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# SCIENTIFIC ARTICLES

# Corrosion of Silver Cones in the Subcutaneous Connective Tissue of the Rat: A Preliminary Scanning Electron Microscope, Electron Microprobe, and Histological Study

# Corrosion de los Conos de Plata en el Tejido Conectivo Subcutaneo de la Rata: Un Estudio Histologico Preliminar con Microscopio de Barrido Electronico y Microsonda Electronica

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**Size 140 silver cones and solid Teflon rods of the same size were implanted in the subcutaneous connective tissue of the rat in an effort to analyze the tissue response to silver cones and their possible Corrosion products at different observation periods. corrosion phenomena and the presence of corrosive by-products on the silver cone surfaces and in the tissues surrounding the implants were studied using the scanning electron microscope and the electron microprobe. Our results showed that despite the silver cones rapidly corroding in the subcutaneous connective tissue of the rat, they appeared to be well tolerated by the tissues. When granulomatous tissues persisted in long observation periods, they seemed to be produced by the presence of corrosive by-products which were released from the cones. However, we feel that more extensive experiences will be necessary to study the possible correlation between the corrosion of the silver cone and the tissue response to silver corrosion products.**

**El objetivo de este estudio es observar si existe alguna correlación entre la respuesta de los tejidos y la presencia de corrosión sobre las superficies de los conos de plata previamente implantados en el tejido conectivo subcutáneo de la rata.**

Despite the fact that silver cones have long been successfully used in endodontics as a solid core filling material, several studies have demonstrated that they may chemically alter when in contact with tissue fluids and rapidly corrode under different experimental conditions (1–4). In addition, corrosive by-products attributed to this corrosion have been shown to be highly cytotoxic in tissue cultures (1). Interestingly, Palmer et al. (5) reported severe inflammatory responses when silver cones plus sealer were pushed through the root apices of monkey teeth. After 155 days of implantation, they found that all of these cones showed a dark discoloration compatible with the corrosive phenomenon. However, other investigators found that silver cones were well tolerated when they were implanted in bone (6–8) or in soft tissues (9–11). Taking into account these contradictory observations, we considered the question of whether or not the corrosive by-products released from silver cones are toxic to the surrounding tissues as one which still remains to be answered.

The intent of this study was to observe if some correlative events occurr between the response of the tissues and the presence of corrosion on the surfaces of silver cones which have been previously implanted in the subcutaneous connective tissue of the rat.

## MATERIALS AND METHODS

Size 140 silver cones, previously cut into sections 10 mm in length, were implanted in the subcutaneous connective tissue of 36 white male Wistar rats that

weighed between 90 and 120 g each. Solid Teflon rods of a similar size and also 10 mm long were used as inert controls. Prior to their implantation, both the silver cones and Teflon rods were autoclaved and stored under sterile conditions until they were used.

The operative procedures were as follows. The animals were anesthetized by intraperitoneal administration of pentobarbital (0.025 g/100 g weight) and the dorsal skin was shaved and disinfected with 5% iodine in alcohol. Two separate incisions were made through the skin using a scalpel. In addition, two subcutaneous pockets were prepared by blunt dissection to a depth of 15 mm. Then, one silver cone and one Teflon rod were carefully placed into the pockets prepared in each rat. After implantation, the wounds were cleaned and sutured.

The animals were killed in groups of 12 each after 30, 90, and 160 days by ether suffocation. The implants, along with the surrounding tissues, were removed in rectangular blocks and then fixed in 10% formalin. After fixation, the tissues were embedded in paraffin and longitudinally sectioned through the implants. In the case of the silver cones, cutting was made just to the metallic surface. The cones were then carefully removed from the tissues and serial sections approximately 7-$\mu$m thick were obtained and stained with hematoxylin and eosin.

