HARD AND SOFT TISSUE REACTION TO FOUR AMALGAM SYSTEMS: A HISTOLOGIC AND ELECTRON MICROPROBE INVESTIGATION

by

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DEDICATION

To my sons, Nathan and Aaron

INTRODUCTION

Satisfactory root canal treatment is completed only when the canal system is obturated by one of many filling techniques and/or materials. There are two basic approaches: (1) an orthograde obliteration of the entire canal system, and (2) a retrograde filling of the apex that requires a surgical approach. The retrograde method, which commonly uses silver amalgam as the filling material, is most frequently used when orthograde treatment has failed.

Such failure is reported to be due to insufficient debridement and/or canal obturation.[25,26] The specific conditions which warrant a surgical approach with retrograde filling include: (1) postoperative failure of the orthograde filling,[51] (2) predictable failure of the orthograde filling (flaring immature apices, tortuous canals, apical root fractures),[41] (3) impracticality of orthograde therapy (presence of porcelain jacket crowns or dowel posts),[28] and (4) procedural accidents.[70] Even though silver amalgam has been the material of choice for retrograde fillings, other materials have been employed. Gutta percha and chloropercha,[63] gold foil,[36] copper amalgam,[21] methyl-5-cyano acrylate,[62] zinc oxide and eugenol,[60] and Cavit(TM)[a] [68] have all been used with varying degrees of clinical success, at least initially. However, relatively few reports have recorded their long term success rates. Fewer authors have reported their sealing properties and the compatibility of these materials with hard and soft tissues.

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[a] Non-eugenol zinc oxide polyvinyl paste, distributed by Premier Dental Laboratories

Since a favorable prognosis for root canal treatment largely depends on an adequate seal of the apical portion of the root canal, this condition is a prerequisite for both the orthograde and retrograde techniques. If the orthograde procedure accomplishes this goal, then surgery is not usually required. It is anticipated that the natural sequelae of tissue repair will occur at the periapical locus. However, judicious roentgenographic examination is the only means of observing hard tissue repair and at least one author[4] has reported that with casual radiographic evaluation, there may be a tendency to underestimate the degree of periapical destruction. The histologic evaluation of root canal treatment may report success (healing) to be as low as 7%[4] compared to a clinical evaluation reporting success as high as 90% or more.[26] Such lack of periapical tissue repair may be due not only to inadequate debridement and obliteration of the canal, but also the toxic nature of apical sealants, whether or not placed by orthograde or retrograde means.

Non-zinc alloys are often recommended for retrograde fillings based on the rationale that moisture contamination, if it does occur, will not result in expansion of the amalgam.[73] On the other hand, non-zinc alloys are perhaps a poor choice for retrograde fillings based on the evidence that they corrode more readily with greater marginal breakdown than do zinc-containing alloys.[85] Recently, a number of new amalgams have been developed which demonstrate greater resistance to corrosion than those in conventional use. Although clinical results seem to indicate minimal tissue reaction to amalgam, the question is raised as to whether the use of a non-zinc alloy or the use of the newly improved alloy formulations may prove more efficacious for apical fillings than the conventional zinc-containing compounds. It was the purpose of this study to attempt to answer this question.

The primary method used for evaluation was the measurement of the in vivo bone response to amalgam made from the various alloys, using dogs as the test animals. In addition, after the in vivo experiments, some of the amalgam implants were removed from the calvarium of the dogs and examined for evidence of corrosion, using an electron probe microanalyzer.

LITERATURE REVIEW

Retrograde root filling

Apicoectomy, the removal of a small portion of the apical tooth root, followed by a retrograde filling, can be considered an extension or continuation of orthograde root canal therapy. This procedure has been an important part of endodontic treatment procedures for many years. Garvin,[20] an early advocate of periapical surgery, treated teeth with large periapical areas by apical root resection and obliteration of the apical root canal with amalgam condensed into an apical preparation. In a later radiographic study,[21] the success rates for such fillings using copper amalgam or silver amalgam were reported. New trabecular bone was radiographically evident near the silver amalgam, but there was no bone regeneration around the copper amalgam filling.

Luks[42] reported that silver amalgam seemed to be an ideal root end filling material, for it hardened quickly and seemed to produce a sound hermetic seal. Herscovitch[28] described an efficient, workable technique for apical silver amalgam fillings and reported that silver alloy was easy to condense into the apical preparation. It also appeared to be clinically well tolerated by the surrounding periapical tissues.

The efficacy of the apical amalgam seal was demonstrated by Herd,[27] using radioisotopes. Orthograde obliteration of the root canal by one of several commonly used root canal filling materials preceded the retrograde seal. He stated that the addition of a retrograde filling provided a more efficient seal than that provided by orthograde filling alone.

Mattila,[52] in a roentgenographic study of apicoectomized teeth, also reported increased success rates for the two-step procedure. His study also supported Brynolf's contention that roentgenological success was much less than clinical success.

Harty, et al,[25] reported that the excellence of the apical seal was the single most important factor in the success of surgical retrograde filling procedures. He observed that amalgam appeared to be well tolerated by the apical tissue, and he noted the lack of postoperative symptoms due to residual amalgam left behind inadvertantly after apical surgery.

Persson, et al,[68] reported the results of 220 retrograde treatments using either silver amalgam or Cavit. Amalgam fillings were more successful after one year than for Cavit fillings. This was interpreted by the authors as a sign that Cavit did not obliterate the canal as well as amalgam. However, the toxic properties of neither material were evaluated. Moodnik, et al,[56] using electron microscopy, demonstrated substantial defects between the amalgam filling and beveled roots of teeth that had been treated with retrograde procedures. This supports Sapone's[70] contention that surgical treatment with an apical filling may invite failure if the main root canal is not completely cleansed and obturated.

Marcotte,[48] using anterior teeth in monkeys, filled some teeth with orthograde gutta percha root canal fillings and followed this with apical surgery but no retrograde amalgam. In other teeth treated with the same orthograde technique, additional retrograde silver amalgam apical fillings were placed. He concluded from his histopathologic examination of the periapical tissues that the degree of healing was only a matter of time, regardless of the filling techniques used. He noted new cementum over the cut dentin, but none over the apical sealants.

Biological response to implants

Any intentionally implanted material that is to lie within hard and/or soft tissue must be compatible with that tissue. Reactions associated with metallic and non-metallic biological implants placed in hard and soft tissue have been extensively reported. Homsy[31] strongly emphasized the need for extremely critical criteria for the appraisal of implant-tissue reactions. In a widely reported study, Laing[37] speculated that various alloys produce a tissue reaction that is proportionate to the amount of constituent elements released by the corrosive process from the pure metal or alloy.

Harris[24] stated that metallic implants, in general, are placed in highly corrosive and immunologically sensitive environments. Furthermore, most dental implant devices are not only expected to coexist with surrounding vital tissues, but also to behave as functioning, integral members of the dentition, even though they are not permanent devices. Any chemical degradation not only changes the physical properties of the implant, but the products so formed may be toxic and/or carcinogenic.

Ceravolo, et al,[6] in a histological study of non-metallic implants, placed ivory rods (2x5 mm.) in the mandible of rabbits. They reported the presence of giant cells, lack of bone induction, and a particular type of fibrous connective tissue capsule that eventually walled off the implants. This reaction could be considered as a classic foreign body reaction.

An interesting observation by Gross[22] was made in association with metallic implants buried in the mandible and long bones of dogs. He demonstrated, histologically, a fibrous capsule barrier around the mandibular implants, but no similar capsule was evident around the long bone implants. He did not speculate as to the significance of his findings.

Histopathologic evaluation of corresponding tissue reactions has been poorly defined with respect to time. Turner, et al,[80] stated that seven days was a satisfactory time period for routine evaluation of rabbit muscle reaction to implanted materials. Maximum acute reactions usually peak at this time, but it was admitted that mild toxic reactions were difficult to distinguish from physical injury. It was suggested that twelve-week implantation periods should be used for the study of all biomaterials which might have contact with human tissues for more than a few days. If the tissue reactions had not stabilized by then, an undefined longer term study was indicated. They also stated that tissue culture techniques were more sensitive indicators of toxicity of implant materials.

Langeland and Spångberg,[38] studying endosseous implants, indicated that tissue culture tests were quite objective and quantitative, but did not necessarily reflect tissue reactions in vivo. Tissue implantation has the advantage of providing insight into the implant-tissue reaction without the influence of external factors. However, tissue implantation is neither quantitative nor does it reflect clinical usage conditions. Mandibular implants can satisfy both hard and soft tissue reactions, and, in the case of endosseous implants, also satisfy usage conditions.

Biological response to dental filling materials

The irritational or toxic properties of dental materials have been reported in studies that used various screening methods. Dixon and Rickert[8] investigated a number of dental products, including copper and silver amalgam. The materials tested were implanted subcutaneously and intramuscularly in rabbits. They particularly noted chronic inflammation, featuring lymphocytes and eosinophils in the tissue surrounding the copper amalgam. Hunter[32] tested dental products in the bone of guinea pig tibias.

He reported complete bone repair around gutta percha and the silver cones.

In a classic study, Mitchell[55] tested tissue reactions to various restorative materials, both freshly mixed and set. He reported very little inflammatory response at four weeks due to silver amalgam. Copper amalgam, however, produced necrosis and abscess formation.

Nagai, et al,[58] implanted various pure metals and alloys, including silver amalgam intramuscularly in rabbits, to relate the vital tissue reaction to the resultant corrosion of embedded metals. Although the amalgam displayed discoloration and corrosion under microscopic examination, no discernible tissue reaction was noted even after a five-month implantation period. They concluded that the apparent lack of tissue reaction was due to the scarcity of soluble toxic metallic ions. Feldmann and Nyborg[14,15] studied the bone response to silver amalgam, gutta percha and AH 26 (a root canal cement). These materials were implanted, preset, into the mandible of rabbits for a period of three months. Histologically, it appeared that there was much less tissue reaction to the amalgam than to either of the other two materials. Fifty percent of the amalgam implants were in contact with new trabecular bone.

Lyon, et al,[45] implanted gallium alloy and silver amalgam subcutaneously in rats for a six-month test period. Their histologic evaluation demonstrated that the soft tissue response to amalgam was innocuous and that the implants were covered by a thin layer of mature fibrous connective tissue. Sperber[76] implanted set, premolded amalgam pellets subdermally in rats. After thirty-two days, histologic evaluation revealed that the pellets were walled off by a fibrous capsule. The presence or absence of zinc in the amalgam appeared to be of no consequence in the tissue reaction.

Friend and Browne[17] studied the irritant properties of some root filling materials and amalgam. In two separate experiments, they compared the toxic properties of each material in soft and hard tissue. At the time of implantation, the materials were placed into polyethylene tubes and then implanted either in the flanks and backs of rabbits or into prepared sites in rabbit tibia. The soft tissue implants were recovered after one year and the bone implants after six months. Their histologic examination found an immediate severe tissue reaction to all materials used. The amalgam implant was gradually surrounded by a thin fibrous tissue capsule. They concluded that the tissue reaction to root filling materials placed in plastic tubes was essentially the same whether the implants were embedded in soft tissue or bone.

Spångberg[74] devised a standardized implantation technique and a method for intra-individual comparisons of the findings. He demonstrated that a polytetrafluorethylene tube was quite suitable as a vehicle for testing the toxicity of implant materials. Using this technique, and implanting the samples in guinea pig mandibles, he tested several root canal pastes and silver amalgam. He reported that silver amalgam implanted for twelve weeks ranked approximately in the middle range of tissue reactions.

Flanders, et al,[16] implanted Cavit(TM) and zinc-free amalgam subcutaneously in the back and in the mandibular bone of rats. They reported that Cavit produced a more severe foreign body response than did amalgam at each postoperative interval from ten days to six months. Martin, et al,[49] tested a non-zinc amalgam and a zinc-containing amalgam. Each type was implanted subcutaneously in rats. Histologically, they noted no difference in tissue response between the two types of implants. They also reported that they could not demonstrate any zinc carbonate

precipitation, a reported sequelae of zinc-containing amalgam retrograde fillings.

A study by Nagen-Filho, et al,[59] reported a precipitate of white powder around the amalgam end of an amalgam-gold rod implanted in soft tissue. Chemical analysis revealed that this precipitate contained salts of silver, tin, and zinc cations and carbonate, sulphate, and phosphate anions.

Ellender,[10] using a sulfide-silver histochemical technique, was able to detect (at the ultrastructural level) corrosion products and disassociated metals derived from silver amalgam implanted subcutaneously in rat tissues. He reported that heavy metals in tissues may be converted to various sulfide forms and that they may act as nuclei on which reduced silver can be deposited preferentially. Examination with an electron microscope appeared to indicate a relationship between corrosion products thus produced, and the ensuing inflammatory reaction. In another study, Ellender and Ham[11] were able to demonstrate that the method used for histologic preparation was critical in the identification of amalgam granules in soft tissue. More recently, Ellender,[12] using a modified sulfide-silver histochemical analysis showed amalgam corrosion products in macrophages, fibroblasts, collagen fibers and in the basement membrane of some small blood vessels.

Several studies using tissue cultures have reported the cytotoxicity of silver amalgam. Keresztesi and Kellner[35] evaluated copper amalgam and silver amalgam and various root filling materials. Using chicken fibroblasts as the bioassay system, they noted that silver amalgam produced an inhibition of growth of approximately twenty percent while copper amalgam inhibited growth 100 percent. When they implanted these materials subcu-

taneously in guinea pigs for thirty days, silver amalgam produced a mild inflammation and the copper amalgam produced necrosis. Leirskar,[40] using human epithelial cells, measured the metal cations released into the culture medium as an indicator of the cytotoxicity of copper amalgam and silver amalgam. He reported that both metals had pronounced cytotoxic effects on this culture system. After three days, no cells were found on the surface nor around the amalgam disks. With the aid of spectrophotometry, he demonstrated that zinc ions were rapidly released from the silver amalgam into the culture medium. He speculated that these ions may contribute to the cytotoxic nature of silver amalgam.

Amalgam properties

The popular and required use of silver amalgam for apical fillings requires that its phases and properties be clearly understood. The conventional silver amalgam alloy system consists essentially of 69 percent by weight silver, 26 percent by weight tin, and 5 percent by weight copper. Zinc, which may be present in amounts of less than 2 percent by weight, is placed in the melt as an oxidizer of impurities, even though this effect has not been proved conclusively. In most conventional systems, the elements in the untriturated alloy are in the form of the following phases:

$$\text{beta (Ag-Sn)} + \text{gamma } (Ag_3Sn) + Cu_3Sn$$

The beta Ag-Sn and Cu_3Sn are present in small amounts. When triturated with mercury, three new phases are formed: Ag_2Hg_3 (gamma 1), Sn_8Hg (gamma 2), and Cu_6Sn_5. About 20 percent by volume of undissolved alloy particles remains. Thus, the reaction is:

$$\text{alloy} + Hg \rightarrow \text{gamma 1} + \text{gamma 2} + Cu_6Sn_5 + \text{alloy}$$

Cu_6Sn_5 is present only in minute quantities in the resultant set mix.

Mahler, et al,[46] studying a new alloy system[a] containing an average copper content of thirteen percent, reported that there was an almost complete absence of gamma 2 (Sn_8Hg) in the set amalgam. This alloy consisted of one part silver-copper spherical particles added to two parts of a conventional chip-cut alloy. When triturated with mercury, the following reaction occurred:

$$\left.\begin{array}{l} \text{alloy} + Hg \longrightarrow Ag_2Hg_3 + Sn \\ (Ag\text{-}Cu) + Hg \rightarrow Ag_2Hg_3 + Cu \end{array}\right\} \rightarrow Ag_2Hg_3 + Cu_6Sn_5$$

The absence of the gamma 2 phase seems to coincide with less corrosion of this amalgam when it is used for oral restoration. Another new single composition system of Ag-Sn-Cu also has thirteen percent copper.[b] This system also does not exhibit gamma 2 in the set amalgam.[1]

Corrosion phenomena

Corrosion is the destructive electrochemical change of a pure or alloyed metal when it reacts with a moist environment. The destruction is brought about by an electrical current flow created between an anode and cathode. All metals corrode *in vivo*, regardless of their degree of corrosion resistance.[37,84] The corrosion products are, in general, cytotoxic. Venables, et al,[82] showed experimentally that the electrochemical reaction between metals caused destruction of bone and soft tissue. Byrne, et al,[5] demonstrated that a single piece of metal may have both anodic and cathodic areas because of the inclusion of different materials in varying

[a] Dispersalloy, Western Metallurgical, Ltd., Edmonton, Canada
[b] Tytin, S. S. White Co., Philadelphia, Pa.

amounts within the metal itself and also because of surface imperfections. Therefore, it is not necessary to have two dissimilar metals present to instigate an electrical current, one hetereogeneous metal could produce current flow within itself.

Corrosion of amalgam

Dental amalgam corrodes and is known to release its constituent elements into the surrounding environment. Schoonover, et al,[71] noted excessive corrosion of amalgam restorations at their margins. The destructive nature of this process was slowed only by maintaining a homogeneous amalgam surface. Jorgensen[33] studied the marginal fracture of amalgam fillings. He established that metallic mercury, though liberated by corrosive action, did not enter the oral environment, but rather diffused back into the amalgam restoration. In an *in vitro* study, Guthrow, et al,[23] demonstrated that the tin-mercury phase, gamma 2, was attacked most actively by the corrosive process. They also reported silver-chloride as a corrosion product. Mueller, et al, [57] reported that the gamma 2 phase had the greatest corrosion potential and the highest corrosive current. They reasoned that the corrosive phenomena in amalgam was the result of micro-anode and microcathode behavior produced as a result of metallurgical and compositional heterogeneity. Mateer and Reitz[50] identified corrosion products from restorations of silver amalgam by x-ray diffraction and chemical analysis. They noted two discreet types of corrosion, a surface corrosion and a deep penetrating form of cracking. The corrosion residue contained large amounts of sulfur, much as tin sulfide. Tobler, et al,[79] noted the reactions of sulfur with amalgam restorations high in silver content. Rapidly tarnished areas contained high concentrations of silver-mercury and tin-mercury.

