A scanning electron microscope examination of silver cones removed from endodontically treated teeth

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Twenty-five silver cones were removed from teeth which had been treated endodontically from 3 months to 20 years previously. Examination by the scanning electron microscope revealed that these cones were moderately to severely corroded. The corrosion patterns were described as ranging from pitting to deep crater formation with globular or spherical agglomerations. Examinations with the electron probe showed sulfur peaks on the corroded portions of the cones. X-ray diffraction analyses indicated that the chemical compounds formed were silver sulfides, silver sulfates, silver carbonates, and silver amine sulfate amide hydrates. Tissue culture studies indicated that the corrosion products were highly cytotoxic. The mechanisms for the formation of the corrosion products have been postulated as being due to plastic deformations and metal transfer to the silver cones, plus contact of the silver with tissue fluids.

Root canal treatment failures have been ascribed to many factors, including poor sealing qualities and the inflammatory potential of various cements, pastes, and hard-core filling materials. Tissue toxicity studies of root canal filling materials have been conducted in tissue culture and in vivo in various laboratory animals, often with contradictory results. The subject has been reviewed comprehensively by Seltzer.1 Comparisons between various root canal filling materials, including the two most popular hard-core filling materials silver and gutta-percha—have been made by Feldmann and associates.2-4 Their results indicated that silver was tolerated better than gutta-percha when

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implanted in the mandibles of rabbits. On the other hand, Engström and Spångberg6 found that gutta-percha was much more irritating than calcium hydroxide when placed in human root canals.

In human endodontic therapy, failures occur with all types of root canal filling materials, but whether a greater percentage of clinical success results from the use of any specific material remains conjectural. A common clinical observation is that when silver cones are removed from root canals in which treatment has failed, they are usually blackened and foul smelling. It would appear to be of clinical significance to ascertain the reasons for the discoloration of the silver cones and to determine whether the blackened surface products are cytotoxic.

The scanning electron microscope is ideally suited for the study of root canal filling materials, especially metals such as silver cones. It is the most versatile surface-studying tool available to date.

In the scanning electron microscope, a high-efficiency electron gun is used to produce electron beams with energies between 1 and 30 kev.6 The electron beam is then reduced in diameter by passing through magnetic lenses. The beam is deflected or scanned over the specimen by electromagnetic scanning coils. They scan the specimen in a raster pattern similar to that used in television.7 The electrons that emerge or are backscattered from the specimen are measured with electron detectors. The electrons are also collected and processed in various ways, one of which is to modulate the synchronously scanned oscilloscope electron beam, producing a scanning electron micrograph.8 The micrograph is characteristic of the surface morphology of the specimen.

**X-RAY MICROANALYSIS**

The x-rays which are emitted from the electron-bombarded specimen also yield characteristic lines of the chemical elements present within the specimen. Suitable x-ray detectors can measure the intensities of the characteristic lines. Micrographs of the specimen, similar to those of the electron scanning micrographs, can be made in terms of the element or elements present.

The use of an x-ray spectrometer scan (the electron probe) covers a wide range of elements from aluminum to barium. The K lines provide a strong analytical signal with a 50 kv. instrument. The energies represent the K or L shells in the atomic structure. The microprobe analysis can be correlated with the morphology of the sample with high spatial precision.

With the electron probe, the full x-ray spectrum can be recorded. From the position of the characteristic peaks, the identity of the elements present can be deduced.

The probe beam can be slowly scanned across the sample along a straight line. Monitoring and recording of the intensity of a given characteristic x-ray during such a line scan results in measuring of the concentration of the element along the line.

An x-ray micrograph can be made of the distribution of an element across an area of sample. The x-ray detection system is set to react to a specific
Table I. Number of root canal failures at varying time periods

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>3 months</th>
<th>1 year</th>
<th>5 to 10 years</th>
<th>11 to 20 years</th>
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<td>2</td>
<td>3</td>
<td>11</td>
<td>5</td>
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characteristic x-ray when the probe beam scans the sample. The x-ray micrograph shows bright regions where there is a high concentration of the selected element and darker regions where there is less of the element.

