Light microscopy, scanning electron microscopy, and microprobe analysis of bone response to zinc and nonzinc amalgam implants

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Freshly mixed, unset zinc-free and zinc-containing amalgam was implanted in the right tibia of 32 rats. Half of the specimens were examined by the light microscope and the other half by the scanning electron microscope and x-ray microprobe analysis. It was found that amalgam is well tolerated by the rat osseous tissue, and there were no histologic reaction differences between zinc and zinc free amalgam. The surfaces of the implants were covered by an organic film at 3 weeks and with bone at later intervals. Very little corrosion products containing sulfur were observed on the amalgam surface at all intervals. Bone adjacent to the amalgam contained tin and sulfur irrespective of the presence of zinc in the alloy, indicating outward migration of specific components of the alloy.

The use of amalgam as a restorative material in operative dentistry is a well-established and accepted technique. The same techniques of amalgamation, asepsis, and retentive cavity form are employed when the material is used in periapical surgery to insure an adequate apical seal of the root canal. Since amalgam is widely used, numerous studies have evaluated its chemical and physical properties and, to a much lesser extent, the tolerance of tissue to it.

The studies concerning biologic compatibility of amalgam have been largely confined to soft tissue reactions in various experimental animals, principally rabbits[2, 3] or rats,[1, 4] or in cell culture reactions.[5] These have shown amalgam to be biologically well tolerated. The material employed was mixed and allowed to set prior to implantation.[1, 2, 4, 5]

Results varied from chronic inflammation at the end of an average implant time of 23 weeks,[2] to the material being walled off with a fibrous capsule at the end of 32 days.[1]

When freshly mixed amalgam is implanted, a severe inflammatory reaction is found which, over the period of 2 days to 4 weeks, changes to a mild response.[3]

Silver amalgam may be less irritating and better tolerated than gutta-percha. Healed bone was observed in a study to be in direct contact with implanted amalgam in more cases than with gutta-percha. However, these investigators used preset and sterilized amalgam.[7]

Copper amalgam was the accepted material for use in apical root resection until it was suggested that the caustic quality of the material may outweigh any benefit derived from its antiseptic properties.[8] As a consequence, silver amalgam routinely employed in operative dentistry came to be the material of choice for apical amalgams. Since 1959 there has been a virtual ban on the use of zinc-containing alloy for apical fillings. This ban is based on the report of one case of surgical failure in which a zinc carbonate precipitate was noted and attributed to the zinc contained in the amalgam or "from the root canal post."[6]

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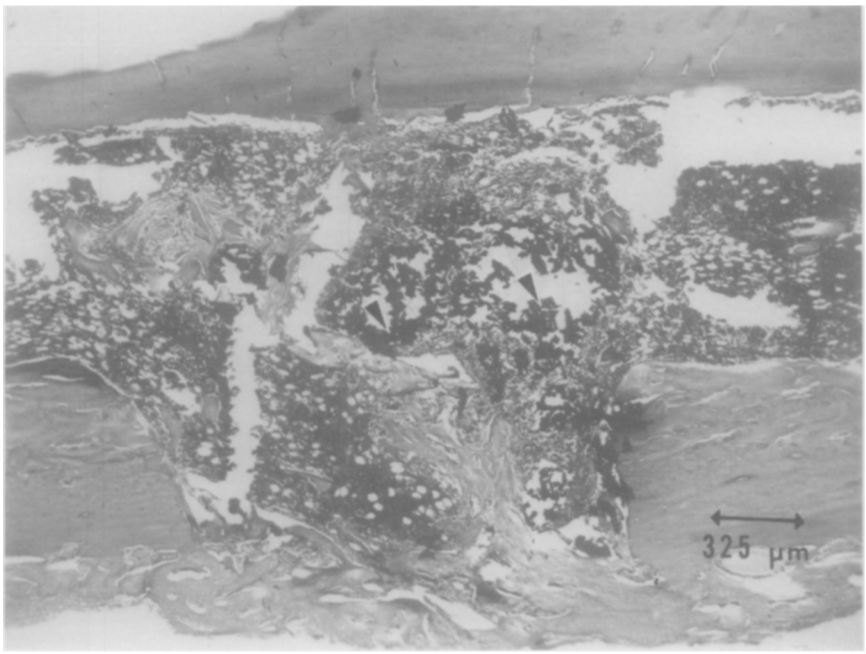

Fig. 1. Photomicrograph of 3-week zinc-free amalgam implant *(arrows)*. Unimpeded osteoid proliferation is evident. (Original magnification, ×40.)

