of Paris is a useful binder for the containment of ceramic hydroxylapatite particles for bone repair. These studies have also shown that plaster binders permit the implantation of particles in several different modes. First, preformed implants can be tailored externally. The hydroxylapatite/plaster of Paris (HA/PP) alloplast can be preformed, sterilized and implanted in a subsequent surgical procedure. Alternatively, the implant consisting of hydroxylapatite and plaster can be tailored or formed at surgery directly in the defect or augmentation site. This approach receives support from our finding that the plaster component of these composite implants is slowly resorbed by the body. It is replaced, however, at the same rate by the infiltration of resilient fibrovascular tissue. This tissue not only maintains the form of the implant, but its vascular and collagen components appear to promote healing and ossification of the ceramic hydroxylapatite particles. The use of these composite implants precludes the difficulties encountered when plaster alone is employed (resorption) or when hydroxylapatite alone is used (particle scatter or nonpenetration by host vascular and connective tissue) for bone repair.

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II. SUMMARY REVIEW OF SCIENTIFIC ACCOMPLISHMENTS

1) Development of the PATS reaction. This reaction demonstrates the type III collagen of the pericapillary reticular network which acts as a scaffold for revascularization in bone healing. With this reaction the microvasculature can be readily observed in demineralized tissues where the classical histological and histochemical stains won't work. An example would be in tissues demineralized under conditions required to remove sintered hydroxylapatite.

2) Plaster of Paris alone, or composite implants consisting of calcium ceramic particles held together by various binders are more efficient than demineralized bone in promoting extensive revascularization and filling by fibrovascular tissue and bone of a 4 mm defect drilled in the relatively avascular ramus of rat mandible. Plaster of Paris alone is more effective than the ceramic particles alone in promoting ossification of the healing defect.

3) A new surgical implantation procedure has been devised for these composite plaster/hydroxylapatite implants which is far superior to any procedure previously used for implantation of either plaster or HA. This process precludes the difficulties encountered when plaster alone is implanted (resorption) or when HA alone is used (particle scatter or nonpenetration by host vascular and connective tissue).

4) Ceramic particles, much more than the demineralized bone particles, become encapsulated by collagenous reticular fibers as a result of a nonimmunologic foreign body reaction which promotes infiltration of the defects by connective tissue cells, blood vessels, and fibrovascular tissue.

5) Extensive incorporation of ceramic implant particles into host bone was observed in rodents and monkeys. The preservation of periosteum was also important in this regard.

6) The absence of infection and inflammatory cells such as neutrophils, monocytes, macrophages and lymphocytes and the presence of large numbers of fibroblasts, as well as multinucleated giant cells and collagen in the capsules surrounding implant particles, suggest that a reparative granuloma aids incorporation of the implants and healing of the defects.

7) Plaster of Paris is bioresorbable and becomes replaced at the same rate by resilient fibrovascular tissue which holds the calcium ceramic particles together. The formation of new cancellous bone on the surface of the ceramic particles was noted where the implants were in contact with host bone.

8) The composite ceramic alloplasts consisting of a mixture of equal parts of hydroxylapatite (HA) and plaster (PP) could be formed and tailored to fit the implantation site either internally or externally. These implants could be reinforced by, and screwed into place with the assistance of, metal (or plastic) wires, mesh or bars.

9) Mixtures of moist calcium ceramic particles and HYDROCAL C base (a dense plaster) could be injected into subperiosteal tunnels in monkeys (after experimental radical alveolectomy and healing) and man to achieve better contour and greater vertical ridge augmentation than could be achieved with HA in saline or water. In Class III or Class IV ridge deficiencies, the need for

additional surgery to obtain autologous iliac bone or rib grafts can apparently be eliminated.

10) The rate of setting of the HA/PP mixtures could be adjusted by the addition of potassium sulfate depending on the reconstructive surgical procedure and the surgeon's preference.

11) Tricalcium phosphate ceramic particles, which are much less slowly resorbed than plaster, can be added to the composites to furnish a constant slow release of calcium ion during the healing process. This apparently stimulates osteogenesis (and could possibly enhance reparative dentine formation).

12) The addition of even even small amounts of calcium hydroxide to the composite implants raises the pH sufficiently to have a bactericidal effect on oral microbes.

13) In addition to the use of these implants to support dentures and other prostheses, it appears they may have significant application in dentistry to restore contour. They could be used for very minor defects as well as for extensive reconstruction after resorption, trauma or tumor.

14) Fluoride administration to newborn mice resulted in significant increases in the amounts of enamel and dentin formed. Although differences in the amounts of alveolar bone could not be discerned, there was a significant decrease in the numbers of osteoclasts.

15) The effects of plaster on revascularization, and subsequent osteogenesis, were clearly shown by the implantation of experimental defects with plaster alone. A more dramatic increase of new cancellous bone resulted than observed when the defects were filled with any other material or combination of materials.

16) Methods have been devised to sterilize prepackaged HA/PP components, mixtures and preformed implants to repair bone defects.

17) Preformed implants sterilized by ethylene oxide were found to have greater tensile strength than those sterilized by dry heat.

18) Blood was found to have certain properties superior to water when employed as the setting fluid for HA/PP implantation for alveolar bone augmentation especially for periodontal defects. With this procedure, the particles can be packed very close together before setting even though the initial HA/PP mixture contains only 50% HA and is very malleable.

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IV. IMPORTANT FACTORS IN BONE REVASCULARIZATION

A. Oxygen Tension

The first morphologically visible step in most cell injury is cloudy swelling. The cloudiness or granularity of the cytoplasm is caused by swelling and disruption of mitochondria due to hypoxia. Since mitochondria are the centers of aerobic oxidation, it is not surprising that they should show the first signs of injury due to hypoxia.

Increased oxygen tension has been shown by Persson (1968) to increase bone growth; changes in vascularization would probably affect bone primarily via oxygen tension although effects on the delivery of crucial factors and nutrients could be important. Inadequate oxygen tension is also necessary for fibroblast proliferation and collagen formation as well as osteogenesis. Adequate oxygen tension is also required for cidal activity against aerobic and anaerobic organisms which are enhanced in ischemic or avascular tissues (Kerley et al., 1981).

Kapp (1981) has reported that hyperbaric oxygen can be important as an adjunct to revascularization in the surgical treatment of occlusion of cerebral arteries.

It is important to realize however that immature vessels are more sensitive to oxygen than mature vessels perhaps because their endothelial cells have not been induced to synthesize the protective enzymes (Weiter, 1981).

B. Effects of Cells & Tissues

1) Cells

When a fracture occurs, an important early contribution of blood (principally for oxygen and nutrients) can come from the arterial system of surrounding tissues (especially muscle). Indeed, blood is considered the prime prerequisite for the "budding" of new capillaries from the preexisting microvasculature (Eriksson and Zarem, 1977). However, the importance of cells of different types in this process is also considerable. It has long been known that new blood vessels can be stimulated to grow only in the presence of tissue such as epithelium (Merwin and Algire, 1956; O'Donaghue and Zarem, 1971). Clark and Clark demonstrated (1939) that new capillaries arise from the preexisting microvasculature--arterioles, venules and capillaries--by endothelial sprouting. This gave rise to the dictum that endothelium begets endothelium. The continued elongation of new capillaries in wounds results principally from mitosis and prior migration of endothelial cells (Ausprunk and Folkman, 1977). The effects of other bone marrow derived or connective tissue cells may also be important, however. Thus activated macrophages (Polverini et al., 1977), lymphocytes (Auerbach and Sidky, 1979) neutrophils (Fromer and Klintworth, 1976) fibroblasts (Eriksson and Zarem, 1977), mast cells (Kessler et al., 1976), and platelets (Zawicki et al., 1981) and even eosinophils may have significant roles. Vracko (1972, 1974) has pointed out the significance of the capillary basement membrane for revascularization.

2) Tissues

A number of adult tissues, principally glandular, appear to stimulate capillary proliferation in host animals (Ausprunk, 1979). These tissues include anterior pituitary, ovary, corpus luteum, adrenal cortex, salivary gland and testis. Adult epidermis and lymph node also apparently induce angiogenesis. Most of this budding of the microvasculature is inhibited by methylprednisolone (Shubik et al., 1976).

3) Cell and Tissue Extracts

Angiogenesis by tumor tissue due to tumor angiogenic factor (TAF) and in a number of other tissues will be discussed under D. Effects of sex hormones and various angiogenesis factors from tumors and salivary glands.

C. Various Ions, Radicals, Biomolecules, Biogenic Amines and Nutrients (other than sex hormones and angiogenesis factors such as TAF discussed below).

1) General

The exudation of fluid, fibrin and debris of leukocytes and erythrocytes takes place into wounds. The sprouting and ingrowth of blood vessels is affected by various ions, radicals and nutrients. In addition, other aspects of healing have special requirements. Thus sulfur-containing amino acids are important for granulation tissue formation (Edwards and Dunphy, 1957). Capillary endothelium and collagen fibril formation, essential to revascularization, are decreased by ascorbic acid deficiency; the latter also increases capillary fragility (Levenson et al., 1957).

2) Corticosteroids

The corticosteroids and ACTH in large concentrations retard wound healing (Altura and Altura, 1974). They dramatically inhibit neovascularization in addition to the proliferation and migration of fibroblasts; they also interfere with the margination and emigration of leukocytes. Vascularization of skin grafts requires twice as much time in steroid-treated animals.

3) Biogenic amines, polypeptides, heparin and proteases including collagenase

Increased vascular permeability in inflammation due to injury is mediated by the biogenic amines histamine and serotonin, the polypeptides such as bradykinin, and possibly proteases (Wilhelm, 1962). To the extent that the endothelial barrier is affected by the formation of clefts between the endothelial cells, there is no doubt that revascularization is also affected. The mediation by histamine of estrogenic stimulation of the rat uterus resulting in vasodilation and capillary permeability is attributed to the action of histamine released in situ by estrogen (Spaziani and Szego, 1959).

The importance of the autonomic innervation in control of the microcirculation is obvious from the vasomotor responses that can be elicited in rich perivascular nerve plexuses in many tissues. These neurogenic components, which contribute to regulation of the microcirculation, could also be expected to have important effects in revascularization in tissue injury

(Fulton et al., 1961). Eosinophils, mast cells, macrophages and even endothelial cells may be involved with heparin and collagenase metabolism. Collagenase, as well as other proteases, may be activated by heparin (Kessler et al., 1976). These proteases could facilitate angiogenesis by modifying stroma and enabling new capillaries to grow through it.

D. Effects of Sex Hormones and Various Neoangiogenesis Factors from Tumors and Salivary Glands

1) Sex Hormones in Wound and Bone Healing

Lindhe and co-workers (1968 a,b,c) examined the effects of different sex hormones on the vascularization of granulation tissue in wound healing in rabbits. After the injection of 500 I.U. of chorionic gonadotropin in female rabbits, there was a marked increase in the number of functioning vessels as well as an increased flow volume and flow rate in these vessels.

The retardation of granuloma formation in wounds in chronic alcoholism (Benveniste and Thut, 1981) and fracture repair in diabetes mellitus (Rosen and Enquist, 1961) could be mediated through effects on the gonads and the hypothalamic-pituitary axis (Van Thiel et al., 1974; Hanker, et al., 1980). It is expected that dramatic differences observed in the autonomic innervation of submandibular gland due to the effects of hormones and diabetes (Hanker et al., 1975, 1980; Carson et al., 1982) might be paralleled by the vasculature of the gland.

2) Tumor Angiogenesis Factor (TAF), Similar Salivary Gland and Miscellaneous Factors

a) TAF

These humoral factors (Folkman and Cotran, 1976), with molecular weights of approximately 200 (Weiss et al., 1979; McAuslan and Hoffman, 1979), are potent in vivo stimulants of neoangiogenesis. They have been characterized primarily in tumor cells where they are present in large amounts. They are probably not unique to malignant cells.

It has been known since the beginning of the century (Goldman, 1907) that extensive formations of new blood vessels surround tumor implants. This results from the continually increasing metabolic needs of the expanding neoplasm. Similar extensive proliferations of capillaries accompany the normal cyclic growth of the uterine mucosa, chronic inflammation, and wound-healing (Ausprunk, 1979). Ausprunk and Folkman (1977) have demonstrated that capillaries proliferating in response to a tumor implant elongate by the same mechanism as wound-induced vessels. In either case, mitosis of the endothelial cells is accompanied by migration of these cells toward the angiogenic stimulus or factor.

b) Salivary factors

Factors resembling TAF which stimulate angiogenesis have been described by Jeney and Toro (1935) in human saliva. Ito (1960) reported a protein factor from bovine parotid gland which induces vascular proliferation. Endothelial growth stimulating factors of molecular weight ca. 80,000 have been isolated

from bovine parotid gland and mouse submaxillary gland (Hoffman et al., 1976); they cause the enlargement of the vascular bed in a number of tissues. The effects produced by these bovine and murine salivary gland factors on the capillary bed, when injected into rats, are strikingly similar to those produced by TAF from Walker carcinoma cells (McAuslan and Hoffman, 1979). They all promote capillary outgrowth and neovascularization.

c) Miscellaneous Factors

Other factors with angiogenic activity have been described in hamster epidermis (Wolf and Harrison, 1973) and retina (Glaser et al., 1980). The presence in normal tissues of substances with a similar biological action to TAF suggests that they may have a significant role in the development and maintenance of the normal vasculature. Low molecular weight angiogenic factors (possibly identical) isolated from both Walker carcinoma cells and mammalian salivary glands have been shown to contain ionic copper (McAuslan et al., 1980). Pure copper salts such as the copper salicylates, copper sulfate and anti-inflammatory drugs which promote endothelial migration, such as indomethacin and aspirin, are also angiogenic. It is noteworthy that aspirin induces neovascularization in mice well within the dose range used therapeutically in man. It is believed that these compounds (including copper sulfate) induce endothelial cell motility and neovascularization as a consequence of inhibiting or modulating prostaglandin synthesis by endothelial cells. Copper metabolism appears to be important for tissue angiogenesis.

Fibroblast growth factor (FGF) is a neurotrophic peptide agent isolated from brain tissue (Gospodarowicz et al., 1978 a,b,c) that has profound implications for the initiation of the wound healing process. Most tissues are innervated by myelinated fibers which release FGF when injured. FGF is mitogenic for myoblasts and chondrocytes as well as a potent stimulant for the growth of vascular endothelial cells in vivo and in vitro. It also stimulates Schwann cell and glial proliferation and there is some suggestion that endothelial cell stimulation may be dependent on the neurotrophic effect. Both FGF and epidermal growth factor (EGF) have been shown to have in vivo neoangiogenic activity (Gospodarowicz et al., 1979) and topical application of EGF has been shown to facilitate healing of skin wounds (Franklin and Lynch, 1979).

3) Angiogenic Capacity of Prostaglandins

Ben Ezra (1978) reported that prostaglandin-E_1 has very strong angiogenic activity -- ten times that of epidermal growth factor. Prostaglandins-E_2 and the F series were much weaker. PGA_1 and PGD_2 were completely inactive. These results receive support from the fact that PGE is produced by cells normally involved in neovascularization such as macrophages, endothelium, platelets and neoplastic cells (Gullino, 1981).

E. The Significance of the Basal Lamina, Especially its Reticulin Component, as the Scaffold for Regeneration of Injured Capillaries

Experiments in our laboratory in conjunction with Beverly Giammara have shown that our PATS stain (periodic acid-thiocarbohydrazide-silver methenamine) is probably staining most intensely the fibronectin of the reticulin component of the basal lamina. This staining appears to be especially prominent in areas

of neoangiogenesis (e.g. breast tumor). These reticulin fibers, constituting the pericapillary reticulin network, support capillaries and their endothelial cell layer. Vracko (1972, 1974) has shown that in areas of ischemia or injury, the basal lamina tends to remain intact and acts as a scaffold upon which the regeneration of pericytes and endothelial cells occurs. In our preliminary studies with the PATS stain in mandibular injury, the reticulin fibers of the capillary basal lamina appear to dramatically demonstrate this function.

Γ. Hydroxylapatite (Calcitite), A Ceramic Bone Implant Material

Work over the past decade (reviewed by Jarcho, 1977, 1981) has resulted in the development of hydroxylapatite, a biocompatible ceramic hard tissue implant. When implanted into bone, this material lacks local or systemic toxicity or an inflammatory or foreign body response. It becomes strongly bonded to bone, without intervening fibrous tissue between host bone and implant. It appears to become an integral part of living bone tissue and is being used clinically for alveolar ridge augmentation.

V. COMPOSITE CERAMIC IMPLANTS FOR MAXILLOFACIAL BONE RECONSTRUCTION

Onlay bone grafting is generally more successful over the cranium than over the facial skeleton where the grafts may undergo progressive resorption (Knize, 1974). Both ilium and rib are widely used to augment or reconstruct mandible but success is variable (Baker et al., 1979; Kent et al., 1983). Although cancellous iliac bone is osteogenic and undergoes early revascularization, it is relatively quickly resorbed (Fonseca et al., 1980). Compact or cortical rib grafts are revascularized slowly, are not osteogenic, and are susceptible to dehiscence and infection (Terry et al., 1974). Donor site complications can occur with both types of grafts. This has led to the study of allogeneic bone but dehiscence, resorption and immunogenicity can be problems (Kelly, J.F. and Friedlaender, G.E., 1977).

The use of calcium bioceramic implants is potentially of considerable importance in preprosthetic oral and maxillofacial surgery (Bhaskar et al., 1971; Ferraro, Plast. Reconstruct. Surg. 63, 634-640, 1979; Jarcho, Clin Orthop. 157, 259-278 (1981); Kent et al., 1982, 1983; Uchida et al., 1984) especially in augmenting areas of the atrophic or deficient alveolar ridge. These materials are biocompatible and even stimulate osteogenesis to some extent but stabilization of these implants can be a problem. Presently available cementing methods are not the answer. On the other hand, variations in implant design can be important in achieving sufficient stability for the implant to withstand all mechanical demands placed upon it. Thus porosity of ceramic implant particles, which permits infiltration by fibrovascular tissues, can increase the shear strength of the host bone/implant interface region. This can be important in implant fixation (Welsh et al., 1971; Predecki et al., 1972).

The process developed in our laboratory enables the use of very dense (non-resorbable) ceramic implant particles inasmuch as the resorbable plaster component is a substitute for the pores. As the plaster is resorbed, it not only permits but promotes the infiltration of fibrovascular tissue and even new bone, to some extent, between the dense apatite particles. Thus this process precludes the difficulties encountered when plaster alone is implanted (resorption) or when HA alone is used (particle scatter or nonpentration by host vascular and connective tissue).

Perhaps the highest incidence of massive bone loss due to resorption, trauma or tumor occurs in the craniofacial region (Bajpai, 1983). Even though grafting of autologous bone (which requires additional surgery and results in additional morbidity) is currently the most widely used surgical procedure to correct these defects, the implantation of biomaterials appears to be the most promising approach for their reconstruction in the future (Bajpai, 1983).

Resorbable tricalcium phophate ceramic particles (Metsger et al., 1982) and nonresorbable sintered calcium phosphate (hydroxylapatite) particles (Kent et al., 1982, 1983) are commercially available and have been proposed for use in periodontal defects, the preservation of alveolar ridge forms, the augmentation of atrophic alveolar ridges and as an interpositional bone substitute in preprosthetic and orthognathic surgery. These materials are employed as slurries of particles in saline or distilled water although large solid cones of hydroxylapatite ceramic are available for rebuilding root sockets (Dennisen and de Groot, 1979). The use of the particles in saline or water presents a problem in containment. There is a tendency for the particles to stray from the implantation site and get trapped in other areas.

Recent studies in our laboratory (Hanker et al., 1983, 1984; Tucker et al., 1985; Hanker et al., in press) have shown that plaster of Paris is a useful binder for the containment of ceramic hydroxylapatite particles for implantation. These studies have shown that plaster binders permit the implantation of particles in several different modes. First, preformed implants can be tailored externally. The hydroxylapatite/plaster of Paris (HA/PP) alloplast can be supported by a metal (or plastic) wire, screen or bar if required. They can be screwed into place. Alternatively, the implant consisting of hydroxylapatite and plaster (and a metal support if required) can be tailored or formed directly in the defect or augmentation site. This approach receives support from our finding that the plaster component of these composite implants is slowly resorbed by the body. It is replaced, however, at the same rate by the infiltration of resilient fibrovascular tissue. This tissue not only maintains the form of the implant but its vascular and collagen components appear to promote healing and incorporation by new bone of the ceramic hydroxylapatite particles.

This newly devised surgical process employing plaster will enable the reconstruction of mandibles and maxillae without the need for secondary surgery to obtain a rib or ilium graft.

The fact that mixtures of HA and HYDROCAL C (a dense form of plaster) with predictable setting times can readily be formed in and administered by syringes simplifies some implantation procedures. However, a new procedure for tailoring and implanting plaster/HA devised by Dr. Bill C. Terry appears even more useful for rebuilding the deficient or atrophic alveolar ridge and other bony defects.

Another potentially very important use for these composite implants would be for alveolar ridge maintenance. Maintenance of ridge height and contour immediately after extraction could eliminate, or at least reduce, the severity of post-extraction atrophy or resorption. The large preformed implants of solid hydroxylapatite currently on the market for this purpose cannot conform to the extraction socket. By using plaster with HA particles, adaptation to the socket and the appropriate height and contour could be readily achieved.

It is expected that maintenance of this height and contour would be no problem because of the osteogenic nature of plaster. Although this project would be only of secondary importance to ridge augmentation, some implants for ridge maintenance will be performed if the appropriate need presents in any of our patients. A newly devised procedure, where blood is the only setting fluid employed in forming the HA/PP implant, might be especially useful in ridge maintenance as well as for infrabony periodontal defects.

Hydroxylapatite (HA) and Tricalcium Phosphate (TCP) Ceramics

At least 5 US manufacturers furnish nonresorbable sintered calcium hydroxylapatite, $Ca_{12}(PO_4)_6(OH)_2$ ceramics with particle sizes of approximately 20-40 mesh for mandibular ridge augmentation. Sizes of 40-60 mesh for filling periodontal defects are also currently marketed. A coralline hydroxylapatite, having 20-40 mesh particles with large 230 micron channels which apparently promote infiltration by new bone, (at first cancellous but remodelling to lamellar) is also available (Holmes, 1979).

All of these materials are biocompatible, stimulate the ingrowth of fibrovascular tissue and, to some extent and under certain conditions, appear to be osteogenic. The ability of histocompatible calcium hydroxylapatite and tricalcium phosphate (TCP) ceramics (with pore sizes greater than 100 microns) to serve as alternative implants to bone and act as scaffolds for the retention of autologous marrow autografts and for the ingrowth of new bone by "creeping apposition", in the case of nonresorbable apatites, and "creeping substitution" for the resorbable TCP ceramics, was suggested by Nade et al. (1982). This was confirmed by other investigators as well as in our own laboratory. The ideal apatite ceramic to be used in these composite alloplasts with plaster may not yet be commercially available. The appropriate ceramic may be a dense sintered hydroxylapatite with the physical form of the coralline hydroxylapatite (230 micron) channels. In addition it should be remembered that the plaster mixtures of the dense nonresorbable apatite ceramics may require the addition of the less dense resorbable ceramics (e.g., TCP) to act as a source of calcium (after resorption of the plaster) to get more of the "creeping substitution" or osteogenic effect.

Brands of all of these calcium phosphate ceramics have been approved by the FDA for implantation in humans.

Plaster

All of the calcined gypsums currently used in our studies are produced from UNITED STATES GYPSUM COMPANY F & P Grade Terra Alba. This material is widely used in the preparation of foods and approved for the compounding of medicines (F & P stands for Food & Pharmaceutical). Although most of our original studies were done with a less dense plaster (β-$CaSO_4 \cdot \frac{1}{2}H_2O$) our current studies employ more dense plasters (resembling dental stone), B-Base HYDROCAL and C-Base HYDROCAL. Both of these calcined gypsums are α-$CaSO_4 \cdot \frac{1}{2}H_2O$. Recently we have switched over almost entirely to the use of C-Base HYDROCAL (which has the lowest known mortar consistency of any plaster) as the binder for the apatite and tricalcium phosphate ceramics. Plaster alone is known to be osteogenic (Mitchell and Shankwalker, 1958) when implanted in canine mandible and other bones including craniofacial bones (Mitchell and Shankwalker, 1958; Calhoun and Blackledge, 1962; Bahn, 1966; Coetzee, 1980; Beeson, 1981; McKee and Bailey,

1984). The osteogenic potential of calcium and magnesium hydroxides has also been reported (Mitchell and Shankwalker, 1958). The osteogenic properties of plaster may depend upon the presence of periosteum and/or bone (McKee and Bailey, 1984). It has been reported, however (Coetzee, 1980), that plaster implanted in bony defects gives results comparable (if not better) to those achieved with autologous bone. The dramatic effects of plaster could be due to the well-known effect of calcium on stimulating endothelial cell proliferation (D'Amore and Shepro, 1977; D"Amore, 1978). This could be responsible for the proliferation or infiltration of fibrovascular tissue or revascularization seen due to plaster (Beeson, 1981).