To analyze if corrosion had occurred on the silver cones, they were mounted on aluminum stubs, coated with 200 Å of gold-palladium, and examined with the scanning electron microscope (SEM; JEOL JSM-25S; Tokyo, Japan) and the electron microprobe (EMP; CAMECA MS46; Paris, France). In addition, 20 unused silver cones from the same lot were also examined for control purposes. In an effort to investigate whether corrosive by-products were present in the tissues, some selected histological sections were also examined with the EMP. After the sections were studied with light microscopy, specific areas of interest were photographed and then the slides were prepared for the detection of chemical elements. After soaking in xylene, the coverslips were removed and the slides were cut into portions which contained the selected areas. The specimens were air dried, coated with carbon, and transferred to the microprobe specimen chamber. All silver cones and silver cone slides were scanned over their entire surface and the distribution of elements at selected sites were mapped on Polaroid microphotographs.

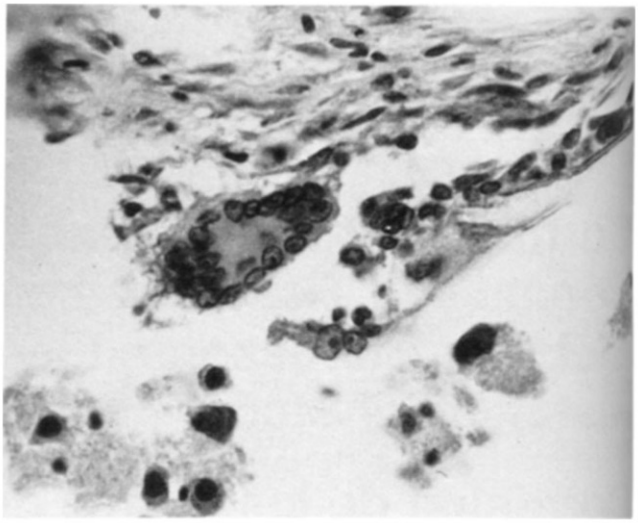

FIG 1. Ninety-day experimental silver cone implant. High-power photomicrograph showing a large multinucleated giant cell and macrophages in direct contact with the implant (hematoxylin and eosin; original magnification ×400).

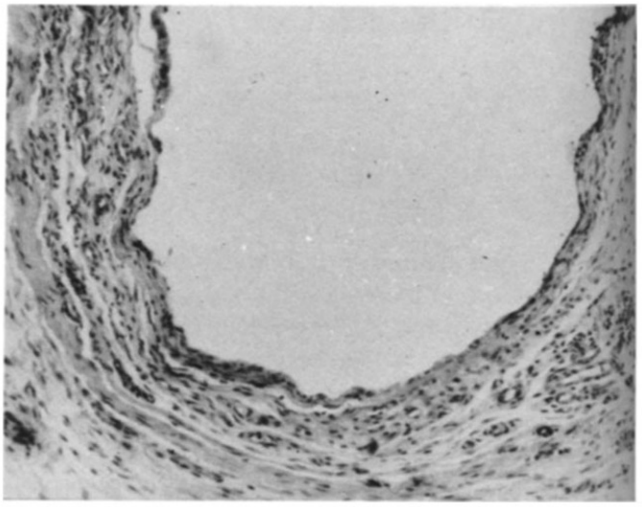

FIG 2. Histological section of a 160-day solid Teflon implant illustrating a fibrous connective tissue capsule surrounding the implant (empty space). Note the presence of numerous wide capillaries and few inflammatory cells (hematoxylin and eosin; original magnification ×70).

TABLE 1. Histological evaluation

| Implanted Material | Time of Death | | | Total Implants |
|---|---|---|---|---|
| | 30 days | 90 days | 160 days | |
| Silver cones | 12 | 10 | 9 | 31 |
| Teflon rods (control) | 12 | 12 | 12 | 36 |

TABLE 2. SEM and EMP examinations

| Implanted Material | Time of Death | | | Total Implants |
|---|---|---|---|---|
| | 30 days | 90 days | 160 days | |
| Silver cones | 10 | 12 | 12 | 34 |
| Unused silver cones (control) | | | | 20 |

## RESULTS

Macroscopic examination showed that wound healing was satisfactory in all instances. A total of 72 specimens containing their respective implants (36 sil-

ver cones and 36 Teflon rods) were obtained. Unfortunately, two histological specimens from the 90-day period and three from the 160-day period in the silver cone group had to be excluded from the study. In addition, two silver cones from the 30-day period were lost. Definitive data from the time periods and the studied materials are shown in Tables 1 and 2.