Holland and Asgar[30] examined amalgam restorations in extracted teeth with the use of a scanning electron microscope and x-ray diffraction analysis. They identified sulfide and chloride anions in the most corroded areas. Tin ions were most prominent in the corrosion residues with copper and silver also identified. Mercury could not be located in the corrosion layers and only a minute quantity of zinc was labelled. The corrosive process was not confined to the superficial structures of the alloy, but was found throughout the entire mass. The gamma 2 phase was selectively corroded, which supports the presence of tin containing corrosion products.

Marek and Hochman,[47] in a recent in vitro study, examined a high copper, low silver amalgam. They concluded that while the corrosive attack on conventional amalgam was localized to the gamma 2 phase, this new amalgam revealed a limited, uniform crevice-corrosive activity despite the fact that no gamma 2 phase was present.

Corrosion types

Galvanic corrosion appears when dissimilar metals are in electrical contact in an electrolyte. Concentration cell or crevice corrosion occurs within a crevice (between an amalgam restoration and a cavity preparation wall filled with an electrolyte, saliva). Oxygen concentration cell corrosion is a particular type of crevice cell corrosion where the oxygen concentration within the crevice is much lower than outside the crevice. Since cathodic reactions require oxygen, the areas outside the crevice become cathodic to those within, and corrosion proceeds until the oxygen concentration is equalized, or the metal destroyed.[33]

Oxygen concentration in an electrolyte is thought to be the key corrosion-producing factor in amalgams placed as restorations *in vivo*. The oxygen concentration cell is formed in the marginal defects found

between the alloy and the cavity wall and the reaction attacks the gamma 2 phase, Sn_8Hg. Tin ions are then released into the electrolyte and they react with non-metallic ions, such as sulfides, chlorides, and oxygen.[23,33,50] The resultant products precipitate as a corrosive residue. The new alloy systems which have no gamma 2 phase should eliminate this major cause of corrosion.

Zinc vs. non-zinc amalgam

Both zinc-free and zinc-containing amalgams have been used for coronal restorations and for apical fillings, but disagreement exists as to which is the superior system. The deleterious effect of moisture incorporated in zinc-containing amalgams as they are condensed has been described by several authors.[9,83] Eames, et al,[9] reported that saliva contamination produced no expansion in zinc-free amalgam. The expansion caused by saliva contamination of zinc-containing amalgam seems to produce an increased tendency for corrosion. Omnell,[64] reporting a case history, noted the formation, postoperatively, of zinc carbonate crystals in the periapical tissues of a tooth previously treated with an apical amalgam filling.

Although much has been written on the physical nature of amalgam and its corrosion process, very little has been reported on the effect of amalgam and its corrosion products on biologic systems.[11] The intraoral behavior of amalgam may not necessarily correlate with the hard and soft tissue reactions when the alloy is implanted. The use of routine histologic techniques in the study of tissue reactions to implants has limited our ability to identify corrosive products and intracellular reactions. If a silver-copper amalgam system reduces the problem of corrosion, attention may be given to other problems of zinc and moisture contamination in such filling procedures.

MATERIALS AND METHODS

Abstract

Seventy-two amalgam implants were placed in the mandible or parietal bones of six dogs. The tissue responses produced in bone and submandibular lymph nodes were examined to evaluate the effect of corrosion of different amalgam systems on such tissues. Samples of amalgam, from each of the four alloy systems, were packed separately into sterile Teflon(TM)[a] tubes. The filled tubes were randomly placed into prepared holes that opened into cancellous bone. Unfilled Teflon tubes and empty surgical defects were used for controls (twenty-four total). One end of the amalgam implant contacted cancellous bone. Two dogs were sedated and killed by perfusion first with saline and then buffered formalin after each of three time periods; six, thirty, and sixty days. Specimens of the mandible and parietal bones containing the defects were decalcified in a solution of EDTA-formalin for an average period of forty-five days. Histologic sections cut eight microns thick, were stained, examined and evaluated. Selected implants were also examined with an electron microprobe[b] for corrosive residues.

Animal preparation

The canine mandibles and parietal bones were used for the *in vivo* evaluation of the four amalgam systems. Six, twelve week old German

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[a] Small Parts, Inc., Miami, Florida Laboratories
[b] Model EMX-SM, Applied Research, Sunland, California

shepherd littermates, weighing twenty-five to thirty pounds were used. All animals were held in the research facility for a minimum of two weeks prior to surgery for acclimation. During this period, all animals were given a complete physical examination by the facility veterinarian. A complete blood count was performed on each animal as well as an eighteen parameter blood chemistry screening for the detection of subclinical anomalies. Fecal material was examined for ova and parasites. All animals were vaccinated for rabies, distemper, hepatitis, and leptospirosis, and each dog was dewormed. When the animals were declared healthy, surgery was performed. Veterinary examinations were repeated every seven to ten days postoperatively to insure the continued health of each dog until it was killed.

Description of amalgam systems

Four selected commercial dental amalgams were analyzed: Alloy #1, a fine cut zinc-containing amalgam[a]; alloy #2, a fine cut zinc-free amalgam[b]; alloy #3, a blend amalgam of silver-copper spherical particles added to a conventional chip in a ratio of one to two[c]; alloy #4, a silver-tin-copper single composition amalgam.[d] (Table 1) Each alloy sample was weighed, before mixing, on an analytical balance and the mercury dispenser was carefully adjusted to produce optimum consistency of the mix and condensation. The amalgam and mercury were triturated according to the manufacturer's directions, immediately prior to implantation. The amalgam was packed by hand into presterilized Teflon tubes approximately 6 mm. long. Each tube

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[a] New True Dentalloy, Pennwalt, S. S. White Dental Division, Philadelphia, Pa.

[b] New True Dentalloy (zinc-free)

[c] Dispersalloy, Western Metallurgical Ltd., Edmonton, Canada

[d] Tytin, S. S. White Dental Division, Philadelphia, Pa.

had an outside diameter of 2.06 mm. and an inside diameter of 1.7 mm.

Anesthesia and surgical approach

Anesthetic procedures included a one milliliter intravenous injection of atropine sulfate[a] (0.5 mg/ml) prior to intravenous administration of Surital(TM)[b] given to effect, followed by oral-tracheal intubation. Closed circuit Ethrane(TM)[c]-oxygen-nitrous-oxide was used to maintain the animals in deep surgical anesthesia throughout the procedure, which varied in time from three to five hours. Post-anesthetic pain was controlled by three grain acetylsalicylic acid suppositories administered immediately after surgery. Strict aseptic techniques were followed throughout the surgery.

The dogs were placed first in a prone position and the parietal bones were exposed via a midline skin incision extending approximately 10 cm. from the sagittal crest to the posterior aspect of the occipital protuberance. The muscles and fasciae were separated by blunt dissection and retracted laterally to expose the parietal bones. Four round holes, 1.5 mm. in depth and 2 mm. in diameter, were drilled 1 cm. apart on each side of the midline. A dental high speed handpiece and an appropriate size bur, calibrated for depth, was used concurrently with large volumes of sterile physiologic saline solution.[2] The implants were then fitted into the prepared holes and the excess implant above the cortical plate was reduced with a sharp blade so that only 1-2 mm. was left protruded to facilitate removal at the appropriate time. The muscles and fasciae were approximated and closed in layers with a continuous suture using 4.0 absorbable gut and the skin was closed with interrupted sutures using

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[a] Burroughs Wellcome Co., Research Triangle Park, North Carolina
[b] Thiamylal sodium - N.F., Parke Davis, Detroit, Michigan
[c] Enfleurane, Ohio Medical Products, Madison, Wisconsin

4.0 braided silk. All skin sutures were removed approximately ten days post-operatively.

The dogs were repositioned in a supine position, and a 12 cm. skin incision was made from the mandibular symphysis to the laryngeal prominence to expose the lateral borders of the mandible. The fasciae were separated by blunt dissection and the periosteum overlying the buccal cortical plate was retracted. Six holes, 2 mm. in diameter and 3 mm. deep were made approximately 1 cm. apart between the mental foramen and the angle of the ramus. The single skin incision provided easy and adequate access to both the right and left borders of the mandible. The placement of the amalgam implants and the closure was completed in a similar manner to that in the calvarium (Figure 1).

Tissue laboratory procedure

Two dogs were killed postoperatively at six, thirty and sixty days. Each animal was sedated intravenously with Nembutal(TM)[a] administered in the amount of 0.6 ml/kgm in a concentration of 50 mg/ml. The animal was then perfused with normal saline followed by 10% buffered formalin, administered via the left ventricle and with the right atrium opened. When the perfusion was complete, the amalgam implants were immediately removed from the calvarium for analysis of surface corrosion. The mandible was disarticulated from the skull and sectioned into twelve separate pieces, each containing an implant or control. The hard tissue specimens with implants in situ, were placed in separate labeled containers of buffered 10% formalin for a period of fourteen days to achieve optimal fixation. A similar procedure was completed for the parietal bone minus

[a] Abbot Labs., North Chicago, Illinois

the implants except that all implant holes were in one piece of bone. After two weeks, the bony specimens were decalcified in a 10% ethylene-diamine tetracetic acid (disodium salt) in 10% formalin solution for an average of forty-five days. Radiographic analysis indicated the end-points of the decalcification procedure.

Before the specimens were embedded in paraffin, the amalgam implants were carefully removed. After paraffinization was complete, eight micron sections were cut parallel to the long axis of the implants. Where possible, every tenth section was selected from a continuous ribbon of serial sections for study. Various sections were stained with one of the following techniques: (1) Harris' Haematoxylin and Eosin,[43] to show general microanatomical detail, (2) Wilder's Reticulum,[7] to show reticular fibers, (3) May-Grunwald Giemsa,[7] to show blood cells, and (4) Heidenhain's Azan,[7] to demonstrate collagen fibers and bone.

The right and left submandibular lymph nodes from each dog were also removed immediately after perfusion, and placed in separate labeled containers of buffered 10% formalin for two weeks. They were then embedded in paraffin and four micron sections were cut parallel to the longest axis of the node. Every tenth section from a continuous ribbon was selected for study. The sections were stained with one of the following techniques: (1) Harris' Haemotoxylin and Eosin[7] for general micro-anatomical detail; (2) Perl's Prussian Blue[7] to show ferric iron pigment; (3) Schmorl's Lipofuscin[7] to demonstrate lipofuscin and melanin; and (4) Masson-Fontana[7] for melanin pigment.

Electron probe analysis

The amalgam surfaces of the cavarium implants that contacted the cancellous bone were examined using an electron microprobe. The specimens

were mounted longitudinally in rings filled with epoxy resin (Figure 2), after the Teflon tubes were removed. The specimens were then ground and polished and the bone contacting surfaces of the implants examined in three ways:

1. A sample current image was taken of the amalgam to differentiate the various phases of the amalgam samples.
2. A secondary electron image was taken to indicate the surface topography of the bone-amalgam interface.
3. X-ray intensity images were taken to demonstrate the various elements close to the alloy surface. In this manner, any corrosive residue or the effect of corrosion on the amalgam could be identified.

The rationale for investigating the surface integrity of the amalgam implants was to determine whether or not and to what extent the amalgam was subject to corrosive breakdown. One effect of such breakdown would be the release of metallic ions into the tissue fluids with a resultant deleterious biological reaction. Another effect would be the formation of corrosion products that could also serve to produce an undesirable biological response. Finally, a severe corrosive breakdown could destroy the structural integrity of the amalgam itself and result in a failure to seal.

To determine whether corrosion processes had been active, the interface area between amalgam and tissue was examined for corrosion products. Based on previous studies of amalgam corrosion,[23,33,50] an attempt was made to identify chlorine, sulfur, and oxygen, as well as iron, a constituent of hemoglobin. In addition, the interface of each amalgam sample was examined for changes in the structure of the constituent phases, which may liberate metallic ions into tissue fluids.

RESULTS - MANDIBULAR IMPLANTS

Six-day implants

Amalgam #1: Two of the four implants were encased in cancellous bone, the tip of the other two penetrated the tissues of the mandibular canal. The bone implants demonstrated various responses, while the tissue reaction to the canal implants appeared similar.

Bone implants: The periosteal response to the surgery was similar to that of the controls. Subperiosteal bone deposition was evident and a layer of fibrous connective tissue had closed the surgical defect and grown over the top of the implant.

In one implant (Figure 3a), a thick layer of polymorphonuclear neutrophils (PMN's) had accumulated endosteally adjacent to the amalgam surface. Peripheral to this zone there was a substantial layer of proliferating connective tissue, infiltrated with blood vessels, macrophages and a few lymphocytes (Figure 5a). The cancellous bone around the implant exhibited both osteoclastic and osteoblastic activity. Bony margins directly in contact with the amalgam exhibited lacunae which were either empty or contained osteocytes with pyknotic nuclei.

The bony response to the other implant was much more intense. PMN's formed a thick layer next to the amalgam. Macrophages, lymphocytes and red blood cells were also present. The acute response was surrounded by cellular connective tissue that contained many branching blood vessels. Osteoblastic and osteoclastic activity was associated with the adjacent hard tissues.

Canal implants: Both implants demonstrated a similar tissue response. PMN's and some lymphocytes were seen in contact with the amalgam. Red blood cells and macrophages were also present.

A thin layer of connective tissue surrounded the inflammatory cells. Arteries, veins, nerves and lymphatics in the mandibular canal showed no evidence of damage or cellular degeneration, even when the implant was very close to the structure involved.

Amalgam #2: All four implants penetrated the mandibular canal. The inflammatory reaction found in one was common to all.

Bone implants: None

Canal implants: PMN's, several layers thick, were found in contact with the amalgam. An area of increased fibroblastic activity, with branching connective tissue fibers, interspersed fibroblasts and a few lymphocytes surrounded this area. The thickness of this reactive tissue layer varied from one implant to the next (Figure 3b).

Fragments of bone created by the surgical procedure were seen encased by soft tissue. Osteoclasts were noted on or near such spicules which had empty lacunae. The bone bordering the defect site was also devoid of osteocytes.

Adjacent and peripheral to the acute response, a varying number of fat-like cells were seen. These cells were similar to those in canal tissue at some distance from the implant site. In some areas the cells appeared to be packed more densely due to the tissue reaction. Nerves and blood vessels of the mandibular canal were microscopically intact. One nerve bundle was in direct contact with the amalgam, and even in this instance no histological evidence of epineurial damage or loss of cellular integrity was apparent.

Amalgam #3: Three of the four implants were placed in cancellous bone; a fourth specimen penetrated the mandibular canal.

Bone implants: All three implants demonstrated similar reactions (Figure 3c). As with the other six-day implants, the layer of cells contacting the amalgam was composed mainly of PMN's with a few macrophages and lymphocytes. Dense cellular connective tissue was present immediately around the inflammatory cells. Extravasated red blood cells were scattered throughout both layers (Figure 5b).

In the bone adjacent to the implants, the lacunae were empty and some osteoclastic activity was noted. In areas of bone farther from the surgical defect, osteogenic activity was seen. Only one specimen exhibited woven bone deposition near the implant.

Bone fragments produced by the surgical procedure were seen in all specimens. They were devoid of osteocytes and surrounded by osteoclasts.

Canal implants: The polymorphonuclear neutrophil layer in contact with the amalgam appeared to be more dense than that observed in the cancellous bone implants. This layer, as before, contained small numbers of lymphocytes and macrophages and was enveloped by a thick layer of fibroblasts and connective tissue fibers. Newly formed blood vessels were prominent in this tissue. Fat cells were abundant at the reactive tissue margins.

Several small nerve bundles lay close to the amalgam. Their sheaths were normal, and cellular integrity was histologically unimpaired.

A small lamella of bone abutted one corner of one specimen. Osteoclastic activity was noted on the contacting surface and osteoblastic activity on the far surface.

Amalgam #4: One implant was embedded completely in cancellous bone, two of the implants penetrated the canal, and the fourth contacted the periodontal ligament of an adjacent tooth.

Bone implant: Polymorphonuclear neutrophils were seen to form a border around the amalgam end of the tube, a finding similar to the other six-day implants (Figure 3d). An area of intense fibroblastic activity, as described for the canal implant of amalgam #2, surrounded the inflammatory cells. Bone adjacent to the implant site contained empty lacunae. Bone spicules lying within the connective tissue layer were surrounded by osteoclasts. Trabecular bone farther away from the tissue-implant interface exhibited normal osteogenic activity.

Canal implants: The inflammatory response to each was similar. Again, PMN's were seen to cover the amalgam surface and one implant showed an increased concentration of inflammatory cells at the corners. A few macrophages and lymphocytes were seen and a layer of thick cellular connective tissue surrounded the inflammatory cells with fat cells noted at the periphery of the reactive area. Although one of the implants had deformed the mandibular nerve, it appeared undamaged with the epineurium intact and its cellular makeup histologically normal.

Periodontal ligament implant: A thin layer of PMN's were in contact with the amalgam. Several pieces of bone, devoid of osteocytes and surrounded by osteoclasts, were observed in the ligament. A few red blood cells, macrophages and lymphocytes were also present.

The connective tissue of the ligament appeared undamaged but somewhat disorganized. The cementum of the tooth was unaffected by the surgery or the implant.