Reparative Granulomas Formed Around Ceramic Particles Implanted for Alveolar Ridge Augmentation

When an alloplastic material is implanted during preprosthetic surgery, bleeding can be a problem. The natural defense is, the same as in any injury, the formation of a clot. The thrombocyte or platelet is the key cell in clot formation--also an important part of the stimulus to fibroplasia and angiogenesis which are so important in wound repair (Greenburg and Hunt, 1978). The roles of neutrophils, monocytes and fibroblasts in wound healing, even in the absence of infection, are now being reassessed. The clot is the scaffolding or original foreign (or at least ectopic) body on which wound healing progresses. It must be replaced before healing can occur. It is now clear (Ross, 1980) that normal wound healing goes through processes of clotting, epithelialization, replacement of clot by granulation tissue and replacement of the latter by mature cross-linked collagen. Any body that occupies space it doesn't normally occupy is a foreign (or at least ectopic) body. True foreign bodies are synthetic materials (or at least of animal origin). Even ectopic materials from the host can cause the presence of foreign body giant cells. Where inflammation is long and intense a granuloma may occur. Many of these can be of the immunologic type (Warren, 1980; Boros, 1978) causing chronic infection or irritation. Synthetic materials generally evoke less inflammatory response. A fibrous tissue capsule (See Results), often with giant cells, will form around them and not only prevent the body from rejecting the implant, but actually aid the implant's incorporation by the body (reparative granuloma). The formation of granulation tissue is required for revascularization which, in turn, is necessary to supply the nutrients and oxygen essential for osseous repair (Urist, 1980).

The significance of multinucleated giant cells seen adjacent to ceramic hydroxylapatite particles after implanting atrophic mandible is not clear. The presence of many fibroblasts and collagen in the area and the absence of inflammatory cells, monocytes and macrophages suggest that these are not true foreign-body giant cells and that this is not a foreign-body granuloma. Indeed, these giant cells could be formed from fibroblasts (See Results following). Even the discrimination of these giant cells from osteoclasts is not clear (Pease, 1982). Giant cell formation is even evoked by undecalcified bone allografts (cancellous or cortical) from the same strain of guinea pig, only about the implant and never in relation to the newly formed bone (Cummine and Nade, 1977). The authors suggest that the early appearance of these cells in a nonsensitized animal suggests that they are nonimmunologic.

Histochemistry and Analytical Electron Microscopy in Evaluating Implant Histocompatibility

Histochemical methods and those of analytical electron microscopy, where one can look directly at the implant-tissue interface, are the best methods for evaluating the biocompatibility of implants (Salthouse and Matlaga, 1980; Salthouse, 1976). Although tissue culture methods are receiving more use for evaluating the toxicity of materials, they provide little information on material/tissue interactions in vivo. Admittedly, biochemists are often better able to follow sequences of complex biochemical and enzyme systems in vitro but histochemistry and analytical electron microscopy are the only means for examining those systems at the implant/tissue interface or in direct relation to particular cells and cellular organization. Raekallio and his colleagues (1970) have shown the importance of enzyme histochemistry in providing information on changes in cell function in tissues in wound healing. Giammara et al., 1983, 1984 have shown the application of analytical electron microscopy to similar problems.

VI. OUTLINE OF EXPERIMENTAL SURGICAL PROCEDURES

A. Healing of experimental fractures and experimental defects of maxilla or mandible of rodents or other laboratory animals.

B. Healing with hydroxylapatite implants, --maxilla or mandible

1) Onlay
2) Interpositional
3) Bridging continuity defects
4) With or without binders such as collagen or adhesives for containment.

C. Nonhealing defects (without implants)

D. Effects of oxygen tension and hyperbaric oxygen on experimental fractures, and healing or nonhealing defects

E. Effects of local instillation of epidermal growth factor and prostaglandin E_1 on revascularization of experimental mandibular fractures or defects.

F. Summary of additional experimental procedures

a). Models for experimental fractures and bone defects

These have previously been described for many animals including dog (Richter et al., Oral Surg. 26, 396-405, 1968) and rat (Kaban and Glowacki, J. Dent. Res. 60, 1356-1364 (1981).

b). Preparation and Administration of Factors

1) Oxygen tension (Persson, Calc. Tiss. res. 2, Suppl. 28, 1968)

2) Hyperbaric oxygen (Kapp, Surg. Neurol. 12, 457, 1979; Kerley et al., J. Oral Surg. 39, 619, 1981).

3) Functional maxillofacial bone strain (Hylander and Bays, Archs. oral biol. 24, 689, 1979).

4) Tumor angiogenic factor (TAF, Cavallo et al., J. Cell Biol. 54, 408, 1972; Folkman et al., J. exp. med. 133, 275, 1971; McAuslan and Hoffman, Exp. Cell Res. 119, 181, 1979; Weiss et al., Br. J. Cancer 40, 493, 1979).

5) Bovine parotid gland angiogenic factor (Ito, Ann. N.Y. Acad. Sci. 85, 228, 1960; mouse submandibular gland factor, Exp. cell Res. 102, 269, 1976; fibroblast growth factor, J. Biol. Chem. 253, 3736, 1978).

6) Epidermal growth factor (Gospodarowicz et al., Exp. Eye Res. 28, 501-514 (1979); Franklin and Lynch, Plast. Reconstruct. Surg. 64, 766-770, 1979).

7) Prostaglandins (Ben Ezra, Am. J. Ophthamol. 86, 455, 1978; Gullino, Handbook Exptl. Pharmacol. 57, 427, 1981).

8) Adhesives (Brauer et al., J. Biomed. Materials Res. 13, 593-606, 1979); Redl et al., The Thoracic and Cardiovascular Surgeon 4, 223-227, 1982).

c. Evaluation of Bone Revascularization

1) This can be caculated by the uptake of ^{51}Cr labeled erythrocytes and new bone formation calculated by ^{85}Sr uptake (Goldberg and Lance, J. Bone and Joint Surg. 54, 807, 1972) or by ^{45}Ca incorporation (Glowacki et al., 1981).

2) Revascularization can be studied with the vital dye Thioflavine-S which renders only the actively functioning blood vessels fluorescent (Schlegel, Anat. Rec. 105, 433, 1949; Stain Tech. 46, 321, 1971) or our new PATS stain (Giammara et al., 1983, 1984).

3) Vascularity can also be studied by horseradish peroxidase administration and staining with a chromogen such as 3,3'-diaminobenzidine or p-phenylenediamine/pyrocatechol after sacrifice, fixation and decalcification (Graham & Karnovsky, J. Histochem. Cytochem. 14, 291, 1966; Hanker et al., Histochem. J. 9, 789, 1977). This technique can also be applied to the light and electron microscopic study of phagocytosis by young capillary endothelial cells.

VII. Method of Approach

All of the experiments will be carried out in laboratory animals - rodents, where feasible. If required, monkeys may occasionally be utilized. All animals for our laboratory are ordered through the Division of Laboratory Animal Medicine of the University of North Carolina. Their housing, hygiene, feeding and medical care are under the supervision of this facility which is directed by Dr. James R. Pick, a veterinarian. This division ensures compliance of all animal experimentation with local and federal policy.

A. Creation of Bone Defects and Fractures in Rats

Generally, bone grafts for repair and reconstruction of maxillofacial defects were studied in mandibular defects or fractures created in an

appropriate number of 90-day-old male, CD strain, Charles River rats according to the methods of Mulliken and Glowacki (see Kaban and Glowacki, 1981 for references). The rats will be anesthetized with ether or with IP sodium pentobarbital (6 mg/kg) supplemented by ether inhalation. The mandibular rami will be exposed bilaterally. Fractures or defects will be created on one side only, the other side will serve as a control. Fractures will be created by sectioning the mandibular ramus from the sigmoid notch to the inferior border with a fine rotary instrument. Bony defects, including continuity defects, and fractures will be created in an appropriate number of dogs by similar methods.

Full thickness, 4 mm round, bony defects will be created with an electric drill and burr. The defects will be rinsed with Ringer's or Ringer's lactate.

B. Preparation of Hydroxylapatite Implants

The implants will be prepared by mixing the commercial Calcitite with various binders such as collagen, bovine serum albumin, plaster or cyanoacrylate or fibrin adhesives as required.

C. Administration of Factors Affecting Revascularization

The administration of various factors affecting wound healing is presented in great detail in the references cited under IV Summary of additional experimental procedures. B. Preparation and Administration of Factors. These factors may be applied by parenteral administration prior to, during, or after creation of the defect and will vary for the factor and type of bone injury or defect. A valuable review of these references is the paper by Gullino (Handbook Exptl. Pharmacol. 57, 427-449, 1981). Hyperbaric oxygen administration as adjunctive therapy during mandibular bone regeneration is described in detail in the references in the paper by Kerley et al. (J. Oral Surgery 39, 619-623, 1981).

D. Experimental Surgical Repair Procedures

Some of the defects in rats will be filled by implantation of various hydroxylapatite mixtures to encourage healing. The control defect will not heal. This study will also include creation of continuity defects in the mandibular bodies, repair by various types of hydroxylapatite implants, and fixation by bone plates - compression and non-compression.

E. Assessment of Revascularization of Traumatized Area

Revascularization will be evaluated by noting the presence or absence of blood-filled capillaries in the marrow spaces. When all such areas contain blood-filled capillaries, then revascularization will be considered complete. In samples in which this process does not extend to all marrow spaces, an estimate will be made of the percentage revascularized.

Revascularization of osseous areas will also be evaluated with the PATS stain.

F. Evaluation of Osseous Repair

Osseous repair will be evaluated by gross examination of the skulls and by histologic studies, including hematoxylin-eosin, safranin-O (for chondroitin sulfate), von Kossa (for mineralization), and van Gieson (for collagen). Some animals will receive oxytetracycline (15 mg/kg) intraperitoneally 24 hours before sacrifice.

Bone synthesis will be quantitated by ^{45}Ca uptake studies at 2 weeks; 100 Ci $^{45}CaCl_2$ per 0.1 ml was administered intraperitoneally 4 hours before sacrifice. The amount of ^{45}Ca incorporated into the acid-soluble fraction (hydroxyapatite) of the implanted defect will be measured by scintillation counting and expressed in terms of counts per minute per milligram of tissue.

New-bone formation will also be estimated by using ^{85}strontium (strontium nitrate-Amersham-Searle) according to the principles of Bauer and Ray. Forty-eight hours prior to death, each rat will receive 2.5 microcuries per kilogram of ^{85}strontium intravenously.

G. Statistical Significance of Experimental Results

The appropriate numbers of animals and controls for each factor or variable to be assessed in these studies will be designated in consultation with Drs. Dennis Gillings and Dr. Gary Koch of the Biometric Consulting Laboratory, Department of Biostatistics, University of North Carolina. The differences between experimental and control animals will be assessed by microscopic examination of specimens, for example histologic sections. The levels of vascularization (e.g.) on each microscope slide will be graded empirically. These empirical grades of activity will then be converted to ridit values, as described by Bross (Biometrics 14, 18-38, 1958). The ridit values will be analyzed for statistical significance by methods such as the Wilcoxon test. The significance of differences between experimental and control groups will be assessed by using either the t-test, nonparametric rank methods or Fisher's exact test as appropriate for the nature of the data under consideration.

VIII. DETAILS OF EXPERIMENTAL PROCEDURES

A. Surgical Procedure for Drilling and Implanting Defects in the Relatively Avascular Mandibular Ramus of Rat

Charles River (CD) male white rats (350-450 g) were anesthetized with Ketamine (10 mg/100g body wt) and the mandibular skin depilated. Under antiseptic conditions the masseter muscle and connective tissues were bluntly dissected down to the mandibular ramus. In some animals care was exercised to preserve one or both layers of periosteum during the procedure while in others the layers of periosteum were not protected from the burr. A four mm through defect was created (Fig. 1) by using a low speed dental drill with irrigation. Bleeding was controlled by pressure while the implant material was being mixed (with a binder if required) to a thick paste. A plug of the implant material was pressed tightly into the defect and this was smoothed (Fig. 2). Any periosteum (in some experiments) and musculature was reapposed and skin sutured closed. Animals were allowed food and water immediately after surgery. Sacrifice times ranged from 2 weeks to 6 months after surgery.

B. Implant Materials

One of the problems encountered when dense hydroxylapatite ceramic particles (Fig. 3) (Calcitite or Durapatite) are employed as a slurry in saline is their tendency to migrate into spaces and tissues adjacent to the implant site. Various agents were, therefore, tested as binders to contain the particles of the implant within the drilled defects; these were collagen, bovine serum albumin (BSA), or plaster of Paris. Mixtures of the individual binders and hydroxylapatite particles (approximately 1:1 by weight) were formed into a thick paste or slurry with a small amount of saline for the protein binders or deionized water for the plaster. The demineralized bone powder implanted in some of the defects was prepared from long bones of rats which had been freeze-dried, powdered, sieved to 75-250 microns and demineralized by the method of Kaban and Glowacki (J. Dent. Res. 60, 1356-1364, 1981). These implants consisted either of the bone powder with a small amount of BSA binder, or a 1:1 mixture of the bone powder and hydroxylapatite with a small amount of BSA binder.

In more recent experiments, plaster of Paris alone has been implanted into the defects.

C. Estimation of Angiogenic and Osteogenic Effects of Administration of Simple Factors

The ability of the relatively simple factors (Table 1) to induce both revascularization and new bone formation can be evaluated by administration of these factors either locally in an implant, or parenterally, or in the case of hyperbaric oxygen, in special chambers. The effects of many of these factors can be determined in the rats with the experimental mandibular defect described above. In addition, however, it is important to evaluate the effects of the angiogenic and/or osteogenic factors in simpler systems than our experimental bone defect. This could aid in elucidating the mechanism of action of the factors. For this purpose it is important to use systems recognized for evaluating the effects of factors on both vascularization and osteogenesis. For the former, the effects of the factors will be determined by observing the increment in the number of capillary endothelial cells in a variety of organs, but particularly in the intertubular spaces of kidney after injection in newborn mice (Hoffman et al., 1976; McAuslan and Hoffman, 1979). The effects of factors, plaster of Paris, and particles of hydroxylapatite will also be determined in the hamster cheek pouch experimental model of corneal vascularization with Dr. Klintworth at Duke (Klintworth, 1973). The tissues from the newborn mice can also be studied for the effects of the factors on osteogenesis. Our acid phosphatase method (Hanker, 1979) for looking at the Golgi apparatus of cells secreting matrix for hard tissue formation will be used to ascertain the effects of factors on odontogenic and osteogenic cell layers of maxillofacial and long bones of these mice (Figs. 4-6).

D. Treatment of Rats with Hyperbaric Oxygen

The rats receiving hyperbaric oxygen therapy are exposed to 100% oxygen at 2 ATA 5 consecutive days each week for a period of 4 weeks starting 3 to 5 days after surgery. They are exposed in 2 small animal chambers. One (Fig. 7) is a super high pressure chamber which was built at Duke and holds four rats. The other (Fig. 8) is a Bethlehem model H-70-A that holds three rats.

Table 1

SIMPLE ANGIOGENIC OR OSTEOGENIC FACTORS

Calcium salts
- Hydroxylapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$]
- Plaster of Paris (PP, $CaSO_4 \cdot \frac{1}{2}H_2O$)
- Tricalcium phosphate [TCP, $Ca_3(PO_4)_2$]

Copper salts or proteins
- Copper salicylate, copper sulfate
- Ceruloplasmin

Anti-inflammatory agents
- Aspirin
- Indomethacin
- Prostaglandin E_1

Platelets, Fibrin and Coagulation Factors
- Platelets
- Platelet-derived growth factors
- Thrombin
- Heparin
- Fibrinogen
- Fibrin
- Fibrinopeptides
- Fibronectin
- ε-Aminocaproic acid

Hormones, Vitamins, ets.
- Androgens
- Progesterone
- Salmon thyrocalcitonin
- Vitamins D & A
- Fluoride
- Fluorapatite

Bone marrow aspirate

Pressurization and depressurization of the chambers are accomplished at standard rates for all of the treatment sessions.

E. Evaluation of Structure, Properties, Clinical Efficacy and Biocompatibility of Implants

1. Characterization of Structure and Properties of Implant Materials

The investigations required the formulation and preparation of a large number of gypsum based materials. When such products are used as tissue implants, the response of the tissues depends on many identifiable characteristics of the materials. These characteristics include such factors as the gross size and shape of the implant as well as the composition, crystal structure, solubility, pH, mechanical properties, porosity, and pore size distribution of the materials themselves. In order to provide adequate experimental control, and to aid in establishing cause-effect relationships between the materials, factors and observed clinical response, it is essential that the materials be fully characterized chemically and physically. Similarly it is essential that thorough histologic evaluations be preformed to supplement the anticipated clinical observations by delineating the details of the tissue response at the cellular level.

The physical-chemical procedures employed for the evaluation of raw and formulated materials were conducted as described below following a two level procedure. All materials received routine first level evaluation. Supplemental second level evaluations were conducted as indicated to address unusual results and to provide more thorough characterization of formulations producing the most favorable or least favorable clinical response.

All raw materials on receipt were transferred to sealable glass containers for storage. Representative samples were taken for testing. A portion of the sample was maintained for reference throughout the study.

All material formulations to be used as implants were sampled for evaluation as well as additional formulations as indicated. When clinical specimens were prepared for implantation or other in-vivo evaluation, standard samples for physical testing were prepared from the same mixes. Additional samples were taken from stimulated implants and, where possible, from recovered post-implantation materials.

Routine screening evaluations includes optical and SEM micrographs and X-ray diffraction patterns for all powders. Specimens from each experimental formulation were tested for crushing strength, diametral tensile strength, Rockwell 15y indentation hardness, bulk porosity, solubility and pH. Where applicable the methods of A.O.A. Specifications 2 and 25 were employed. Porosity was estimated by bulk density, calibrated by point count measurements of resin infiltrated specimens. Pore size was estimated from line count determinations on optical micrographs. Solubility was measured by the method of A.D.A. Specification 8, and equilibrium pH was determined on the supernatant liquid.

Supplementary methods for use as needed included: SEM-EDAX analysis in a scanning mode for overall analysis, followed by point determinations on identified non-standard areas, sieving and inert fluid elutriation for

determination of particle size distribution, permeability measurements (gas flow), and mercury porosimetry for determination of pore size distribution.

2. Clinical Efficacy and Biocompatibility of Implants

Clinical protocols were designed for each project--maxillofacial surgery, operative dentistry, endodontics, periodontics, orthopaedic surgery and cancer chemotherapy. These protocols were designed in conjunction with the involved clinicians for both the experimental animal and human studies. Acquisition of human specimens were approved by the human subjects committee. In addition to physical examination of the animals and patients, the implants were examined during the course of healing by conventional radiographs, computerized tomography or photodensitometry as required to assess their success.

Biopsy samples of tissues from humans and monkeys could be obtained upon recontouring or vestibuloplasty. Biopsy specimens from rodents, dogs or monkeys were obtained by proper surgical procedures under sterile conditions. Necropsy specimens were also obtained from the experimental animals at appropriate times to assess the success of the implant procedure, and the accommodation and biocompatibility of the implant at a microscopic and ultrastructural level.

a. Histological and Histochemical Methods

All of the rats were sacrificed without prior perfusion of fixative inasmuch as one of the principal histochemical methods we intended to use to visualize the microvasculature (Figs. 9, 10) was based on the demonstration of the peroxidatic activity of erythrocyte catalase. This method showed the capillaries by virtue of their red cell content.

At sacrifice the mandibles were excised and separated into halves. In earlier studies, some were fixed in 2.5% glutaraldehyde/2% (para)formaldehyde, 0.1M in cacodylate buffer, pH 7.4, for demonstration of the blood vessels via erythrocyte staining. After this histochemical method was found to be undesirable for the demonstration of tne microvasculature of decalcified specimens, a fixative containing 4% formaldehyde/1% glutaraldehyde, 0.1M in phosphate buffer (pH 7.4) was employed. This fixative was used for the PATS reaction which demonstrates the pericapillary reticular network of the microvasculature for all modes of electron microscopy including analytical modes as well as conventional light microscopic histochemistry. This newly developed histochemical method (Giammara et al., 1983) was also found extremely useful in demonstrating neutrophil absence (by virtue of their glycogen) and lack of acute inflammation in these specimens. A fixation time of 3 hours at 0-4° was generally employed, followed by rinsing in the appropriate buffer in the refrigerator for a period of overnight to several weeks. During this period radiographs were made of each mandibular defect. With care exercised to preserve the bone-implant relationship, additional muscle and connective tissue were removed exposing the implant. Each specimen was then photographed and the degree of defect filling, position of the defect, and quality of the surrounding tissues were noted. For erythrocyte catalase demonstration, decalcification of specimens was effected by immersion for 24-48 hours in 5% EDTA, 0.1M in cacodylate buffer (pH 6.8) with sonication in a cold room; rinsing in several changes of cacodylate buffer was performed during a period of at least 16 hours. For the PATS reaction, decalcification could be carried

out by treatment with RDO (DuPage Kinetic Laboratories, Plainfield, Ill.) for two hours with sonication in a cold room. Several rinses with distilled water were required over a period of 3 to 4 hours prior to immersion in cacodylate buffer overnight. Cryostat sections (10 microns) of the decalcified, rinsed tissues were collected and dried on albuminized coverslips.

For electron microscopy, 30-50 micron Vibratome sections of some fixed, decalcified, implanted defects were made for treatment with the PATS reaction.

b. Histochemical Staining Reactions

The reaction employed to demonstrate the microvasculature by virtue of the peroxidatic activity of erythrocyte catalase was a modification of the method of Novikoff and Goldfischer (J. Histochem. Cytochem. 17, 675, 1969). This modification (Hanker and Romanovicz, Science 197, 895, 1977) replaces postosmication with intensification by posttreatment with 0.5% copper nitrate. The PATS reaction (Giammara et al., 1983) is a modification of the PAS reaction which employs thiocarbohydrazide and silver methenamine instead of the Schiff reagent after periodic acid treatment. In addition to demonstrating glycogen, by both light and electron microscopy, it demonstrates collagens due to their argyrophilia. Especially prominent are fibers of the pericapillary reticular network and glycogen of neutrophils. The latter are readily discriminated from eosinophils with the PATS procedure. Specimens to show erythrocytes are dehydrated and infiltrated with "Medcast" epoxy resin (Ted Pella, Inc.). They are then embedded in this resin in the form of an epoxy microscope slide. Areas selected with a light microscope can be photographed, (Fig. 10) cut out, glued onto a blank block and sectioned for electron microscopy. These ultrathin sections are poststained on grids with uranyl acetate and lead citrate and viewed on an AEI 801 electron microscope.

Routine H & E procedures did not impart stains to sections of defects that had been decalcified with RDO. It was found, however, that if the sections were immersed for a much longer period of time in the hematoxylin component of the Gill modification of the Papanicolaou trichrome procedure, that sufficient staining could be obtained to discriminate the microvasculature. However, this staining did not give as much information as the PATS reaction.

All of the biopsy and necropsy specimens were fixed in appropriate glutaraldehyde and formaldehyde solutions as soon as feasible. Each specimen was examined grossly and photographed. Specimens from control and experimental animals were photographed together as well as individually. Attempts were made to assess the extent of ossification in the unsectioned specimens by X-ray, computerized tomography or photodensitometry as required.

Undecalcified specimens were sectioned (0.75-2mm) with a diamond saw (Gillings-Hamco Thin Sectioning Machine). They were examined by polarized light microscopy to assess incorporation of the implant or implant particles by new host bone. The new bone can be stained on these undecalcified sections by Alizarin red S or the von Kossa silver method which show bone by virtue of its calcium or phosphate, respectively. These thick sections could then be examined by reflected light microscopy.