Supposedly, by eliminating the zinc, the phenomenon of electrolytic inflammation caused by the zinc ion can be avoided. However, the critical level of concentration of zinc ions to produce clinically apparent changes is unknown.[9]

In endodontics it is of paramount importance to minimize irritation, so as not to impede healing and to yield increased assurance of successful resolution of the bony lesion. This study was undertaken to compare the inflammatory reactions in rat osseous tissue of freshly mixed zinc-containing amalgam to zinc-free amalgam.[10] The inflammatory response was evaluated, and the surface changes were examined by light microscopy, scanning electron microscopy, and the x-ray microprobe analyzer.

METHODS AND MATERIALS

Freshly mixed, unset amalgam was implanted in the right tibia of 32 200 to 250 gm. Walter Reed Strain rats. In half of the animals zinc-containing amalgam* was used; zinc-free amalgam† was used in the other half. The amalgam implants were prepared in a $^{5}/_{64}$-inch K-G retrofilling amalgam carrier.† The left tibia was used as the control; it was surgically prepared, as was the right tibia, but no implant was imbedded. Eight rats, four with implants of zinc-containing amalgam and four with zinc-free amalgam, were sacrificed at intervals of 3, 6, 9, and 12 weeks.

Half of the specimens were prepared for light microscopy and the other half for scanning electron microscopy (SEM). The light microscopic specimens were fixed in 10 percent buffered formalin solution, decalcified, double-embedded in paraffin, sectioned at 6 μm, and stained with hemotoxylin and eosin. The specimens for scanning electron microscopy and x-ray microanalysis were fixed in 2.5 percent glutaraldehyde in cacodylate buffer at pH 7.4, dehydrated, and coated with carbon.* Specimens were examined in the SEM,† fractured through the amalgam-bone interface, recoated with carbon, and reexamined. X-ray analysis of the implant surface was performed with an EDAX analyzer equipped with the EDIT II computer software.‡

RESULTS

Light microscopy

Three weeks. Microscopic findings at the 3-week interval demonstrated vigorous osteoid formation near the amalgam implant, coupled with evidence of cal-

*L. D. Caulk Co., Milford, Del.
†Union Broach Co., Long Island City, N. Y.

*Hummer II Sputterer Coating Apparatus Techniques, Inc., Alexandria, Va.
†Model 1000 Scanning Electron Microscope, Advance Metals Research Corp., Burlington, Mass.
‡Energy Dispersive X-Ray Analyzer, Model 707-A, EDAX Int., Prairie View, Ill.

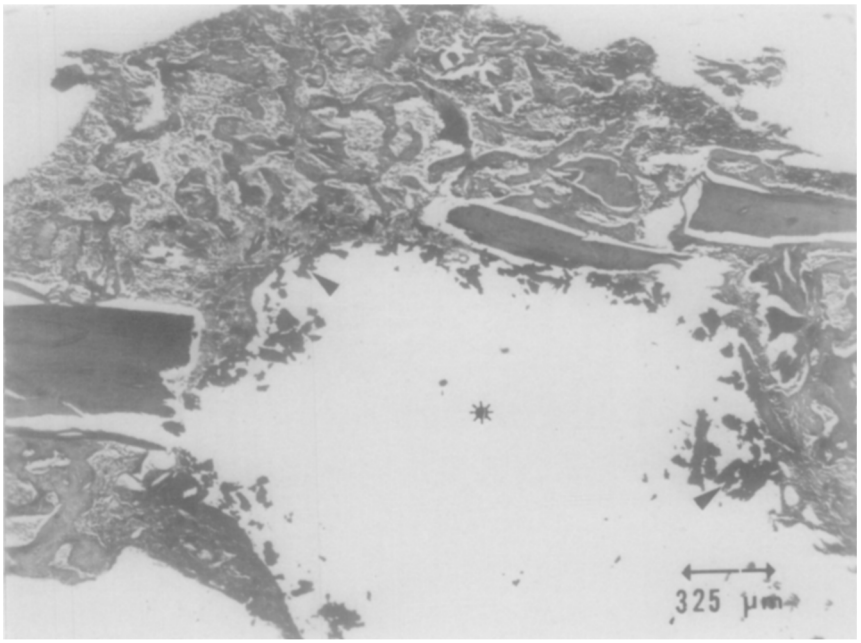

Fig. 2. Photomicrograph of 3-week zinc-containing amalgam implant. (*), Amalgam-occupied space. Arrows show surface of bone contact. (Original magnification, ×40.)

louslike formation over the surgical site for both the zinc-free and the zinc-containing experimental groups. Polarized light revealed fibrous, feathery-fringed trabeculae of calcifying bone matrix and chronic inflammatory cells. Lymphocytes were predominant with fewer histiocytes and plasma cells present in both groups.