### Histological Observation

At the 30-day observation, histological sections of both test and control Teflon samples showed a thin granulomatous layer in direct contact with the implants. This layer was generally surrounded by fibrous connective tissue differentiation with a few inflammatory cells and numerous wide capillaries containing red blood cells. This picture was common to all of the implants, except for two cases in the silver cone group and three in the control group. In these five cases, the granulomatous band was thicker than that observed elsewhere and some multinucleated giant cells could also be seen in contact with the implants.

At the 90-day observation, the implants were surrounded by healthy fibrous connective tissue of irregular thickness with the presence of an occasional giant cell (Fig. 1). Only scattered inflammatory cells, mostly lymphocytes and macrophages, persisted in contact with the silver cone implants. In this group some dark

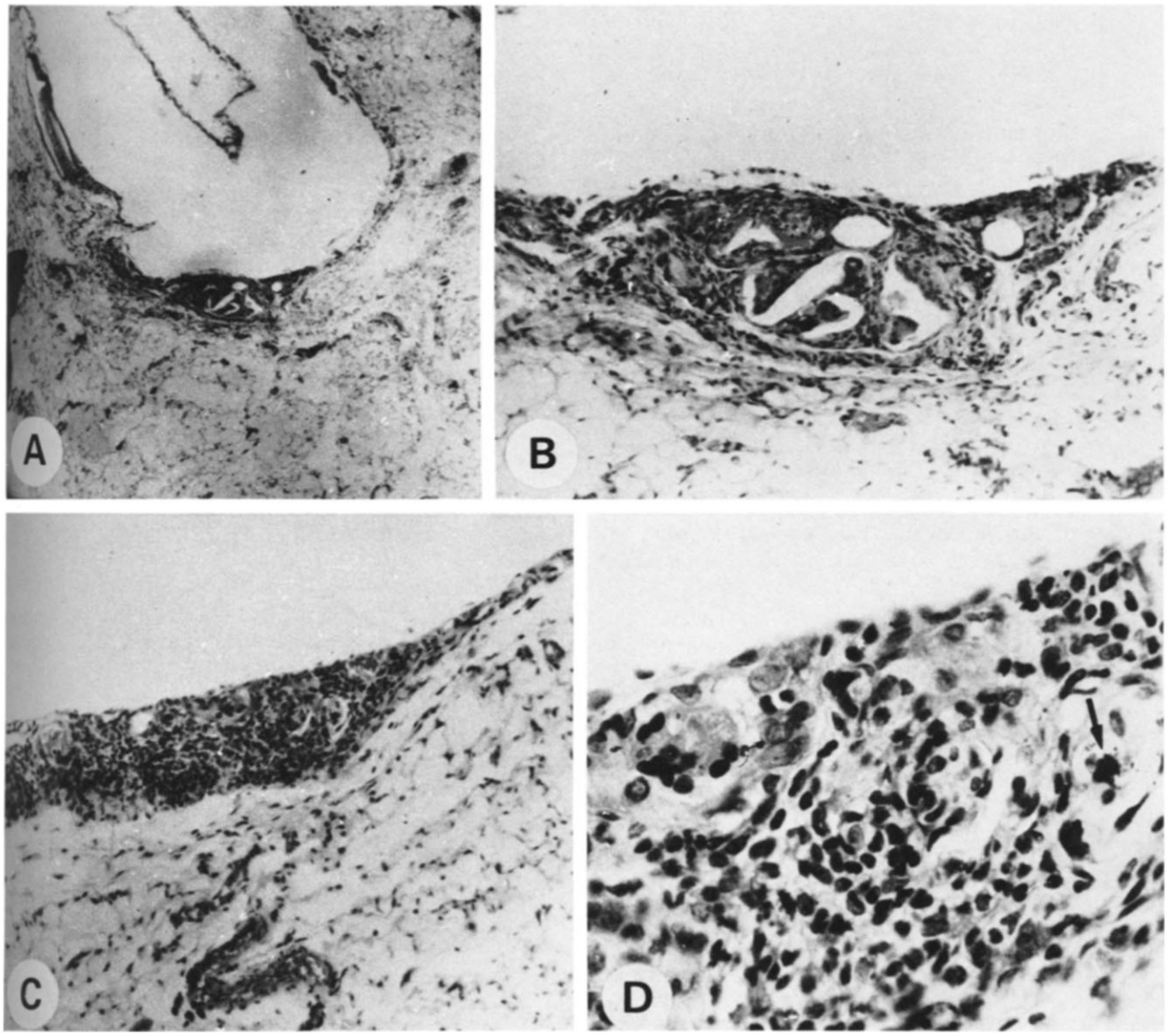


Fig 3. *A*, Low-power photomicrograph of a 160-day experimental silver cone implant. Note the presence of granulomatous areas in direct contact with the implant (empty space). In addition, some inflammatory cells are present in the surrounding tissues (hematoxylin and eosin; original magnification ×70). *B*, Higher magnification of *A* showing a well-localized granulomatous tissue containing lymphocytes, plasmocytes, multinucleated giant cells, and macrophages (original magnification ×250). *C*, Inflammatory granulomatous tissue in direct contact with a 160-day experimental silver cone implant (hematoxylin and eosin; original magnification ×250). *D*, Detail of an area of the granulomatous tissue shown in C. There are lymphocytes, plasmocytes, and some fibroblasts. Note the presence of dark particles (*arrow*) and many macrophagic cells (original magnification ×400).

particles which were surrounded by macrophagic cells were observed in the tissues, but this was not common to all implants. When these slides were studied with the EMP, silver and sulfur were detected.

At the 160-day observation, all control and four of the silver cone implants were totally surrounded by a noninflamed irregular connective tissue capsule (Fig. 2). However, some multinucleated giant cells persisted in contact with some control and experimental implants. The remaining five specimens of the silver cone group showed occasional disruption of the connective capsule with the presence of granulomatous tissue at these areas (Fig. 3). This was infiltrated by lymphocytes, plasmocytes, and macrophages. In addition, dark particles which appeared to be phagocytosed by the multinucleated giant cells could be frequently observed