Summary

Although the inflammatory response appeared to spread farther in the large marrow spaces than in the small spaces, the reaction was considered moderate, as to extent, and consisted primarily of polymorphonuclear neutrophils together with a small number of macrophages and lymphocytes. The reaction to all of the implants in the mandible at this time period, regardless of site, was similar. No difference in reactions could be related to the type of amalgam used.

RESULTS - PARIETAL BONE

Six-day implants

Amalgam #1: Both implants were confined within bone and the reaction was similar (Figure 4a). One specimen exhibited a dense lymphocytic infiltrate which was in contact with the amalgam and also spread laterally into the marrow spaces surrounding the surgical site. PMN's and a few macrophages were also observed. The cell response to the other specimen was also mainly lymphocytic but appeared less intense and was confined to a smaller area.

Pieces of bone, extravasated blood cells, and other debris were evident at the tissue-amalgam interface and in the medullary spaces bordering both implants. Osteoclasts surrounded these pieces.

The bony walls bordering the surgical defect contained empty lacunae. Osteoblasts were seen in the marrow cavities removed from the implant site.

Amalgam #2: One implant was confined to bone (Figure 4b). The other specimen completely penetrated the calvarium and appeared only as an empty hole.

An intense, widespread inflammatory cell response bordered the end and sides of the implant and also penetrated the marrow spaces adjacent to the defect. The majority of the cells seen were lymphocytes and plasma cells. Macrophages lay adjacent to the debris present. Fibroblasts and connective tissue strands were intermingled with the cellular response.

The lacunae of the bone approximating the implant contained no osteocytes. Osteoblasts were only seen in areas some distance away from the surgical site.

Amalgam #3: Both implants exhibited a similar tissue response (Figure 4c). No inflammatory cells were noted, and the amalgam rested directly against the bone. In the marrow spaces adjacent to the implant a few lymphocytes and polymorphonuclear neutrophils were found.

Osteoclastic activity was very intense and a majority of the marrow cavities contained these cells. Necrotic debris was also apparent in these spaces, together with macrophages. Bone surrounding the implant site was devoid of osteocytes.

Amalgam #4: One of the two implants penetrated a large marrow cavity and this area was entirely filled with inflammatory cells. The majority of cells present were lymphocytes and plasma cells, with only a very thin layer of polymorphonuclear neutrophils in contact with the amalgam (Figure 4d). Several large fragments of bone that had empty lacunae and that were bordered by osteoclasts were also seen.

The other implant showed a very mild inflammatory cell response. A small number of polymorphonuclear neutrophils bordered the base of the tube. These cells were surrounded by extravasated red blood cells and necrotic tissue. The medullary spaces contained few lymphocytes.

The bone approximating the surgical defects contained empty lacunae. There was no histologic evidence of repair.

Summary

When the implant penetrated cancellous bone with large marrow cavities, the inflammatory response appeared to be more intense than it

did when the amalgam directly penetrated compact bone with small marrow spaces. The marrow cavities contiguous with the implant site appeared to allow a greater inflammatory cell infiltration or an easier spread of the reaction. The more extensive the reaction, the greater the number of lymphocytes observed. Lymphocytes were far more numerous in the parietal bone responses than they were in the six-day mandibular reactions. The small number of polymorphonuclear neutrophils present, however, usually approximated the amalgam.

RESULTS - MANDIBULAR IMPLANTS

Thirty-day implants

Amalgam #1: Three of the four implants penetrated the mandibular canal. The fourth implant was confined within cancellous bone, except for a small perforation of the mandibular canal. Only one canal implant will be described as the quality of the tissue sections from the other two implants was poor.

Bone implant: An intense inflammatory infiltrate approximated the amalgam surface (Figure 6a). Though primarily composed of lymphocytes, PMN's formed a thin layer directly at the amalgam surface. PMN's were especially numerous at the perforation site. The marrow spaces adjacent to the defect were also packed with lymphocytes and plasma cells.

Fibroblasts were seen to be intermingled with the round cells. Both were surrounded by strands of collagen to form a loose connective tissue layer. Within this soft tissue were several bone spicules surrounded by osteoblasts. Such cells were also seen on the bony margins of the defect, and on the trabecular bone that touched the end of the implant. Osteoclasts were few in number and were only found in medullary spaces.

The inflammatory reaction at the site of perforation into the mandibular canal was quite intense. Although lymphocytes were the predominant cells here too, there were additionally a large number of PMN's present. Numerous blood vessels were observed in the inflammatory tissue. Two pieces of trabecular bone, apparently separated from the rest of the hard tissues, were enveloped by osteoblasts.

Canal implants: A thin layer of fibrous connective tissue was seen in contact with the amalgam surface. Outside this layer was a marked lymphocytic infiltrate. The connective tissue capsule was apparently confluent with the connective tissue which surrounded the Teflon tube itself. Macrophages, containing refractile foreign material in their cytoplasm were found beyond the area of connective tissue and lymphocytes. Numerous blood vessels penetrated the lymphocytic and fibrous tissue.

A small wedge of bone lay adjacent to one corner of the amalgam surface. Both osteoblastic and osteoclastic activity were apparent around it. Evidence of new bone deposition was present on the bony walls of the canal. New bone was also apparently being laid down over bone adjacent to the surgical hole, which had empty lacunae.

Amalgam #2: Two amalgam implants partially perforated the mandibular canal wall, while the other two implants penetrated into the canal. Because of the similarities, only one example of each condition will be reported.

Implant in bone and canal: A thin layer of regularly arranged fibrous connective tissue contacted the amalgam surface (Figure 6b). This tissue appeared to be confluent with connective tissue encasing the Teflon tube. Outside this fibrous tissue layer an area of cellular connective tissue was found, composed mainly of fibroblasts. Within this cellular layer were a large number of inflammatory cells, primarily lymphocytes and plasma cells. This round cell response was particularly intense at the site of perforation into the mandibular canal.

Bone splinters and other debris were clustered near the center of the tissues contacting the amalgam. Macrophages, PMN's, and osteoclasts were intermingled within the debris.

Bone near the implant walls was devoid of osteocytes, and osteoclastic activity was noticeable. Bone farther from the implant site appeared to be undergoing osteogenic activity. All the soft tissues were well vascularized.

Canal implants: A fibrous connective tissue layer, contiguous with the endosteum of the canal, extended across the amalgam surface. Its thickness, however, varied and was different for each implant. There was a loose, more cellular connective tissue layer directly outside the fibrous tissue. A dense, thick inflammatory response containing lymphocytes, plasma cells and a few PMN's was seen as a third layer. The connective tissue was well vascularized.

The major vessels and nerves of the mandibular canal which were near the implant appeared to exhibit no histologic changes in their cellular integrity. The endosteum of the canal wall demonstrated normal cellular activity.

Amalgam #3: Only one implant was completely in contact with cancellous bone; the other three totally or partially entered the mandibular canal. These three exhibited a typical tissue response with only minor variations.

Bone implant: The cancellous bone, in this instance, was composed of large marrow spaces (Figure 6c) in contrast to the more commonly seen cancellous bone with small marrow cavities. No fibrous connective tissue was seen, and the tissue near the amalgam surface exhibited only a slight inflammatory response. Lymphocytes, some PMN's and macrophages composed the inflammatory infiltrate. Some necrotic debris from the surgical procedure was noted directly beneath the end of the implants.

Osteoblastic activity was evident on the bony walls adjacent to the tube. A cellular connective tissue layer containing fibroblasts

lined the marrow spaces. Many blood vessels and a few lymphocytes were observed in the connective tissue.

Canal implants: A thin layer of fibrous connective tissue containing chronic inflammatory cells was seen contacting the amalgam surface of one implant. Outside this region was a much broader layer of cellular connective tissue, containing a considerable quantity of structureless debris and necrotic bone. Osteoclasts, PMN's, and macrophages were seen throughout this material. Between the implant and the mandibular canal, a thick layer of connective tissue filled with a dense infiltrate of lymphocytes and plasma cells was present. Numerous blood vessels were found within the soft tissues.

The tissue response to the second canal implant was quite similar to the one just described. A thick, regularly arranged fibrous connective tissue layer was also seen contacting the amalgam surface, except in one corner where an intense lymphocytic reaction had occurred. As before, the inflammatory reaction was separated from the canal contents by additional connective tissue.

Approximately one half the amalgam surface of the third implant was in direct contact with a thin layer of regular fibrous connective tissue. Necrotic material abutted the other half, with a dense population of lymphocytes and plasma cells bordering the debris. A few macrophages and PMN's were present among the round cells.

Thick strands of connective tissue separated the inflammatory reaction from the contents of the mandibular canal. These tissue bundles were continuous with the endosteum and the connective tissue encapsulating the implant.

Amalgam #4: These four implants were all located in the mandibular canal. Three specimens had comparable tissue responses and will be described. The fourth specimen, because of poor quality of the histologic sections, is omitted.

Bone implants: None.

Canal implants: A fibrous connective tissue layer (Figure 6d) covered the amalgam surface of all three implants. Surrounding this tissue layer, in two implants, a mild inflammatory response was found, consisting primarily of lymphocytes and plasma cells. Some macrophages and PMN's were also evident. The third canal implant demonstrated a very intense lymphocytic reaction at one corner of the implanted specimen. This phenomenon was not noted in any tissue sections from the other implants.

Lymphatics and blood vessels were very prominent. Fat cells in varying quantities bordered the soft tissue reaction area. As was seen with other specimens, either a major blood vessel or nerve lay adjacent to or was deformed by the implants. No damage to the walls or sheaths of these structures was evident.

Summary

A lymphocytic response to the bone implants was observed, but no well-defined fibrous tissue encapsulation was apparent. Bony deposition on the walls adjacent to the implant site was a universal finding, and osteoclastic activity was confined to a few marrow spaces.

The canal implants generally showed a thin layer of regular fibrous connective tissue covering most of the amalgam surfaces. Outside this layer there was usually a marked inflammatory reaction of varying thickness and extent. Lymphocytes and plasma cells made up the majority

of the cell populations. PMN's and macrophages were present only in small numbers. In the six-day results this was reversed. Of particular note was the reactive tissue continuous with the endosteum of the mandibular canal, which appeared to support or wall off the inflammatory response. As was reported for the six-day implant, no differences were observed that could be related to the type of amalgam used.

RESULTS - PARIETAL BONE

Thirty-day implants

Amalgam #1: Each of the two implants demonstrated dissimilar tissue responses. One implant was enveloped at the amalgam surface by an intense lymphocytic infiltrate. Macrophages and PMN's were also present together with a large quantity of necrotic debris. A very pronounced fibroblastic proliferative state was apparent. Many branching blood vessels were present in the mesh of loose fibrous connective tissue around the implant site. Some evidence of bone repair was noted on the walls of the defect, demonstrated by the presence of osteoblasts and reversal lines.

The other specimen exhibited none of the inflammatory cell reaction noted above (Figure 7a). It appeared, however, that most of the adjoining marrow cavities were filled with an immature bony matrix. A few osteoclasts were noted on the walls of the marrow spaces.

Amalgam #2: The end of one of the amalgam specimens was positioned in a large marrow cavity (Figure 7b). The resultant lymphocytic and plasma cell reaction not only covered the end of the implant, but also extended into the medullary spaces that were contiguous with the defect site. Extravasated red blood cells, macrophages, PMN's, and fibroblasts composed the remainder of the resident cell population. A large volume of structureless debris was trapped within this cellular tissue.

The majority of bone bordering the defect was undergoing osteogenic activity. A few osteoclasts were present, but only in isolated areas.

The other implant exhibited a well-defined layer of lymphocytes and plasma cells at the amalgam surface. The volume of inflammatory cells was not as large as noted in the other specimen. There appeared to be some repair of the bony walls approximating the Teflon tube. The lacunae of the bone near the end of the implant contained intact osteocytes.

Amalgam #3: The tissue response to one implant was very mild. A few inflammatory cells, lymphocytes and scattered PMN's abutted the tip of the implant. Several foreign body giant cells were observed adjacent to the implant. The medullary cavities approximating the implant contained an immature bony matrix. The bone close to the amalgam-tissue interface was covered with osteoblasts. A limited osteoclastic reaction was observed.

The reaction to the other implant was more severe (Figure 7c). A dense infiltrate of lymphocytes and plasma cells was demonstrated near the amalgam surface, a reaction similar to that described for amalgam #2. This cellular response filled the marrow spaces branching from the surgery site. Few osteoclasts were apparent but osteoblastic activity was prevalent on the walls and floor of the defect.

Amalgam #4: Both amalgam implants produced a mild inflammatory reaction. Lymphocytes and plasma cells, together with macrophages and fibroblasts composed the majority of the cellular population at the base of the implant (Figure 7d). The marrow cavities of the bone contacting the tube showed evidence of osteoblastic activity. Many medullary spaces contained an immature bony matrix. Osteoclastic activity was restricted to a few isolated areas and around the necrotic bone spicules.

Summary

As was reported for the six-day implants, the inflammatory response

appeared to be more intense when the amalgam surface penetrated a large marrow cavity. Similar to the mandibular thirty-day bone implants, lymphocytes and plasma cells composed the majority of the inflammatory cell population. Osteoblastic activity was apparent on the bony walls adjacent to the surgical site, while osteoclastic activity was very limited.

RESULTS - MANDIBULAR IMPLANTS

Sixty-day implants

Amalgam #1: The tissue response to two of the four implants will be described. The other two specimens were not found in bone, but were resting on top of the cortical plate and their surgical holes could not be located.

Bone implants: None

Canal implants: Both implants perforated the mandibular canal. The tissue response to each, however, was slightly different.

A thick layer of cellular connective tissue contacted the amalgam surface. Within this tissue were large numbers of lymphocytes and plasma cells. Of particular interest was the dense population of PMN's in several areas along the tissue-amalgam interface. The inflammatory reaction appeared to have traveled along the side of the Teflon tube to the periosteal surface.

The other implant (Figure 8a) demonstrated a much less intense inflammatory response, except at one corner where a large number of lymphocytes and plasma cells were confined (Figure 11a). Thick, unorganized connective tissue strands were observed to ramify throughout the inflammatory reaction. No obvious capsule formation was apparent, a similar situation to that of the other implant.

Foreign body giant cells were very conspicuous (Figure 9a). Many were located at the border of the inflammatory tissue, directly facing the amalgam surface or the Teflon tube.

Numerous thin- and thick-walled blood vessels were present within the inflammatory site. Fat cells were located at the connective tissue margins. Several small nerves were also incorporated within the inflammatory locus but were apparently undamaged.

Bone adjacent to the sides of the implant tube was covered with a new hard tissue matrix. A thin fibrous connective tissue layer separated the tube from the surrounding bone. Pieces of bone, scattered beneath the implant, were seen to be undergoing osteoblastic and osteoclastic activity simultaneously, typical of remodeling.

Amalgam #2: Four specimens were examined. Three were found implanted in the mandibular canal and one primarily in cancellous bone. This latter implant perforated the canal wall at one point only.

Bone implant: A very dense accumulation of lymphocytes completely encased the end of the implant. At the perforation, the round cell reaction was even more intense. Generally, fibroblasts were scattered throughout the inflammatory cells, but no definite connective tissue layer was evident. Several foreign body giant cells were located along the implant-tissue interface.

The surface of cancellous bone bordering the end of the implant exhibited osteoblastic activity. Osteoclasts were present in several of the marrow spaces. Tissues surrounding the end of the implant were well vascularized, and several of the vessels possessed multi-layered walls typical of arterioles or small arteries.

Of interest was the tissue response to the tube, a thin layer of regular fibrous connective tissue enveloped the Teflon implant in its entirety. It was, however, impossible to discern whether or not this capsule was confluent with the periosteal tissues because of the poor

quality of the tissue sections.

Canal implants: Similar tissue reactions were noted around all three implants. A very dense infiltrate of lymphocytes and plasma cells was common to all, and the reaction was even more intense at the corners of the Teflon tubes (Figure 8 b). Numerous fibroblasts were seen among the inflammatory cells, and one specimen which lay against the canal wall demonstrated thick bands of fibrous connective tissue. These tissue strands appeared to isolate the specimen from the elements of the canal. This particular histological picture was similar to some canal implants at thirty days. Substantial amounts of necrotic bone were incorporated within the cellular milieu and osteoclastic activity was very evident around these fragments.

The degree of vascularity was similar to that reported for other sixty-day implants. Numerous blood vessels of various sizes traversed the tissues near the amalgam surface. Major vessels and nerves of the mandibular canal appeared normal in all respects despite their proximity to the implants.

Amalgam #3: Two of the four implants were embedded in cancellous bone, while a third penetrated the canal wall in one small area. The other implant lay outside the cortical plate and will not be described.

Bone implants: The inflammatory reaction was intense around the ends of all implants (Figure 8c), including the specimen that penetrated the canal. Lymphocytes and plasma cells, mixed with fibroblasts, composed the cellular population of the tissue response (Figure 11b). No demonstrable connective tissue capsule was evident for two of the implants, a situation similar to most other sixty-day bone implants. The third implant, which penetrated the canal wall,

displayed a tendency for encapsulation, demonstrated by a layer of fibrous tissue which had been formed on one side, facing the perforation. Foreign body giant cells were common to all three specimens. The majority of these cells lay adjacent to the implant, contacting both the Teflon walls and the amalgam surface.

Osteoclastic activity was intense for the bone that directly bordered the amalgam-tissue interface. Bone down the side of the implant exhibited normal osteoblastic activity, and was comparable to sites farther removed from the defect.

Normal cellular connective tissue filled those medullary spaces not occupied by inflammatory cells. Numerous blood vessels were evident.

Canal implants: None.

Amalgam #4: Three of the implants will be described. A fourth implant was not found in cortical plate. This specimen may have been pushed out by bone ingrowth of the surgical site or may have been removed (accidentally) at the time of surgery.