Areas of sequestrated or devitalized bone from the effects of the surgical procedure were not apparent.

The control-side surgical sites exhibited normal healing with a callous formation featuring a classic filling pattern (Figs. 1 and 2).

Six weeks. The 6-week group presented a similar picture with the exception that histiocytes were no longer observed. Lymphocytes were present in relatively fewer numbers than in the 3-week specimens. Osteoid continued to be laid down, and the chronic inflammatory response appeared slightly more severe in zinc-free as compared with zinc-containing group. In the control side healing was almost complete.

Nine weeks. The 9-week specimens of both groups were considered to be completely healed even though some slight inflammation persisted. In some areas a fibrous capsule ranging from four to eight cells in thickness and walling off the implant material was seen. In adjacent areas the stimulation of bone formation around the amalgam implant was noted, as evidenced by the presence of large plump osteoblasts rimming immature osseous trabeculae. The callous formation was complete and appeared to be mature bone. Polarized light demonstrated normal lamellations within the newly formed bone. There were minimal differences in healing or inflammation between the two groups. Healing was complete in the control specimens.

Twelve weeks. At 12 weeks, healing was complete in both groups and the implants were walled off by a fibrous connective tissue capsule within the bone. Some bone was noted in direct contact with the amalgam (Figs. 3 and 4), with no evidence of incompatability at the interface.

Scanning electron microscopy

Examination of the control surfaces in the SEM revealed the irregular surfaces of both zinc-containing and zinc-free amalgams (Figs. 5 and 6). Electron microprobe analysis in the SEM revealed characteristic x-ray spectra of mercury, silver, tin, copper, and zinc (Fig. 7). (Figure legends explain the different x-ray lines.) The zinc-containing alloy had a higher concentration of tin, as seen in the comparison of the tin L series of spectra in Fig. 7. Copper was present in the zinc alloy but undetectable in the zinc-free alloy. At various intervals after implantation of the alloys, the implantation sites were removed and fractured through the bone-implant interface, and the surfaces of the alloy and bone were studied with SEM and x-ray analysis.

After 12 weeks the surfaces were covered by a proteinaceous covering obscuring the surface features (Fig. 8). In the later intervals of healing, bone was

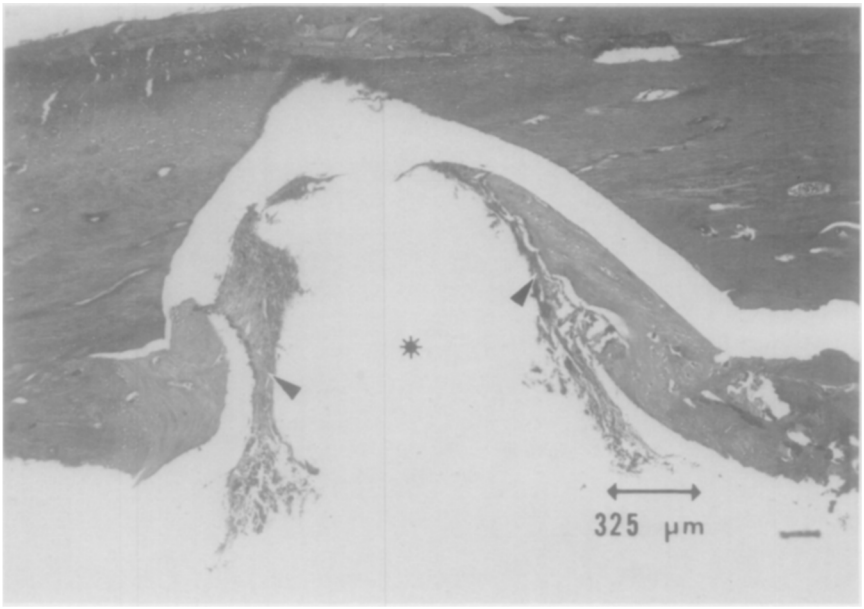

Fig. 3. Photomicrograph of 12-week zinc-free amalgam implant. (*), Amalgam-occupied space. Arrows indicate fibrous capsule enveloping implant. (Original magnification, ×40.)