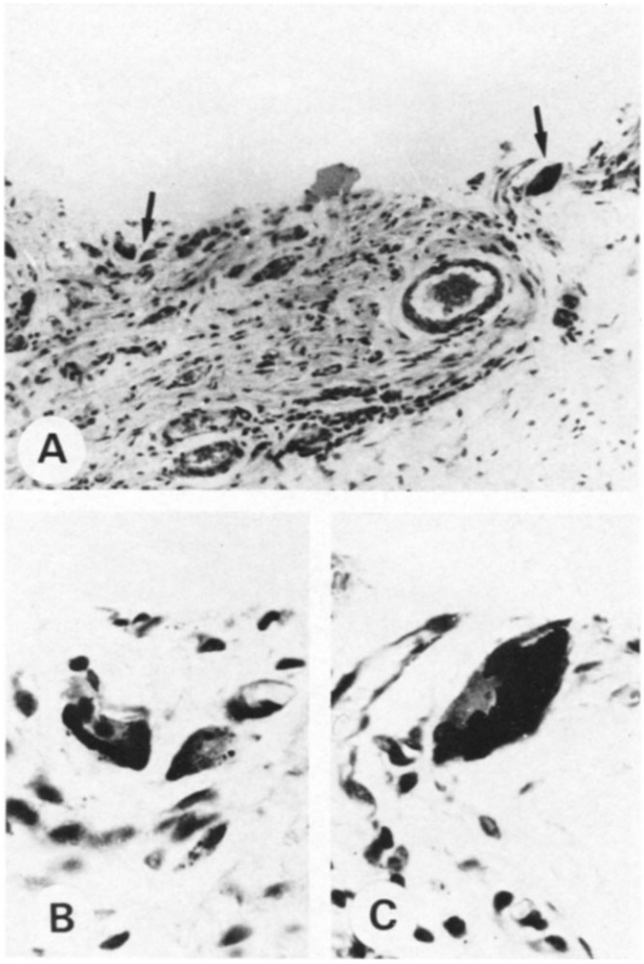


FIG 4. Histological section of a 160-day experimental silver cone implant. *A*, Low-power photomicrograph showing fibrous connective tissue in direct contact with the implant (empty space). Note the presence of foreign body cells and macrophages (*arrows*) within a localized granulomatous area (hematoxylin and eosin; original magnification ×100). *B* and *C*, Detail of the same areas indicated by *arrows* in *A* showing foreign body cells and macrophages containing dark particles in their cytoplasm (original magnification ×400).

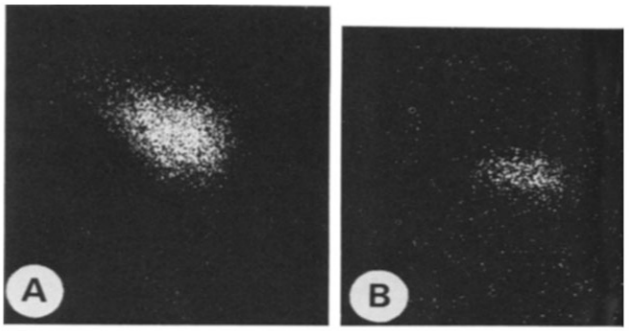


FIG 5. Electron microprobe analysis of the same histological section of Fig. 4. Elemental mapping of elements showed silver accumulations (*A*) and sulfur (*B*) in the same area. Elements are concentrated in white masses (original magnification ×750).

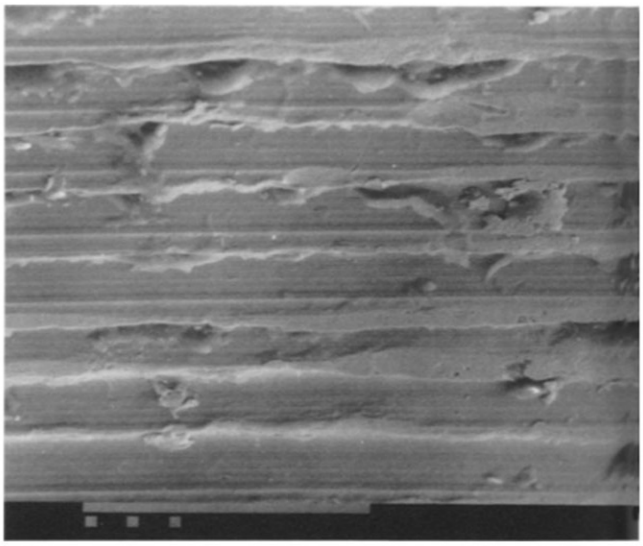

FIG 6. Scanning micrograph of surface material from an unused recently purchased silver cone. There are roughly parallel horizontal striations with many irregularities and globular protuberances on the surface. *Bar*, 100 μm (original magnification ×450).

(Fig. 4). Silver accumulations and sulfur were disclosed in these areas when the EMP was used (Fig. 5).