For histological, histochemical and autoradiographic studies of the bone- or bone and implant-tissue interace, specimens must be decalcified as rapidly

as possible prior to staining. Formic acid (Morse) or EDTA (except when autoradiography is intended) are preferred decalcifying agents. Specimens containing dense calcium hydroxylapatite ceramic particles were decalcified rapidly under sonication in RDO. Just before staining, the acid in the bone sections was neutralized by brief immersion in 1% aqueous lithium carbonate.

Bone formation was evaluated qualitatively and quantitatively in experimental animals. Qualitatively, by administration of oxytetracycline (Pallasch, 1968) and subsequent fluorescence microscopy of sections. Quantitatively by administration of ^{45}Ca (Glowacki et al., 1981) or ^{85}Sr (Goldberg and Lance, 1972) prior to sacrifice. The amounts of ^{45}Ca or ^{85}Sr incorporated are measured by scintillation counting of the excised implant, defect or control areas.

The cytochemical activity of periosteum (principally the preosteoblastic and osteoblastic layers) was shown by our improved light and electron microscopic cytochemical procedures for acid and alkaline phosphatases and ATPases (Hanker, 1979). The PATS stain may also be valuable for showing the glycogen present in preosteoblasts and osteoblasts of periosteum. Our acid phosphatase procedure is especially important in that it is a marker for the Golgi apparatus of cells secreting matrices for hard tissue formation (Figs. 11, 12).

Osteoblast activity at the bone-implant interface was studied by histochemical methods which show their functional activity via energy metabolism (Coleman et al., 1976; Hanker et al., 177a) or horseradish peroxidase ingestion (Hanker et al., 1977b).

The activities of inflammatory cells (neutrophils, eosinophils, lymphocytes and macrophages) are most dramatically shown by our peroxidase staining technique for blood developed originally (and widely used) for the detection of acute myeloid leukemia. This test enables the rapid detection and differentiation of all leukocyte and inflammatory cell types in tissues as well as in blood specimens. It can also be used for various modes of electron microscopy (Berman et al., 1983) as well as light microscopy. A new variation of the PAS reaction known as the PATS reaction developed in our laboratory (Giammara et al., 1983) is especially useful in differentiating eosinophils (chronic inflammation) from neutrophils (acute inflammation) by virtue of the high glycogen content of the latter. This reaction is also useful for light and electron microscopy of collagen type III-containing reticular fibers.

Changes in reticular fibers, which contain especially large amounts of collagen type III, are dramatic in healing tissues probably because fibroblasts in granulation tissues synthesize more collagen type III (Bailey et al., 1975; Gabbiani et al., 1976). In addition to showing reticular fibers and revascularization, new bone synthesis is also shown clearly with this staining procedure. Classical stains including H & E, Os-TMEDA and safranin-O for chondroitin sulfate, von Kossa for mineralization and van Gieson staining for collagen were used if required.

Thin sections of the composite ceramic alloplasts, taken at different times after surgical reconstructive procedures in monkeys, were made with a diamond impregnated wire saw. They were studied by the backscattered electron imaging (Becker & Sogard, 1980) and X-ray microanalysis (Chandler, 1979) modes of

scanning electron microscopy in order to follow resorption of the plaster of Paris and ossification of the implants.

The evaluation of histochemically-stained cryostat tissue sections of a particular implant versus tissue sections from another implant (or a control defect) were done as described by Salthouse and Matlaga "An approach to the numerical quantitation of the acute tissue response to biomaterials." Biomaterials Med. Devices Artif. Organs 3, 47 (1975).

Rating System for Tissue at Animal Implant Sites (From Black, 1981). This system, based upon earlier studies (Gourlay et al., 1978; Sewell et al., 1955) involves measuring the capsule thickness, evaluating the local cellular response, and then assigning weighting factors to arrive at an overall rating.

c. Estimation of Size of Unossified Area in Sections of Implanted Defects

Ossification of the implanted defects proceeds from the periphery (at the interface with host bone) inward. Two different methods are utilized to calculate the size of the area of unossified defect at various intervals during the healing period. Both methods are applied to the same microscope slides of 10 micron PATS stained cryostat sections. Three to five sagittal sections are taken through the maximal area of decalcified implanted defect. The first method employs a dissecting microscope with a gridded 1X ocular that measures directly in tenths of millimeters. On each section, measurements are taken along two parallel axes to determine the longest (major axis) and shortest (minor axis) distances across the defect. Data from all of the sections is averaged to give X and Y measurements. If both axes are approximately equal (X=Y) the defect area is calculated as a circle ($A=\pi r^2$). If the average axis measurements are unequal ($X \neq Y$), the defect is considered elliptical and the formula for the area of an ellipse, $A=\frac{\pi XY}{4}$, is used.

Another method to determine the size of the area of unossified defect: The sections are photographed at a standard magnification on 35 mm color transparency film. Each color slide is then printed to a standard size on a Polaroid Polaprinter. Computer planimetry may then be used to estimate the area of remaining unhealed defect. On the prints the borders of the defects are traced with a cursor of a Houston Complot digitizing tablet. This information is then fed to a DEC PDP 11-60 computer which has been programmed to calculate the area within the closed tracings.

When required, ossification may also be estimated by X-ray diffraction studies as described below.

d. Electron Microanalytical Studies

The information obtained by light microscopy of these tissues can be supplemented at higher magnification by the various modes of analytical electron microscopy including electron microprobe analysis, X-ray microanalysis and the backscattered electron imaging mode of scanning electron microscopy. SIMS (secondary ion mass spectrometry) can also be used. These studies can be done on light microscopy specimens, when required, by techniques developed by B. Giammara.

Ridit analysis (Bross, 1958) was applied to quantitate information on electron micrographs obtained by backscattered electron imaging and X-ray microanalysis modes of scanning electron microscopy. In addition, by use of a computer and the Kevex system, quantitative information about certain elements can be obtained directly by X-ray microanalysis of specimens in the SEM.

3. Radiographic Techniques for Bone-Gypsum Density Measurements

a. Conventional Radiography - Standardized periapical or bitewing films, optically scanned by a densitometer. Readout relates to amount of light that penetrates radiograph. Sensitivity is to two decimal places. Same technique can be used for Panoramic radiography.

b. Computerized Tomography - This technique could be utilized for regions of several centimeters, but not single well-defects of periodontium. Computerized Tomography numbers can be generated for each density reading. It is reliable, accurate, and expensive.

c. Computation of defect area, HA fill or new bone formation with a LaMont Image Analyzer System.

4. In Vitro Studies of Composite Implants

The implants are formed under sterile conditions from different plasters manufactured by USG which meet F & P specifications. Presently USG HYDROCAL C is being used almost exclusively as the resorbable binder for the nonresorbable calcium hydroxylapatite ceramic (Calcitite or Durapatite) particles. Potassium sulfate is being added to the HYDROCAL C powder, wetting solution, and applied to the surface of the implant during formation as needed to hasten the setting rate. Sodium citrate is used, if required, to slow this rate. The stability of HA/PP implants in saline was studied. Further details on in vitro studies of composite implants will be presented under Results of Studies.

F. Experimental Surgery with HA/HYDROCAL C Implants in Rabbits, Dogs and Monkeys

All of this work was done in our very well equipped operatories either in the Dental Research Center or in the Department of Laboratory Animal Medicine of the University. All animals are properly anesthetized and surgery and implantation are performed aseptically. Heat sterilization is preferred and used where possible on the HA and PP or on the preformed implant. In the rabbits and dogs and one monkey, defects were created on the inferior border of the mandible. Only implants of HA/PP as well as implants reinforced (and screwed in) by stainless steel wires were used. Seven monkeys were subjected to radical alveolectomy (Figs. 13-15) resulting in the creation of an edentulous mandible (Fig. 16). After healing, subperiosteal tunnels were created (Fig. 17) on both sides; one side was injected with HA alone and sutured. The other side was injected with HA/PP. After forming and contouring to the appropriate height, the HA/PP side was sutured. In the first monkey dental stone was employed as the plaster and instead of HA alone (by injection) on the control side, an onlay of HA/PP was implanted. In the later monkeys HA was used alone and in combination with HYDROCAL C (Figs. 18-26).

PROCEDURES FOR AUGMENTATION OF MONKEY ALVEOLAR RIDGE WITH HA ALONE VS HA/PLASTER AFTER RADICAL ALVEOLECTOMY

RADICAL ALVEOLECTOMY. The Macaca fascicularis monkeys included in this experiment were taken to the Department of Laboratory Animal Medicine (DLAM) operating room, where they were anesthetized with Surital, intubated and maintained on inhalation agents during the operating procedure. Xylocaine with epinephrine was injected into the mandibular vestibule for homeostasis and postoperative comfort of the monkeys. A circumvestibular incision was made at the depth of the vestibule and a full thickness mucoperiosteal dissection was completed, exposing the lateral aspect of the mandible. A subapical osteotomy was then performed from the most posterior body area to the symphysis area bilaterally. The lingual mucoperiosteum was then reflected from the lingual aspect of the mandible. The entire dentoalveolar segment was then removed totally. The underlying mandibular bone was then smoothed with a bone file and irrigated copiously with saline. Primary closure of the wound was then obtained with 3-0 chromic suture in a horizontal mattress fashion. Postoperatively the monkeys were maintained on a soft monkey biscuit diet for the three month period prior to the time of hydroxylapatite augmentation.

HYDROXYLAPATITE AND HYDROXYLAPATITE PLASTER OF PARIS AUGMENTATIONS OF THE MANDIBLE. The monkeys were brought to the DLAM operating rooms 3 months following radical alveolectomy and were anesthetized in a similar manner to the above described procedure. Bilateral vertical incisions were made in the area just anterior to the mental foramen on both sides of the mandible. A subperiosteal dissection in a tunneling fashion was then completed, exposing the superior aspect of the mandible. In all monkeys there appeared to be good cortical bone coverage of the mandible in all areas. On the right side hydroxylapatite mixed with saline only was injected into the subperiosteal tongue. On the left side a combination of hydroxylapatite and C-base HYDROCAL in 50% proportions were combined and injected into the left subperiosteal tunnel. Both incisions were closed with 3-0 chromic and the monkeys were again maintained on a soft monkey biscuit diet until the time of sacrifice.

POSTOPERATIVE COMPLICATIONS. All monkeys did well except for the monkey sacrificed at the 20 week postoperative period. This monkey developed significant intraoral trauma in the incisioned areas, resulting in bilateral infections in the augmentation areas.

SACRIFICE. Monkeys were sacrificed at 3, 6, 12, 20, and 24 weeks. The monkeys were brought to DLAM necropsy area and anesthetized with pentobarbital. An open thoracotomy was then performed and a large catheter was inserted into the ascending aorta and secured in place with 2-0 silk suture. The aorta grout was then clamped and perfusion of the head and neck area was completed with a formaldehyde/glutaraldehyde solution. The mandibles were excised and immersed in fixative.

HISTOLOGIC EVALUATION

Cross sections of the undecalcified mandible were obtained with the diamond impregnated wire saw and stained as described above.

G. Implantation of HA/HYDROCAL C in Patients

These patients signed an informed consent and release from (attached next page). Again, the full details of these procedures will be furnished at a later date by the surgeons and prosthodontist as applicable. The HA and PP were used by Drs. Tucker and Holland for the reconstruction of contour defects of the maxillary alveolar ridge in 2 humans. In one case, this was done principally for cosmetic or esthetic effect rather than for denture support. In the other cases it was done to augment the maxillary alveolar ridge after augmentation with HA alone was unsuccessful. The cases by Dr. Baker and Dr. Terry involved augmentation of 1 or 2 sides of the mandibular alveolar ridge which were very deficient. Dr. Reynolds A. Carnevale, a periodontist in Fayetteville, has used the HA/PP mixture in approximately 60 patients with severe infrabony defects. As many as 13 teeth were done in a single patient. The procedures will be described in greater detail under Results of Studies.

IX. RESULTS OF STUDIES

A. Efficacy of Staining Procedures

A classical stain for demonstrating the microvasculature in the nervous system with benzidine (Doherty et al., 1938) by selective staining of the red blood cells in the vessels (Fig. 9) could not be used because of the carcinogenicity of the reagent benzidine. An alternative procedure developed in our laboratory demonstrates the erythrocytes in capillaries by virtue of their catalase activity with 3,3'-diaminobenzidine and copper intensification (Hanker and Romanovicz, 1977). Although this procedure was excellent in soft tissues such as salivary glands (Fig. 10) and in hard tissues before mineralization was completed, it did not always stain capillaries effectively after RDO decalcification. This was especially true in surgically created or healing defects (Fig. 28). Moreover, the erythrocytes were frequently displaced from the vasculature due to sectioning. The PATS stain (Fig. 27) was much more useful in demonstrating the microvasculature. It was found especially useful to demonstrate the pericapillary reticular network (Fig. 29) in vessels in demineralized bone specimens. It also showed the type III collagen of the fibrovascular tissue infiltrating and filling the implanted defects (Fig. 30). The elastic collagen fibers in the arterioles which invade the implanted defects were also shown (Fig. 31).

The capsules formed by a foreign body reaction around ceramic implant particles were shown by the staining of their supporting reticular fibers with the PATS reaction (Figs. 31, 32) Giammara et al., 1984. The new blood vessels forming between these ceramic particles were also clearly seen with the PATS stain in the implanted defects (Figs. 30, 33). The PATS stain was found very useful in distinguishing neutrophils and neutrophil pseudoplatelets in acutely inflamed specimens from other leukocytes. The neutrophils and neutrophil pseudoplatelets (Hanker and Giammara, 1983) could be discriminated from other leukocytes by their relatively high glycogen content. This permitted an assessment of acute inflammation in necropsy specimens from our experimental animals.

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INFORMED CONSENT FORM FOR PLACEMENT OF COMBINATION HYDROXYLAPATITE AND PLASTER OF PARIS

While plaster of Paris and hydroxylapatite have both been used as implant materials, there has been no report of combined use of these materials in humans. Evidence from animal research suggests that there is no increased inflammatory response or other adverse reactions which might occur using a combination of these materials.

The proposed advantages of the use of a combination of approximately 50% hydroxylapatite and 50% plaster of Paris is to allow for better handling capabilities which will allow this material to be molded to the proper size and shape at the time of surgery. This will allow improved access to the surgical site, better contouring of the material and an overall improved functional and esthetic result. This mixture may also promote healing.

Disadvantages and risks of these materials (individually or combined) include possible swelling, discomfort, infection, loss of the implanted material and loss of bone or overlying soft tissue. It is also possible that long-term unforeseen reactions could occur to this combination although this does not appear to be likely from results in animal experiments.

The alternative to a combination implant would be to use hydroxylapatite alone. This may decrease the amount of augmentation and the ability to properly contour the material at the time of surgery.

I understand the potential advantages, risks and alternative treatment. I agree to proceed with the surgery for implantation of hydroxylapatite and plaster of Paris in combination.

_____ _____
Signature Date

_____ _____
Witness Date

The modified Gill Papanicolaou trichrome stain was also found useful in demonstrating the microvasculature in the implanted mandibular defects in rats and in specimens obtained at vestibuloplasty from humans after mandibular ridge augmentation with hydroxylapatite (Fig. 34).

B. Revascularization After Implantation of Experimental Defects with Plaster of Paris or Ceramic Particles

Calcium compounds such as plaster of Paris itself or mixtures of hydroxylapatite ceramic particles with various binders such as collagen, bovine serum albumin or plaster of Paris were found to be much more effective than demineralized bone in promoting vascularization and filling by fibrovascular tissue of the experimental defects drilled in this poorly vascularized area of the ramus of the mandible (Figs. 35-39).

A nonimmunologic foreign body reaction (not affected by the administration of corticosteroids or aspirin) resulted from implantation of the defects with hydroxylapatite particles. This reaction was highlighted by the conspicuous capsules demonstrated by the PATS reaction around each implant particle (Figs. 31-32). The capsules contained both Langhans and multinucleated giant cells as well as fibroblasts and collagen fibers (Figs. 40-44). This foreign body reaction and the resulting infiltration of fibrovascular tissue and capsule formation was much more prominent in defects filled with ceramic hydroxylapatite particles than with demineralized bone. Indeed, when mixtures of both types of particles were employed, capsules were more prominent around ceramic particles than bone particles (Fig. 45). Indeed ossification (Table 2) was pronounced around many of the hydroxylapatite particles (as well as demineralized bone particles) especially at the periphery of the defect and only when plaster of Paris was used as the binder, Table 2. When care was taken with the periosteum during these procedures, the incorporation of the particles into bone was also more pronounced. In addition to the formation of new cancellous bone around ceramic particles at the periphery of the defect, it was occasionally observed around particles and blood vessels in the center of the implanted defects (Fig. 46). It is important to note (Table 2) that demineralized bone particles, although incorporated into bone at the periphery of the defects, did not act as a stimulus for complete filling of the defects by fibrovascular tissues (Fig. 36). Indeed plaster of Paris alone or implants of hydroxylapatite particles with various binders led to much better filling of the defects and their infiltration by fibrovascular tissues (Figs. 37-39). Moreover, it appeared that the calcium compounds not only acted as a source of calcium for ossification but that they also stimulated revascularization, possibly by an effect on endothelial cells. Thus, even calcium ceramic implants which showed poor ossification around particles or incorporation into bone, displayed 100% filling of the defects by fibrovascular tissue (Table 2). In sections of necropsy specimens from defects implanted in this manner or in biopsy specimens from humans who had undergone ridge augmentation with Calcitite alone, extensive revascularization was observed (Figs. 34, 47).

C. Facilitation of Healing by Formation of a Nonimmunologic Foreign Body Granuloma in the Defects Implanted with Hydroxylapatite

The PATS reaction stains neutrophils by virtue of their high glycogen content. Thus infiltration by neutrophils or polymorphs (and neutrophil pseudoplatelets) can readily be observed (Fig. 48) in areas of acute

Table 2

INCORPORATION OF IMPLANT PARTICLES INTO BONE

Implant Material	Time of Sacrifice	% Defects Completely Filled	Number of Implants	Number of Defects Showing Particle Ossification	% Defects Showing Particle Ossification
Calcitite/BSA	16 wks	100	4	0	0
Calcitite/Collagen	"	100	1	0	0
Calcitite/Plaster	"	100	3	1	33
	3 wks	100	12	6	50
Durapatite/Plaster	3 wks	100	12	1	8
Demineralized Bone/ BSA	5 wks	50	2	2	100
	10 wks	33	3	1	33
	16 wks	66	3	2	66
Demineralized Bone/ Calcitite/BSA	6 wks	100	3	1	33
	10 wks	100	5	2	40

inflammation. Other leukocytes, including eosinophils, are not stained by this procedure. With the PATS reaction and our trichrome stain, the absence of macrophages and acute or chronic inflammation was noted in the tissues from our experimental animals taken at 2 weeks or later after implant surgery. The administration of cortisone or acetylsalicylic acid did not affect giant cell or capsule formation around the particles. Therefore, this reparative granuloma was classified as nonimmunologic. Both Langhans (Fig. 40) and multinucleated foreign body (Figs. 41-43) giant cells were observed in the capsules. The observations of many fibroblasts and collagen fibers (Fig. 44) in the capsules as well as giant cells, and the relative absence of macrophages, suggested that the giant cells could be formed by fusion of fibroblasts rather than monocytes or macrophages. This suggestion received support from our observation of syncytial cells (Figs. 49-51) in our cultures of mouse L-929 fibroblasts.

D. Effect of Hyperbaric Oxygen Treatment on Infiltration of Experimental Mandibular Defects with Fibrovascular Tissue

The administration of hyperbaric oxygen to rats after implantation of the defects with hydroxylapatite ceramic particles resulted in a very significant reduction in size of the defect (Tables 3,4, Figs. 52-55) due to the formation of new cancellous bone (Figs. 56-58). However, no difference in the number of blood vessels could be observed in sections of the implanted defects taken six weeks after implant surgery. This could have been due to the fact that the ossification of the defects occurred so rapidly that the stimulation of revascularization was masked.

TABLE 3

Comparison of Defect *Area at Sacrifice Using Implants with or without Hyperbaric Oxygen (HBO)

Plaster of Paris	Hydroxylapatite/ Bovine Serum Albumin		Hydroxylapatite/ Plaster of Paris	
NO HBO	HBO	NO HBO	HBO	NO HBO
7.2	9.1	10.7	9.4	12.5
4.7	8.5	12.5	6.1	10.7
			4.1	11.0
			5.1	11.5
			5.7	6.6

Defect size at time of surgery was 12.6 mm^2

*Defect areas are in mm^2

TABLE 4

Comparison of Defect Areas Calculated Directly with Areas Computed by Planimetric Image Analysis (mm^2)

Hydroxylapatite/Plaster of Paris

HBO		NO HBO	
Direct Measurement	Image Analysis	Direct Measurement	Image Analaysis
5.1	3.4	11.5	8.4
4.2	3.3	10.4	8.6
4.5	2.8		

E. Statistical Analysis of Factors Affecting Healing of Experimental Defects in Rat Mandibles

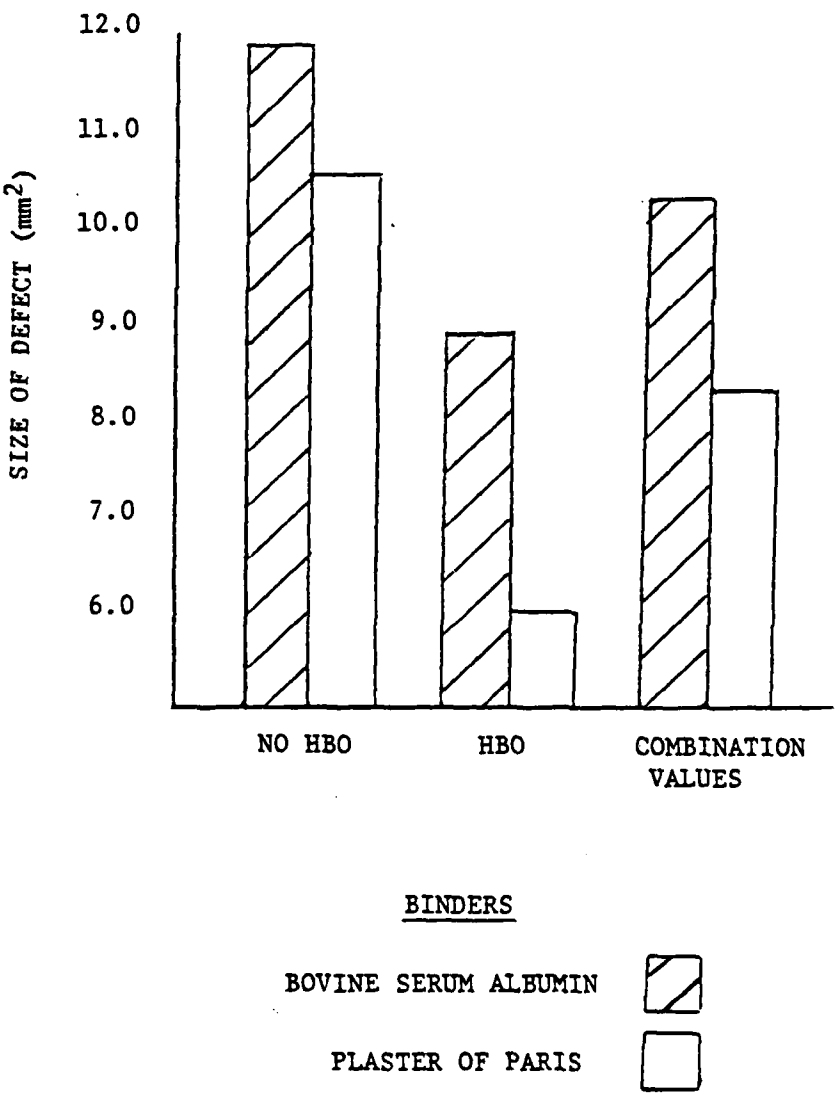
The two factors analyzed below are implant binders (Plaster of BSA) and the effect of hyperbaric oxygen during healing. The mean values for each treatment group are shown in Table 5. These values from Tables 3 and 4 are graphed in Figure 59. The statistical analysis and the pairwise comparisons follow.