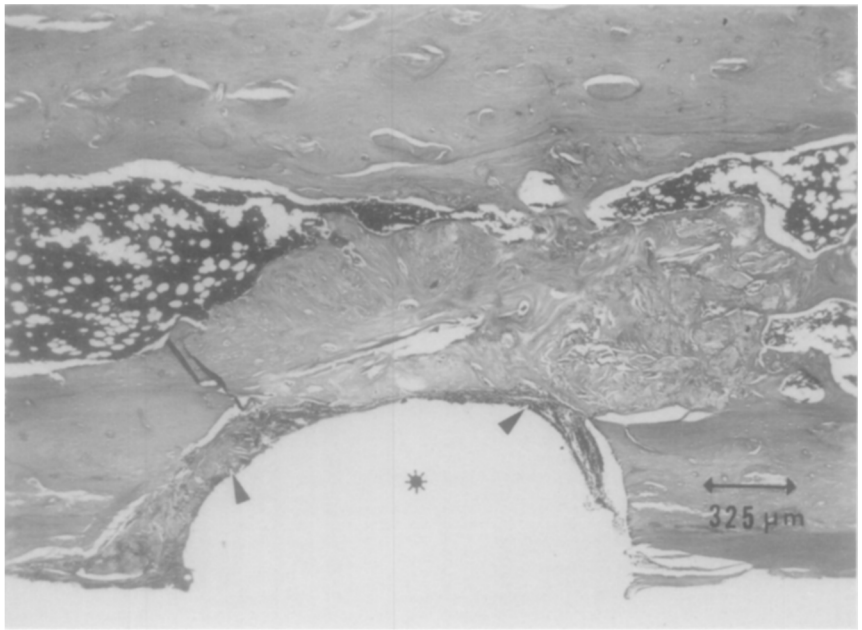

Fig. 4. Photomicrograph of 12-week zinc-containing amalgam implant. (*), Amalgam-occupied space. Arrows show enveloping fibrous capsule. (Original magnification, ×40.)

observed in direct apposition to the amalgam surface (Fig. 9). Very little corrosion products (sulfur and chlorine) were detected upon the alloy surfaces at any of the time intervals studied. All implant surfaces exhibited firm attachment of bone, demonstrative of the universal presence of calcium and phosphorus x-ray spectra, and the close adaptation of new bone to the alloy surface as seen in the SEM. Sulfur was present and chlorine absent in all 13 of the implant bone sites. Tin was present in 11 of the 13 (85 percent) bone sites, and silver, mercury, or copper were detected in none. Zinc was present in only one of the bone sites (at 12 weeks), and the site did not contain either copper or mercury (Fig. 10). In relating sulfur to tin concentration on the bone surface, some degree of relationship was indicated, since 11 of the bone sites (85 percent)

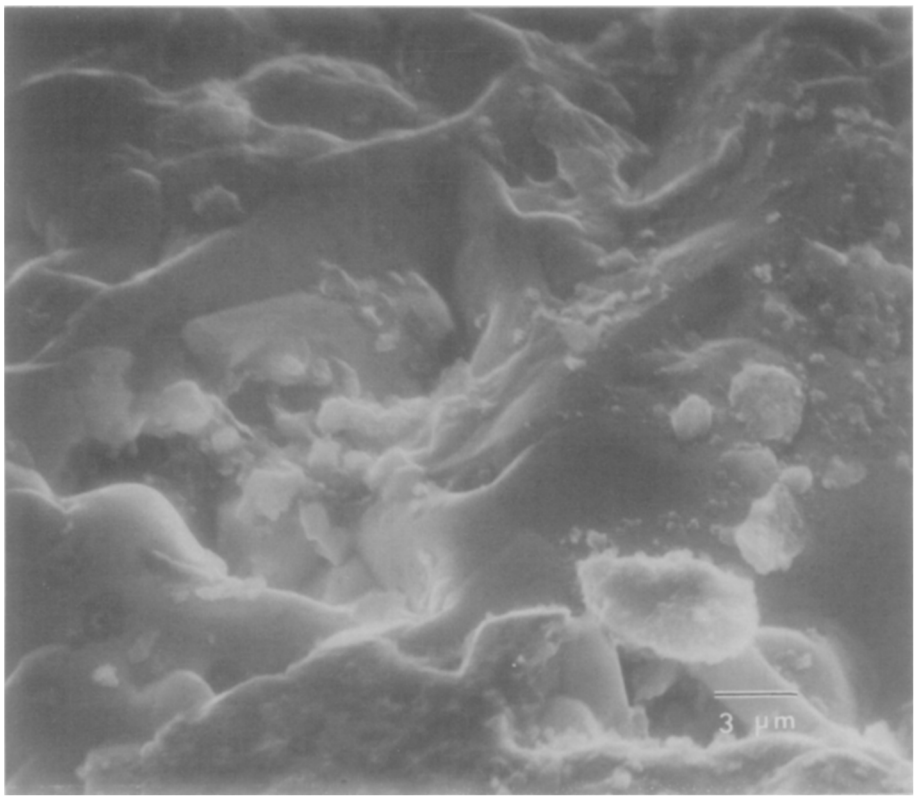

Fig. 5. SEM of zinc-amalgam surface of unimplanted specimen. (Original magnification, ×5,000.)