Bone implants: None.

Canal implants: The two implants displayed comparable tissue responses (Figure 8d). An inflammatory cell reaction of lymphocytes and plasma cells, similar to that described for other sixty-day implants, predominated at the amalgam-tissue interface. The reaction was even more intense at the corners of the Teflon tube. Fibroblasts were abundant and some strands of unorganized fibrous connective tissue were noted. Several foreign body giant cells were seen in adjacent areas.

The third implant was also located in the canal, but the tissue response was the most prominent. A thick, multi-layered connective tissue capsule enclosed the end of the implant completely. No comparable reaction

of this type was seen in any other specimen, regardless of the time period or amalgam sample.

Macrophages, their cytoplasm filled with particles of refractile debris, were very evident at various distances from the capsule (Figure 9b). The inflammatory reaction was limited to an occasional grouping of lymphocytes at the border of the fibrous connective tissue.

As in other implants, the tissue surrounding the capsule demonstrated numerous thin and thick walled vessels. Neuro-vascular components within the canal proper appeared to be histologically normal.

Summary

The canal implants, in general, demonstrated a similar inflammatory response. Lymphocytes and plasma cells were the predominant cells involved. They were observed to form a concentrated and thick layer adjacent to the amalgam surface, with a more extensive response at the corners. Foreign body giant cells were common, a finding not reported for earlier time periods. One implant was encapsulated by a very thick fibrous connective tissue.

The inflammatory response surrounding the bone implant was quite comparable to the canal implants. Lymphocyte and a few plasma cells were common to all the reactive sites with foreign body giant cells also seen. Generally, the tissue response to the sixty-day implants was more chronic in nature than the six- or thirty-day implants.

RESULTS - PARIETAL BONE

Sixty-day implants

Amalgam #1: Only one specimen will be described (Figure 10a). The other implant was placed in a surgical hole which completely perforated the cranium.

An intense and extensive lymphocytic response was evident. The round cells also formed a cellular layer between the walls of the defect and the connective tissue immediately against the sides of the implant.

New bone deposition had occurred on all walls surrounding the implant. Osteoclasts were only found in a few marrow spaces. Bony fragments observed in the tissues adjacent to the amalgam surface were devoid of osteocytes.

Evidence of vasculature structures were noted in the inflammatory tissue. Blood vessels of varying size ramified throughout the cellular reaction.

Amalgam #2: The Teflon tube was still present in the tissue sections, apparently only the amalgam sample being removed prior to sectioning. A thick layer of fibrous connective tissue had formed between the tube and the adjacent bony walls. This tissue layer encapsulated the end of the implant, but it was much thinner at the amalgam surface. Lymphocytes were clumped together in several areas outside the fibrous tissue and one large area in a medullary cavity was filled with round cells. The inflammatory reaction was generally very mild.

New bone deposition was evident on the bony walls abutting the implant. A few osteoclasts were observed in some marrow cavities.

A more intense lymphocyte infiltrate was seen contacting the amalgam surface of the second implant (Figure 10b). Interspersed with these cells was a large quantity of necrotic debris. Osteoblastic activity was similar to other sixty-day implants, and again, only a few osteoclasts were demonstrable.

Amalgam #3: Only one specimen will be described as the second site perforated the cranium and no data was available.

It appeared that this implant was also placed in a through and through surgical hole (Figure 10c). However, the implant did not go all the way, and a thick layer of fibrous connective tissue covered the amalgam surface. A few lymphocytes and foreign body giant cells were seen within this tissue. In a large marrow cavity at the corner of the implant, many lymphocytes and plasma cells were also present. A smaller number of round cells were also noted in the opposite corner.

The bony margins adjacent to the connective tissue bridge showed evidence of new bone deposition. Osteoblasts covered these hard tissue edges and reversal lines were apparent.

Amalgam #4: Both specimens will be described separately as their corresponding tissue response was quite distinct from each other. One implant revealed a well defined, localized infiltrate of lymphocytes and plasma cells contacting the amalgam surface. However, between this inflammatory cell population and the amalgam surface was a layer of fibrous connective tissue containing necrotic debris. The marrow spaces adjacent to the surgical site were free of inflammatory cells.

The bony tissue surrounding the inflammatory reaction exhibited reversal lines and evidence of new bone deposition. Osteoblasts lined the walls of the medullary cavities approximating the implant.

The cellular response to the second implant was more intense, with lymphocytes and plasma cells concentrated near the amalgam surface and in the marrow spaces extending a short distance from the surgical hole (Figure 10d). Fibroblastic activity formed a connective tissue mesh containing round cells.

Bone around the implant demonstrated new hard tissue deposition. Osteoblasts were numerous but osteoclastic activity was limited.

Summary

The sixty-day inflammatory responses were quite similar to the thirty-day responses. The reactions did not appear to be as widespread, however, and inflammatory cells generally did not extend far from the amalgam surface. Only lymphocytes and plasma cells appeared to be involved in the cellular response in addition to the fibroblastic activity. Foreign body giant cells were observed in only one specimen.

Bone repair was observed on all surfaces bordering the implant site. Osteoclastic activity that was seen was confined mainly to nearby marrow spaces.

RESULTS - CONTROLS

Six-day control - Teflon tubes

Four empty Teflon tubes were placed in the mandibles of two dogs. Upon sectioning, it was found that only one tube had been implanted in cancellous bone, two tubes ended in the mandibular canal, and one tube ended in the periodontal ligament. A histologic description is as follows:

Empty tube in cancellous bone: A fibrin blood clot filled the middle of the tube for approximately one half the total length (Figure 12a). At the periosteal aspect, there appeared to be an enlargement and multiplication of cells of the cellular layer of the periosteum (Figure 13b). This inner proliferation had occurred at some distance from the defect, and lifted the fibrous layer of the periosteum off the surface of the cortical plate. New trabecular bone was forming beneath the cellular layer, but not into the defect. Long, slender trabeculae extended at right angles from the cortical plate and were covered with osteoblasts. The trabeculae appeared to be connected by shorter bony bridges lying parallel to the surface of the cortical plate. The periosteum covered the end of the tube and fibroblasts with associated collagen strands could be seen passing from the inner aspect of the periosteum into the tube, forming an unorganized fibrous callus. This cellular network of the callus appeared to penetrate the edge of the fibrin clot. Many individual fibroblasts were incorporated in the blood clot itself. At the surface of the Teflon tube facing the endosteum, it appeared that a similar fibrous callus had been formed, but this had

penetrated the tube further than the subperiosteal callus. Blood vessels were clearly demonstrated amongst the fibrous tissue. Both the endosteal and subperiosteal callus contained many macrophages.

New trabecular bone was evident at the lower aspect of the endosteal callus. The cancellous bone adjacent to the endosteal end of the tube showed marked osteoblastic activity.

The bony walls approximating the Teflon tube contained many empty lacunae, but no osteoclasts were present. There was no inflammatory reaction at either end of the tube.

Empty tube in mandibular canal: Both of the control tubes inadvertently placed into the canal showed a similar histologic appearance. Most of the tubes were filled with blood clot, the cellular layer of the periosteum demonstrated marked hyperplasia under the fibrous layer which was lifted away from the cortical plate. New trabecular bone was in the process of being formed subperiosteally. A fibrous callus migrated down the tube from the periosteum, within which were visible at least six small blood vessels.

The opposite end of this tube was situated across the canal, and could be seen deforming the mandibular nerve against the canal wall. Neither the surgical trauma nor the Teflon tube appeared to have damaged the nerve or its coverings.

As the nerve did not completely obliterate the end of the tube, a fibrous callus was seen to originate from the endosteal aspect of the canal and invade the blood clot of the tube. Several blood vessels were seen in the callus, but no new trabecular bone was visible. A thin fibrous capsule lined the outer aspect of the tube with a loose network of fibrous connective tissue and free floating necrotic red blood cells lay within.

No inflammatory cells were visible at either end of the tubes or along their walls. Macrophages were present along the border of the blood clot within the tube and throughout the soft tissue of the tubes. The poor quality of histologic sections from this particular specimen made it impossible to describe the morphologic changes of the bone through which the tubes passed.

Empty tube lying in the periodontal ligament: Histologically, the tube appeared empty. The periosteal layer showed similar activity to that of the other six-day Teflon controls. However, no subperiosteal fibrous plug could be found within the tube. At the opposite end, the tissue of the periodontal ligament was filled with red blood cells. There appeared to be a thin layer of connective tissue lying parallel to the end of the tube, which probably separated the tube from the periodontal ligament proper. No fibrous callus invaginated the tube from the ligamental tissues.

Bony lacunae, devoid of osteocytes, lined the walls adjacent to the implant. Numerous osteoclasts appeared in the medullary areas next to the tube.

An inflammatory cell infiltrate was not apparent. No inflammatory cells were evident at any area in or near the Teflon tube.

Six-day control - empty defect

Four holes were also made in the mandible and were not filled with Teflon tubes. Two of these surgical sites penetrated into the mandibular canal and one site lay completely within cancellous bone. The fourth site will not be reported as the histologic sections were of very poor quality.

Defect into mandibular canal: A fibrous plug had completely filled the surgical defect. There was no evidence of blood clot, although a few scattered necrotic red blood cells were present in the soft tissue.

New trabecular bone was present subperiosteally. Fingerlike projections were found on both sides of the defect and appeared to travel down the lateral walls of the hole, connecting with the endosteal hard tissue. What contributions the endosteal cells made to this new bone formation could not be evaluated.

The inflammatory reaction was negligible. No inflammatory cells of any quantity were demonstrable. Macrophages were scattered in small numbers throughout the plug.

Defect in cancellous bone: The histologic picture was consistent with the other six-day controls. The inner proliferation of the cellular layer of periosteum had lifted the periosteum from the cortical plate. New trabecular bone could be seen subperiosteally around the surgical site and some new bone was growing into the defect (Figure 13a).

A thick fibrous tissue callus filled the majority of the cavity and many blood vessel walls were clearly demonstrated. A few red blood cells were present, as was an occasional macrophage. There was no blood clot. At the endosteal end of the defect, a new bony matrix was seen extending periosteally. The adjacent cancellous bone exhibited vigorous fibroblastic activity. Osteoclastic activity was limited to an occasional marrow space adjacent to the defect wall.

The bone lining the hole was devoid of osteocytes, and this was a phenomenon common to all six-day controls. There was no inflammatory cell infiltrate in any of these control specimens.

Thirty-day controls - Teflon tubes

Four empty Teflon tubes were implanted in the same manner as the six-day controls. All controls, however, extended into the mandibular canal. Three controls exhibited a similar tissue response and therefore the typical histologic picture of one implant control will be reported as common to all. One corner of the fourth control penetrated into cancellous bone, however, and demonstrated a slightly different reaction.

Empty tube in mandibular canal: At the periosteal level, a bony callus surrounded the surgical site (Figure 13c). The new bone was well demarcated from the hard tissue of the cortical plate by prominent reversal lines. New bone formation was also noted lining the surgical defect. This bone appeared to overlie older bone, devoid of osteocytes.

A thick callus of fibrous connective tissue filled the upper one-quarter of the tube implant toward the periosteal surface. Blood vessels and fibroblasts were present in abundance, together with a few scattered macrophages. No evidence of fibrin clot could be found.

A similar tissue callus was present at the opposite end of the tube, a thick connective tissue capsule encircled it. The tissue of this capsule was confluent with the tissue of the fibrous callus, which in the fourth control contained new bone trabeculae (Figure 12b).

Nerves and blood vessels of the canal appeared to be intact with no apparent damage or breakdown. There were no signs of an inflammatory reaction near or in the tube at either end.

Thirty-day control - empty defect

Four surgical defects, left unfilled, were also examined as control sites. One hole was found to have been placed mostly in cancellous bone,

two holes were drilled into the mandibular canal, and one site could not be located in the tissue specimens.

Defect into cancellous bone: At the periosteal aspect, new trabecular bone had completely bridged the surgical site. This was continuous with newly formed bone at the margins of the surgical site.

At the endosteal end, soft tissue filled the majority of the area. One large sliver of new trabecular bone extended from the underlying cancellous bone to the periosteal bridge. Due to poor quality of this particular tissue specimen, further description of cellular material and activity was impossible.

One interesting feature of this specimen was a small penetration of the mandibular canal by the surgical bur. There seemed to be no bony regeneration of this hole. Only a plug of loose fibrous connective tissue filled the wound.

Defect into mandibular canal: These surgical sites were filled with fibrous connective tissue penetrated by numerous blood vessels. There was no apparent demarcation between endosteal and periosteal tissues. The tissue area was composed of fibroblasts and organized collagen strands. In one specimen, several small bony spicules could be seen within the tissue strands of the fibrous plug. They appeared to be acting as centers of ossification for they were covered with numerous osteoblasts. Viable osteocytes were evident within these hard tissue pieces.

That new bone was being laid down on the sides of the defect was evidenced by easily defined reversal lines. Even though there was generalized osteoblastic activity along the walls of the wound, osteoclasts could be found in marrow spaces adjacent to the surgical site.

There were a limited number of inflammatory cells and these were confined to the periosteal end. They appeared to be mostly lymphocytes and plasma cells.

Sixty-day controls - Teflon tubes

Four Teflon tubes were implanted as controls, of which three were placed into cancellous bone and one into the mandibular canal. One typical example of the cancellous bone implant will be described.

Empty tube in bone: At the periosteal surface, the tube was filled with strands of fibrous connective tissue (Figure 13d). As this tissue migrated down the tube, large islands of newly formed bone were present, and these were covered with osteoblasts. This bone not only filled the central area of the tube, but lay adjacent to the tube wall itself (Figure 12c).

Bone appeared to be deposited in a similar manner at the endosteal end. There was no thick plug of connective tissue, however, as was so evident at the periosteal surface. New bone was also being laid down on the cancellous bone supporting the base of the tube. Some small bony spicules appeared to be undergoing osteoclastic resorption.

Along the bony walls, adjacent to the outer surface of the implant, new bone was being deposited. Osteocytes were present throughout, unlike the six-day sections.

In the center of the tube, a small cluster of lymphocytes was present, the significance of which is not known.

Sixty-day control - empty defect

Four surgical defects were made and left empty with no Teflon tube implanted. Three of the sites showed such evidence of complete repair

that they could not subsequently be located in the sections with any degree of certainty. One site, placed into the mandibular canal, is reported next.

Defect into mandibular canal: The width of this defect was significantly smaller than those made at earlier time periods. A plug of fibrous connective tissue traversed the defect, connecting the periosteal and endosteal surfaces. This tissue was filled with numerous thick and thin walled blood vessels. Thick, organized bands of connective tissue intermingled with loose strands of immature tissue. There was no evidence of bone deposition within the plug. Inflammatory cells were absent. New bone was actively being laid down along the walls of the hole, growing progressively inward. Osteoblasts lined the edge of the newly deposited bone and viable osteocytes were numerous.

RESULTS - ELECTRON MICROPROBE ANALYSIS

Analysis by incident light microscopy (40X) and electron microprobe scanning showed no discernible difference in the surface integrity of the samples due to time or due to sampling. Therefore, only representative sixty-day samples for each amalgam type will be reported.

Amalgam #1

The polished longitudinal sections were examined overall using a light microscope and showed uniform microporosities dispersed evenly throughout the mass of the implant (Figure 14). The topography of the bone-amalgam interface was pitted and irregular. One specimen showed a rather large crack extending from the bone contacting surfaces vertically into the amalgam.

When examined by the electron probe microanalyzer, the sample current images demonstrated the expected amalgam phases and porosities (averaging 5μ across) throughout. Sample current photographs showed that the center of the amalgam surface was much more irregular than the corner of the amalgam contacting the Teflon tube.

The light background of the image represented the amalgam matrix, gamma 1 (Ag_2Hg_3) (Figure 15a). Grayish, irregular sized particles interspersed within the matrix represented uncombined alloy and/or the gamma 2 phase (Sn_8Hg). A tin scan, utilizing x-ray intensity images (Figure 15b) differentiated the gamma 2 phase from the free alloy particles. The gamma 2 phase was present in normal amounts and appeared similar to that

in freshly prepared conventional amalgam. Cu_6Sn_5 was detectable in only minute quantities.

The tin scan was not able to demonstrate any corrosion products which would have been seen in or on the surface of the pits and porosities. X-ray intensity scans for sulfur, chlorine, oxygen, and iron were negative.

Amalgam #2

Light microscopic examination showed uniformly sized porosities within the mass of the implant as well as an irregular, pitted surface at the bone-amalgam interface. No cracking was evident.

Probe analysis demonstrated that the amalgam matrix was very similar to that of amalgam #1. The sample current images showed distinct phases and porosities. The gamma 1 and gamma 2 phase orientation was, as expected, similar to amalgam #1.

X-ray intensity images revealed traces of sulfur, chlorine, and sodium within some of the void areas. Iron and oxygen ions were not detected and a tin scan revealed no tin complexes within the voids occupied by the sulfur and chlorine ions.

Amalgam #3

Light microscopy revealed a similar structure to amalgam implants one and two. Numerous porosities were visible throughout, and the bone-implant interface was rough and pitted.

Electron probe examination showed not only the typical gamma 1 matrix formation and free alloy particles, but also the Ag-Cu dispersant (Figure 15c). These particles were clearly recognizable as dark areas surrounded by a lighter reaction ring, which collaborates a previous study.[46] The amalgam is essentially devoid of any corrosion prone gamma 2 phase, as previously noted.

X-ray intensity images detected no corrosion residue in the sample. Sulfur, chlorine, oxygen, and iron scans were negative.

Amalgam #4

The optical photographs revealed the typical picture of surface irregularities and porosities. No horizontal or vertical cracks were seen.