Table 5. Sample Sizes and Mean Values for Treatment Groups

	Treatment Group		
	Plaster	BSA	Total
No HBO	$n = 5$	$n = 2$	$n = 7$
	$\bar{x} = 10.46$	$\bar{x} = 11.80$	$\bar{x} = 11.13$
HBO	$n = 5$	$n = 4$	$n = 9$
	$\bar{x}$ 6.08	$\bar{x} = 8.93$	$\bar{x}$ 7.50
Total	$n = 10$	$n = 6$	
	$\bar{x}$ 8.27	$\bar{x} = 10.36$	

Figure 59

Histogram of Healed Defect Size After Implantation
With Hydroxylapatite Particles And Different Binders
With and Without Hyperbaric Oxygen (HBO)



Because the test of binder effect suggested some differences and because the HBO effect was significant, pairwise tests were carried out in order to determine the subsets of the data for which differences might exist. The P--values in Table 6 indicate that a significant difference in defect size exists between plaster and BSA binders. Fig. 60 shows this graphically.

The results in Table 7 are the same as shown previously--there is a significant difference between HBO groups.

Table 6. Pairwise Comparison of Treatment Groups

Comparison	P-value	Conclusion
Plaster vs BSA	.0411	significant difference

Table 7. Pairwise Comparison of HBO Groups

Comparison	P-value	Conclusions
HBO vs non HBO	.0080	highly significant difference

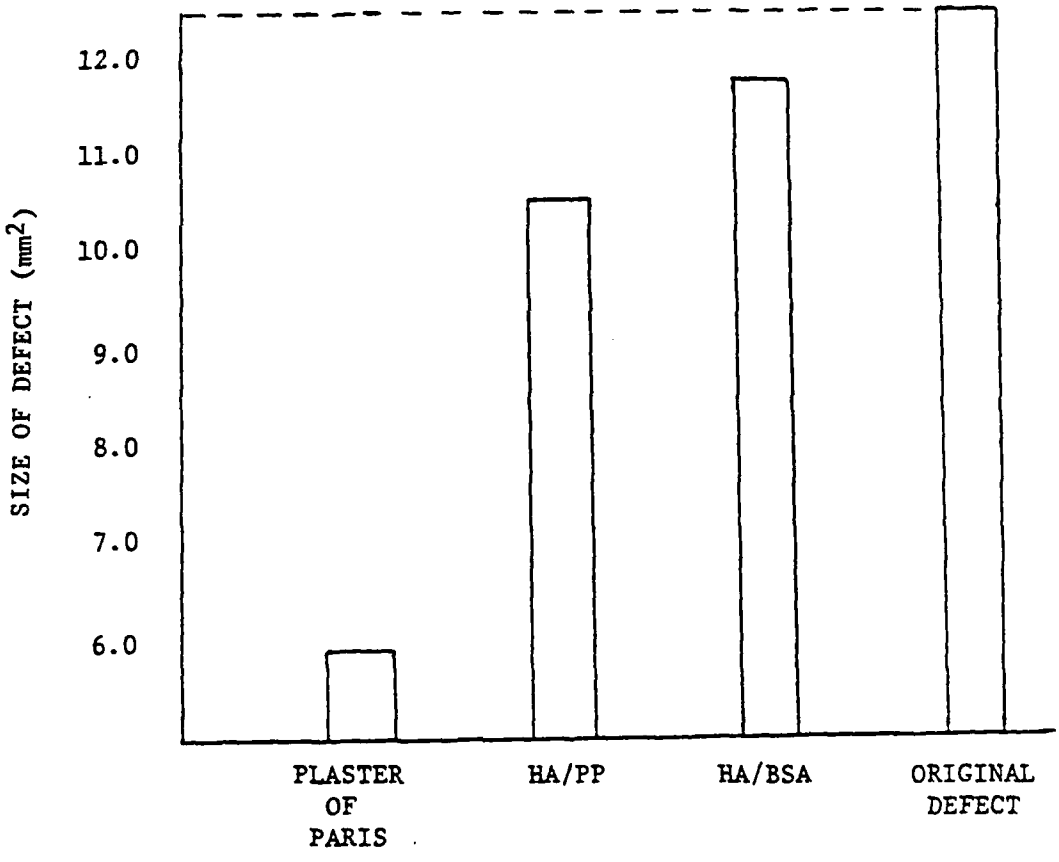
Additional factors analyzed below are implants, binders (Plaster alone, HA/PP or HA/BSA) and the length of healing time on experimental defect diameter ($\bar{x}$). The mean values are shown in the table below, and are shown graphically in figure 60.

Table 8. Sample Size (n) and Defect Diameter ($\bar{x}$) for Treatment Groups

	Implant Group		
	Plaster only	HA/PP	HA/BSA
3 weeks	n=2 $\bar{x}$=5.0	n=11 $\bar{x}$=7.8	
6 weeks	n=4 $\bar{x}$=6.8	n=7 $\bar{x}$=5.7	n=2 $\bar{x}$=11.8
Total	n=6 $\bar{x}$=5.9	n=18 $\bar{x}$=6.8	n=2 $\bar{x}$=11.8

Figure 60

Comparison of Healed Defect Size after Implantation with Plaster of Paris Alone or Hydroxylapatite (HA) and Different Binders



BINDERS

PP = Plaster of Paris
BSA = Bovine Serum Albumin

Because the test of binder effect suggested some differences, pairwise tests were carried out in order to determine the subsets of the data for which differences might exist.

Table 9. Pairwise Comparison of Treatment Groups (3 weeks only)

Comparison	Conclusion
Plaster only or Plaster as binder vs. BSA as binder	highly significant difference
Plaster only vs. Plaster as binder	suggestive significant difference

Also note that appreciably more healing (new bone growth) has occurred after six weeks than after three weeks.

F. Antibacterial Study of $CaSO_4/Ca(OH)_2$ for Cavity Lining, Periodontics, Endodontics, Oral and Orthopaedic Surgery

Research performed here showed that $Ca(OH)_2$ dramatically eliminated or reduced decay bacteria when it was placed under amalgam fillings used to restore decayed teeth. (King, Crawford, and Lindahl, 1965).

Calcium hydroxide produces an alkaline pH in water (about 12.5) which apparently accounts for its antagonistic activity against bacteria found in decayed and diseased teeth. This antimicrobial activity appears to contribute to the therapeutic quality of calcium hydroxide and to the remarkable lack of infections of teeth treated with $Ca(OH)_2$.

Because of the ability of calcium sulfate to be resorbed and to aid bone formation, and because its lower pH may be less traumatic to tissues, it would be desirable to investigate the following questions:

a. Is $CaSO_4$ antagonistic or lethal for a representative strain of oral bacteria that infect teeth and surrounding tissues, and at what concentrations?

b. At what concentrations does $Ca(OH)_2$ retain its activity against such bacteria, when mixed with $CaSO_4$?

c. Is activity pH-dependent?

d. Are the effects of these combinations of $Ca(OH)_2$ and $CaSO_4$ the same against various other aerobic and anaerobic species of bacteria that are recognized in infections of the pulp and tissues that surround the teeth?

Answers to these questions should help guide the clinician in choosing the most protective and therapeutic concentration and combination of $CaSO_4$ with $Ca(OH)_2$.

For our pilot screening tests, we selected a strain of Streptococcus faecalis, a bacterium that is commonly found in infected teeth and we have found in severe mandibular infections.

We tested concentrations of 1%, 4%, and 30% $Ca(OH)_2$ against a heavy inoculum of the bacteria in an optimal growth medium (TSB), using a method employed for antibiotic testing.

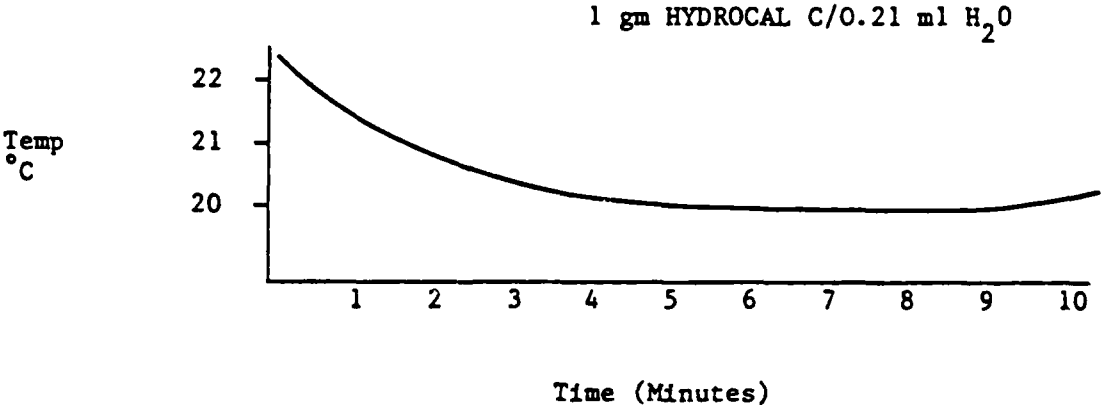
All concentrations of $Ca(OH)_2$ used were found to be inhibitory and lethal for the bacterial strain tested (Table 10). This implies that $Ca(OH)_2$ has antibacterial capabilities at various concentrations that help explain its in vivo effectiveness. What pH and other variables are necessary for $Ca(OH)_2$ to retain its antibacterial activities in combination with $CaSO_4$, or indeed, whether $CaSO_4$ has antibacterial properties of its own, remain to be determined, as do antibacterial effects of these chemicals against a spectrum of other orally infectious bacterial agents.

G. Results of In Vitro Studies of Composite Implants

Fig. 61 shows that the denser of the two plasters holds up better to 24 hour immersion in saline at room temperature. Figure 62 shows the effects of different concentrations of K_2SO_4 and sodium citrate on the setting rate and consistency of HYDROCAL C.

H. Cooling During Setting of Small Samples of Hydrocal C

Temperature was analyzed as a factor in setting times of Hydrocal C implants. Using small samples of from one to five grams in vitro to simulate the size of a monkey or human implant, it was found that the hydrated mix (paste) actually cooled during initial setting.



Apparently the heat released during hydration increases the rate of evaporation from the surface which causes a net cooling of the small sample. This cooling effect of an implant would probably be overcome by body heat in an animal or human and would not be a limiting factor.

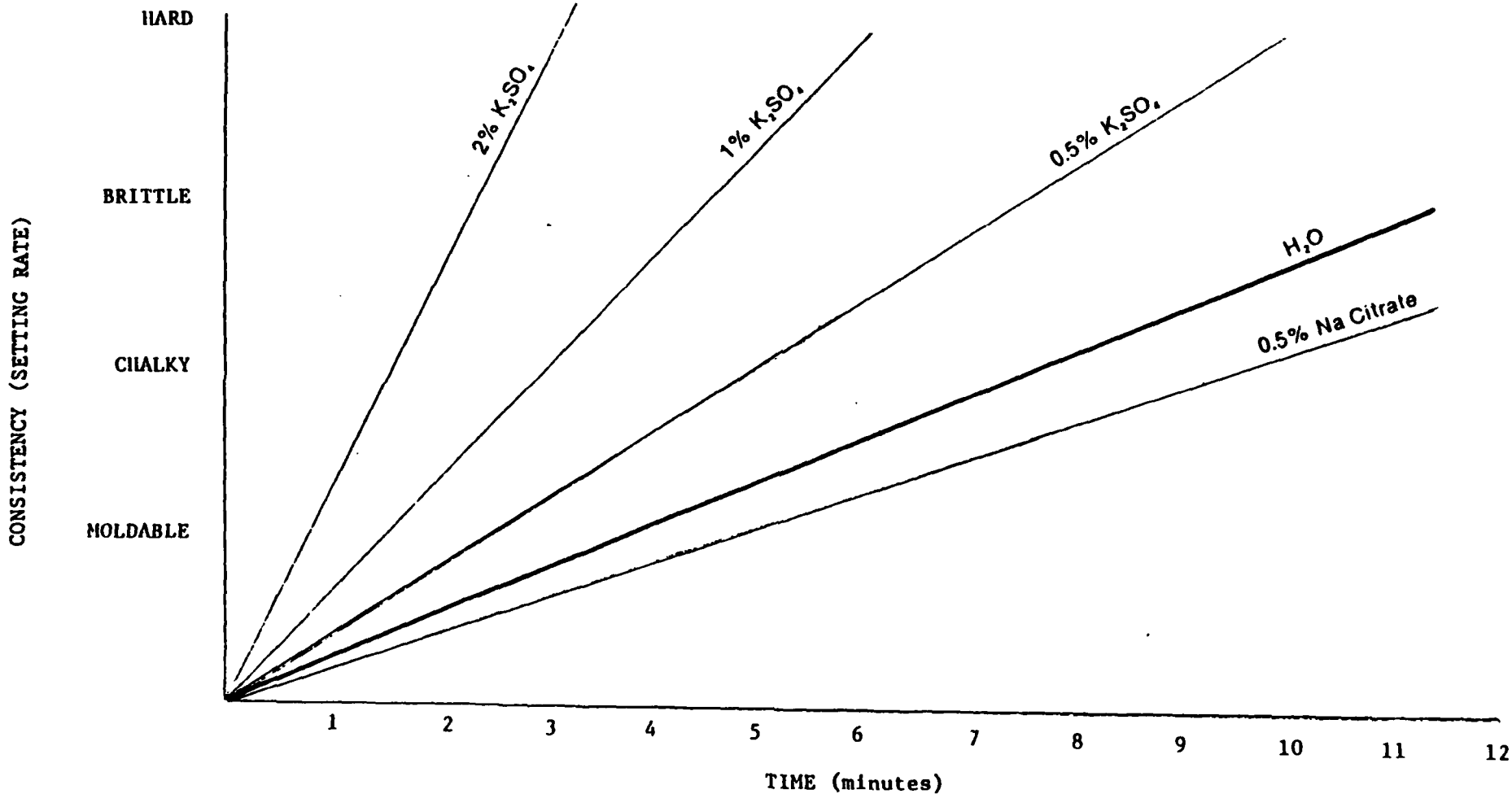
TABLE 10

BACTERICIDAL ACTIVITY OF SUSPENSIONS
OF CALCIUM COMPOUNDS

pH of Suspension	Results of 24 hr. Subculture	% Composition $CaSO_4 \cdot \frac{1}{2}H_2O$	% Composition $Ca(OH)_2$
12.35	Neg.	0	30
12.40	Neg.	15	15
12.40	Neg.	4	2
12.40	Neg.	2	4
12.45	Neg.	4	4
7.45	growth	2	0
7.20	growth	4	0
6.80	growth	30	0

Fig. 62

Effects of Ions* on Setting Rate and Consistency of HYDROCAL C**



*Dissolved in wetting solution

**All samples consisted of 1gm HYDROCAL C in 0.21ml wetting solution

I. Effects of Experimental Surgery with HA/HYDROCAL C Implants in Rabbits, Dogs and Monkeys

It is important to state that although a granuloma accompanied the implantation of HA with or without HYDROCAL C, it did not accompany the implantation of HYDROCAL C itself. The granuloma accompanying the implantation of HA appeared to be a nonimmunologic reparative granuloma which actually helps the body incorporate and tolerate the implant. Unlike usual granulomas, there were no inflammatory cells apparent. Fibroblasts, however, were prominent (Fig. 44) in the capsules surrounding HA particles. Studies with cultured fibroblasts by B. Giammara in our laboratory have shown that fibroblasts (as well as macrophages) can fuse (Figs. 49-51) to form syncytial or multinucleated cells. The radiograph of a mandibular inferior border onlay implant of HA/PP (reinforced by stainless steel) in a monkey shows that it is well tolerated after 5 months (Fig. 63). Injection of HA alone (in saline or water) into subperiosteal tunnels of monkey mandible (Figs. 19, 20) showed scattering of some particles throughout the surgical field in addition to containment of some within the tunnel. On the other hand, injection of HA/HYDROCAL C into subperiosteal tunnels (Fig. 25) resulted in much better control of particle placement (Fig. 26). Greater vertical heights could generally be achieved (Figs. 64, 65) when the HA was mixed with HYDROCAL C. In addition, contouring was facilitated. Actual numbers cannot be put on the heights achieved with HA alone and HA/PP in the monkeys until vestibuloplasty is performed or measurements are made on the radiographs. Five of six monkeys were sacrificed at periods ranging from 3 to 24 weeks after implantation. After excision, the mandible was divided and the two sides compared radiographically and physically (Figs. 66-69). Both the radiographic and physical examination showed that, generally, significantly greater augmentation height was achieved on the side implanted with HA/P that with HA alone (Figs. 66-69). In addition, containment of the implant particles (Tables 11, 12), or prevention of their scattering (Figs. 66-69) was at least twice as good on the side in which the HA was implanted with plaster as a binder. Sections of undecalcified mandible obtained with a diamond saw (Figs. 70-75) and stained with Alizarin Red S or von Kossa stain showed that new cancellous bone eventually incorporates the HA particles into the alveolar bone of the monkey mandible. These sections also showed that containment by HA/PP was better than with HA alone.

J. Results of Ridge Augmentation with HA/HYDROCAL C in 5 Patients

The first 2 human cases where HA/HYDROCAL C was implanted were for the correction of maxillary defects due to trauma. The very first case was for esthetic effect and phonetic function. These results have already been achieved. Whether or not new bone forms around the particles is immaterial inasmuch as the prosthesis can be anchored on teeth adjacent to the reconstructed defect.

The second case is a facial trauma patient with severe compromise of the alveolar ridge associated with the loss of maxillary anterior teeth and alveolar bone. Augmentation with HA alone was unsuccessful, but success was achieved by the subsequent application of HA/HYDROCAL C.

The third case involved atrophy of the posterior and midbody regions of the mandible to the extent that a soft tissue procedure alone would be inadequate to provide a suitable denture-bearing area. The use of HA alone in this case would have resulted in a less substantial augmentation secondary to soft tissue

Table 11

RIDGE HEIGHTS AND PARTICLE CONTAINMENT ATTAINED WITH HA/P AND HA ALONE IN MANDIBULAR AUGMENTATION

Monkey No.	Post-Surgical Time of Necropsy Specimen (Weeks)	
3-49	3	Much better height and less scatter with HA/P
3-50	6	HA/P not quite as high as HA alone but much less particle scatter
828-E	12	Greater augmentation and less particle scatter with HA/P
3-51	16	HA/P Infected and showed loss of implant. HA alone showed scattering
3-52	24	Excellent augmentation with HA/P and less scatter than HA alone
3-53	Not sacrificed	

TABLE 12

COMPARISON OF RIDGE AUGMENTED WITH
HA/PLASTER TO RIDGE IMPLANTED WITH HA ALONE

Monkey No.	Height	Particle-Containment
3-49	+++/+	++/+
3-50	++/+++	+++/+
828-E	+++/++	++/+
3-51*	/++	/+
3-52	+++/+++	+++/+
Overall Values	11/9	10/4

*HA-plaster side infected and, therefore, not rated.

tension. Soft tissue tension was not a problem when we used the combination of HA/HYDROCAL C since the material could be contoured and allowed to set, eliminating soft tissue influence on the augmentation material. The fourth case was very similar. The fifth case was done by our new procedure where the implant was isolated from blood and other tissue fluids during its formation at the augmentation site in a sterile finger cot. It was taken out to set (approximately 8 minutes after formation) and then smoothed and implanted (Figs. 76-82).

K. Results of Filling Infrabony Periodontal Defects in 65 Patients

Dr. Reynolds A. Carnevale of Fayetteville has done 60 of these patients and the other five were done in the graduate periodontal clinic of the UNC School of Dentistry. Dr. Carnevale's experience is obviously much more extensive and he has done as many as thirteen teeth in an individual patient. He has found a dramatic decrease in tooth mobility in almost all of his patients. Although he has not recorded probe depths before and after the surgery, there is radiographic evidence (Figs. 83-87) of good retention of the hydroxylapatite particles in the defects up to a year after implantation. He is currently attempting the repair using blood alone as the setting liquid for plaster.

The results in the periodontal clinic at UNC have, to date, not been as satisfactory. On the other hand, the radiographs in some of Dr. Carnevale's patients suggest that the induction of osteogenesis may have occurred at the implant site.

L. Augmentation of Rat Mandibular Ramus Defect and Deficient Monkey Alveolar Ridge with Plaster (HYDROCAL C) Alone vs. HA/HYDROCAL C

The implantation with plaster (HYDROCAL C) alone of the defect in the ramus of the rat mandible led to more cancellous bone formation (Figs. 106-108) than with HA alone or even HA/HYDROCAL C (Fig. 53 and Table 8). It was, therefore, of interest to see whether HYDROCAL C alone could be as effective as the composite ceramic (HA/HYDROCAL C) implants. The 4 mm defects on one side of the mandible of 6 rats were filled with HYDROCAL C alone. Defects on the opposite side were filled with the HYDROCAL C/HA mixture. Immediately after implantation one side of the mandible was indistinguishable from the other by X-ray (Figs. 88, 89). One week later, however, the resorption of almost all of the plaster was evident (Figs. 90, 91).

The result of augmentation of a monkey alveolar ridge (after radical alveolectomy and healing as described above) with HYDROCAL C alone was compared with augmentation (of the deficient ridge on the opposite side) by the injection of HYDROCAL C/HA in the subperiosteal tunnel.

Physical examination of the monkey 2 weeks post-surgery indicated that resorption of the HYDROCAL C had occurred on the right side. Comparison of the radiographs (Figs. 92, 93) taken at this time showed that less than 20% of the C base HYDROCAL implanted remained. On the other hand, the ridge on the left side augmented with HA/HYDROCAL C was as high and as firm after 2 weeks as it was at the time of implantation and the radiograph (Fig. 93) showed that the particles did not stray.

M. Effect of Fluoride Administration on Alevolar Bone, Dentine and Enamel Formation in Newborn Mice

Inasmuch as fluoride could be added to the HA/plaster composite to stimulate revascularization or osteogenesis, and fluorapatite is a possible substitute for hydroxylapatite, the effects of fluoride on hard tissue formation in newborn mice was assessed.

Newborn mice were injected once daily with 20 micrograms sodium fluoride (in 10 microliters of water). They were sacrificed on day four or day five by decapitation. The heads were fixed in calcium formol solution, cryostat-sectioned, and incubated for acid phosphatase activity (Hanker, 1979) to show ameloblasts, odontoblasts, osteoblasts and osteoclasts. Some sections were treated with the PATS reaction (Giammara et al., 1983) to show the collagen in the dentine and bone matrix. To estimate the effect of fluoride administration on the formation of the hard tissues, four to seven sections were cut from the mandible of each animal at approximately the same level through the first lower molar. Table 13 shows that there were significant increases in the overall relative amounts of dentin and enamel in the fluoride-injected animals. When the widths of dentin and enamel present were estimated at the end of the Stage I Segment, they were more than twice as wide in fluoride-injected mice. Differences in the amounts of alveolar bone present could not be readily discerned. However there was a very significant decrease (Fig. 94) in the numbers of osteoclasts associated with the alveolar bone of the fluoride-treated mice.

N. Sterilization of HA/Plaster Components, Mixtures and Preformed Implants to Repair Bone Defects

Inasmuch as the HA/C-Base HYDROCAL mixtures are being employed on a fairly regular basis in the surgical suite at NC Memorial Hospital and the Graduate Periodontic Clinic in the UNC School of Dentistry, it was necessary to have preweighed amounts of the sterile plaster to mix in the OR or perio clinic with the standard-size packaged amounts of sterile HA particles that are commercially available. It was found that preweighed plaster could be put in glass vials having polypropylene screw caps and sterilized by dry heat at 160° for 2 hr without any apparent problem. These vials could then be placed (under sterile conditions in a laminar flow hood) into ethylene oxide (ETO) sterilized sterilization bags or sterilizer pouches (Fig. 95). These could be taken into the OR or sent to practioners who found it necessary to employ our procedure where the implant is formed at surgery.

On a number of occasions our surgeons have preferred to tailor preformed HA/plaster implants prior to surgery. It was found that these implants could readily be formed and shaped on casts made from tissue displaced impressions or moulages. These implants were formed by the thoroughly mixing the HA with C-base HYDROCAL (containing 0.75% K_2SO_4). Water was mixed in (water/plaster ratio - 0.3) and the implant formed on cast. After hardening, the implants were sawed in half; one half was subjected to our usual dry heat sterilization process. The other half was not sterilized. The sterilized portions of the implants discolored (brown) and appeared to be brittle.