The purposes of this study were (1) to examine morphologically, with the scanning electron microscope, silver cones removed from teeth in which endodontic treatment had failed, as well as a few from successfully treated teeth, (2) to study the corrosion of those cones when such corrosion was observed, (3) to study, by means of electron probe microanalysis, the elements present on the surface of the corroded cones and to map the distribution of those elements, (4) to identify, by means of x-ray diffraction, the exact nature of the corrosion products, and (5) to study the cytotoxicity of corroded cones in tissue culture as compared to controls.

MATERIALS AND METHODS

Twenty-five silver cones were removed from thirteen teeth which had been treated endodontically from 3 months to 20 years previously (Table I). All of these cases were considered to be endodontic treatment failures by virtue of the fact that symptoms of pain and/or swelling had developed, necessitating intervention and treatment.

In addition, five silver cones were removed from three teeth for which treatment had not failed. One tooth, a lower premolar, had received endodontic therapy 25 years previously. The other four cones were removed from lower molars in which the occlusal restorations had fractured and needed to be replaced. One of those silver cones had been in the tooth for 7 years, and the other three had been present for unknown periods of time.

Thirteen silver cones, made by two different manufacturers,* were purchased at two different times on the open market. Five of these cones had been purchased approximately 5 years previously and had remained in a vial during that time but were otherwise unhandled. Both ends of these cones had a brownish black appearance. The other eight cones were purchased a few months prior to examination and had been stored in their original vials. They exhibited no discoloration.

All of the cones were mounted on aluminum stubs for examination with the scanning electron microscope and the electron probe. The specimens were placed in the electron beam column under a vacuum of $5 \times 10^{-5}$ mm. Hg with an accelerating voltage of 25 kv. and a beam current of $2 \times 10^{-11}$ amperes.

The cytotoxicity of twenty-five corroded silver cones and ten fresh, unused

Fig. 1. Unused, recently purchased silver cone. (Magnifications: A, x180; B, x1,800; C, x6,000; D, x10,000.)

Cones to mouse fibroblasts, strain L-929, was determined by the agar-diffusion method of Guess and his co-workers. Monolayers of cells were prepared by pipetting a volume of cells (approximately $2 \times 10^6$ cells in 10.0 ml. of Eagles Minimal Essential Medium [MEM]) into 100 ml. Petri dishes. These cultures were incubated at $37^\circ$ C. in an atmosphere of 10 per cent carbon dioxide and air until full monolayers of cells formed on the bottom surface of the plates. Following the incubated period, the media were removed from the culture dishes by aspiration and the cell layers were washed once with phosphate-buffered saline solution containing 1.0 per cent calf serum.

Eight milliliters of MEM containing 1 per cent agar and 1 per cent calf serum were then pipetted into each culture dish, and the agar overlay was
allowed to solidify at room temperature on a level surface. The monolayer of cells was then stained in the following manner: 10 ml. of 0.01 per cent neutral red solution was pipetted into the center of the agar surface, and the plate was rotated so that the stain would be equally distributed. The stain was allowed to remain on the plates for 15 minutes and then was removed.

**TESTING METHOD**

The specimens to be tested were placed gently but firmly on the agar overlay. Following implantation, the plates were inverted and incubated at 37° C. The plates were removed after 24 hours, and the cells surrounding the cones were
examined both macroscopically and microscopically for signs of injury or death. Gross changes were observed by placing the plates on a white surface. Obvious zones of cell death were indicated by the presence of a clear colorless zone around the silver cones, resulting from the leaching-out of the neutral red stain from the cells. Nontoxic samples were distinguished by the even pink color of the cell monolayer surrounding the silver cones.

**RESULTS**

**Scanning electron microscope observations**

All of the silver cones were scanned over their entire surfaces, and photomicrographs were taken at magnifications ranging from x60 to x10,000.