The sample current images displayed a slightly different phase pattern (Figure 15d). Significant amounts of Cu_6Sn_5, represented by gray, circular particles of varying sizes, were intermixed within a light colored matrix of gamma 1. As evidenced in amalgam #3, the gamma 2 phase was not present in discernible amounts.

There was no evidence of any corrosion activity on the implant surface or within the voids. X-ray intensity scans for sulfur, oxygen, chlorine, and iron were negative.

RESULTS - SUBMANDIBULAR LYMPH NODES

Four micron sections from the submandibular nodes of each dog were examined by light microscopy. In all haemotoxylin and eosin stained specimens, macrophage containing intracellular dark brown pigment granules were seen in the sinusoids. The other stains gave positive results for ferric iron, lipofuscin and melanin.

DISCUSSION

Previous in vivo studies[3,8,16,17,49,55,75] have been conducted to determine the irritational quality of dental amalgam. Except for two investigations,[17,75] the experiments were not designed to simulate clinical application. Amalgam was implanted simply to compare its toxic reaction with that of other root canal filling materials.

This study was designed to test the tissue inflammatory response to four amalgam systems, two conventional types and two newly formulated. As amalgam is almost exclusively the material of choice for retrograde fillings, it was anticipated that useful data regarding the tissue toxicity of the two new systems would result. In vivo studies have demonstrated the irritational effect of corroding metal implants, but only one attempt[10] has been made to identify which corrosion products were produced by dental amalgam. No investigations have examined the effect of such corrosion in hard tissue. It was therefore anticipated that an electron microprobe analysis of the amalgam surface contacting bone might identify specific products that could be related to particular results noted histologically. These combined observations might then be helpful in predicting clinical success for amalgam used as retrograde fillings.

No discernible differences were found, however, when histologic studies and electron microprobe analysis of the amalgams were evaluated. Because of this lack of difference and also difficulties experienced in control of the variables, no attempt was made to quantitate the results

for statistical analysis. Control problems were due partly to the difficulty in positioning of the implants, as a large number of them were found to have penetrated the mandibular canal, a problem also encountered by Feldmann and Nyborg.[14,15] Variation in the density of the cancellous bone which approximated the amalgam surface would have made any quantitative analysis difficult. This observation was particularly significant in the calvarium, which showed extensive inflammatory reactions spreading into the marrow spaces from the implant site when the implant surface lay within a large medullary cavity.

Any quantitative assessment of the inflammatory response would have had to take into account the effect of the Teflon tubes.[53,65,74] Although it was apparent that the tubes did not cause an appreciable inflammatory response by themselves, many implants demonstrated layers of inflammatory cells which were migrating endosteally along the tubes towards the periosteum and vice-versa. It has been reported previously[53] that Teflon is nonwetting and self-lubricating, and that a direct "attachment" between bone and tube was therefore impossible. Thus, any gap between the Teflon and the hard tissue walls would provide easy access for inflammatory cell movement and migration.

The final variable to be considered was fixation artifact and technical difficulties encountered during tissue preparation. The handling of large specimens of bone and teeth presented certain problems in decalcification and sectioning which were only solved by experience as the study progressed. Tissue sections from some specimens were torn or stained poorly. It was obvious in other sections that the implant holes were not of correct width or diameter, probably as a result of improper angulation of the specimens during embedding and/or sectioning. Experienced help

was only available on a consultation basis, and this was a detriment to preparation.

Although there were no apparent differences created by the implant materials used, there were obvious differences due to time and location of the implant. It was clearly shown that the inflammatory reactions in the mandible were acute in nature at six days and that parietal implants were not. Gradually, however, this histologic picture changed, and at sixty days lymphocytes and plasma cells were more prominent than before, and this response was common to both parietal and mandibular implants.

At six days, all mandibular implants produced a similar tissue response, unrelated to the type of amalgam used. A common finding, however, was the presence of a PMN layer, usually just several cells thick, adjacent to the amalgam surface in both canal and bone implants. These cells were supported by a layer of cellular connective tissue composed of fibroblasts and a few round cells. Bone surrounding the hard tissue implants contained empty lacunae, reported by others[14,17] as being due to the surgical procedure. The parietal bone implants produced mainly a chronic inflammatory response of lymphocytes and plasma cells. This marked difference was either a more rapid replacement of the acute cells in the calvarium versus the mandible due to blood supply, an immune response not preceded by PMN's, or some other factor.

Examination of the empty drill-hole controls and of the Teflon tubes alone showed, as expected, delayed healing around the tubes due to suppression of the repair process in the adjacent marrow spaces.

At thirty days, an organized layer of fibrous connective tissue, or capsule, covered the amalgam surface of most canal implants. Adjacent to this layer was an intense inflammatory cell response of lymphocytes and

plasma cells, with many macrophages and a few PMN's. A similar cell response was observed around the end of the bone implants, but usually without the presence of the connective tissue capsule. Lymphocytes and plasma cells were in contact with the amalgam surface of the parietal bone implants, and extended into the marrow spaces adjacent to the implant site.

Although bone was observed in the Teflon tube controls and filled the empty drill holes as well, perforations of the mandibular canal were covered only with fibrous connective tissue. This tissue connected in many instances with the supporting tissues of the neurovascular bundle. Any attempt at bone regeneration may have been affected by this difference in tissue type.[69]

At sixty days, four of the mandibular implants lay directly on the cortical plate. According to Friend and Browne,[17] who observed similar conditions, the implants were apparently pushed out by new bone growth. However, it is also possible that the implants could have been dislodged during the remaining surgical procedures. Unlike the six-day specimens, the remainder of the implants, both in mandibles and parietal bone, exhibited a dense chronic inflammatory reaction near the amalgam surface. Macrophages with cytoplasmic inclusions were more prominent than in earlier time periods, and foreign body giant cells were numerous in the mandibular implants but scarce in the calvarium. This distribution of giant cells agrees with that reported by Browne and Friend,[3] but not with Feldmann and Nyborg.[14,15] It is interesting to speculate on their role here, inasmuch as few investigators agree on the role of these cells.

Tissue encapsulation was much less pronounced around these implants than in the thirty-day experiments, except for one canal implant which possessed the thickest capsule of any observed.

Two interesting phenomena characterized capsular formation; (1) they were more apparent around mandibular canal implants than in bone, (2) they apparently degenerated or decreased in size from thirty to sixty days. Most studies of implants demonstrate capsule formation except around the most toxic root filling substances. However, disagreement exists concerning the exact significance of this fibrous tissue layer. Weismann[84] stated that a thin fibrous membrane indicated little effect of the tissue environment upon the metal implant and that conversely the thin membrane indicated good corrosion resistance. A thick membrane was indicative of a higher corrosion rate and possible mechanical irritation.

Lyman[44] speculated that the connective tissue capsules, depending on their thickness, may act as a barrier to the normal immune responses and that this tissue may be a preferred site for the survival and growth of antigenic tumors. Stinson,[77,78] in his studies with rats and guinea pigs, noted that even though the implants in both animals were surrounded by tissue capsules of varying thicknesses, the capsules became thinner as time passed in the guinea pigs and thicker in the rats. He postulated that increased surface area of the implant bore a direct relationship to the increased incidence of "foreign body tumors." He also postulated that as the capsule thickened in rats, the metabolites produced in the inner layers of the tissue would probably be removed more slowly than in the thinner capsules of the guinea pigs. The result would be an accumulation of cell metabolites in the inner capsular layer which may be important in the production of tumors.

Feldmann and Nyborg[14, 15] emphasized that capsular thickness was directly correlated to the degree of acute inflammation produced by the implant. The stronger the irritant, the thicker the capsule. This

explanation does not satisfy the tissue reactions as noted in the sixty-day implants of this study. The lack of capsule formation, or at least the lack of a regular fibrous connective tissue layer across the amalgam surface, could signify a very low toxic characteristic of the implants. The dense inflammatory infiltrate adjacent to the amalgams, however, appears to signify the presence of some significant irritational qualities of the metal alloys used. This cannot be explained.

The sixty-day control reactions were almost identical to those of thirty days, but more pronounced with repair almost complete. However, the mandibular canal perforations were not healed. Such lack of healing raises the question of the role of the endosteum in bone repair, noted by Friend and Browne[17] and Hjørting-Hansen and Andreasen.[29] These authors credit the endosteum being mainly responsible for repair. If this is so, the lack of repair here may be due to neural damage such as that postulated by Retief,[69] even though histologically none was evident in this series of experiments.

Ferguson, et al,[18,19] reported that after spectrochemical analysis of soft tissue surrounding various metallic implants, certain metallic ions native to the implants were found in concentrations which were significantly greater than was normal for the area. He stated further that as both metal and non-metal implants become more frequently used, it will be necessary to study the toxicity of both the alloy and its liberated ions when selecting an ideal alloy. Instances have been reported[18] where acute inflammation, without evidence of bacterial infection, has developed in the tissues surrounding implants after years of apparent quiescence.

These findings may be important when amalgam is used in retrograde

fillings. Although only a small surface area is involved, and thus only a low concentration of irritating products result, years of perpetual release of corrosive products into a local, confined environment may eventually cause the breakdown of the periapical tissues.

The histologic examination of the submandibular nodes revealed foreign material within numerous macrophages, but, as in the bone studies, showed no difference due to the amalgam used. The various staining methods suggested that this foreign material could be one of several substances, such as lipofucsin, melanin, or breakdown products including hemosiderin. Since a surgical procedure was performed on each animal, the possible composition of these granules does not evoke surprise. There were no traces of particulate alloy. As was indicated by Ellender,[10,11,12] special tissue preparation and special stains may be required for identification of corrosive products in soft tissue. Ferguson, et al,[19] was able to trace the spread of metal ions to the liver, kidney, spleen, and lung in rabbits after various metals were implanted, but a spectrochemical analysis was required for his investigation. The methods used in this study may not have been sophisticated or sensitive enough to identify metallic ions and/or compounds that are released by the various amalgams.

The other part of this investigation dealt with electron microprobe analysis of the amalgam surfaces. It was anticipated that if corrosion products could be identified, a possible correlation with subsequent inflammatory reactions could be made.

The electron microprobe examination demonstrated no recognizable differences of the surface integrity for the various samples of amalgam used. This lack of corrosion did not change with time either. No

corrosion products were revealed and the respective phases of each amalgam system appeared to be intact. The probe analysis was carried out because it was postulated that amalgam in bone might react differently than amalgam used for oral restorations and that sixty-day implants would show the difference.

One mechanism of corrosion that could not be measured by the electron microprobe was the loss of soluble metallic ions from the amalgam surface. These ions in a biologic system, sensitive to any antigen, may indeed affect living tissue. However, the experiment was neither designed to investigate the ion concentration in tissues adjacent to the implants, nor was the probe sensitive enough to detect the loss of such small quantities of constituent ions from the amalgam surface.

X-ray intensity images revealed traces of sulfur and chlorine in voids of one sample of amalgam #2. These anions were apparently released from the contacting soft tissue.[72] No cations, such as tin, were revealed by the x-ray intensity images in the same voids as the anions. Therefore, as tin cations are only released from the breakdown of the gamma 2 phase of the alloy, and since this phase was apparently intact, no corrosive residue could have existed at this time period, or if it did it was too small to detect.

In reviewing the procedural difficulties and the lack of quantitative data from this study, questions could be raised as to the value of the particular methodology followed in this investigation. Although the final results are mostly negative, the procedures outlined should be useful in the future, with modification such as smaller blocks to section and absolute orientation of the hole and reaction tissue. Even though many implants penetrated the mandibular canal, their overall tissue

response was similar to the implants embedded completely in bone and in long term studies could be used if in sufficient numbers. The implanting of specimens into hard tissue for a comparative study may only be worthwhile when combined with tissue culture examinations. Though even this combination may fall short of true in vivo studies.

The fact remains, however, that a tissue reaction to the amalgam systems was quite evident. Unfortunately, the long term affect on periapical tissues in humans can only be speculative. It is possible that an autogenous material such as dentin chips, as suggested by Pappin, et al,[67] may be a better retrograde filling, particularly if dentin fragments induce cementum growth. This could provide a permanent seal of the apical end of the root canal without toxicity.

SUMMARY

Four different amalgam systems condensed into Teflon tubes were randomly implanted in the mandible and parietal bones of six dogs in order to determine the bony tissue reactions to each. An empty tube and an unfilled hole served as controls. Two dogs were killed by perfusion at six, thirty, and sixty days and the implants removed. The bony specimens were prepared for sectioning and histologic examination. The calvarium implants were removed prior to fixation and examined by an electron microprobe analyzer for surface corrosion. The submandibular lymph nodes were also removed and examined for the presence of corrosion products.

Although some of the mandibular implants penetrated into the mandibular canal, a similar reaction was noted for both these implants and those actually embedded in bone. At six days the predominant inflammatory cells were polymorphonuclear neutrophils, but the histologic picture changed to that of a more chronic response with lymphocytes and plasma cells at sixty days.

The only major difference discovered between the canal implants and those situated in mandibular bone proper was that a fibrous tissue capsule covered the amalgam surface of the canal implants. This was seen to occur more frequently at thirty days than at six or sixty days. Connective tissue capsules were not apparent around the amalgam surface of the mandibular implants, except in one experiment of sixty days duration.

The parietal bone implants were never found to demonstrate a similar

acute stage of inflammation with polymorphonuclear neutrophils at six days as was observed with the mandibular specimens. The thirty- and sixty-day implants, however, produced a similar tissue reaction to those in the mandible. No capsular formation was ever found in these parietal bone specimens.

Electron microprobe analysis demonstrated no corrosion products on the amalgam tissue interface of any implants from any time period. No evidence of corrosion products were noted in the submandibular lymph nodes.

CONCLUSIONS

1. The tissue response, at six days, to all four amalgam systems implanted in the mandibular bone was that of an acute inflammatory reaction. A chronic inflammatory response characterized the tissue adjacent to all the thirty- and sixty-day implants.

2. Tissue reactions in parietal bone implants were similar to those exhibited in the mandible except at six days, when a chronic reaction with round cells was observed.

3. Connective tissue capsule formation was seen adjacent to the thirty-day canal implants. A capsule was also present around the amalgam surface of one sixty-day implant.

4. The controls in bone demonstrated the occurrence of a normal repair process. Controls perforating the mandibular canal demonstrated none or minimal bone regeneration.

5. No corrosion products were identified by electron probe micro-analysis of the amalgam surfaces or in tissue sections from the submandibular lymph nodes.

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TABLE 1

NATURE AND COMPOSITION OF THE TEST AMALGAM ALLOYS

ALLOY		NATURE	COMPOSITION (WEIGHT %)			
			Ag	Sn	Cu	Zn
#1		Conventional lathe cut with zinc	69	27	3	1
#2		Conventional lathe cut without zinc	70	27	3	
#3	A	Conventional lathe cut alloy (2 parts)	69	27	3	1
	B	Spherical Ag-Cu eutectic (1 part)	72		28	
#4		Single composition high copper spherical alloy	60	27	13	

MANDIBULAR IMPLANTS

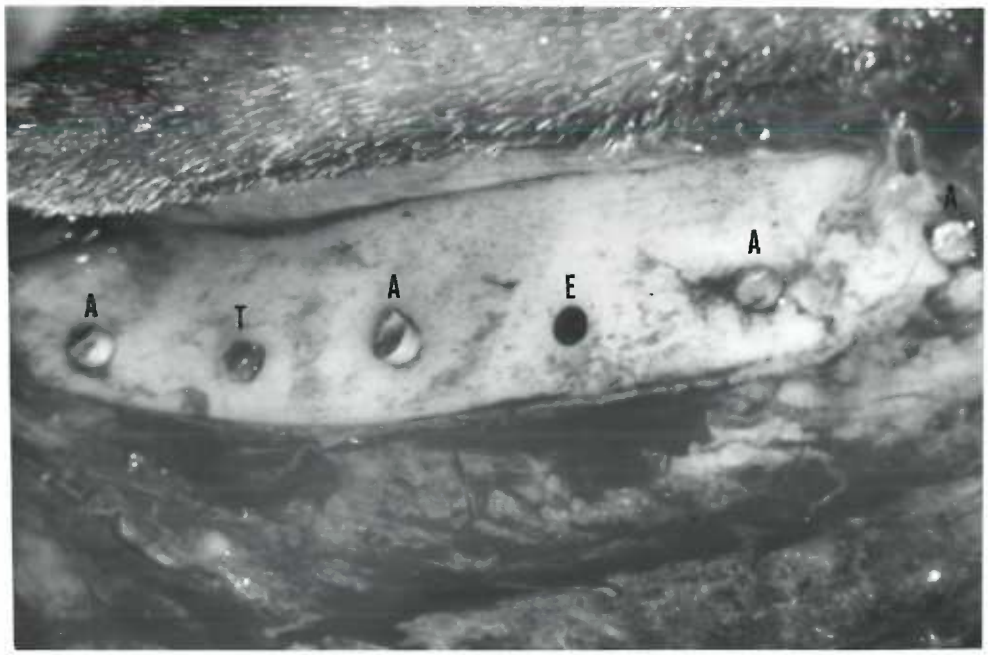


Figure 1. Lateral aspect of the mandibular amalgam implants and controls immediately after placement. Note the four different amalgam implants (A), Teflon tube control (T), and empty defect (E). (X3)