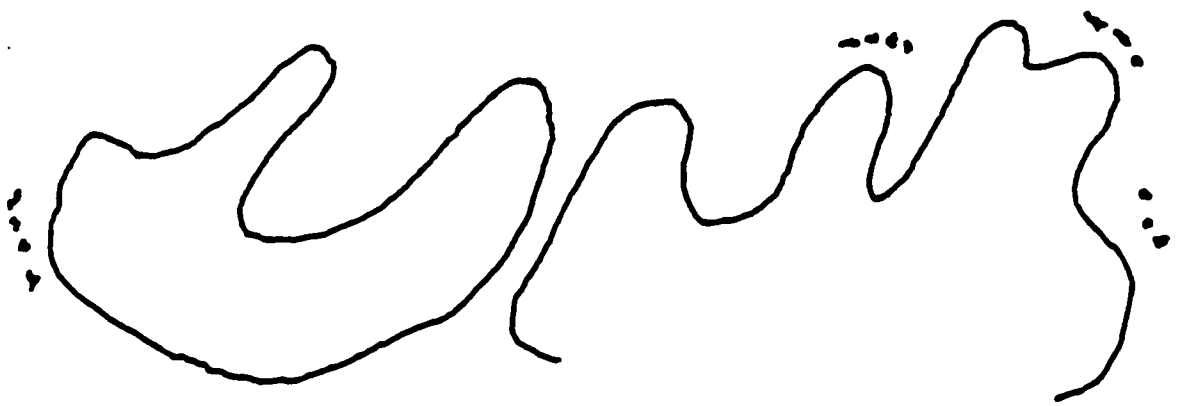
TABLE 13

EFFECT OF FLUORIDE ON DENTIN AND ENAMEL FORMATION
IN FIRST LOWER MOLARS OF NEWBORN MICE

	Age (in Days) at Sacrifice	Overall Relative Amount		Estimated Width at End of Stage I Segment	
		Dentin (1-6)	Enamel (1-4)	Dentin (1-7)	Enamel (1-4)
Controls	4,5	2.9	1.4	2.3	1.2
	4	2.7	1.2	2.0	1.0
	4	2.7	1.3	2.0	1.0
	5	3.4	1.8	3.0	1.5
Fluoride-Injected	4,5	5.0	3.0	5.5	2.9
	4	4.8	2.8	5.0	3.0
	4	4.6	2.5	4.0	2.8
	5	5.3	3.5	6.0	2.5
	5	5.1	3.3	7.0	3.5

Diagrammatic Representation Of Decrease In Osteoclast Population In Alveolar Bone Surrounding Molars Of Four-Day-Old Mouse Due To Fluoride Administration

Control



Fluoride-Treated

O. Tensile Strengths of Preformed HA/Plaster Implants Sterilized by Dry Heat or Ethylene Oxide

As mentioned above, although dry heat sterilization (160 deg C, 2 hr) was suitable for the calcined gypsums prior to mixing with water, this same treatment appeared to render the preformed composite implants brittle. Autoclaving might also make the implants brittle due to loss of water of hydration from the plaster component. Therefore, the effects of dry heat and ethylene oxide (ETO) sterilization on the plaster component were compared.

A silicone rubber mold (Fig. 96, 97) was made in which discs of USG C-base HYDROCAL could be cast and removed with minimal stress. One group of discs was sterilized with dry heat, one with ETO, and one group served as a control (Fig. 98). Using a diametral compression test, the tensile strengths were evaluated with an Instron testing machine (Figs. 99, 100) at a crosshead speed of 0.1 cm/min.

Compared to samples sterilized with dry heat (2770±186 kPa), both the control specimens (6760±922 kPa) and the ETO group (7600±324 kPa) were much stronger. Although no significant differences was noted between the control and the ETO groups ($p > 0.20$), the dry heat group was significantly weaker than the other groups ($p < 0.001$).

The complete results of the diametral compression tests on the C-base HYDROCAL discs are shown in Table 14 and in the corresponding graph (Fig. 101).

P. FACTORS AFFECTING THE SETTING OF PLASTER IN BLOOD AND TISSUE FLUIDS

When the implants were formed at surgery it was noted that blood and tissue fluids interfered with the setting of the implants. This problem could frequently be circumvented by Dr. Terry's procedure where the HA/plaster was isolated in a sterile finger cot or membrane during formation of the implant in the surgical site or by preforming it. On the other hand in perio patients, the sterile membrane procedure was not practical. Much success could be achieved however by packing in a moist 50/50 HA/HYDROCAL C mixture and then tightly pressing the HA particles into the defect with a sterile gauze which aided in removing blood, XS water and XS plaster. On certain occasions, however, the perio field is so bloody that the above procedure is not practical. In studies with blood, we have found that we can get plaster to set in blood alone if appropriate quantities of K_2SO_4 or NaCl are added. Figs. 102, 103 show implants formed from C-base HYDROCAL alone or C-base HYDROCAL/HA where whole blood was the only liquid used to moisten the powder. The scanning electron micrographs (Figs. 104, 105) show how close together the HA particles can be packed in the implant by this procedure even though the starting mixture contains only 50% HA.

Fig. 101

EFFECTS OF STERILIZATION ON TENSILE STRENGTH OF C-BASE HYDROCAL IMPLANTS

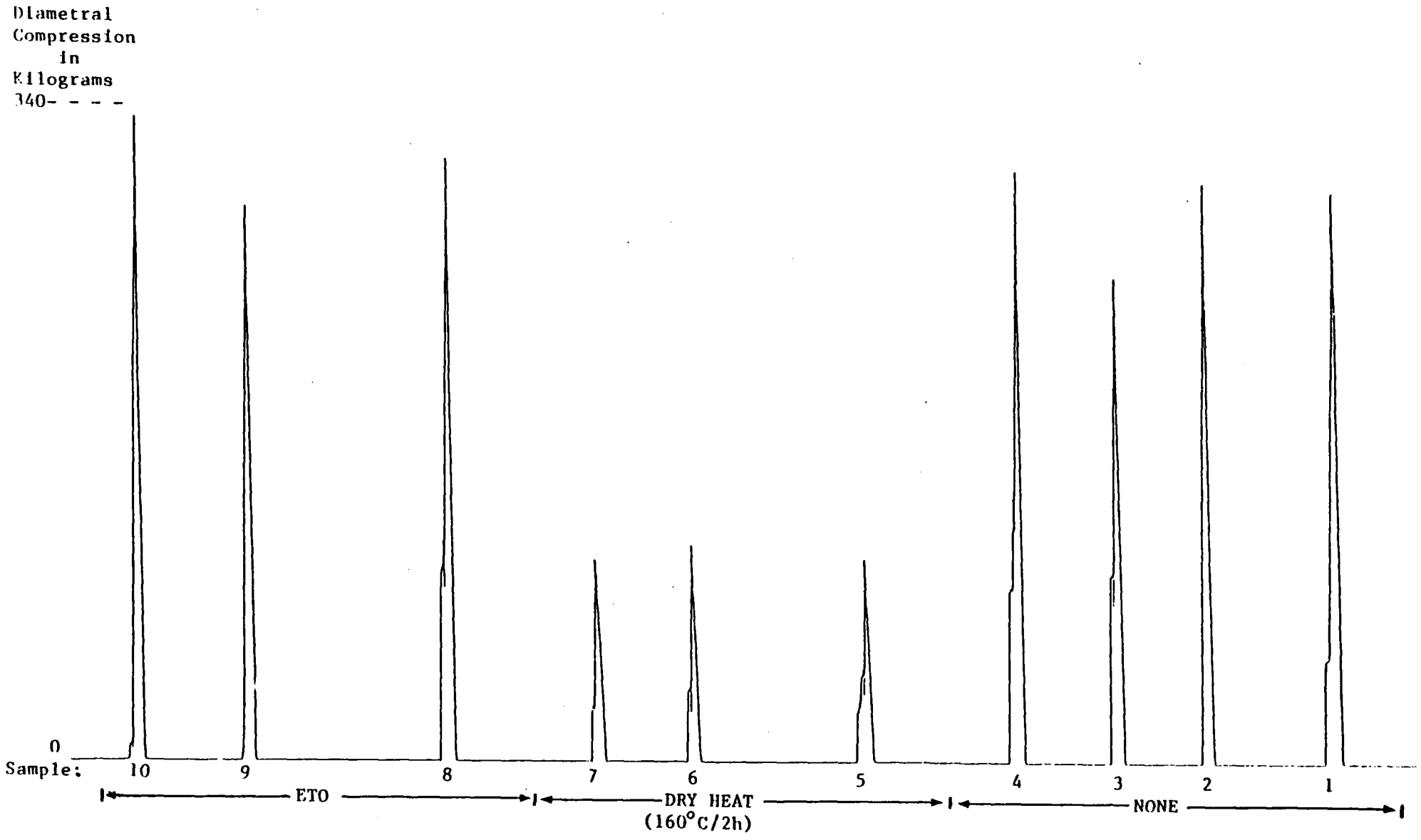


TABLE 14

DIAMETRAL COMPRESSION TESTS

EXPERIMENTAL DESIGNATION: GYPSUM-1

10 samples tested using the 5000 kgs. load cell.
C.H.S. = .1 cm./min. and C.P.S. = 1 cm./min.

SAMPLE	TREATMENT	MF(kg)	D(in)	L(in)	DC(kg/cm^2)	DC(kPa)
1	None	302.0	1.240	0.320	75.1	7365
2	None	307.0	1.241	0.340	71.8	7041
3	None	257.0	1.242	0.372	54.9	5383
4	None	312.0	1.240	0.337	73.7	7225
5	160 deg C/ 2h	108.0	1.229	0.300	28.9	2835
6	160 deg C/ 2h	116.0	1.233	0.314	29.6	2899
7	160 deg C/ 2h	107.0	1.235	0.328	26.1	2556
8	ETO	318.0	1.237	0.337	75.3	7382
9	ETO	293.0	1.235	0.308	76.0	7454
10	ETO	340.0	1.239	0.333	81.3	7974

Q. Calcification Induced by Implantation of Plasters Subdermally

Previous studies (Mitchell and Shankwalker, 1958; Binnie and Mitchell, 1973) have shown the ability of calcium compounds like calcium hydroxide and plaster to stimulate the formation of calcified, bonelike tissue in the fibrous capsule formed around small pellets of the calcium compound implanted under the skin in subpannicular pouches. It is important to note that the mineralized material induced in this model is histochemically, but not histomorphologically, compatible with bone (Mitchell and Shankwalker, 1958; Binnie and Mitchell, 1973). This is apparently due to the absence of collagenous bone matrix. Pellets of calcium hydroxide, No. 1 Moulding Plaster, B-Base HYDROCAL and C-Base HYDROCAL were formed and implanted subdermally in pouches on the back of a rat as previously described (Mitchell and Shankwalker, 1958; Binnie and Mitchell, 1973). Radiographs (Figs. 109, 110) showed the presence of radio-opaque bone-like material 32 days after implantation. The plasters were apparently not resorbed in this situation to the extent that occurs when implanted in a NHDRRM*. The differences in radiopacity at the sites of implantation of the different plasters subdermally are not significant because a statistically significant number of implants was not done with each calcium material.

*Non-healing defect in the ramus of the rat mandible.

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XI. LEGENDS FOR FIGURES

Fig. 1. 4mm non-healing (control) defect created by drilling through the relatively avascular area of the ramus of rat mandible.

Fig. 2. Implantation of the defect with a moistened 50:50 mixture of plaster of Paris/hydroxylapatite (PP/HA).

Fig. 3. Particles of sintered hydroxylapatite, a calcium phosphate ceramic.

Fig. 4. Acid phosphatase in Golgi apparati (not nuclei) of ameloblast layer (A) and odontoblast layer (O) of developing mouse molar.

Fig. 5. Same histochemical procedure shows Golgi apparati (not nuclei) of osteoblasts in area of developing mouse mandible and osteoclasts (arrows) with negative nuclei.

Fig. 6. Higher magnification of osteoblast area of same specimen.

Fig. 7. Super high pressure chamber used to administer HBO to surgically-treated rats.

Fig. 8. Bethlehem H-70-A chamber used for the same purpose.

Fig. 9. Sections of mouse cerebral cortex stained with benzidine to show microvasculature by virtue of red cell catalase.

Fig. 10. Vibratome section of mouse submandibular gland stained for catalase activity shows microvasculature in Cast-A-Slide embedment.

Fig. 11. Notice how acid phosphatase activity in and the size of the Golgi apparati (not nuclei) in osteoblasts increase in the area of bone formation. Note decreased acid phosphatase activity in osteocytes (arrows) surrounded by newly formed bone.

Fig. 12. An area of Fig. 11 enlarged. Arrows indicate osteocytes.

Fig. 13. Monkey prior to experimental mandibular ridge augmentation.

Fig. 14. Radical alveolectomy to remove lower dentition.

Fig. 15. Mandibular dentition excised.

Fig. 16. Edentulated monkey mandible resulting from radical alveolectomy.

Fig. 17. After healing, a subperiosteal tunnel was created.

Fig. 18. Diagrammatic representation of mandibular ridge augmentation with hydroxylapatite.

Fig. 19. Injection of HA alone for ridge augmentation.

Fig. 20. Injection of HA alone into subperiosteal tunnel.

Fig. 21-22. In addition to HA particles in the subperiosteal tunnel, others can be seen throughout the surgical field due to lack of containment.

Figs. 23, 24. Preparation of 50:50 C-Base Hydrocal/HA mixture for implantation by injection.

Fig. 25. Injection of C-Base Hydrocal/HA mixture into subperiosteal tunnel in monkey mandible.

Fig. 26. C-Base Hydrocal/HA mixture is completely contained within the subperiosteal tunnel.

Fig. 27. PATS stain (left) shows pericapillary reticular network of microvasculature in healing defect. Red cells are only lightly stained.

Fig. 28. Adjacent section stained with DAB and copper barely shows vessel structure although red cells are intensely stained.

Fig. 29. Red cells (arrow) are barely visible in bone capillary delineated by PATS staining of reticular fibers (double arrows) in adjacent section.

Fig. 30-32. PATS stain on sections of decalcified healing defect implanted with hydroxylapatite and protein binder. Capsules (double arrows) are clearly seen around spaces resulting from demineralization of hydroxylapatite. Reticular fibers around blood vessels or capillaries are clearly seen (arrows).

Fig. 33. Arterioles (arrows) shown by PATS stain in fibrovascular tissue infiltrating healing defect.

Fig. 34. Blood vessels (arrows) which have infiltrated biopsy specimen obtained from human at vestibuloplasty subsequent to ridge augmentation with HA. Trichrome stain.

Fig. 35. Control nonhealed unimplanted defect.

Fig. 36. Notice nonhealing or only partial healing (filling) obtained on defects implanted with demineralized bone alone.

Figs. 37-39. Healed defect after implantation with 37) plaster of Paris alone; 38) Calcitite and plaster of Paris binder; 39) Calcitite and demineralized bone.

Figs. 40, 41. Methylene blue stain shows multiple nuclei of syncytial Langhans (Fig. 40a) and multinucleated foreign body (Fig. 40b) giant cells from a single capsule (Fig. 41) surrounding an HA particle.

Fig. 42. Epon semithin section stained by methylene blue shows foreign body multinucleated giant cell from capsule (arrow).

Fig. 43. EM of capsular foreign body multinucleate giant cell, PATS stain.

Fig. 44. EM showing fibroblasts and collagen fibrils in a different area of PATS stained capsule. No counter stain was used.

Fig. 45. PATS stained section of defect filled with hydroxylapatite/demineralized bone mixture shows capsules more prominently around all ceramic particle spaces than some demineralized bone particles (arrows).

Fig. 46. Cancellous bone forming around blood vessel (BV) as well as hydroxylapatite space (S) in center of healing defect implanted with Calcitite/plaster of Paris and treated with HBO. PATS stain.

Fig. 47. Biopsy specimen obtained from human at vestibuloplasty subsequent to ridge augmentation with HA stained by PATS reaction. Many blood vessels (arrows) are clearly seen.

Fig. 48. Neutrophils seen by virtue of glycogen in PATS stained section of inflamed rat uterus. Note negative nuclear lobes of neutrophils (arrows).

Figs. 49-51. Cultures of L929 mouse fibroblasts stained with methylene blue showing syncytial (multinucleated) cells (arrows).

Figs. 52, 53. Sections of Calcitite/plaster of Paris filled defect stained with PATS.

Figs. 54, 55. Defect in another animal implanted with same mixture and treated with hyperbaric oxygen (HBO). Image analysis by computerized tomography showed (Tables 3-5 and Fig. 59 in text of report) that defects in animals treated with HBO were significantly smaller due to extensive filling with new cancellous bone (arrows).

Figs. 56 a,b. Higher magnification micrographs of sections of same defect show infiltration with fibrovascular tissue (FT) and envelopment of ceramic particle spaces by new cancellous bone (CA). Compact bone (CP).

Fig. 57 a,b. Scanning electron micrographs of areas of mineralized tissues showing a more woven or immature form (Fig. 57a) versus a more compact or mature form (Fig. 57b).

Fig. 58 a,b. X-ray microanalysis with a Kevex system of the areas of mineralized tissues of Figs. 57a and b shows that the calcium/phosphorous ratio is considerably lower (Fig. 58a) in less mature than in the more mature (Fig. 58b) bone area.

Fig. 59, 60. See body of report, pages 36, 38.

Figs. 61 a,b. Alloplasts (61a) of plaster alone, plaster HA, or plaster and HA reinforced by stainless steel after 24 hour immersion in saline show more disruption than alloplasts (61b) of stone alone, stone and HA or stone and HA reinforced by stainless steel. Dental stone is a denser form of plaster.

Fig. 62. See body of report, page 42.

Fig. 63. Radiograph of mandibular onlay implant (HA/PP reinforced by stainless steel bar) five months after implantation.

Figs. 64, 65. Mandibular ridge augmentation with HA/HYDROCAL C mixture results in greater contour (and vertical augmentation) than can be achieved with HA alone.

Figs. 66, 67. Radiographs showing left side of monkey mandible implanted with HA/P compared to opposite (right) side implanted with HA alone. Note especially poor containment of HA particles on right side of both monkeys, one sacrificed at 12 weeks, one at 24 weeks.

Figs. 68-69. Photographs of corresponding alveolar ridges.

Figs. 70-75. Stained sections of monkey mandible obtained with a diamond saw show particles of HA (arrows) which have been incorporated into bone. Light micrographs of Alizarin Red S stained (70, 71) or Von Kossa stained (72-74) sections.

Fig. 72. Subperiosteal tunnel was filled with HA/PP. Note that particles (arrows) are solely on top of the ridge.

Figs. 73, 74. Tunnel filled with HA alone. Although particles at top of ridge are clearly incorporated into alveolar bone, particles (arrows) can be seen which have strayed to the lingual side of the ridge.

Fig. 75. Scanning electron micrograph of a section shows where 2 particles (arrows) have been incorporated into host bone at the tissue/implant interface.

Figs. 76-82. Augmentation of a deficient mandible with HA/HYDROCAL C by new procedure where implant was isolated from blood and tissue fluids in a sterile finger cot. Patient had a hemimandibulectomy at Sloan-Kettering for osteogenic sarcoma. Then received an iliac crest graft restoring only the body of the mandible.

Fig. 76. Surgical bed demonstrating the previous bone graft-host mandible juncture at the left parasymphysis and the superior surface of the grafted bone.

Fig. 77 a,b,c. Formation of the HA/HYDROCAL C implant.

Fig. 77 a. The hydroxylapatite (HA)-plaster mixture was placed in a sterile finger cot and formed in the surgical bed providing an exact replica of the exposed bony surface. After setting, the implant was removed from its isolated environment.

Fig. 77b. The surface of the implant which duplicates the adjacent bone form.

Fig. 77c. Final implant form after being placed in the wound for final confirmation of desired shape and bulk.

Fig. 78a. HA - Plaster implant in position secured with one 0-chromic circum-implant - mandibular suture. Residual defect noted (arrow) at implant residual mandible juncture.

Fig. 78b. The bone graft-implant juncture with the host mandible has been filled and contoured by direct application of the HA-plaster binder mixture. This area was kept free of blood and/or tissue secretions while this was accomplished.

Figs. 79 a,b. Mandibular ridge prior to and after HA/HYDROCAL C augmentation.

Figs. 80 a-d. Radiographs: a, prior to surgery; b, postsurgery; c, 3 mos. postsurgery; d, composite of b (upper) and c (lower).

Fig. 81 a. Patient prior to augmentation.

Fig. 81 b. Patient with temporary denture after augmentation and vestibuloplasty (about 6 mos. postsurgery).

Figs. 82 a,b. Patient prior to (a) and after (b) surgery.

Figs. 83-87. Radiographs prior to and after filling of infrabony periodontal defects with HA/HYDROCAL C.

83a, prior. 83b, 5 mos post.
84a, prior. 84b, 5 mos. post. 84c, 8 mos. post.
85a, prior. 85b, 5 mos. post.
86a, prior. 86b, 3 mos. post.
87a, prior. 87b, 3 mos. post.

Figs. 88, 89. Rat mandibular ramus defects filled with HA/HYDROCAL C (88) or HYDROCAL C alone (89) cannot be distinguished immediately after surgery.

Figs. 90, 91. One week later, the composite implant is still intact (90) whereas the defect filled with HYDROCAL C alone (91) is practically radiolucent.

Fig. 92, 93. Two weeks after filling the monkey's subperiosteal tunnels with HYDROCAL C alone (92) and HA/HYDROCAL C (93) less than 20% of the plaster (HYDROCAL C) remains (92, arrow) whereas the HA/HYDROCAL C implant (93, arrow) is intact.

Fig. 94. See body of report, page 48.

Fig. 95. Dry-heat sterilized HYDROCAL C ready for delivery to the OR.

Figs. 96-98. Preparation of discs of C-base HYDROCAL to evaluate effects of different sterilization modes on implant tensile strength.

Figs. 99,100. An Instron testing machine (99) and compression testing of a HYDROCAL C disc (100).

Fig. 101. See body of report, page 52.

Fig. 102. HYDROCAL C that was set in rat blood as the only liquid.

Fig. 103. HA/HYDROCAL C that was set in human blood as the only liquid.

Fig. 104. Scanning EM of HA/HYDROCAL C that was set in human blood as the only liquid.

Fig. 105. Higher magnification SEM of HA/HYDROCAL C that was set in human blood shows that the HA particles can be packed together so tightly that they are touching. There is still enough space, however, for the infiltration of fibrovascular tissue as the HA is resorbed.

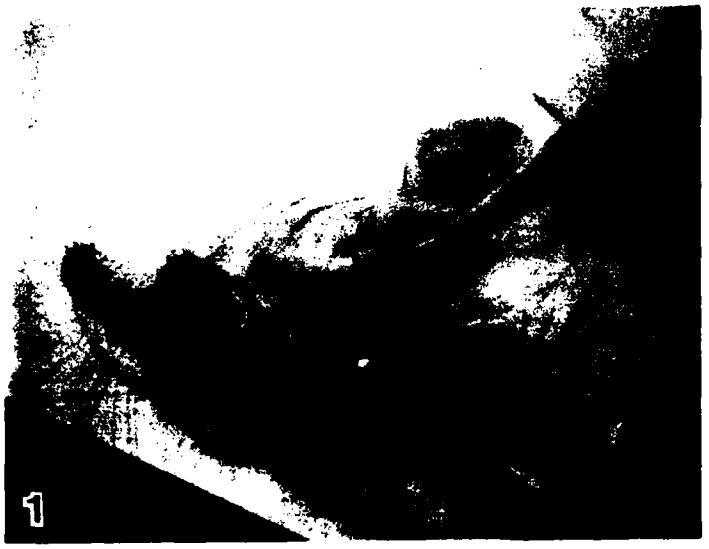
Figs. 106-108. Defects in ramus of rat mandible that were filled with plaster alone (106, 107) or unfilled (108) which served as a control defect.

Fig. 106. Defect that was filled with plaster only shows infiltration of fibrovascular tissue (FV), a rim of new cancellous or trabecular bone (CA) and the outer layer of host bone which is compact bone (CP).

Figs. 107 a,b. Higher manification of an area of interface of fibrovascular tissue (FV) with cancellous bone (CA).

Fig. 108. Note absence of cancellous (or trabecular) bone in the control (unfilled) defect, where only compact bone (CP) is prominent.