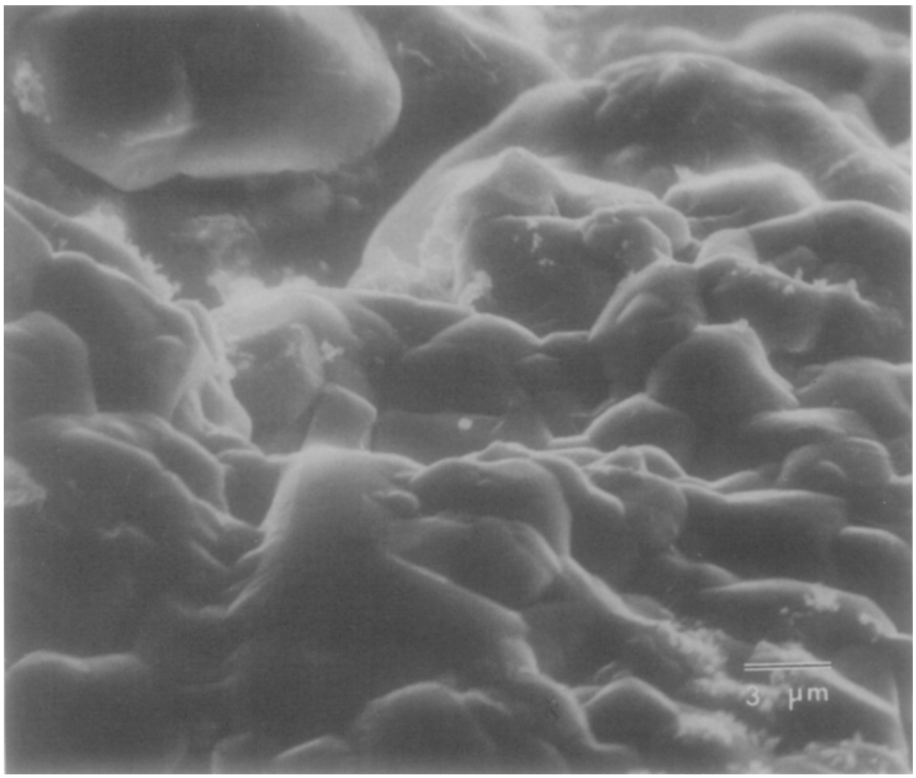

Fig. 6. SEM of zinc-free amalgam surface of unimplanted specimen. (Original magnification, ×5,000.)