*Unused, recently purchased silver cones.* At low-power magnification (x100, x180), the silver cones exhibited roughly parallel, horizontal striations, apparently from the tooling (Fig. 1, A). A number of globules or protuberances were noted randomly dispersed on the surface. Magnified 1,800, 6,000, and 10,000 times, ridges and depressions were prominent, with irregular balls of material attached to some of the ridges (Fig. 1, B and C). Minute, irregularly dispersed cracks were observed on the surface in some areas.

Other unused cones which were fractured by repeated bending back and forth with the fingers appeared similar to the unfractured cones when examined at x100 magnification. When magnified 3,000 times, however, microscopic crevices, several microns in width or diameter, became evident and the ridges were plastically deformed. At x10,000 fine cracks which were randomly dispersed between the ridges became apparent (Fig. 1, D).

The 5-year-old, air-exposed silver cones, when viewed at magnifications of x60, x180, and x10,000, exhibited beginning corrosion in the form of pitting at their blackened ends (Fig. 2, A, B, and C).
Fig. 4. Silver cone removed from mesiobuccal canal of unsuccessfully treated upper molar, 5 years posttreatment. (Magnifications: A, x60; B, x110; C, x180.)

Used silver cones (from nonfailure cases). None of the silver cones removed from treated root canals, whether successful or unsuccessful, were unchanged. The description of a silver cone which was removed from a palatal canal of an upper molar 7 years after treatment is typical of those removed from successfully treated teeth. At the apical portion, which appeared to have been cut, the surface was smooth and compatible with that of an unused cone at a magnification of x60 (Fig. 3, A). When viewed at x600, the surface appeared irregular with distortion of the ridges. Numerous irregularly shaped pores or pits, varying in diameter from 0.1 to 0.6 microns, were evident. Minute cracks were randomly dispersed throughout the entire surface (Fig. 3, B).

Grossly corroded silver cones. The cones were examined at various magnifications, (x60, x120, x180, x420, x600, and x1,200). Occlusal, middle, and apical
portions of the cones were examined, scanned, and photographed. Some of the cones had particles of cement adhering to their surfaces.

All silver cones removed from endodontic failure cases were found to be corroded. The cones were altered in several ways, and various types of corrosion pattern were observed:

1. A rough, more uniform surface cavity or crater and numerous globules or spheroids (Fig. 4, A, B, and C).
2. An intensely pitted, rough, nodular surface containing irregular balls or agglomerations of material (Fig. 5, A, and B).
3. A rough, pitted surface with beginning shallow craters (Fig. 6, A, B, and C).
4. Craters from which silver had been removed in massive quantities, surrounded by surfaces which were pitted. Higher magnifications revealed that the base of the crater contained balls or agglomerations of material. Small cracks were also evident, dispersed throughout the surface of the cone (Fig. 7, A, B, C, and D).

In general, extensive corrosion tended to remove the striations which were originally present and caused the formation of pits, spherical agglomerations and, eventually, large craters (Fig. 8, A and B).

**Microprobe analyses**

A number of areas of all of the silver cones were chemically analyzed by means of the electron probe. These areas included the apical, middle, and coronal portions of the silver cones. Included were the cones from those cases which were considered to be successfully treated as well as five silver cones which had been exposed to air for 5 years. The latter cones had darkened areas at both ends and exhibited a brownish black appearance.
Characteristically, the spectra of all of the corroded cones revealed sulfur, as well as silver, peaks. In five cases, iron peaks were also detected. Of the five cones which were removed from the successfully treated cases, one cone, which had been in the mouth for 25 years, also exhibited a slight sulfur peak in its apical portion. The other four cones from successfully treated cases showed no sulfur peaks.

The air-exposed cones also showed small sulfur peaks at both their darkened ends—areas which had been cut.
Corrosion products were present predominantly in areas which were apparently in contact with tissue fluids or saliva.

In an upper molar which exhibited a roentgenographic area of rarefaction only over the mesiobuccal root 7 years after completion of endodontic therapy, sulfur was found in the coronal portions of the two buccal cones (Fig. 9). However, sulfur was absent in the lingual cone (Fig. 10). The micrograph also indicated a corroded appearance of the two buccal cones but not of the palatal cone.