Figs. 109, 110. Radiopacity clearly indicates the presence of mineralized deposits in subpannicular pouches 32 days as well as 4 days after implantation of various plasters.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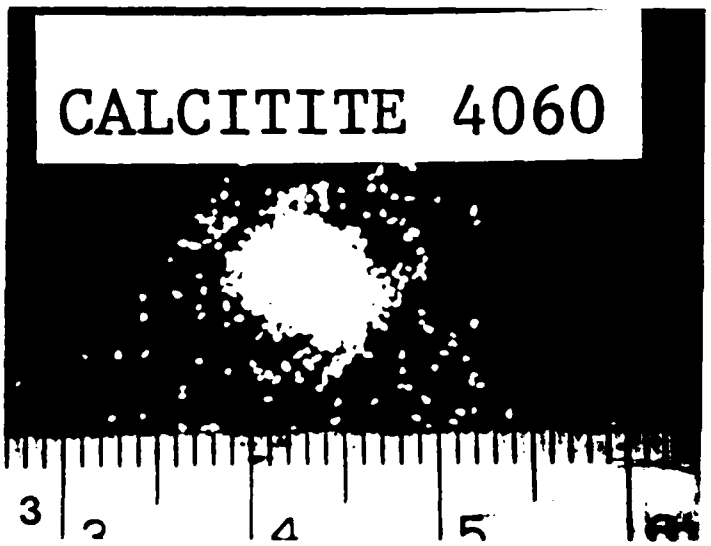
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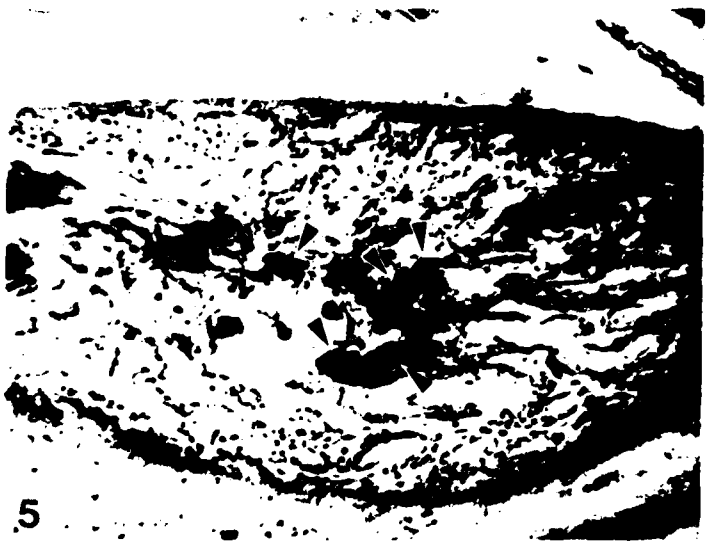
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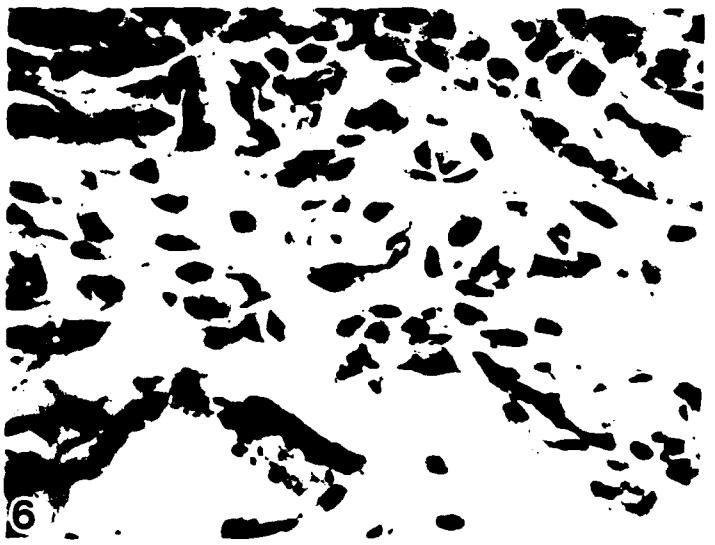
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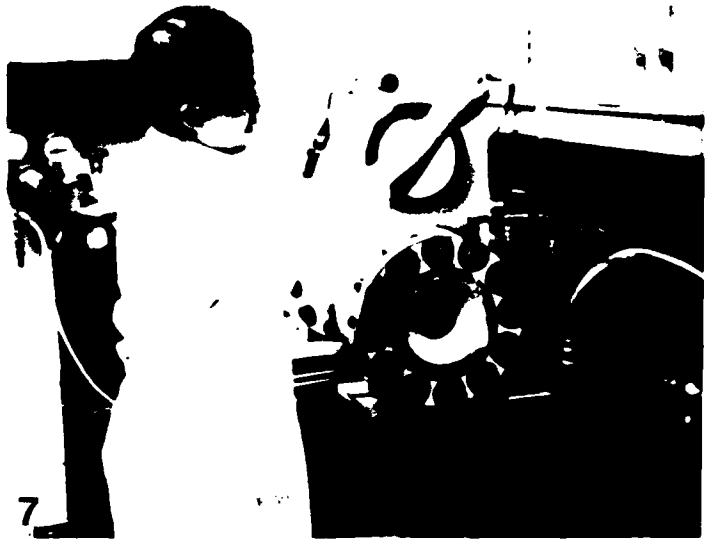
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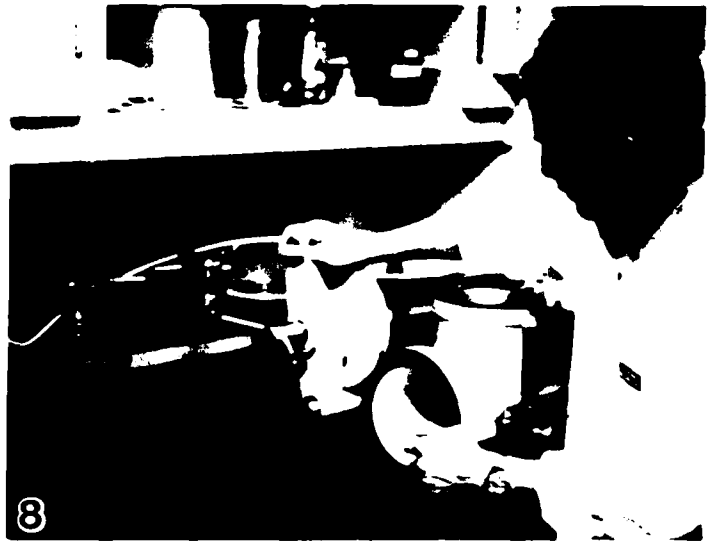
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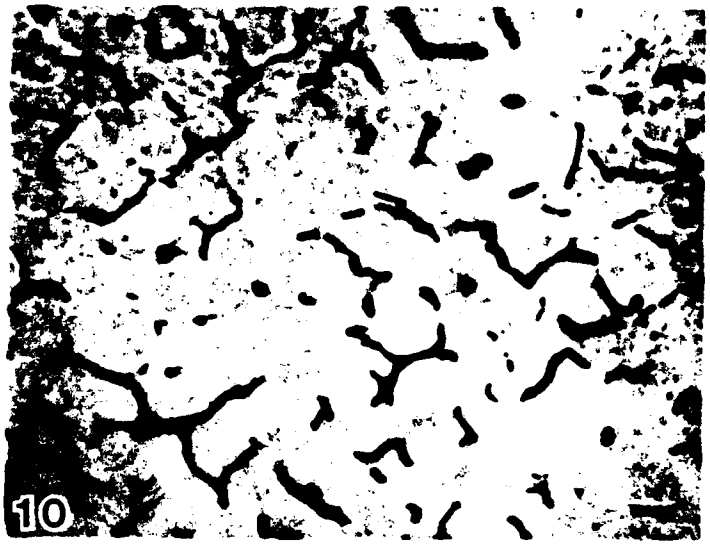
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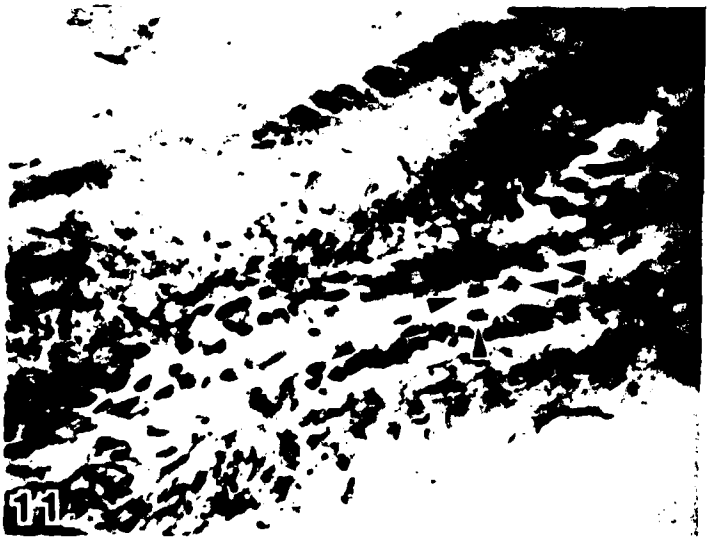
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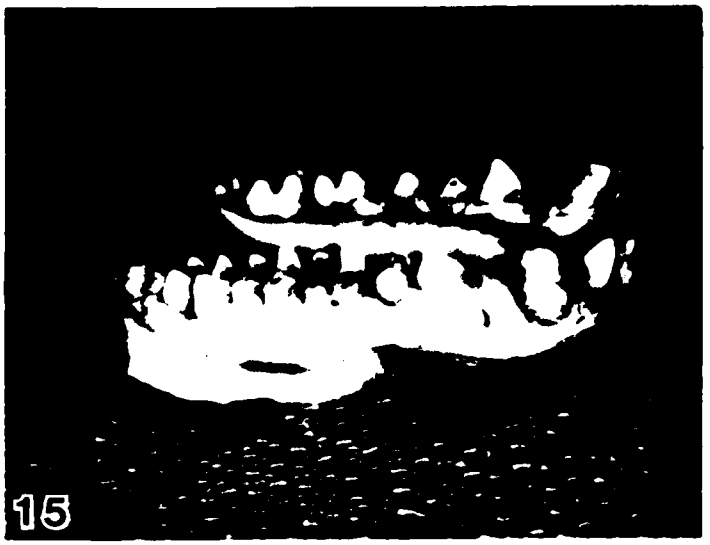
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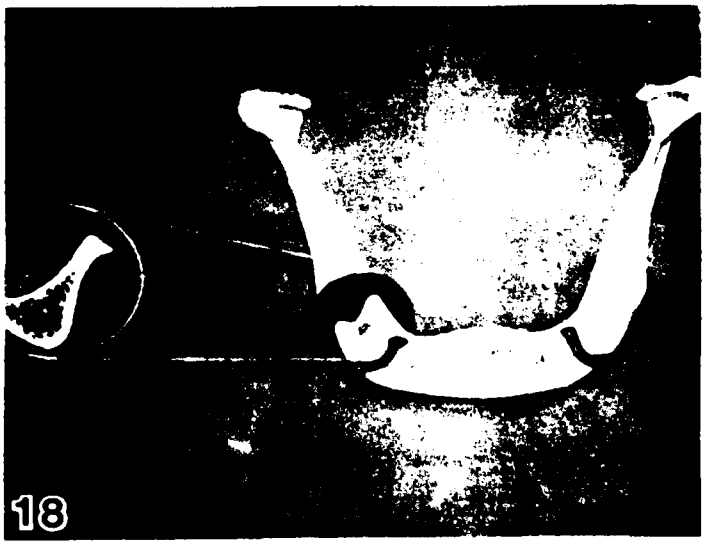
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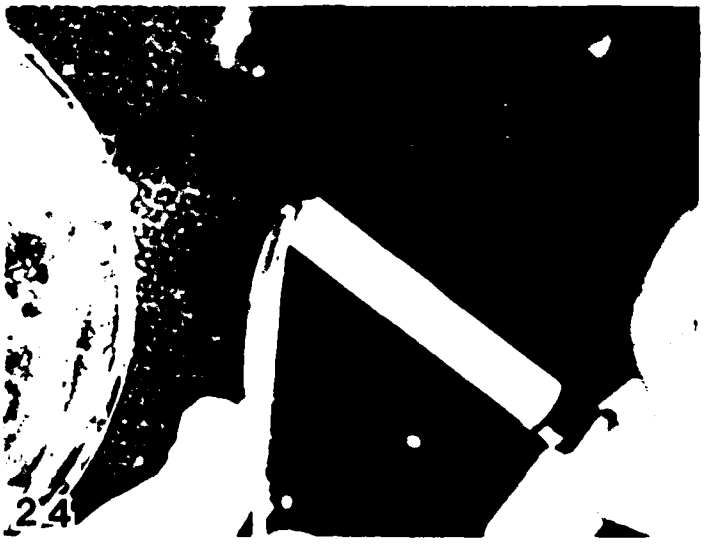
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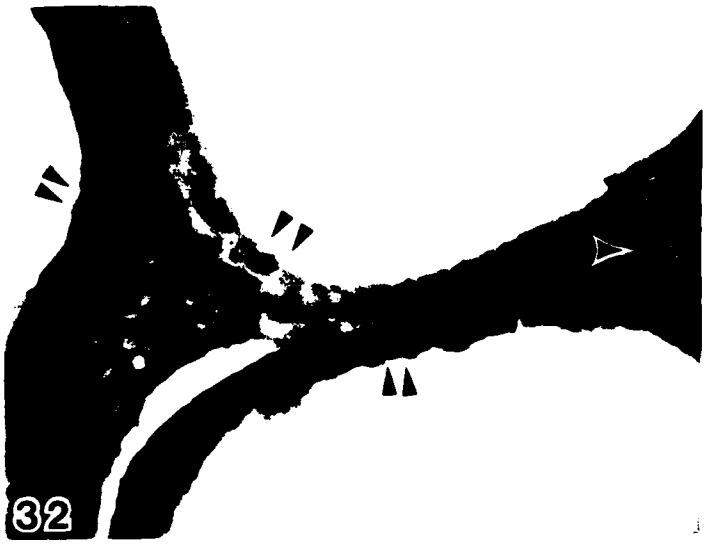
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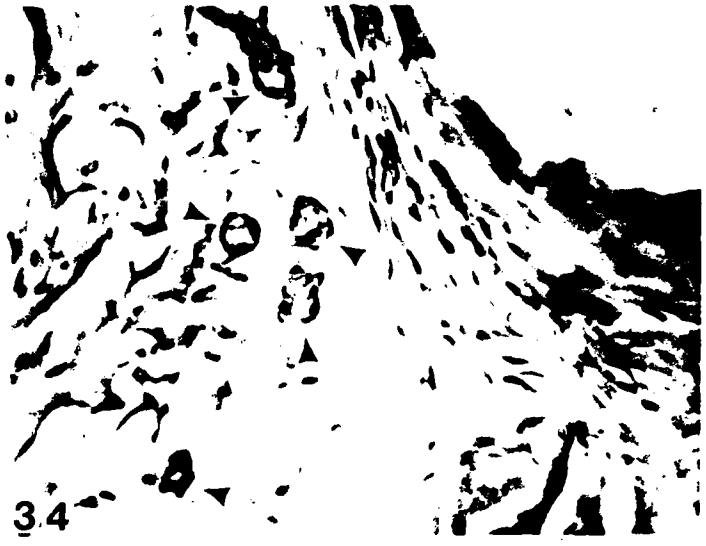
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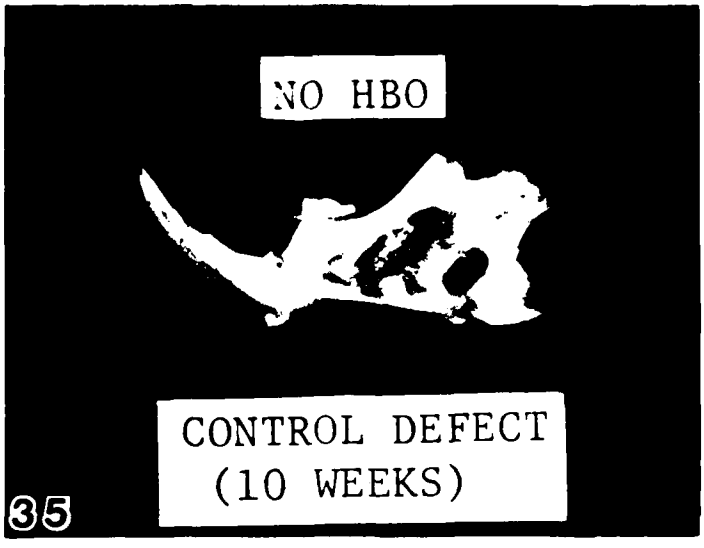

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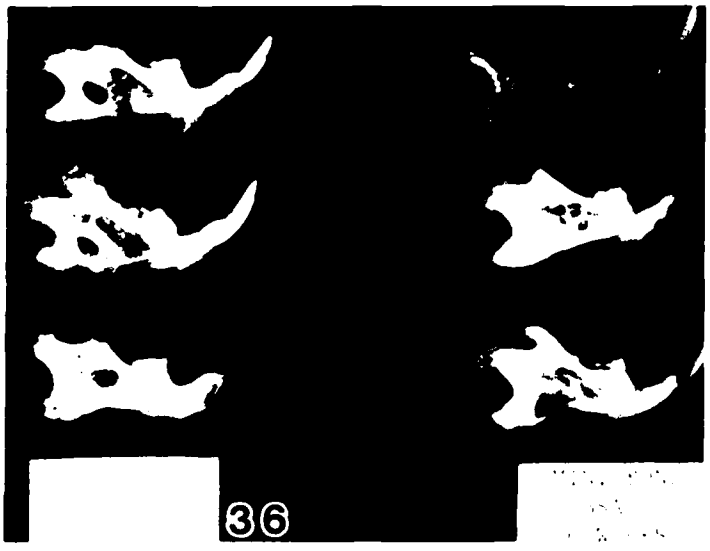
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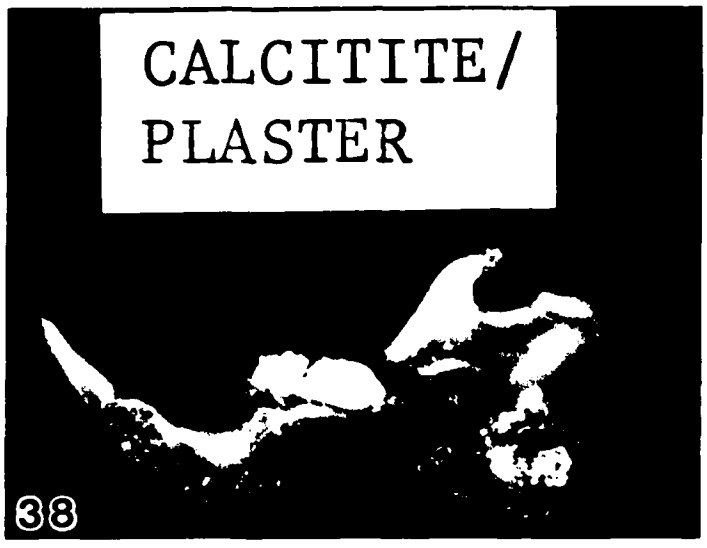
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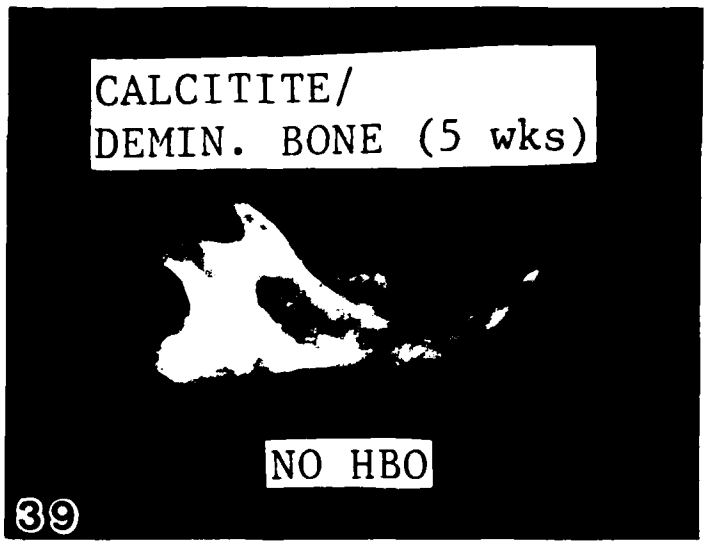
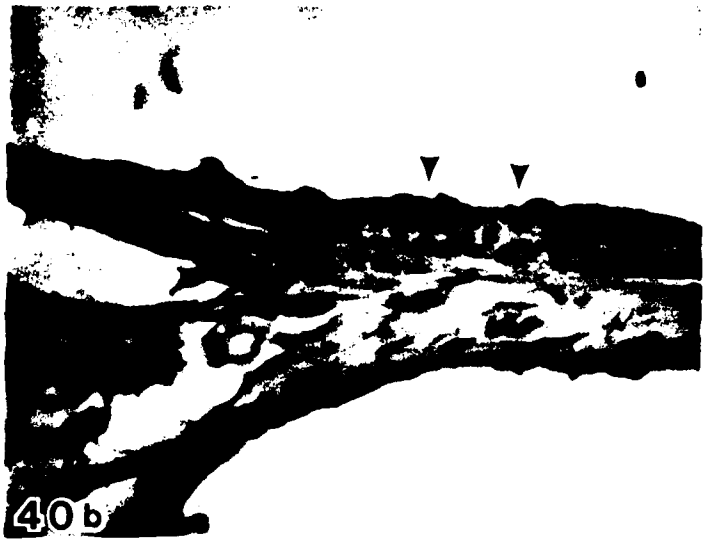

CALCITITE/
DEMIN. BONE (5 wks)
NO HBO
39


40a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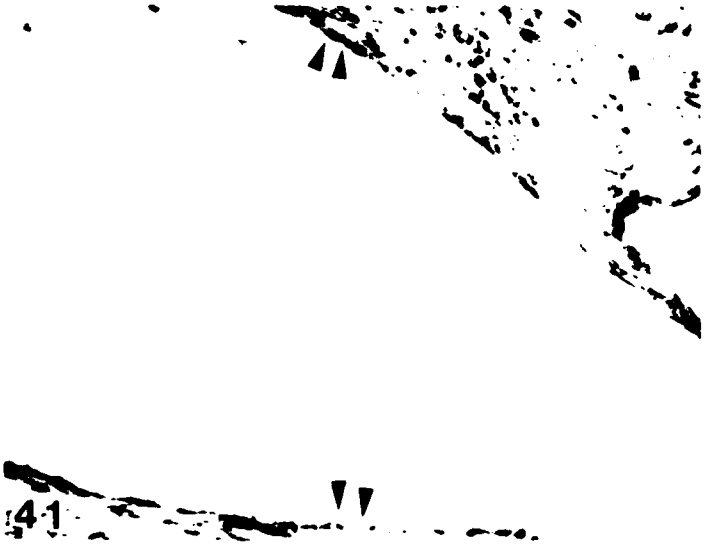
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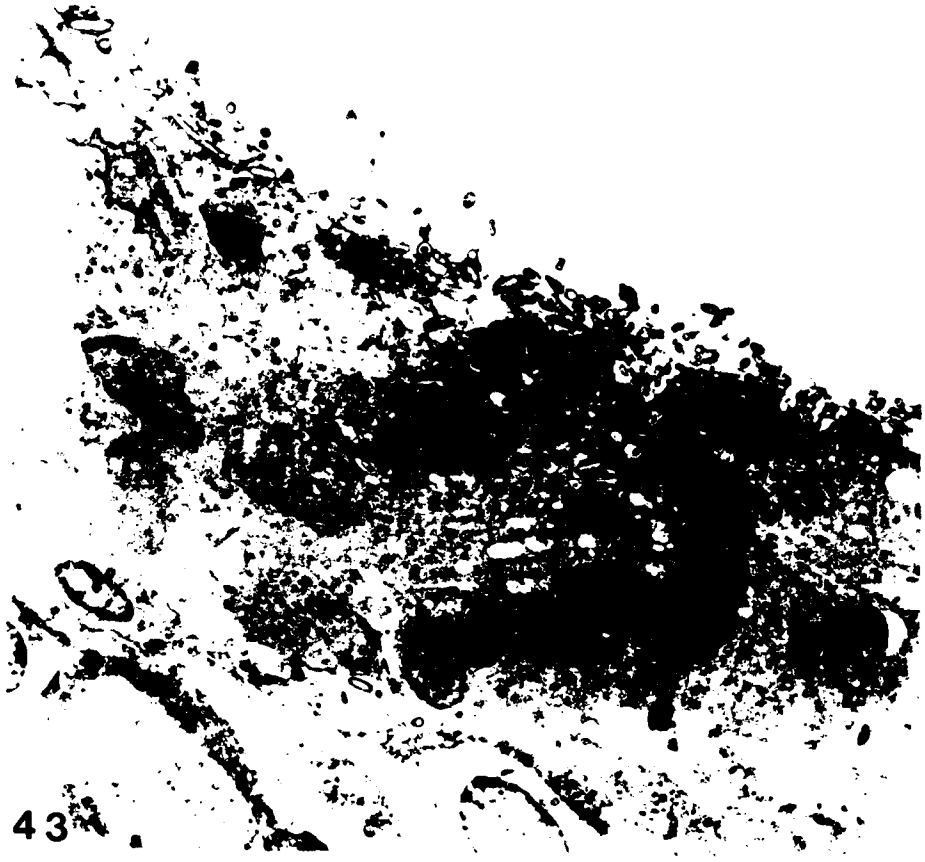