IMPLANTS MOUNTED IN EPOXY RESIN SUPPORT
FOR ELECTRON MICROPROBE ANALYSIS

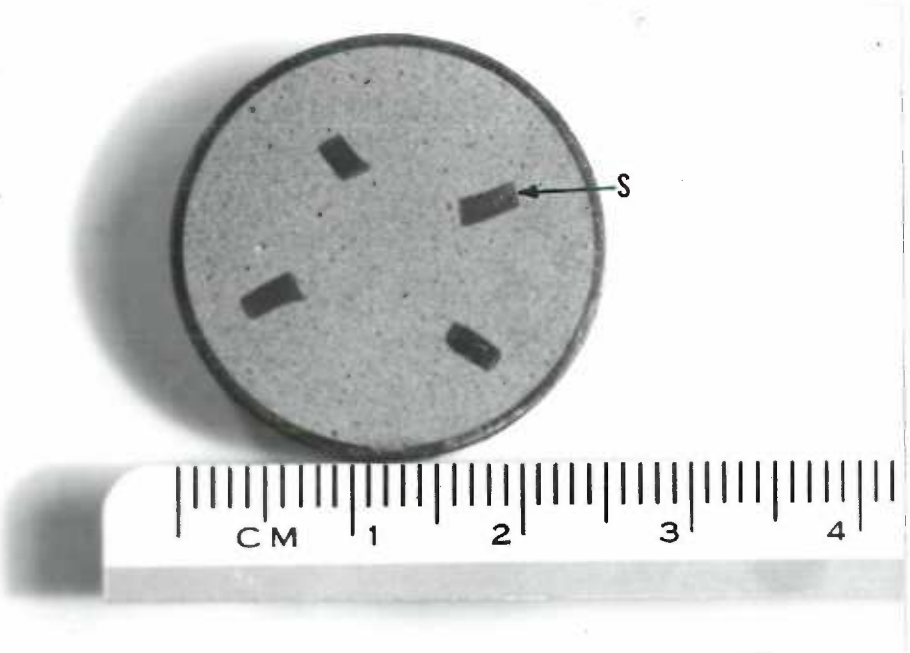
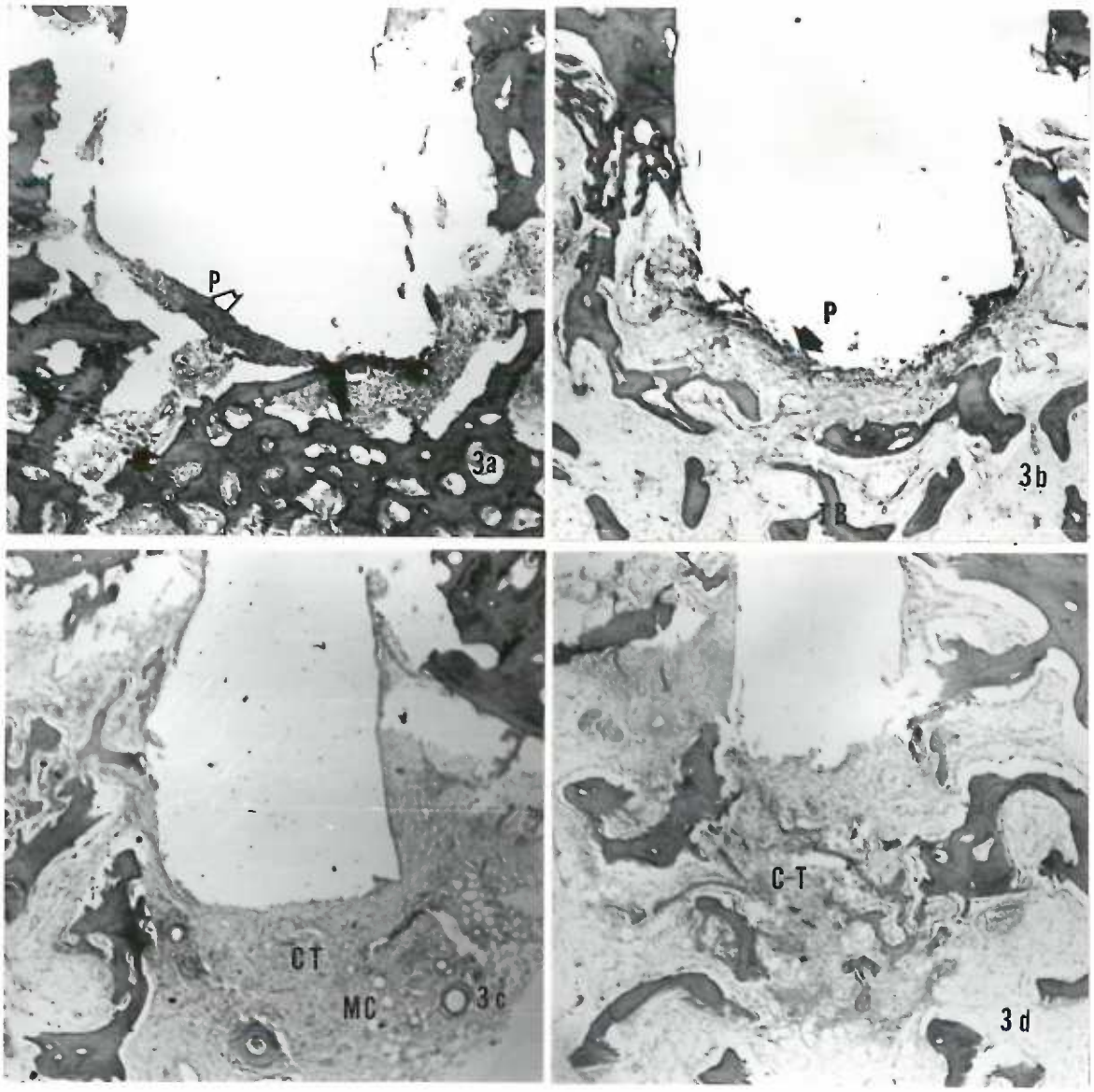


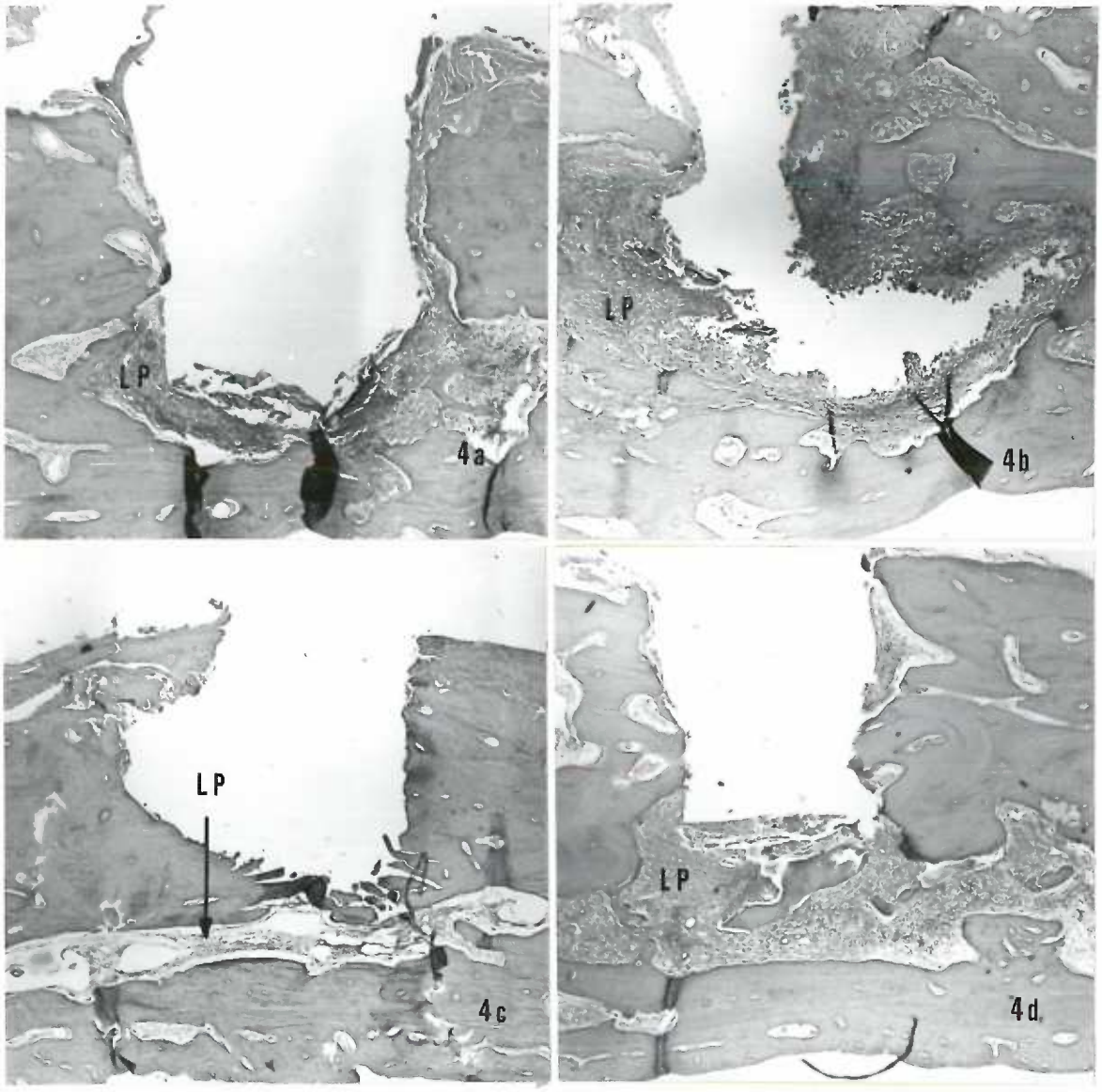
Figure 2. Four different sixty-day amalgam implants embedded in epoxy resin. The amalgam surface (S) which contacted bone is directed peripherally in each instance.

SIX-DAY MANDIBULAR IMPLANTS



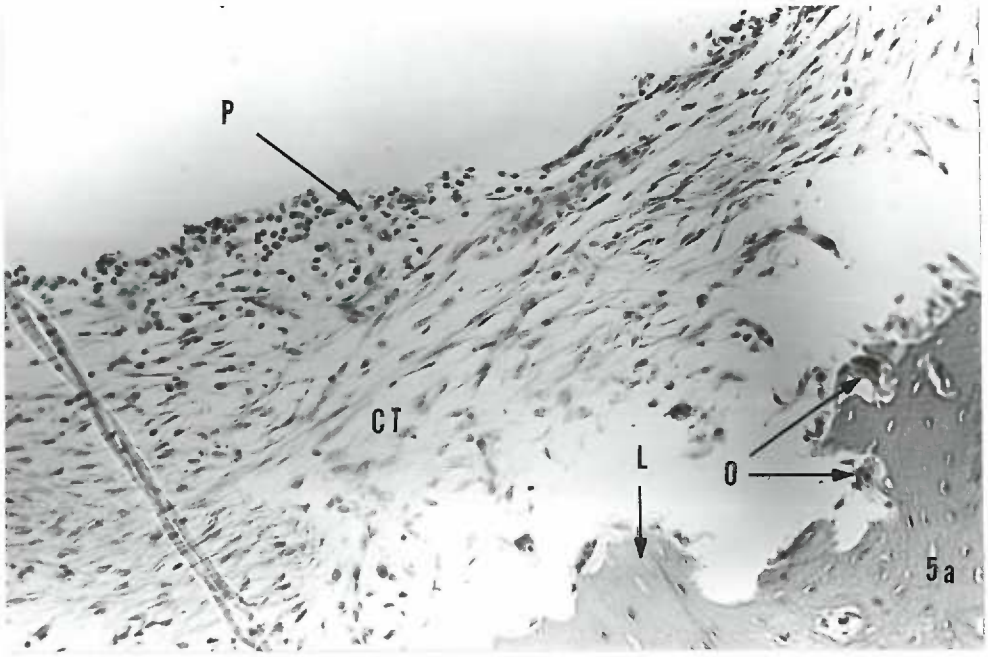
Figures 3a, 3b, 3c, 3d. Longitudinal sections through implant sites. In Figure 3a note thick PMN (P) layer, in Figure 3b note the density of the trabecular bone (TB) compared to Figure 3a. The amalgam implant in Figure 3c has entered the mandibular canal. Note loose connective tissue (CT) surrounding implant in Figures 3b, 3c and 3d. (X 26)

SIX-DAY PARIETAL BONE IMPLANTS



Figures 4a, 4b, 4c, 4d. Longitudinal sections cut through implant sites in the calvarium. Lymphocyte and plasma cell infiltrate (LP) is conspicuous in marrow spaces ramifying from implant defect. It is apparent that the tissue section representing Figure 4b was not cut through the middle of the implant hole. (X 26)

TYPICAL SIX-DAY ACUTE REACTIONS



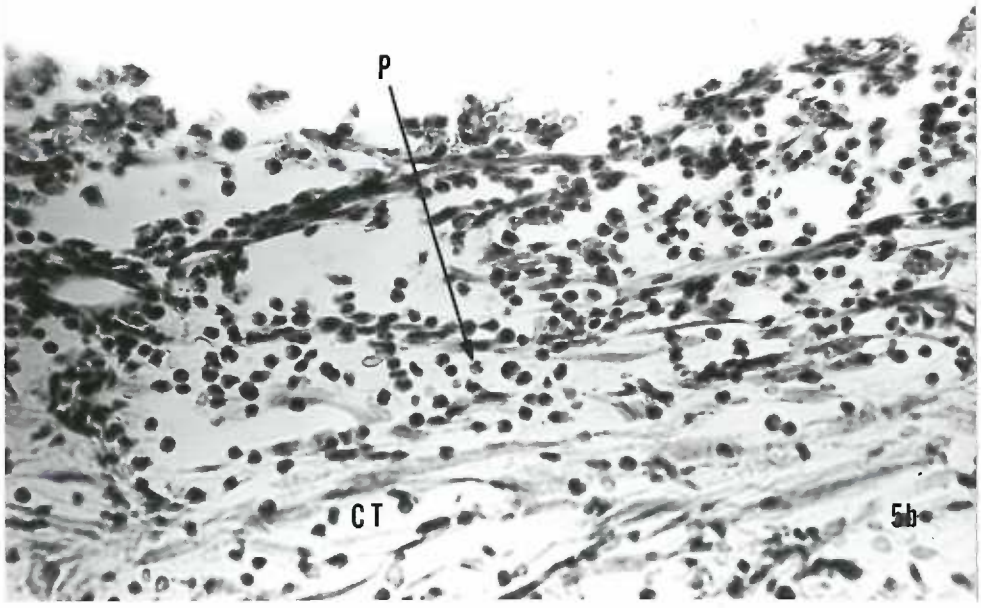
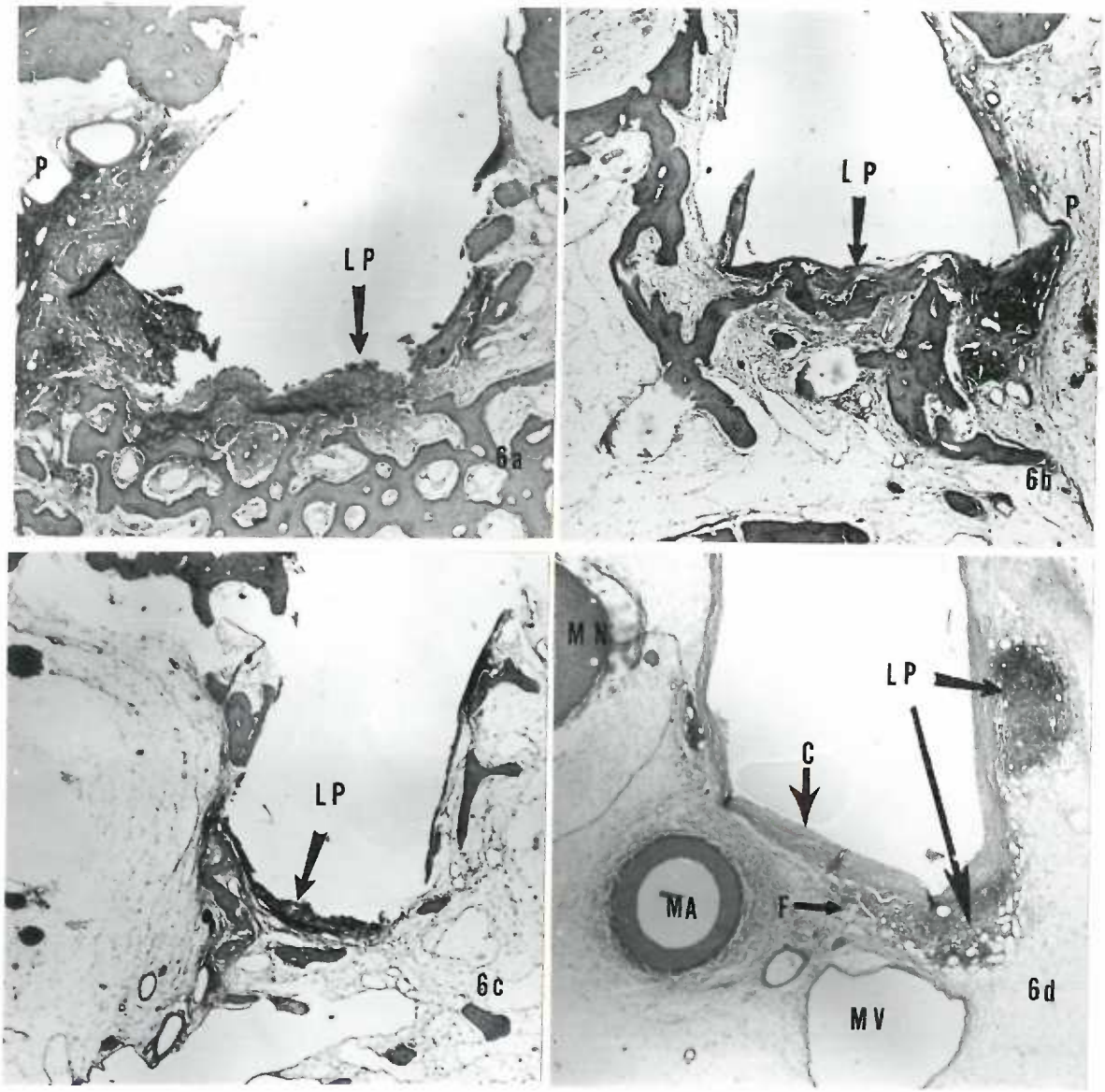


Figure 5a is a higher magnification (X 204) of the acute reaction in Figure 3a. Note PMN's (P), osteoclasts (O), and lacunae (L) either empty or with pyknotic nuclei.