Where cements were found adherent to the surfaces of the cones, the microprobe analysis revealed the elements contained in the cement formula. Included among these elements were zinc, calcium, bismuth, and titanium.
Table II. Analysis of new, unused silver cone

<table>
<thead>
<tr>
<th>Exp. (d) spacing</th>
<th>Silver</th>
<th>Silver sulfide</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.59</td>
<td>2.359 (100)</td>
<td>2.606 (100)</td>
</tr>
<tr>
<td>2.35*</td>
<td></td>
<td>2.21 (45)</td>
</tr>
<tr>
<td>2.17</td>
<td>2.044 (40)</td>
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<td>2.03*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.59</td>
<td></td>
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</tr>
</tbody>
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Ag—ASTM # 4-783.
AgS—ASTM # 14-72.
Intensities in parentheses.
*Most intense experimental lines.

(Fig. 11). Inasmuch as information was lacking, in most instances, as to the nature of the cements which had been employed, a notation of the elements found yielded no significant correlative information.

X-ray diffraction studies

One new and two corroded silver cones were examined by x-ray diffraction. Analysis of the new, unused silver cone indicated the presence of only silver with a trace of silver sulfide (Table II). This correlates with the report of one of the manufacturers (Young Dental Manufacturing Company) that "the silver used in Young's cones is '999 plus' fine silver. We are unable to get the exact analysis of the minute impurities in that it is less than one thousandth of a per cent." The trace of silver sulfide indicates the beginning of corrosion.

Analysis of one of the corroded cones indicated the presence of the following compounds: silver, silver sulfide (Ag,S), and silver sulfate (AgSO₄). The other possible compounds are listed with the \(d\) spacings (Table III).

Table IV shows the match between the observed diffraction pattern and the ASTM standard patterns for silver, silver amine sulfate amide hydrate.
Fig. 9. Microprobe analysis of a mesiobuccal cone of a 7-year failure. The peaks are representative of aluminum, sulfur, silver, and zinc (left to right).

Fig. 10. Microprobe analysis of lingual cone of tooth shown in Fig. 9. The smaller peak on the left is aluminum. The larger peak is silver.

Fig. 11. Representative printout of Kα peaks of corroded cone with adherent cement. The peaks represent aluminum, sulfur, silver, zinc, and titanium.
Fig. 12. Tissue cultures. A. Control silver cone exhibiting no zone of inhibition. B, Corroded silver cone with zone of inhibition.

(Ag(NH₃) · NSO₄ · (Ag (NH₃))₂ · 2H₂O), and silver carbonates (AgCO₃) for the second corroded cone. The silver amine hydrate would appear to be the predominant species in the corrosion, with some uncertainty in the silver carbonate because of overlapping lines.

Of the two corroded cones, the last was visually more corroded. The greater quantity of corrosion products accounted for the higher intensities of the diffraction lines in Table IV as compared to those in Table III. Such higher intensities provide for greater accuracy in identification.

Tissue culture studies

The control silver cones exhibited no observable signs of cell toxicity. On microscopic examination, the cells immediately adjacent to the cones appeared normal and retained their staining characteristics (Fig. 12, A).

Around all the corroded cones, signs of cytotoxicity were evident by the presence of zones of inhibition. Damage to the cell membranes was demonstrated by the rupture of the cell membrane, resulting in the leaching-out of the neutral red stain (Fig. 12, B).

DISCUSSION

Ferguson has defined corrosion as the natural tendency of most metals to revert to their lower (natural) form by oxidation. Most metals are not static and durable; they have been purified from the form found in nature. Ferguson
Table III. Analysis of first corroded cone