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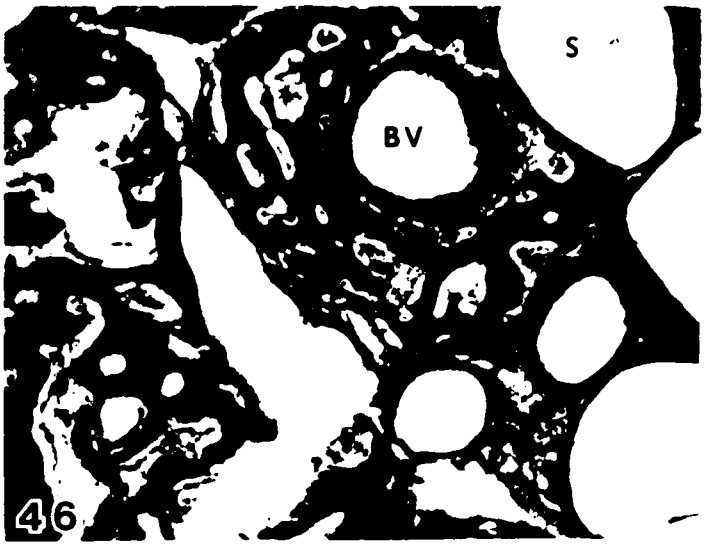

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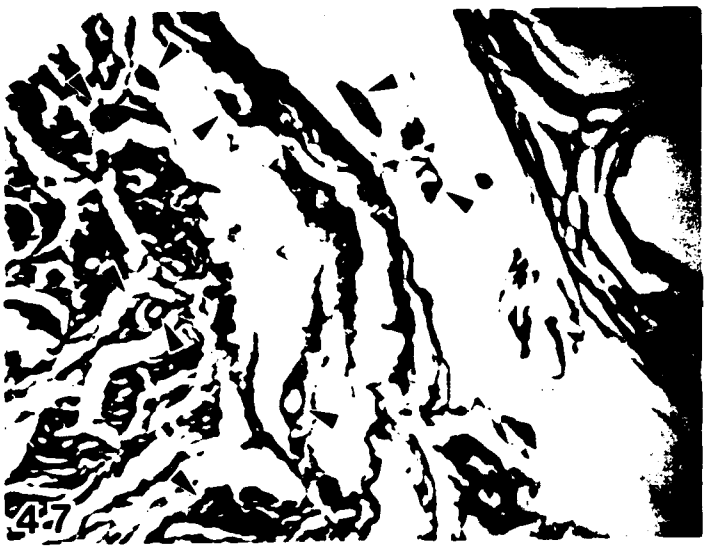

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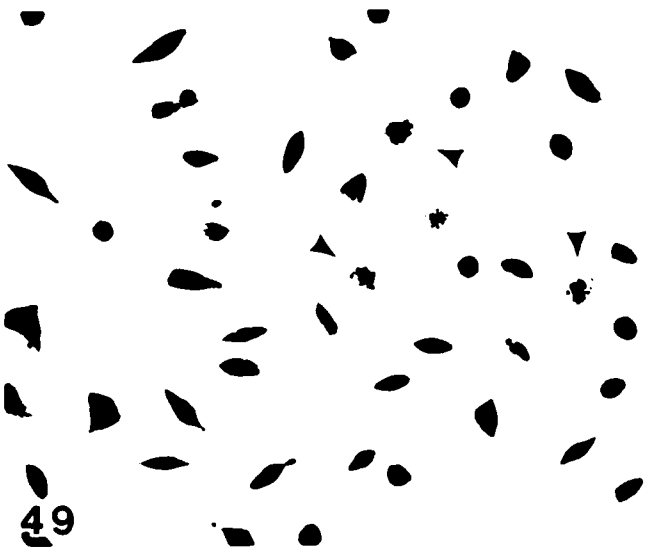

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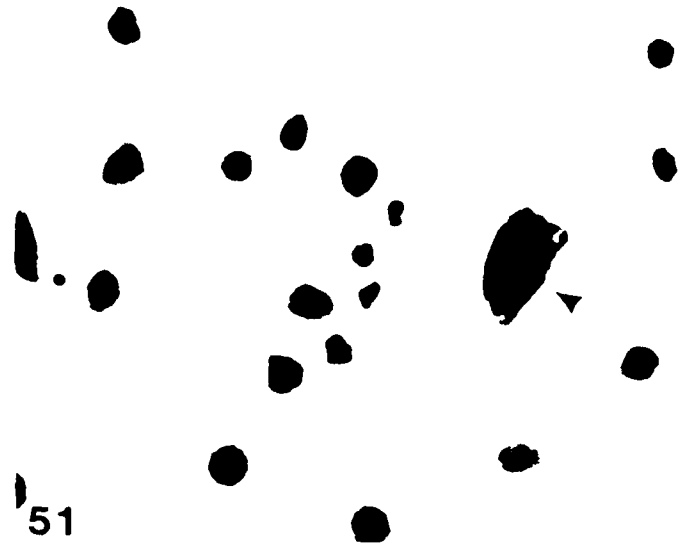

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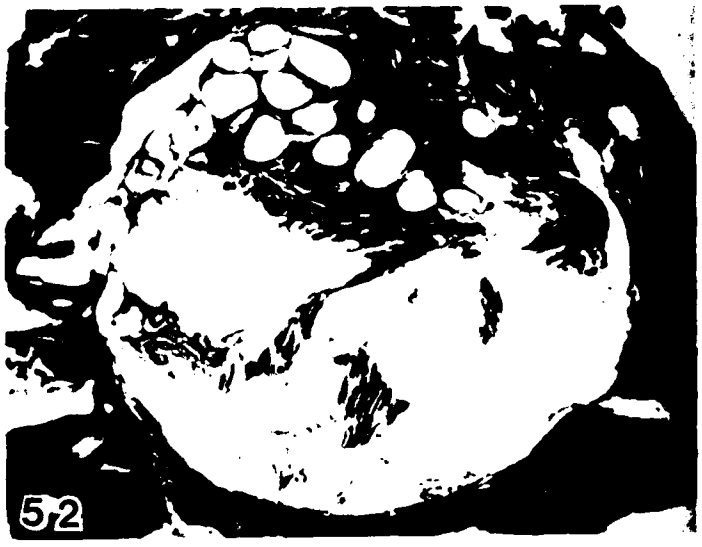

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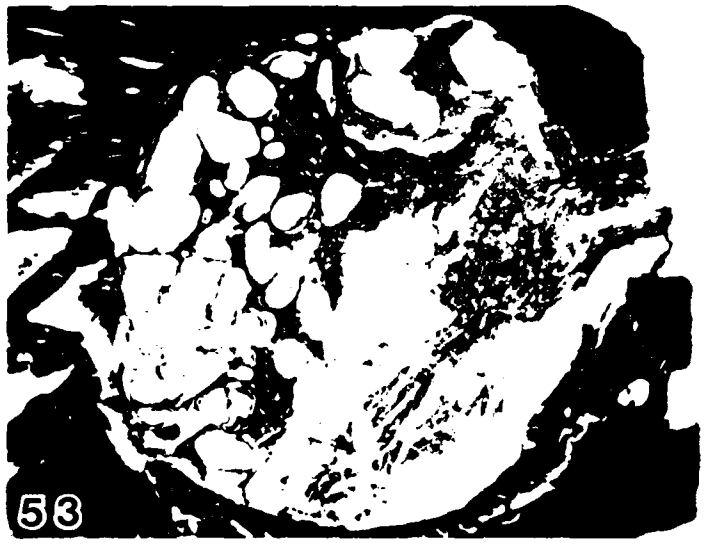

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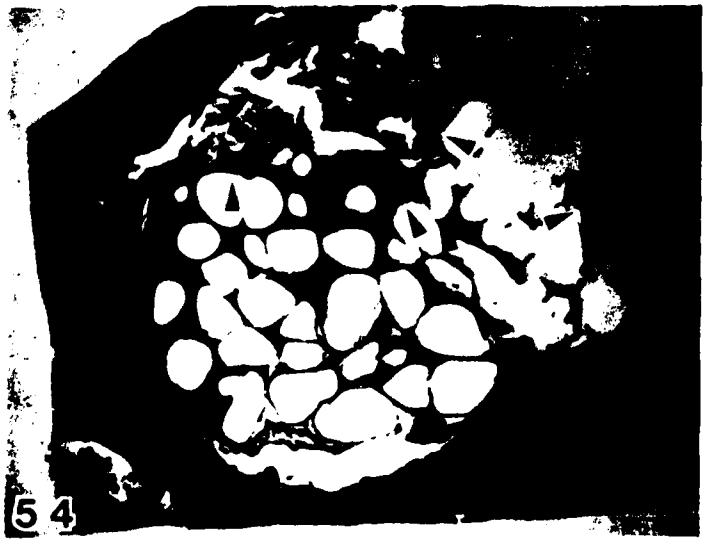

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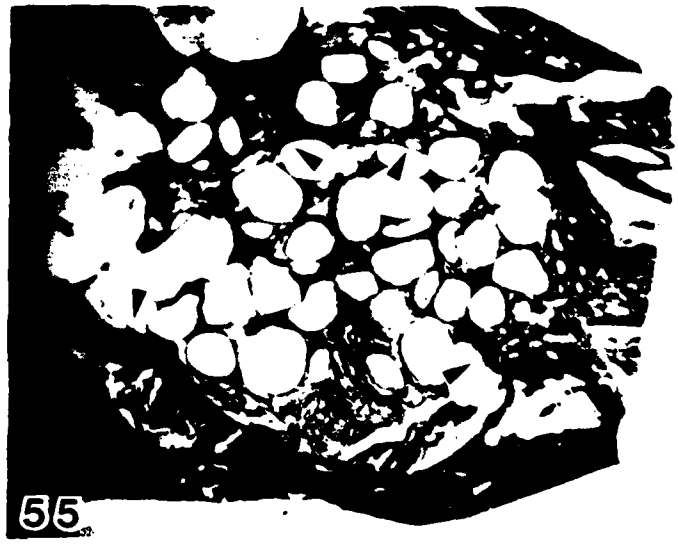

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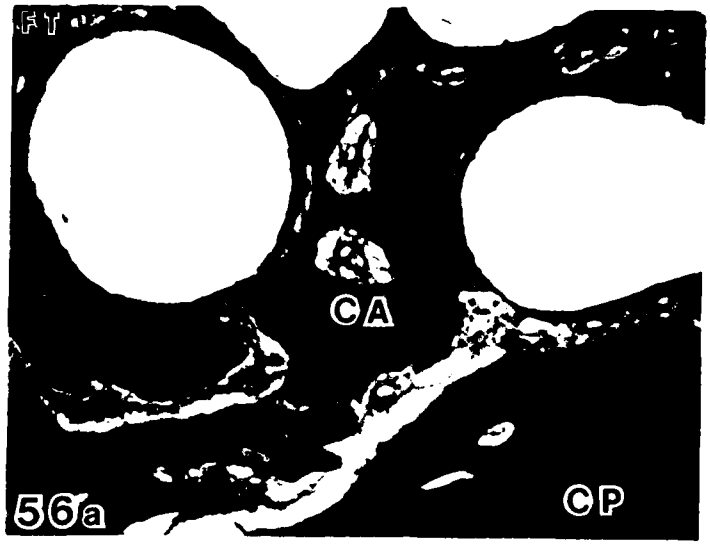
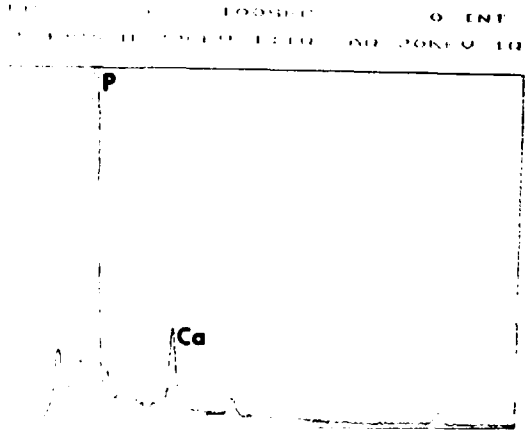


56a

56b

57a

57b



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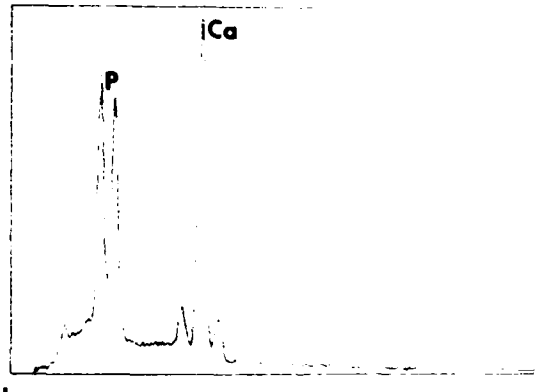

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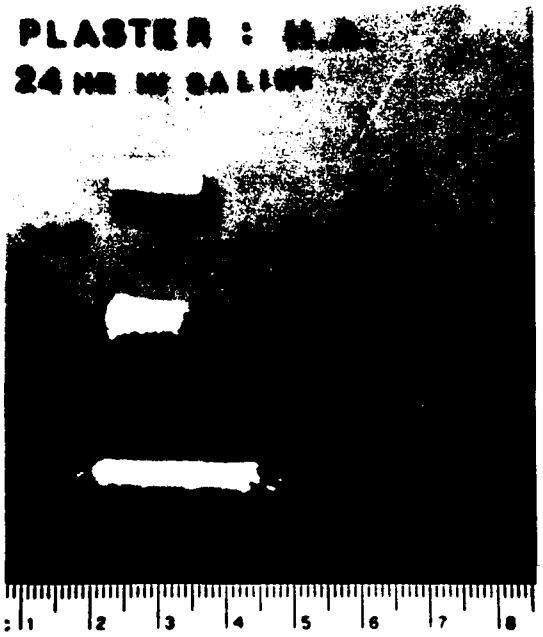

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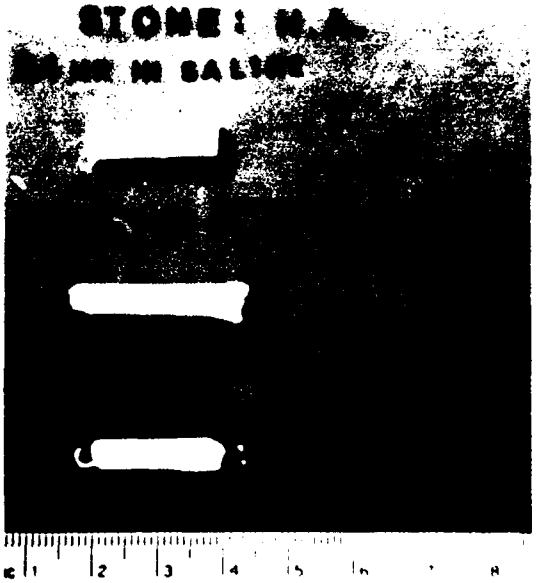

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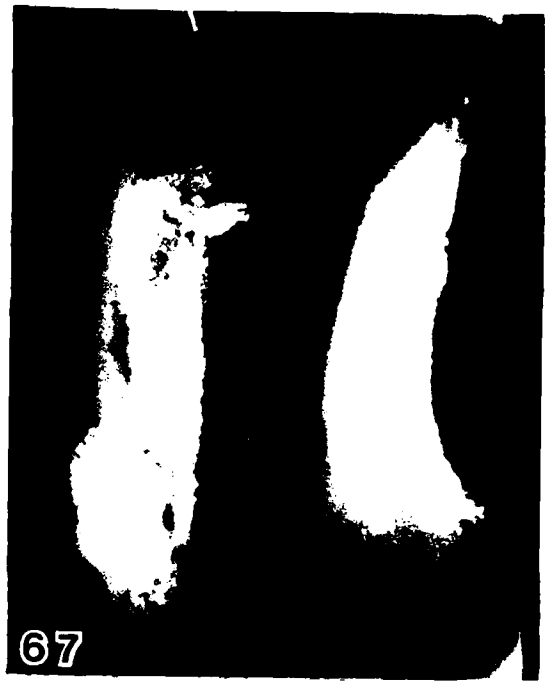
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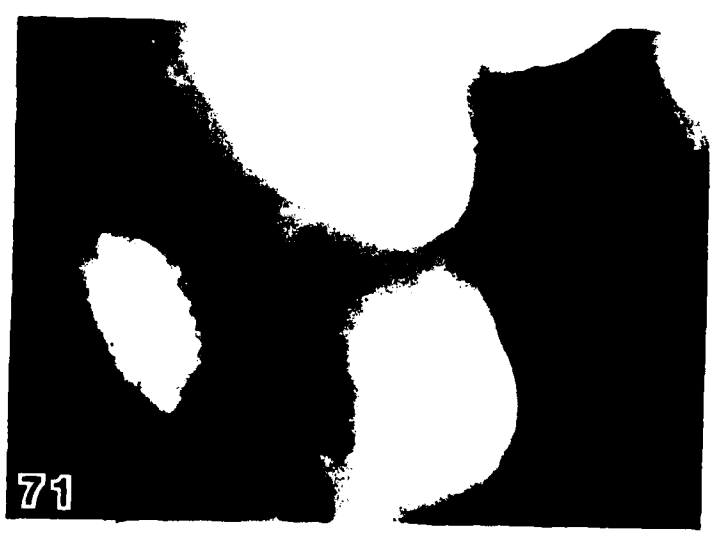
828-E
Twelve Weeks
68


3-52
Twenty-four Weeks
69


70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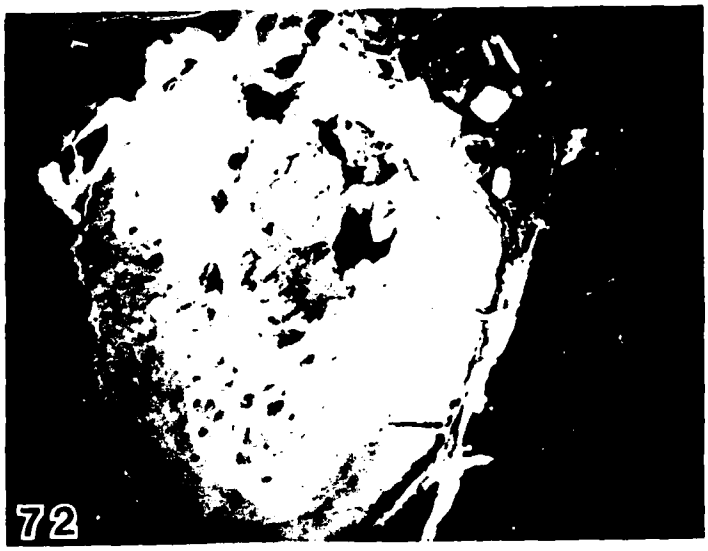
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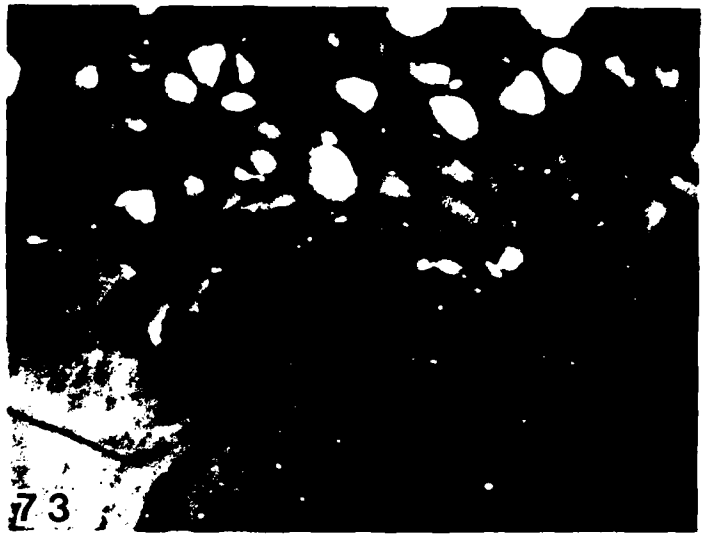
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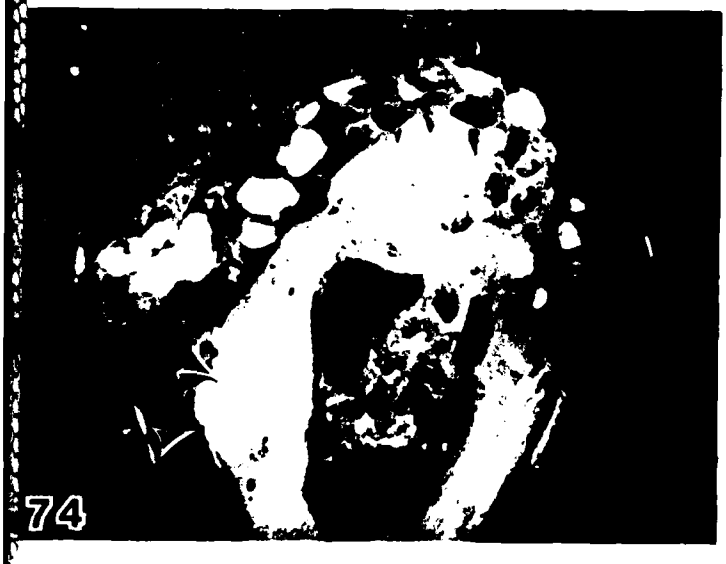
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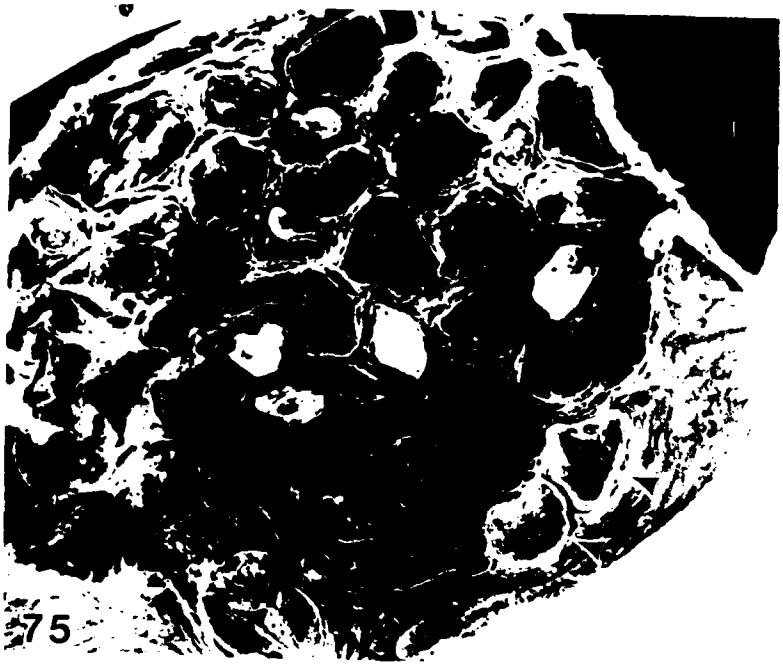
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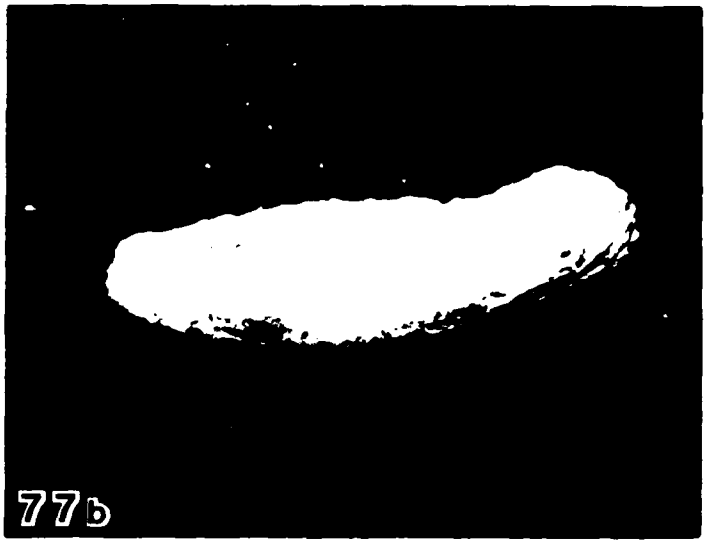
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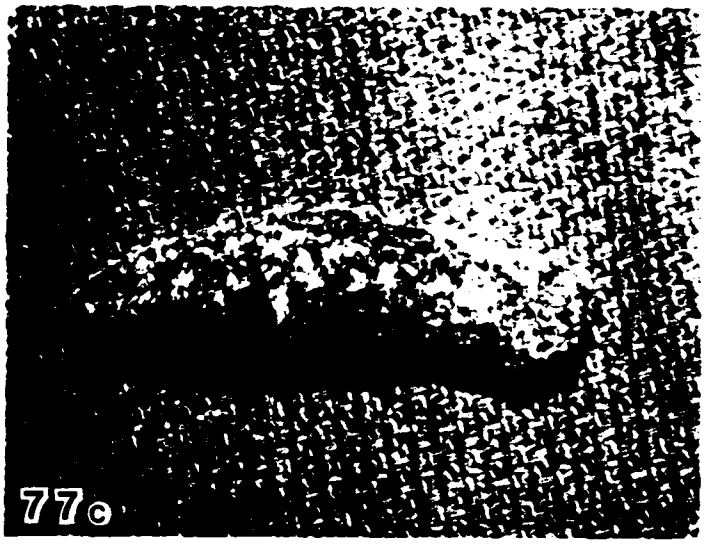
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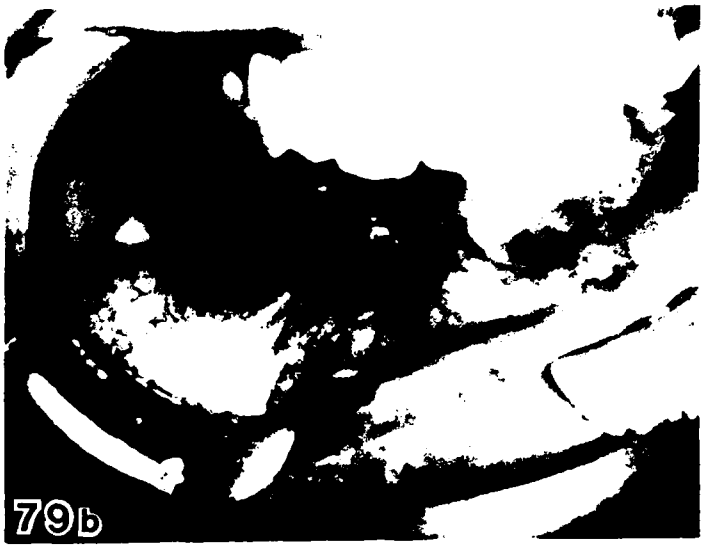
78a