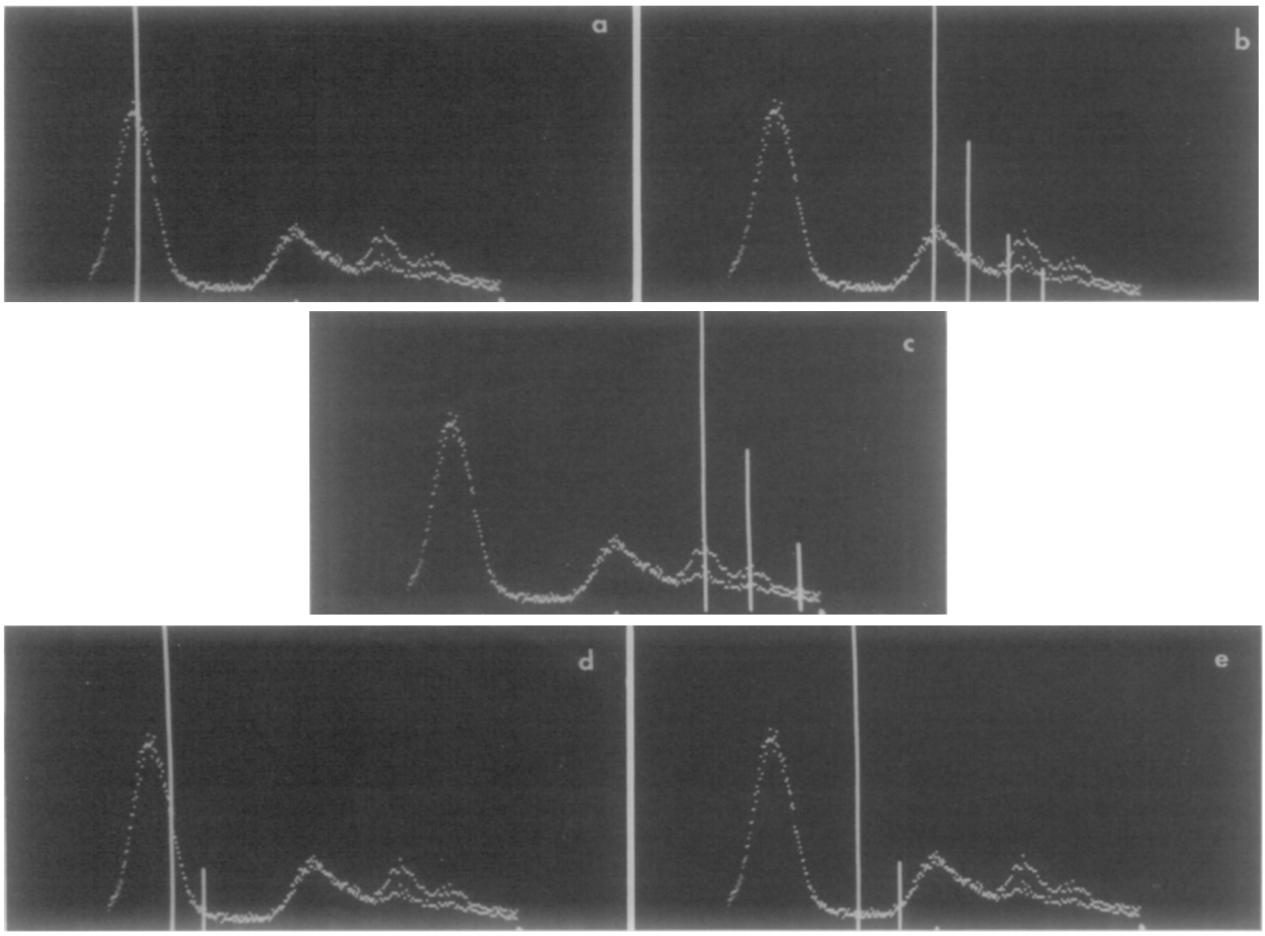

Fig. 7. X-ray spectra of unimplanted amalgams, with principal emission lines for *a*, Mercury M (2,230 ev), *b*, Silver L (2,900 ev), *c*, Tin L (3,440 ev), *d*, Sulfur K (2,310 ev), and *e*, chlorine K (2,620 ev). Note difference in intensity of tin L series x-rays between zinc-containing (higher concentration of tin) and zinc-free amalgams (spectrum c).

had equal or higher peak intensities of sulfur over tin, whereas in only two cases was the tin higher than the sulfur intensity.

An interrelation of sulfur with the presence of zinc was more difficult to demonstrate, but slightly greater sulfur intensities were present in the zinc-containing alloys than in the zinc-free alloys.

DISCUSSION

The histologic results confirmed what has been previously assumed and reported. Amalgam is a biologically well tolerated material. Even though at 3 and 6 weeks there appeared to be a slightly more chronic inflammatory response in the zinc-free specimens, this difference was not considered significant. Osteoid formation followed by new osseous maturation adjacent to the amalgam was indicative of the high degree of tolerance of the implant by the rat osseous tissues. Phosphorus and calcium were present, indicating the close proximity of bone formation to the implant surface. One implant displayed only calcium and phosphorus peaks, the result of bone being so closely in contact with the amalgam that a mechanical lock was formed. When this specimen was split, the bone separated from the underlying tissue rather than at the bone-amalgam interface.

It is of interest to note that a similar corrosive process was found in both amalgams. The microprobe studies of the interface between the alloy and the bone indicate that not much difference in elemental migration occurs when the zinc and zinc-free alloys are compared. Very little sulfur and no chlorine were detected on either alloy, whereas at the bone surface tin and sulfur were almost universally present, regardless of the presence of zinc. The presence of both zinc and tin in the amalgam alloy may be related to a higher concentration of sulfur in the adjoining bone, indicating a more corrosive process in the zinc alloy. However, quantitation