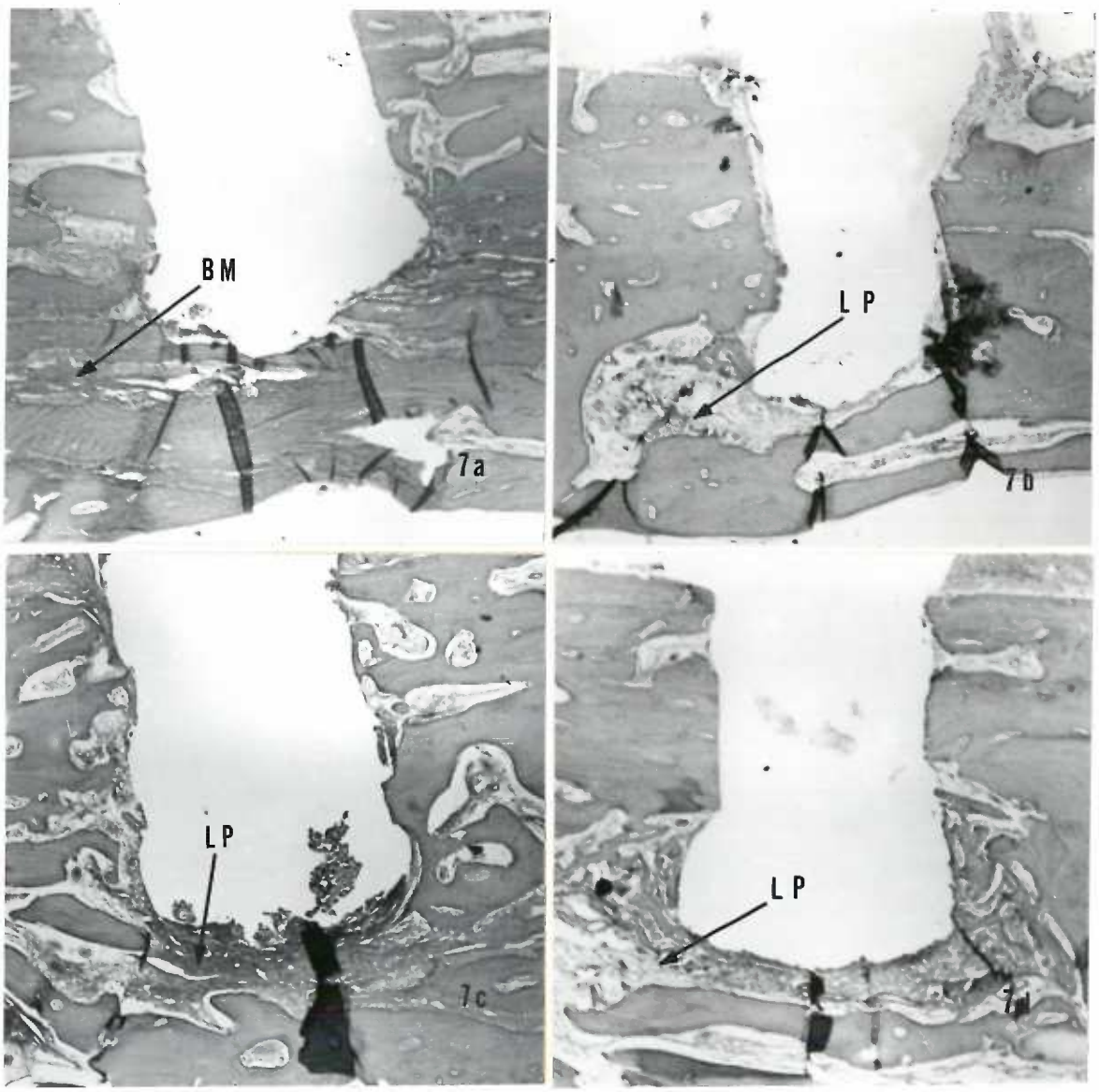
Figure 5b is a higher magnification (X 400) of the reactive tissue of 3b. Although Figure 5a represents the inflammatory reaction of a bone implant, it strongly resembles that in Figure 5b which is tissue adjacent to a canal implant. Fibrous connective tissue (CT) is apparent in both.

THIRTY-DAY MANDIBULAR IMPLANTS



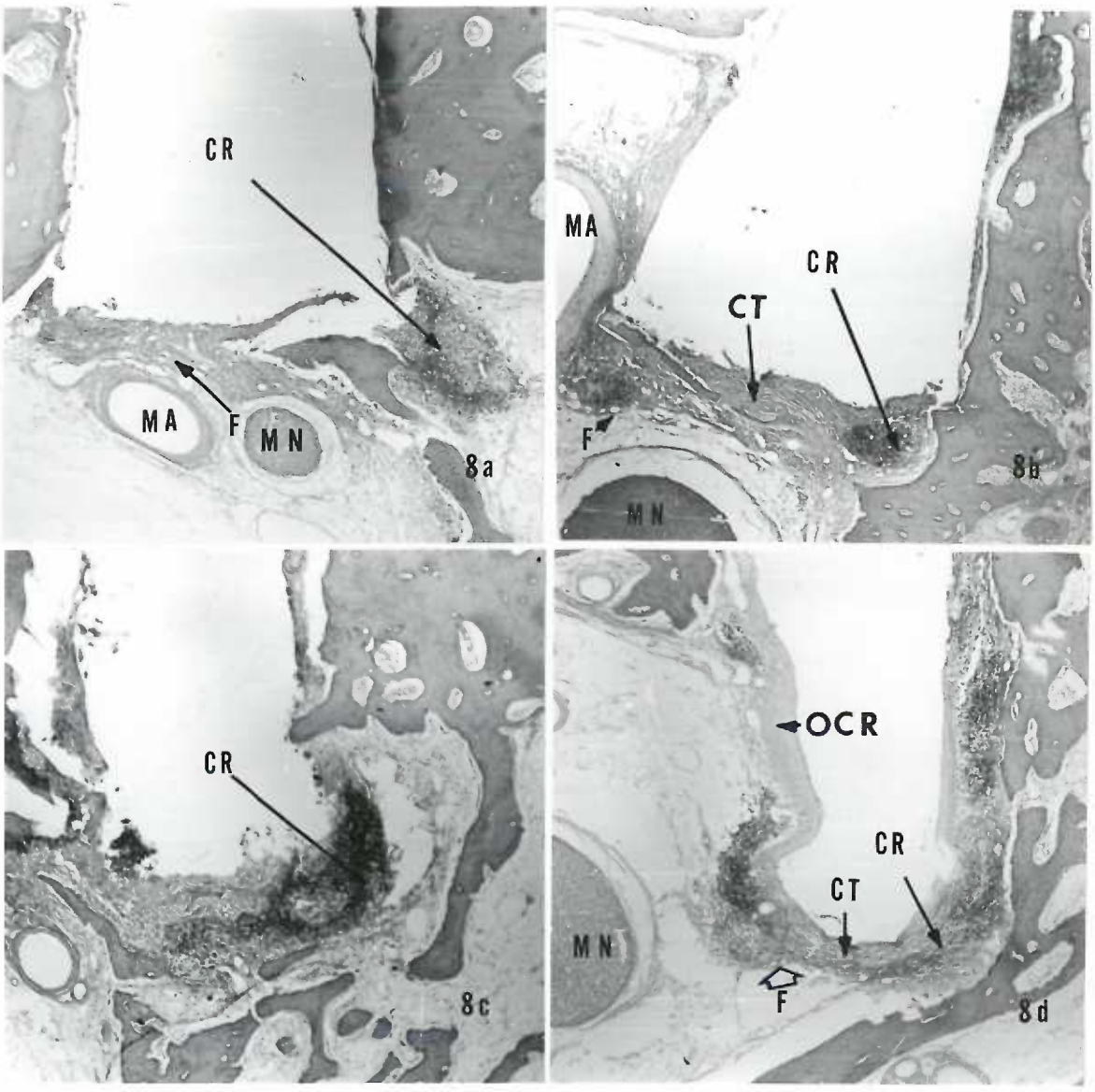
Figures 6a, 6b, 6c, 6d. Longitudinal sections through mandibular implant sites. Note intense lymphocyte and plasma cell infiltrate (LP) surrounding amalgam surfaces of all implants. Note fibrous tissue capsule (C) around canal implant in Figure 6d, which is representative of capsules around most thirty-day canal implants. Note also in Figure 6d the mandibular nerve (MN), mandibular artery (MA), mandibular vein (MV) and fat cells (F). Implant in Figure 6a had perforated the mandibular canal at (P). (X 26)

THIRTY-DAY PARIETAL IMPLANTS



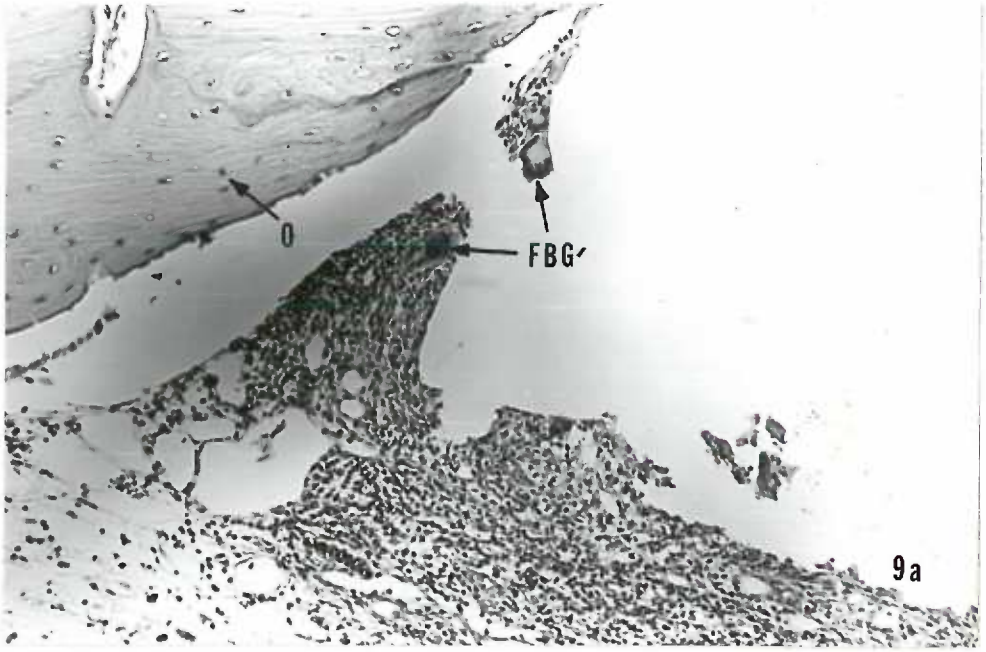
Figures 7a, 7b, 7c, 7d. Longitudinal sections through parietal implants. Note generalized lymphocyte and plasma cell infiltrate (LP) which is shown to border amalgam surface in Figures 7b, 7c and 7d. Figure 7a exhibits immature bone matrix (BM) in medullary spaces adjoining implant site. (X 26)

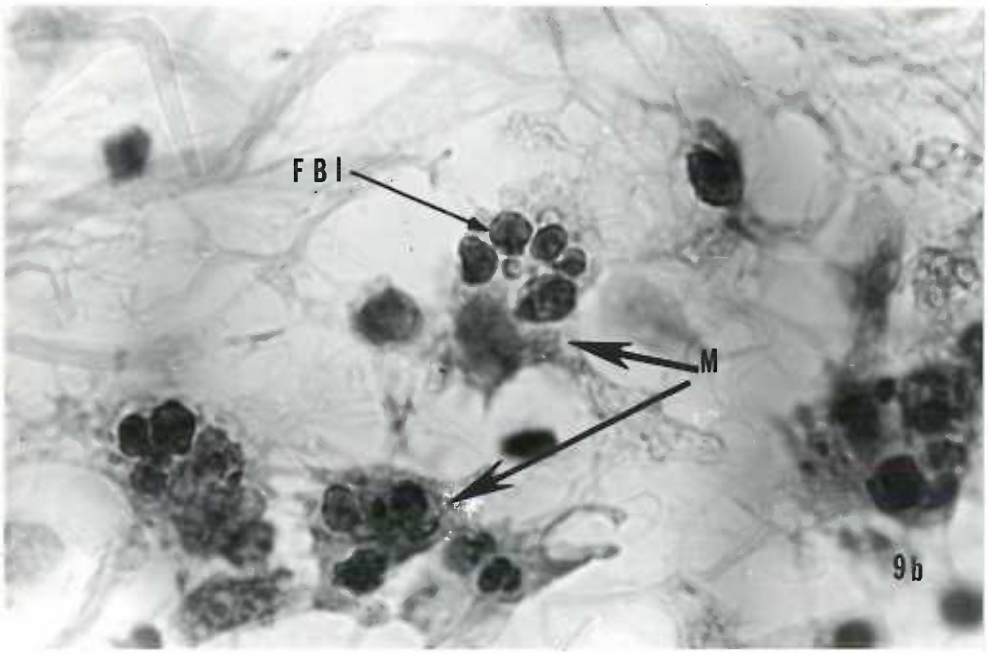
SIXTY-DAY MANDIBULAR IMPLANTS



Figures 8a, 8b, 8c, 8d. In these longitudinal sections an intense chronic inflammatory reaction (CR) borders the amalgam surfaces, especially at the corners of the implants. Note mandibular nerve (MN), mandibular artery (MA) and fat cells (F). No connective capsules are present, though bands of unorganized strands of collagen (CT) are demonstrated bordering the amalgam surfaces of Figures 8b and 8d, and organized strands (OCT) approximate the Teflon walls. (X 26)

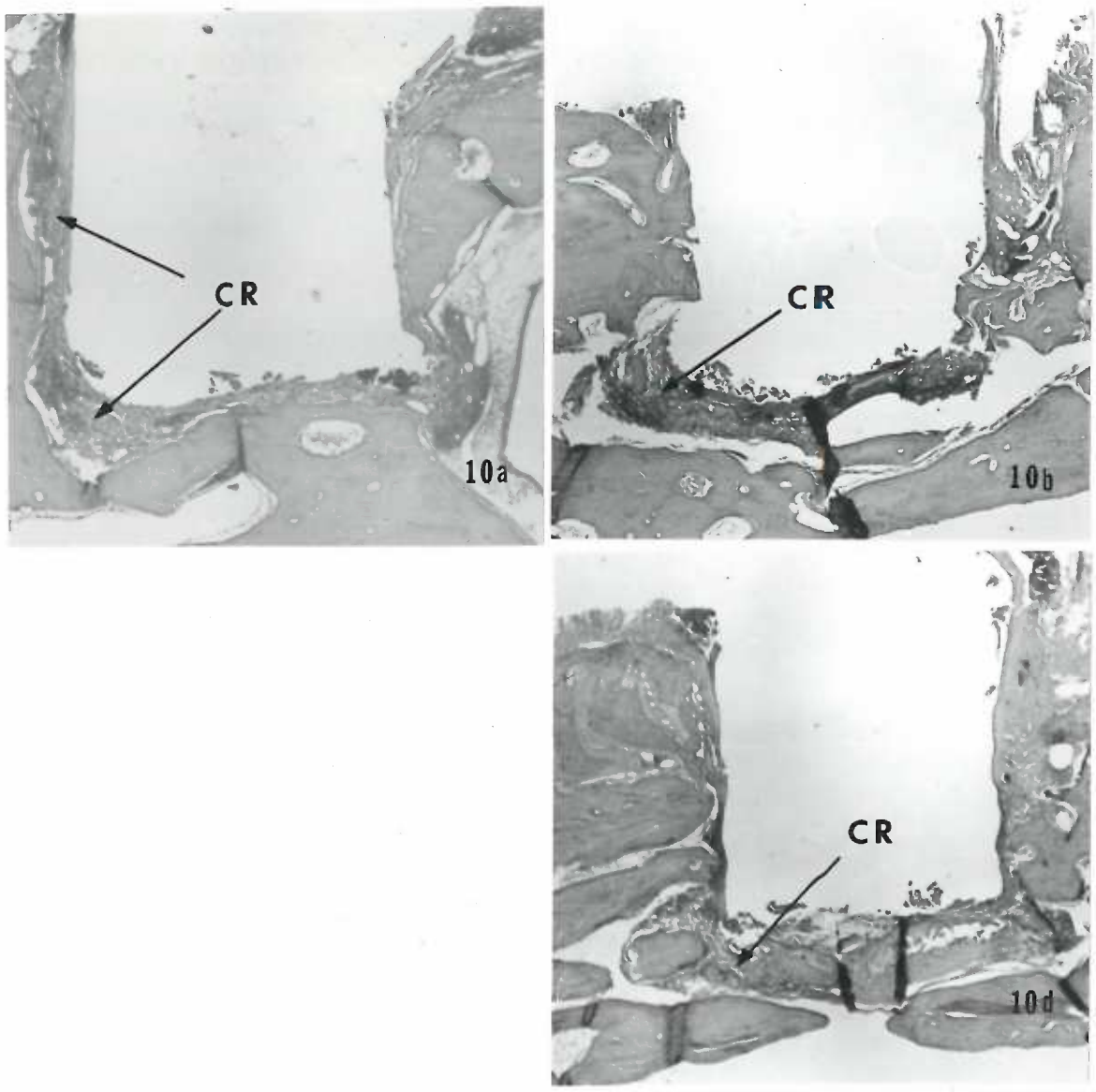
FOREIGN BODY GIANT CELLS AND MACROPHAGES





Figures 9a and 9b. Figure 9a is a higher magnification of foreign body giant cells (FBG) from Figure 8a (X 40). Note all bone lacunae with osteocytes (O). Figure 9b is a higher magnification of macrophages (M) from the connective tissues of 8d. Note pigmented inclusion (FBI).