78b

79a



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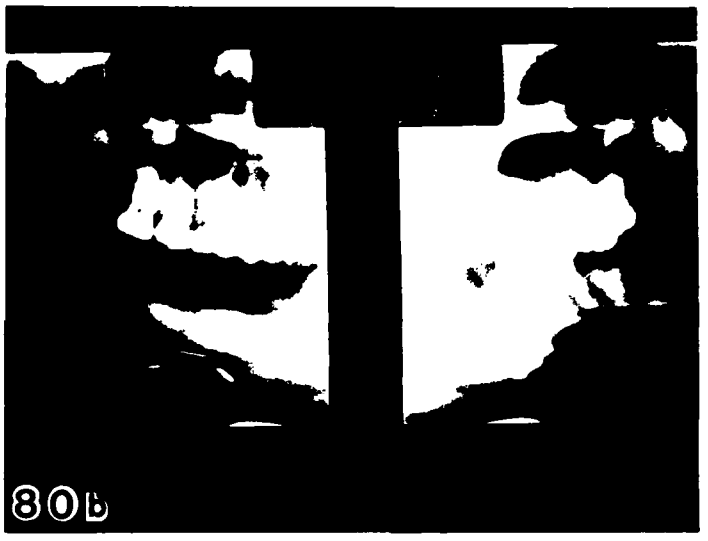

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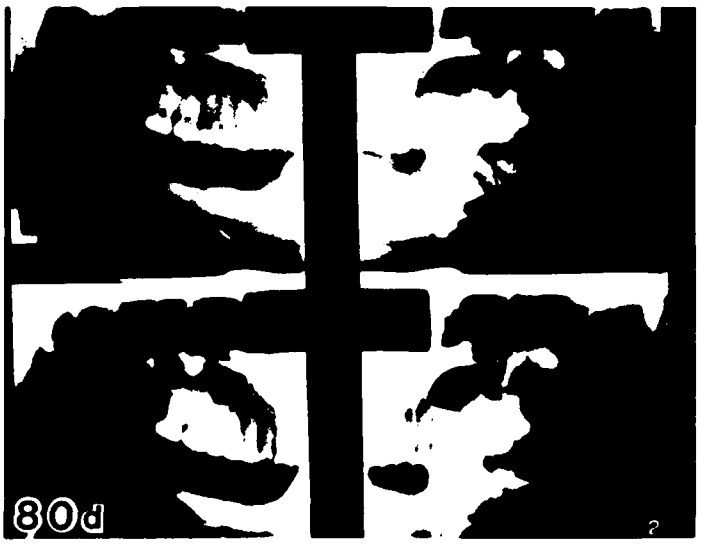

80d


81a


81b


82a


82b

83a

83b

84a

84b

84c

85a

85b

86a

86b

87a

87b

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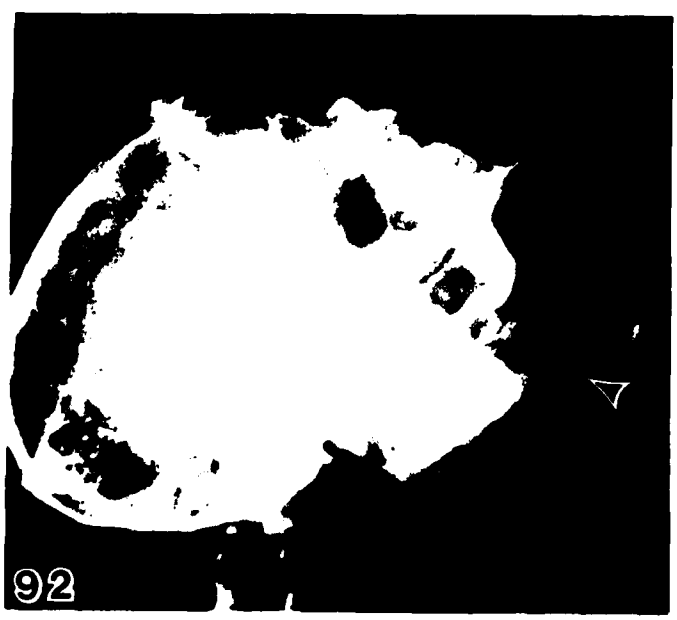
91



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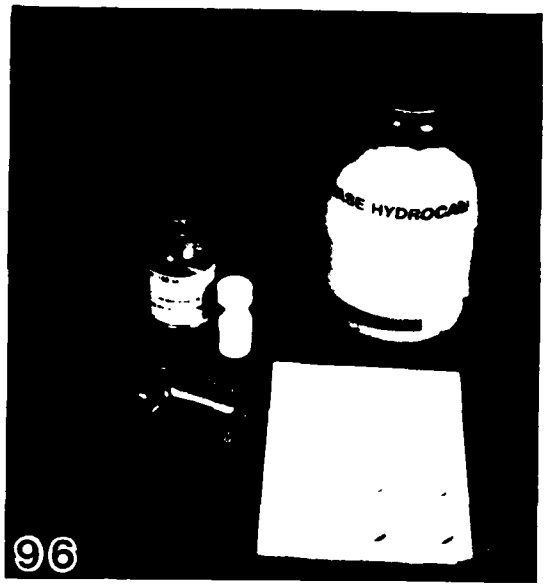

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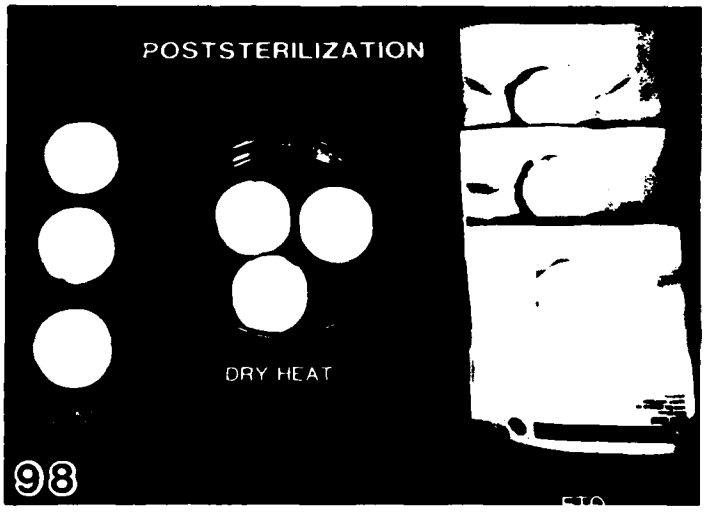

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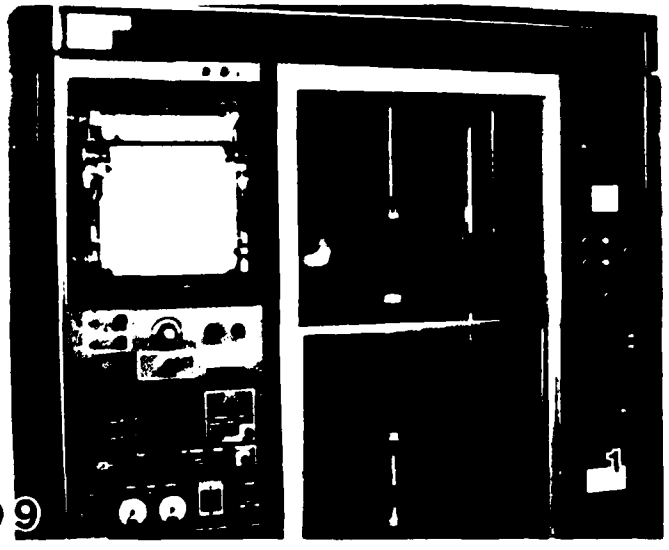
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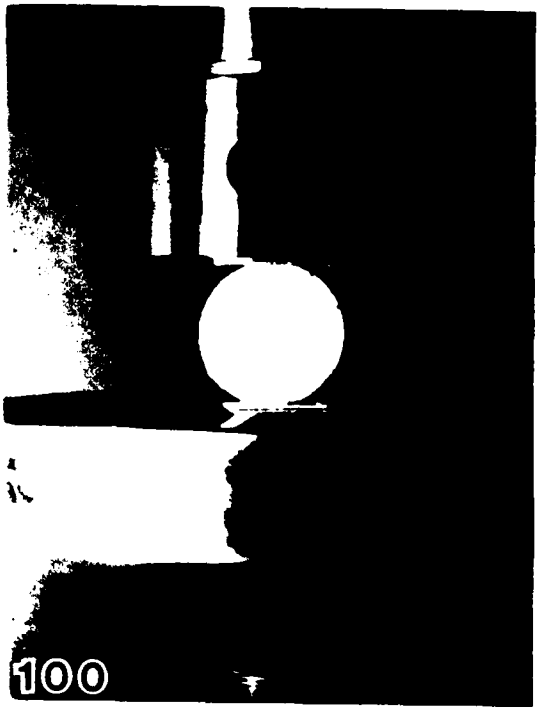
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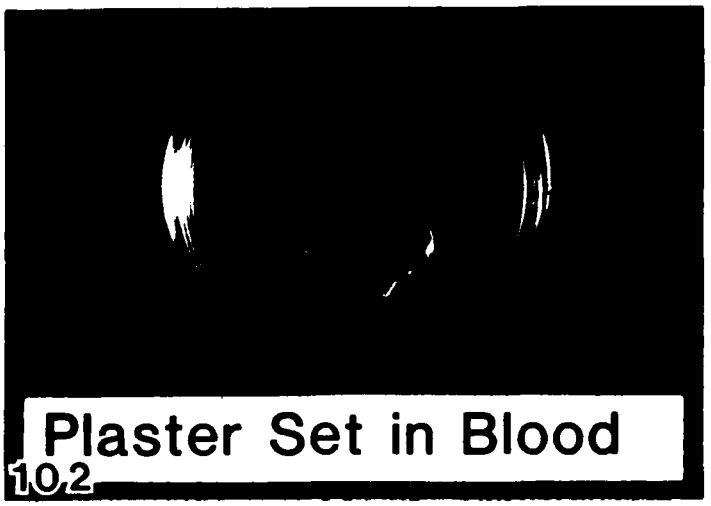
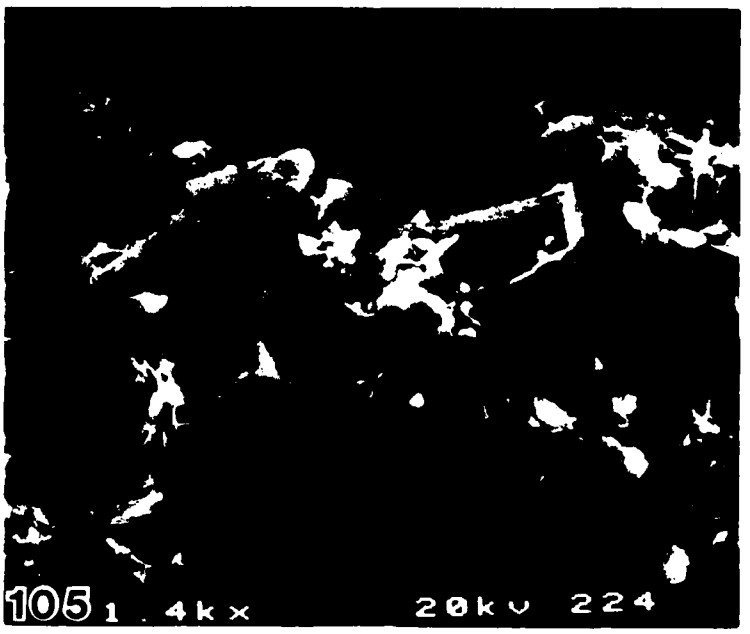
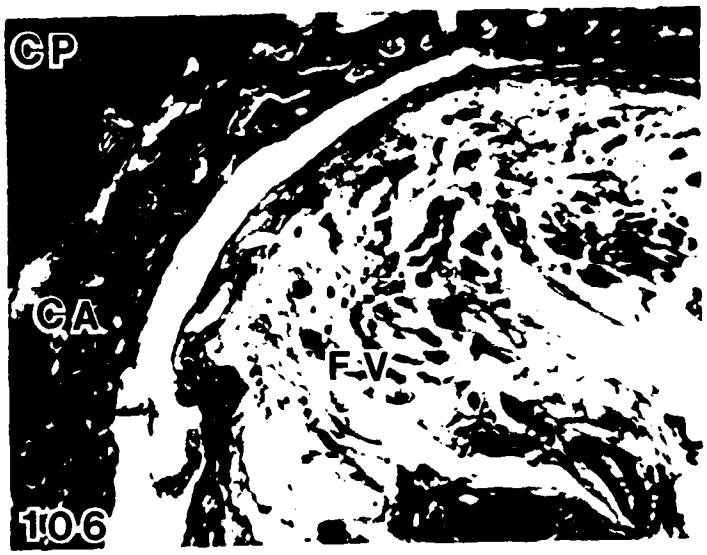


HYDROXYLAPATITE/HYDROC SET IN BLOOD





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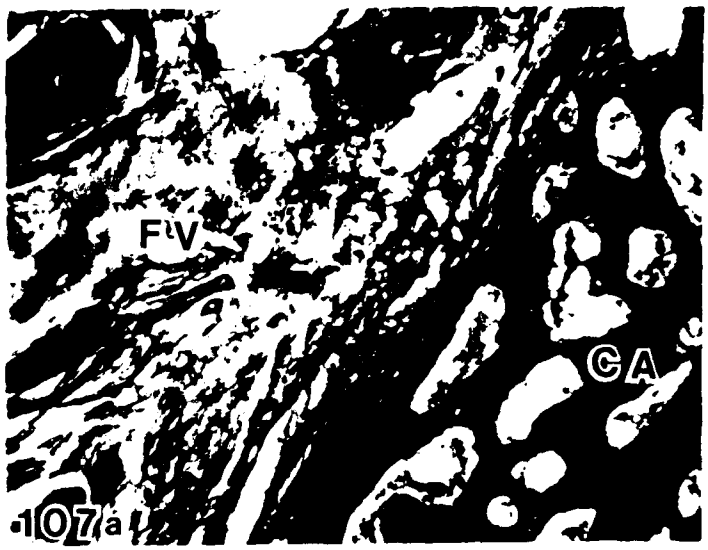

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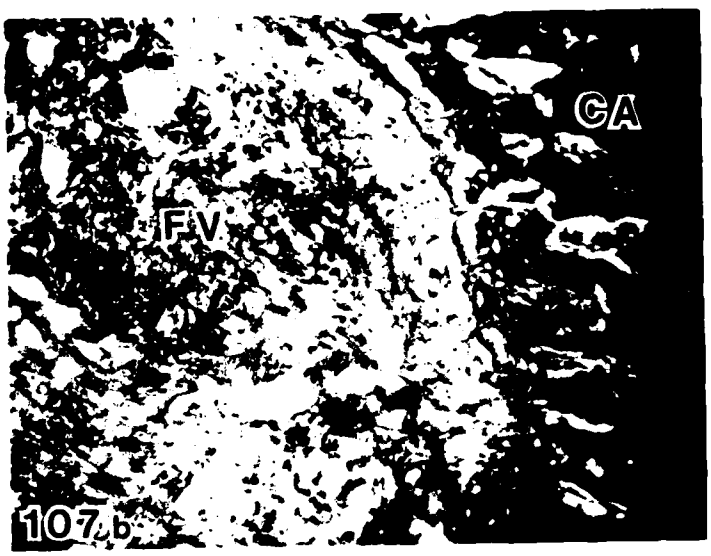

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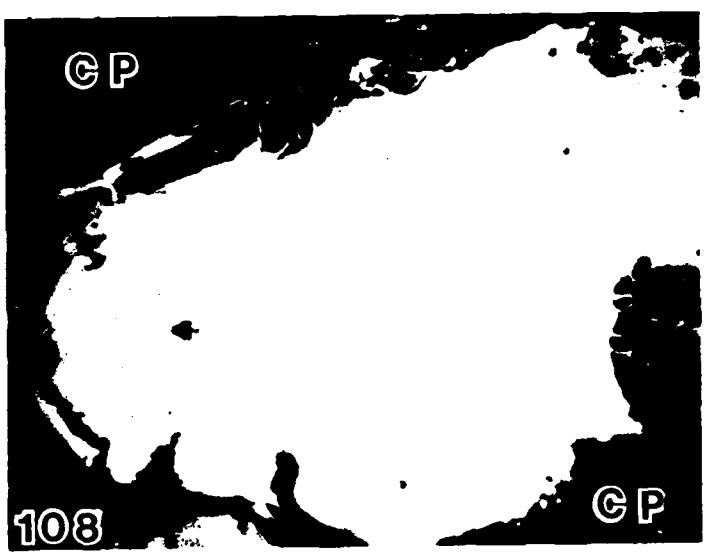

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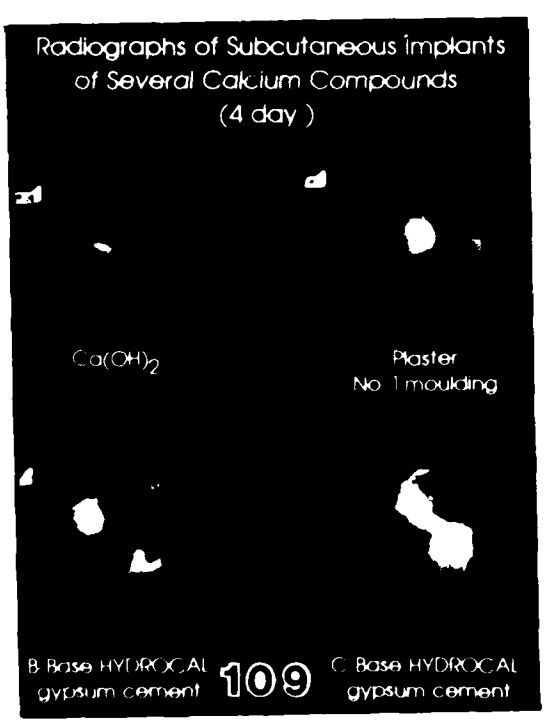

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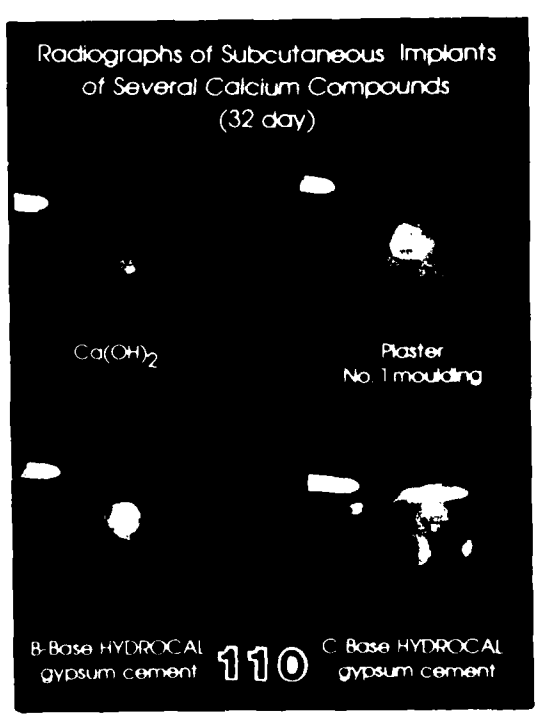

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XIII. APPENDIX (U.S. Patent Applications)

Two applications for United States Patents were filed as a result of work done and supported solely by ONR Contract N00014-82-K-0305:

1) Title: SILVER METHENAMINE STAINING REACTIONS
Serial No. 574,108
Filing Date: January 26, 1984

Inventors: Beverly L. GIAMMARA
Jacob S. HANKER

The University has entered into a licensing agreement with Sigma Chemical Company, St. Louis, MO, to sell a kit for the methodology. The PATS kit will be marketed by Sigma Diagnostics in January, 1986.

2) Title: PLASTER OF PARIS AS A BIORESORABLE SCAFFOLD IN IMPLANTS FOR BONE REPAIR
Serial No. 574,168
Filing Date: January 26, 1984

Inventors: Jacob S. HANKER
Wallace W. AMBROSE
Bill C. TERRY
Cecil R. LUPTON

The University has entered into a licensing agreement with USG Corporation, Chicago, for the marketing of the materials for this process. USG Corporation is currently negotiating with the Dental Products and Orthopaedic Products Laboratories of 3M for funding for filing an IDE with the FDA for the process.

XIV. ACKNOWLEDGEMENTS

The principal investigator is indebted to: Peggy E. Yates for laboratory assistance; Wallace A. Ambrose for laboratory assistance; Lawrence C. Hanker for photographic assistance; and Renee B. Williams for typing and collating this report; Drs. Duane F. Taylor and Robert P. Kusy for assistance in materials research; Dr. James J. Crawford for the design and evaluation of the study for assessing the antibacterial effects of calcium hydroxide; and Dr. Stephen L. Fredette for assistance in the animal surgery.

END

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