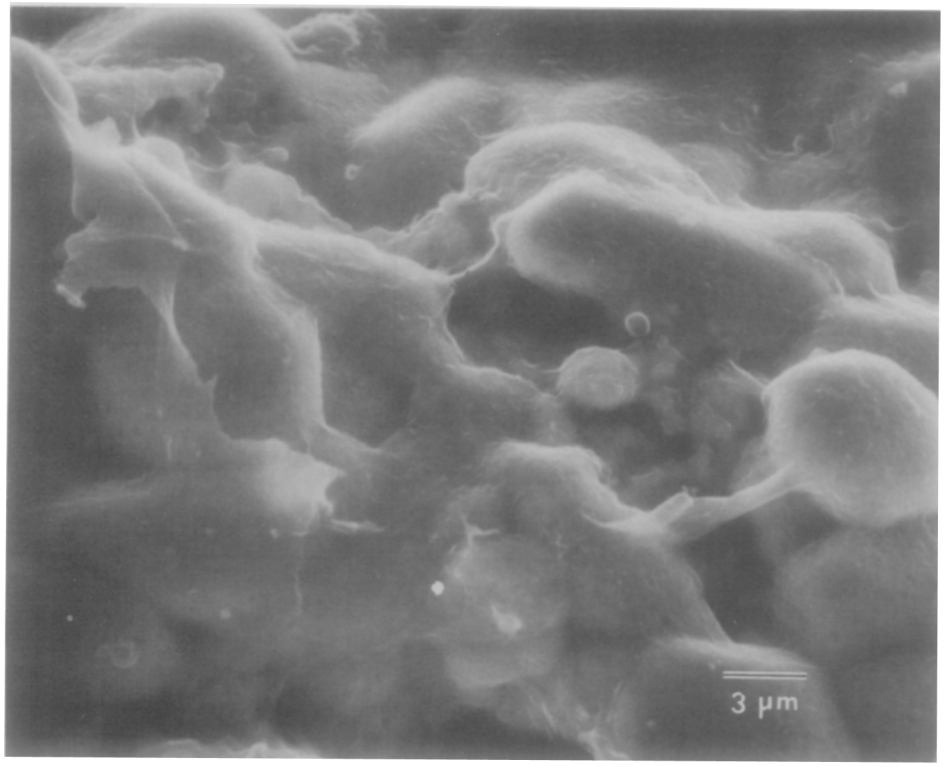

Fig. 8. SEM of zinc amalgam after 12 weeks implantation. (Original magnification, ×5,000.)

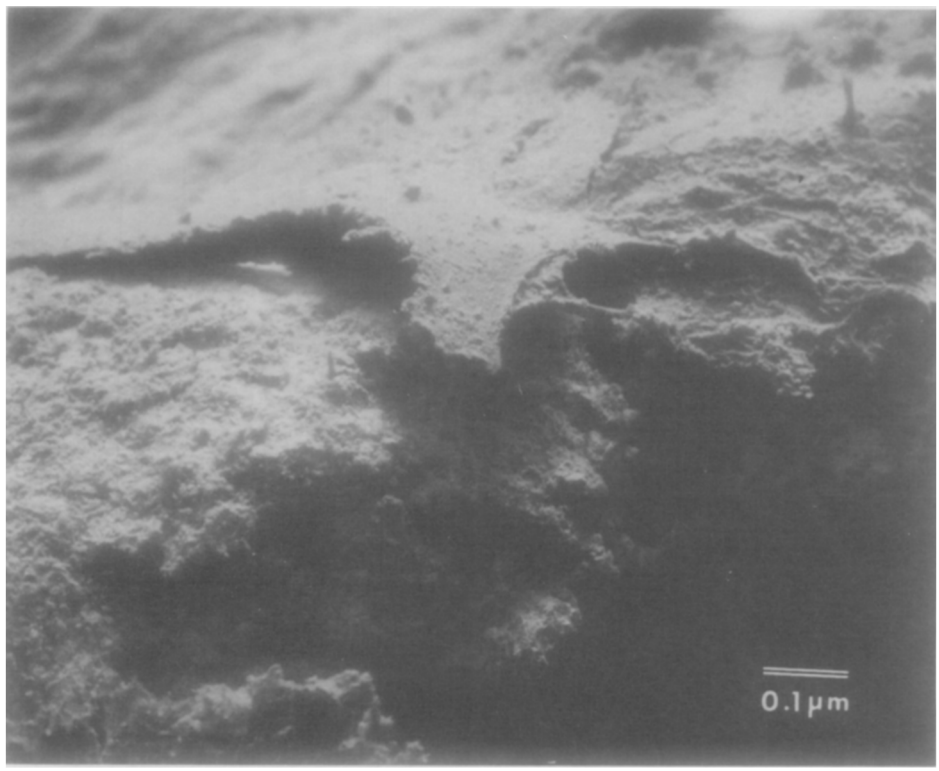

Fig. 9. SEM of whole tibia containing zinc-free amalgam at 12 weeks. Note bone partially covering implant socket. (Original magnification, ×100.)