<table>
<thead>
<tr>
<th>Exp. d spacing</th>
<th>Silver</th>
<th>Silver sulfide</th>
<th>Silver sulfate</th>
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<tr>
<td>3.493</td>
<td></td>
<td></td>
<td>3.17 (75)</td>
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<tr>
<td>3.178</td>
<td>3.08 (60)</td>
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<td>2.87 (100)</td>
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<tr>
<td>2.839</td>
<td>2.836 (70)</td>
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<td>2.591</td>
<td>2.606 (100)</td>
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<td>2.340*</td>
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<td></td>
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<td>2.242</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2.033*</td>
<td>2.044 (40)</td>
<td>2.08 (45)</td>
<td></td>
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<tr>
<td>1.589</td>
<td>2.591</td>
<td>1.443 (100)</td>
<td></td>
</tr>
<tr>
<td>1.443*</td>
<td>1.445 (25)</td>
<td></td>
<td></td>
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Ag—ASTM #: 4-783.
AgS—ASTM #: 14-72.
AgSO₄—ASTM #: 7-203.
Intensities in parentheses.
*Most intense experimental lines.

Table IV. Analysis of second corroded silver cone

<table>
<thead>
<tr>
<th>Exp. d spacing</th>
<th>Ag</th>
<th>A</th>
<th>AgCO₃</th>
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<tr>
<td>3.66</td>
<td>5.95 (80)</td>
<td>3.08 (8)</td>
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<tr>
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<td>3.05 (100)</td>
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<tr>
<td>2.799*</td>
<td>2.76 (80)</td>
<td>2.74 (60)</td>
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<td>2.644</td>
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<td>2.587*</td>
<td>2.57 (50)</td>
<td>2.56 (6)</td>
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<tr>
<td>2.414*</td>
<td>2.40 (20)</td>
<td>2.42 (20)</td>
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<tr>
<td>2.361*</td>
<td>2.32 (100)</td>
<td>4 lines between 2.38 and 2.27</td>
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<td>2.194*</td>
<td>2.21 (10)</td>
<td>2.16 (12)</td>
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<td>2.065*</td>
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<td>1.93 (16)</td>
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<td>1.890</td>
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<td>1.88 (6)</td>
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<td>1.708</td>
<td>1.71 (20)</td>
<td>1.70 (4)</td>
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A—Silver amine sulfate amide hydrate Ag(NH₃) • NSO₄ • (Ag[NH₃]₉) • 2H₂O; ASTM #: 19-1159 (Z. Anorg. Allgeneine Chem. 337: 325, 1967).
Ag—ASTM #: 4-783.
AgCO₃—ASTM #: 12-766.
Intensities in parentheses.
*Most intense experimental line.

has further pointed out that corrosion is an electrochemical process, requiring a solution of electrolytes in an aqueous medium. An anodic area is set up. At the anode, electrons are released and metal ions are formed by oxidation and distintegration of the metal. Ions of the metal pass into solution. A cathodic area must exist in relation to the anode, so that negatively charged electrons may pass to the cathode from the anode. The anode and cathode may exist in the same piece of metal. As a result of disintegration of the metal, pitting, blemishing, and surface deterioration of the metal appear.

In the case of silver cones, the probe studies revealed that the corrosion
of the cones was associated with sulfur and that the chemical compound formed was predominantly a silver amine sulfate amide hydrate. The origin of the sulfur is not difficult to postulate, inasmuch as all cells of the body contain sulfur, primarily in the cell protein. The main sources of sulfur for the body are the two sulfur-containing amino acids, cysteine and methionine. Other organic compounds of sulfur are heparin, glutathione, insulin, thiamine, biotin, coenzyme A, lipoic acid, ergothioneine, taurocholic acid, the sulfocyanides, sulfur conjugates, and the condroitin sulfuric acid in cartilage, tendon, dentine, cementum, and bone matrix. In addition, small amounts of inorganic sulfates, with sodium and potassium, are present in the blood and other tissues. Thus, the sources of the sulfur in the corrosion of silver cones in root canals may be blood, dentine, cementum, bone, or saliva.

Our studies have demonstrated that the main areas of corrosion of silver cones are the apical and coronal portions. Both regions may contact tissue fluids, either periapical exudates or saliva, in the event of leakage.