SIXTY-DAY PARIETAL BONE IMPLANTS



Figures 10a, 10b, 10c, 10d. In these longitudinal sections an intense chronic inflammatory reaction (CR) borders the amalgam surface of the implants. This reaction appears to migrate along the walls of the Teflon tubes. In Figure 10c note the thick fibrous connective tissue layer (CT) separating the amalgam surface of the implant from the endocranium (EC). (X 26)

SIXTY-DAY CHRONIC REACTIONS

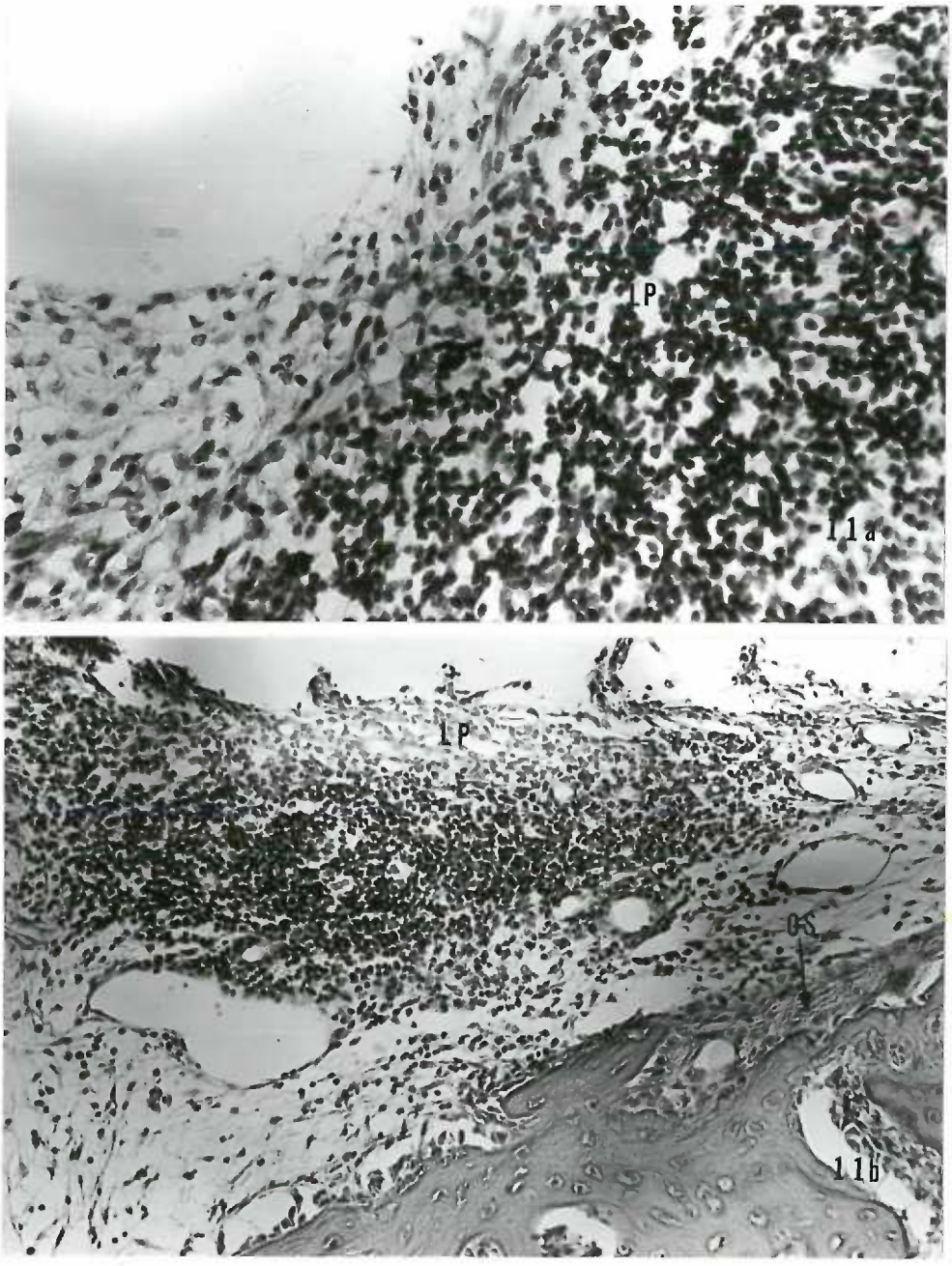
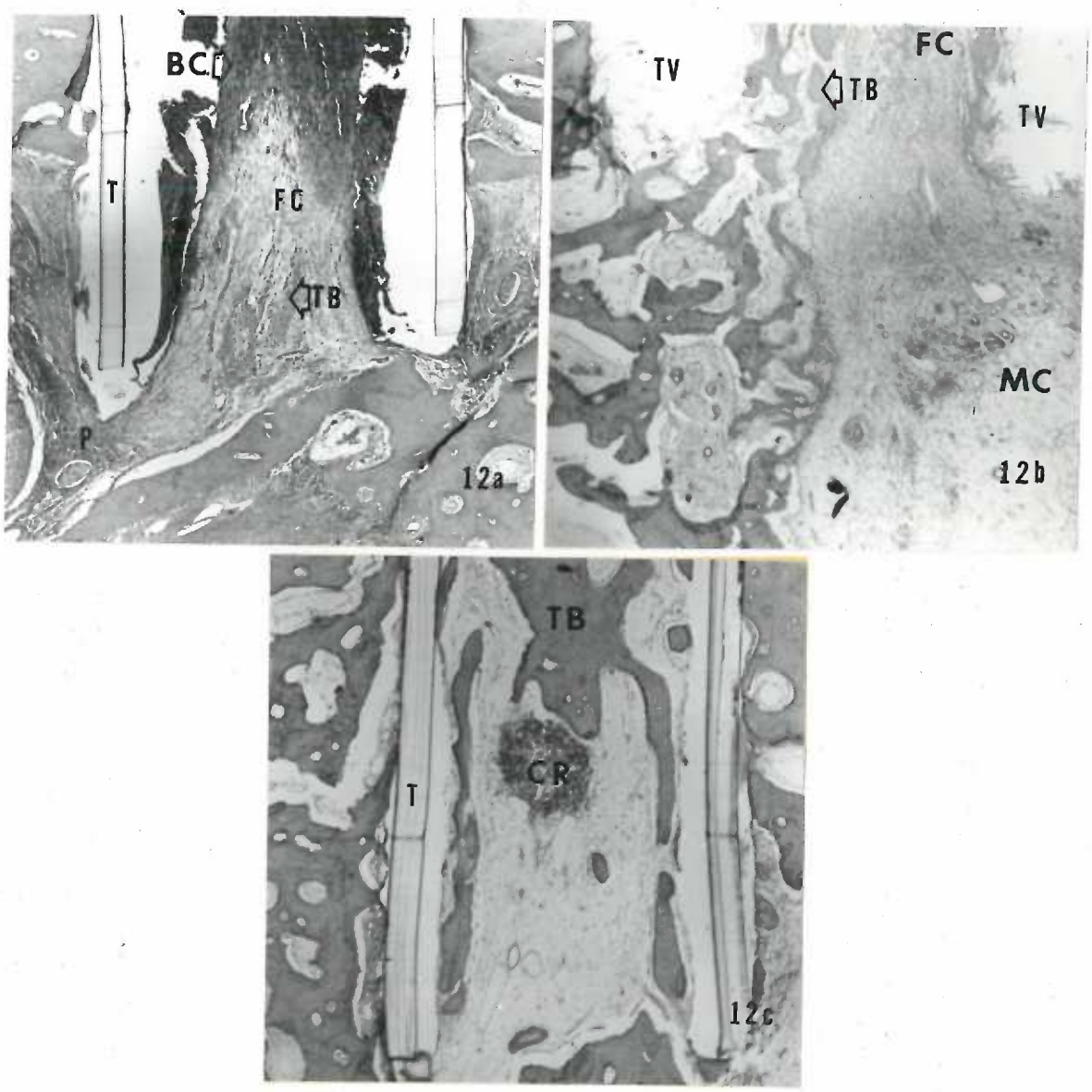


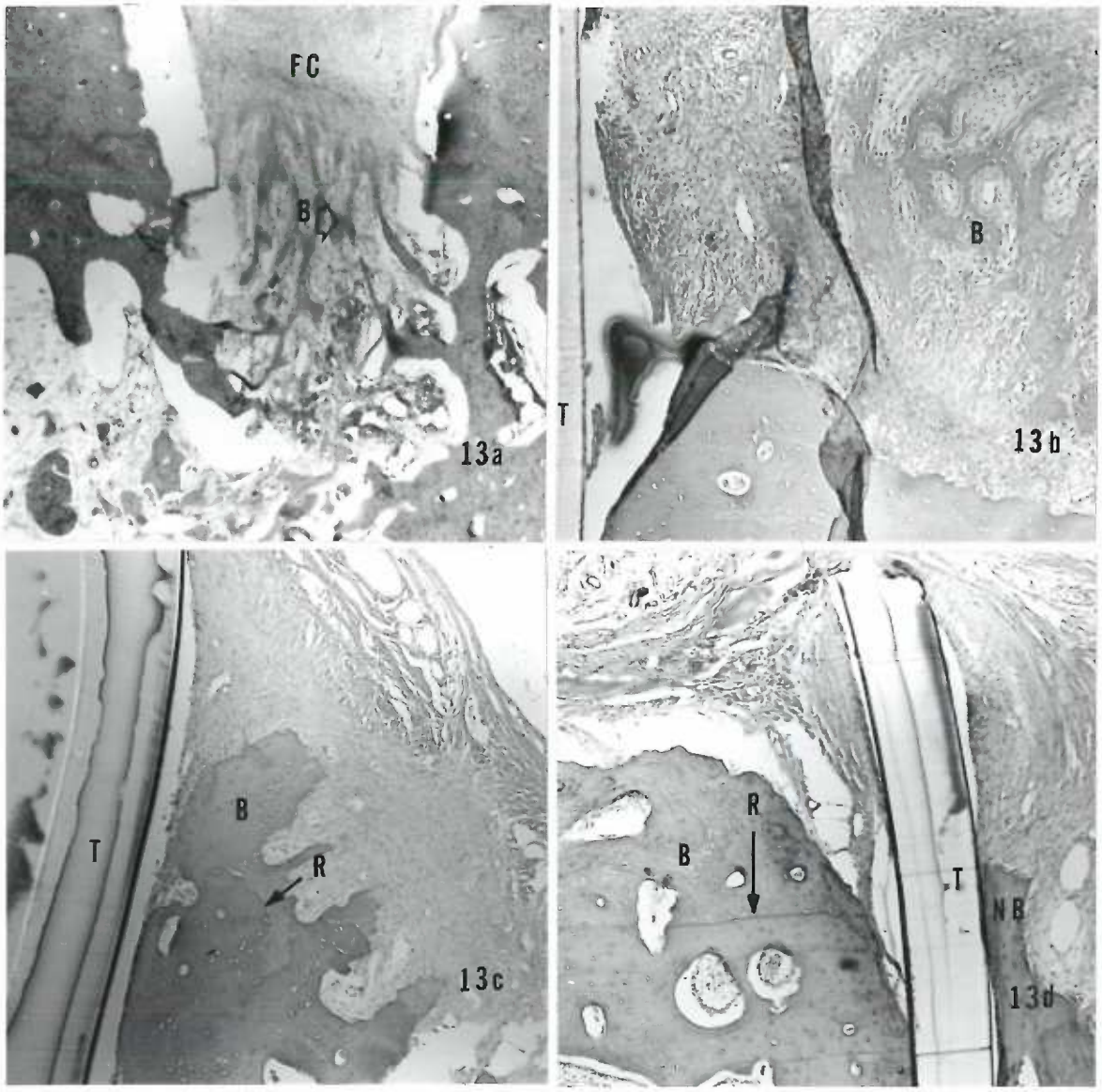
Figure 11a is a higher magnification (X 400) of the chronic inflammatory reaction bordering the amalgam surface in Figure 8a. Note the dense infiltrate of lymphocytes and plasma cells (LP). Figure 11b is a higher magnification (X 204) of the chronic reaction shown in Figure 8c. Note also the intense osteoblastic/endosteal reaction indicating new bone formation (OS).

TEFLON CONTROLS
(SIX, THIRTY AND SIXTY DAYS)



Figures 12a, 12b, 12c. Figure 12a demonstrates the Teflon tube (T) after six days in bone. Note perforation (P) into mandibular canal, blood clot (BC) in tube, fibrous callus (FC), and new trabecular bone (TB) (X 26). Figure 12b represents Teflon tube implant site, demonstrated by the two void areas (TV), after thirty days. Note mandibular canal (MC) and trabecular bone within callus. (X 60) Figure 12c represents sixty-day Teflon tube filled with bone. Note cluster of round cells (CR). (X 26)

SIX-DAY CONTROL DEFECT AND SIX-, THIRTY- AND SIXTY DAY
PERIOSTEAL REACTIONS OF CONTROL DEFECT



Figures 13a, 13b, 13c, 13d. Figure 13a, a simple defect, demonstrates new bone deposition (B) in endosteal portion of fibrous callus (FC) after six days. (X 26) Figure 13b demonstrates subperiosteal bone trabeculae (B) in callus at six days adjacent to Teflon tube (T). (X 60) Figure 13c demonstrates deposition of subperiosteal bone (B) next to Teflon after thirty days. Note reversal lines (R). Figure 13d demonstrates remodeled bone on cortical plate after sixty days. Note new bone (NB) within Teflon tube. (X 60)

LONGITUDINAL SECTION OF AMALGAM IMPLANT

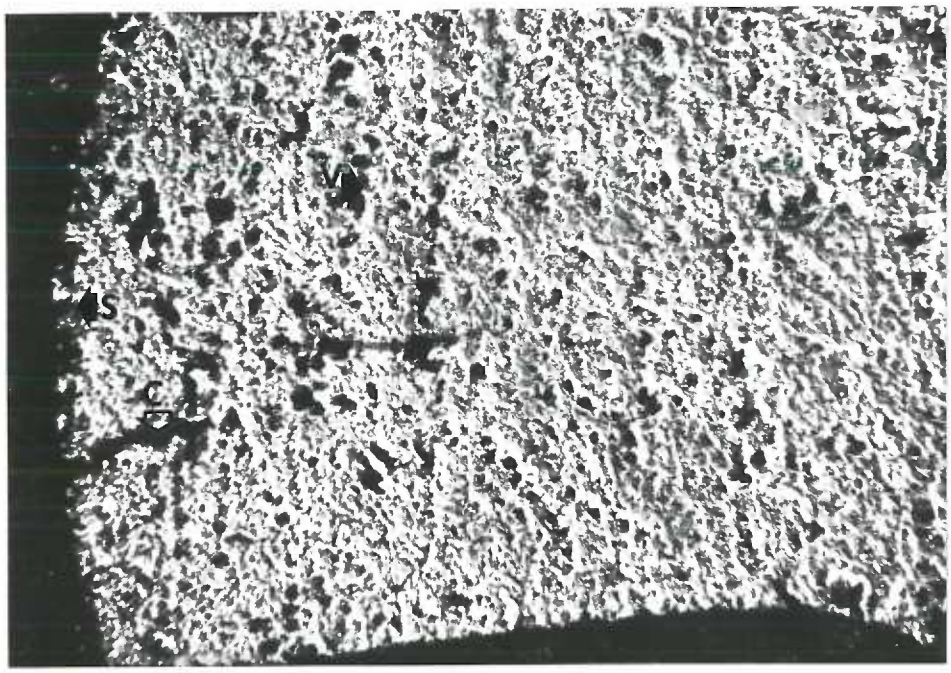


Figure 14. A longitudinal polished section of a typical sixty-day amalgam implant (X 160). Note numerous voids (V) and cracks (C) extending from the surface which was in contact with parietal bone (S).

Figure 15a is a sample current image of the tissue contacting surface of amalgam #1 after sixty days. The gamma 1 (G1) phase is represented by the light background; the gamma 2 (G2) phase appears as discrete islands having clearly defined boundaries. The gamma (G) phase appears as large, irregularly shaped particles, and many voids (V) are present. (X 930) Amalgam #2 demonstrated a similar picture and is not included in this series of photographs.

Figure 15b is a tin scan utilizing x-ray intensity images. Note tin (T) of gamma phase and tin of the gamma 2 phase (T2). Note also the absence of tin in voids, indicative of an insignificant amount, or lack of, tin corrosion products. (X 930)

Figure 15c is a sample current image of amalgam #3. Note the gamma and gamma 1 phases are similar to amalgam #1. The added dispersant, designated phase (d), is shown as dark particles surrounded by a lighter reaction ring, Cu_6Sn_5, designated phase (r). There is no evidence of gamma 2. (X 930)

Figure 15d is a sample current image of amalgam #4. Note the gamma and gamma 1 phases. The large gray circular particles are the unreactive alloy particles (G). A small amount of Cu_6Sn_5 (CS) is also present. (X 930)

ELECTRON MICROPROBE EXAMINATION OF SIXTY-DAY SAMPLES