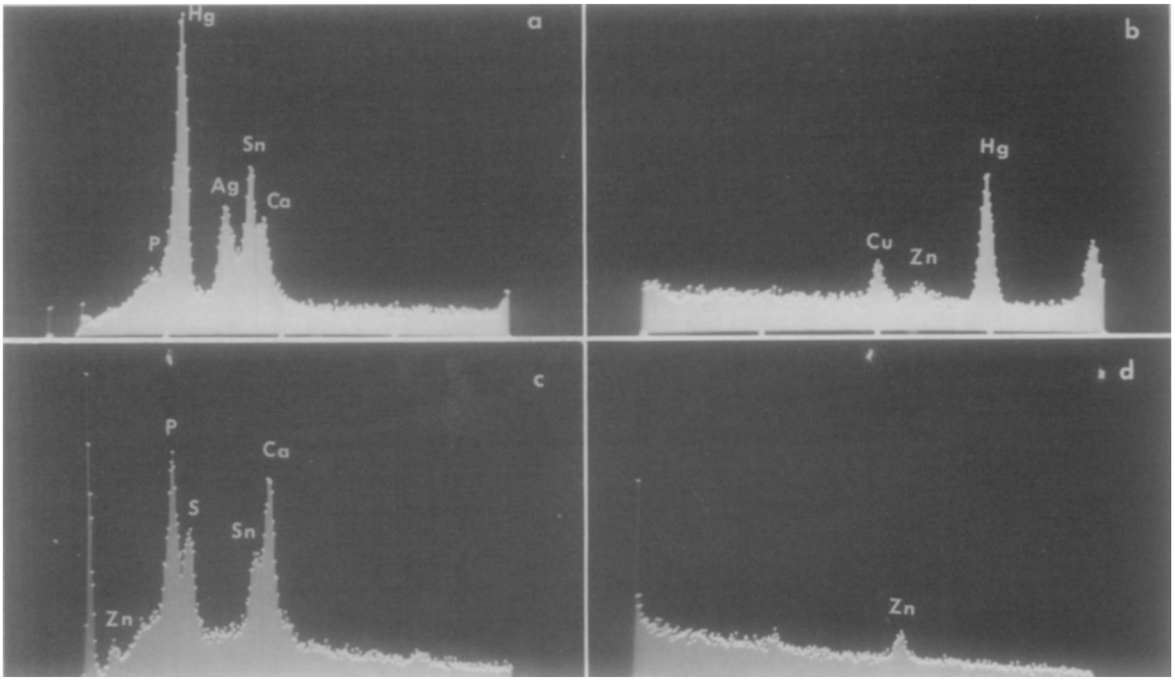

Fig. 10. X-ray spectra of zinc-amalgam at 12 weeks. *a* and *b*, Implant spectra. *c* and *d*, Bone site facing implant.

measurements were not possible because of the uncertain geometry of the x-ray detection conditions. Results suggest a relationship between tin and sulfur in the bone, possibly one of chemical combination. However, in two instances sulfur was present in the absence of tin. Chlorine and zinc, except in one specimen, were not detected in the bone, discounting the importance of these elements in the corrosion process. Corrosion may well be of greater importance than the presence of absence of zinc to the ultimate success or failure of an endodontic case requiring the placement of an apical amalgam. The failure of many endodontically treated teeth whose canals were obturated with silver points has been suggested to be due in part to the corrosion of that point when tissue fluids contact it.[11–13] Tbe corrosion process may not be similar when amalgam alloys and silver points are compared, since contrary to x-ray studies of silver points, no silver was detected at the bone surface in amalgam; this element seemingly affixed in the alloy to the mercury, which also was not found in the adjacent bone. It is possible that some apical amalgam failures might be attributed to the amalgam placed at the apical end of the root coming into direct contact with body tissue fluids and corroding. However, further studies of long-term implantation of amalgam should be undertaken in order to clarify success or failure in relation to corrodibility of amalgam, since in this study inflammation was absent in the presence of corrosion.

From the results of this investigation and from other studies, it is apparent that reaction to apically implanted amalgam in humans is a multifaceted problem. The initial inflammatory response should be considered a separate entity from response to the later corrosion. Corrosion of the amalgam, seen in 9- and 12-week samples in this study, occurred in the presence of a successfully healed implant site and did not appear to initiate an inflammatory response. Finally, long-term response of tissue to the metallic and corrosion products of amalgam has yet to be studied. Certainly elements such as mercury, zinc, tin, copper, and silver in such large concentrations at a periapical site are bound to eventually interact with the surrounding bone cells and connective tissue capsule. Effects may be measured not in months but in years, and this is part of a major concern in dentistry, namely a reexamination of all dental materials for cytotoxic and mutagenic effects over the lifetime of the dental patient, the recipient of such implants.

CONCLUSIONS

1. Amalgam with or without zinc is biologically well tolerated by rat osseous tissue.
2. There were no discernable histologic differences between zinc-containing or zinc-free amalgam implanted in the tibia of the rat.
3. Specific components of the amalgam alloy, tin, and in one case, zinc, appear to migrate into the tissue

surrounding and are accompanied by sulfur, a corrosion product.

4. Long-term corrosion of amalgam should be considered as a possible cause of failures involving apical amalgams but has yet to be studied.

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