A homogeneous interface between the metal and its environment reduces the chances for corrosion. As Ferguson has pointed out, corrosion is enhanced by differences in strain, crystalline structure, and oxide and metallic impurities on the surface. Thus, when used in the root canal, silver cones should be uncontaminated by microorganisms, other metals, or damaged surfaces. In addition, new stresses or design changes should be introduced into the cone after its manufacture. Unfortunately, when used in root canals, silver cones must be manipulated and cut, and hence stressed, during handling and fitting. The manufacturer also must sever both ends of the silver cone before packaging. As demonstrated in this study, cutting of the cone with scissors, plus holding with pliers or hemostats, plastically deforms the metal, resulting in microscopic cracks and changes in crystalline structure. Even placing the cone in the root canal under pressure causes deformation. Such defects enhance corrosion.

Venable and his co-workers, and Laing have demonstrated that whenever one piece of metal comes in contact with another, transfer of small particles from one to the other probably occurs. Thus, cutting and handling of silver cones results in metal transfer to the surface of the cones. Corrosion currents begin at the site of the metal attachments.

Bowden and his co-workers have shown that the most highly polished surface of metal is very rough terrain when viewed under the electron microscope. We can reasonably postulate that when root canals are reamed and filed with carbon or stainless steel instruments, minute specks of metal are deposited on the wall of the root canal. When a silver cone contacts the wall of the root canal, these minute particles of metal from the instruments become “cold welded” together at the points of pressure. When the cone is removed by sliding for final cementation, the welds do not break. Instead, fractures occur through the base of one or other of the metal peaks. (In the probe studies, iron peaks were found on five silver cones.) Corrosion is more marked around the fracture sites, probably because the surface of the silver is seeded with pieces of an alloy having different composition.
Earlier studies had indicated that silver is not highly toxic to tissues. However, our tissue culture studies revealed that the sulfur corrosion product of silver is highly cytotoxic. It has long been known that heavy metals are very toxic since they cause precipitation of the protoplasmic proteins which are the seat of metabolic processes. Generally, toxicity is affected by the solubility of the metal, the level of integration into tissue, the portal of entry, and the efficiency of the detoxification mechanism of the body. All corroding metals set up a reaction, ranging from mild, with relative tolerance, to severe, resulting in rejection.

All currently used metals, when implanted in the human body, give off ions to the surrounding tissues. The degree of tissue reaction is generally proportionate to the amounts of constituent ions released by corrosion of pure metals and alloys. Silver cones, especially those pushed beyond the apex of the tooth, apparently are severely toxic to the periapical tissues. However, the critical concentration at which these damaging ions reach the point of causing clinical damage is unknown. Thus, silver cones in root canals may remain dormant for many years without obvious cell damage. Yet, clinical experience indicates that a long-dormant area around an implanted silver cone may "flare up" suddenly years later with a severe inflammatory response and liquefaction necrosis. Such inflammatory responses may be aseptic. All of the corroded silver cones in this study were associated with acute clinical "flare-ups" years after completion of endodontic therapy. Thus, when corrosion-prone metals are used in root canals, the possibility of corrosion and cytotoxicity is always present, especially when those metals contact tissue fluid or saliva.

SUMMARY

Twenty-five silver cones were removed from teeth which had been treated endodontically from 3 months to 20 years previously. Examination of these cones by the scanning electron microscope revealed that they were moderately to severely corroded. The corrosion patterns were described as ranging from pitting to deep crater formation with globular or spherical agglomerations. Examinations with the electron probe showed sulfur peaks on the corroded portions of the cones. X-ray diffraction analyses indicated that the chemical compounds formed were silver sulfides, silver sulfates, silver carbonates, and silver amine sulfate amide hydrates. Tissue culture studies indicated that the corrosion products were highly cytotoxic. The mechanisms for the formation of the corrosion products have been postulated as being due to plastic deformations and metal transfer to the silver cones, plus contact of the silver with tissue fluids.

We gratefully acknowledge the assistance of Miss L. Marchant, Research Scientist, and Dr. John Meakin, Manager, Physics Laboratory, of the Franklin Institute Research Laboratories for their guidance and technical assistance.

REFERENCES

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