### SEM and EMP Observations of the Silver Cones

All of the unused silver cones showed parallel horizontal striations with numerous irregular protuberances and depresed areas that appeared to be manufacturing characteristics (Fig. 6). The EMP observation revealed a high concentration of silver. Only scattered minute areas showed a low concentration of this element, hence the material was essentially homogeneous. Silver cones removed from animals that were killed 30 days after implantation showed features similar to those of unused cones. However, small portions of the surrounding tissue were observed to be attached to the metallic surface. Occasionally, the beginning of corrosion was detected in the form of surface erosions, minute cracks, or pits (Fig. 7). The EMP analysis re-

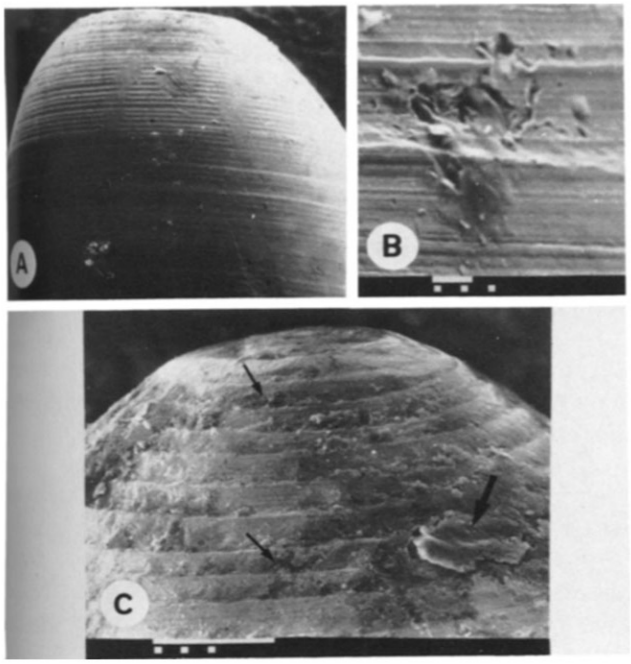


FIG 7. Scanning micrograph of the apical areas of two experimental silver cones removed 30 days after implantation. *A*, Low-power photomicrograph showing parallel striations and tissue particles on the surfaces (original magnification ×200). *B*, Detail of the same cone shown in *A*. Note the presence of pitting formations within the ridges. *Bar*, 10 μm (original magnification ×700). *C*, Photomicrograph of a silver cone showing surface erosions within the ridges (*fine arrows*) and tissue remnants on the surface (*gross arrow*). *Bar*, 100 μm (original magnification ×300).

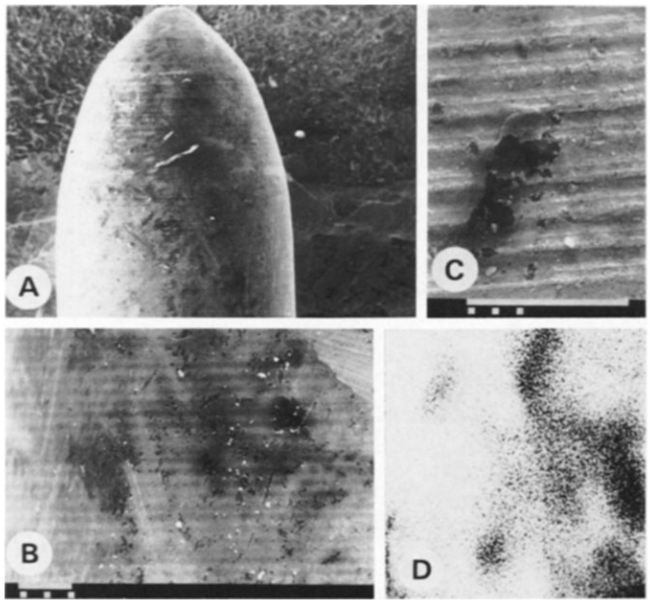


FIG 8. *A*, Scanning micrograph of a silver cone removed 90 days after implantation. Note horizontal parallel striations and numerous irregular pitted areas (original magnification ×70). *B*, Higher magnification of *A* showing an extensive pitting formation. *Bar*, 100 μm (original magnification ×150). *C*, Detail of the same area shown in *B*. Note extensive erosion on the surface and within the ridges. *Bar*, 100 μm (original magnification ×450). *D*, Elemental mapping of silver in the same areas of *B*. Note less concentration of this element in the dark areas (original magnification ×750).

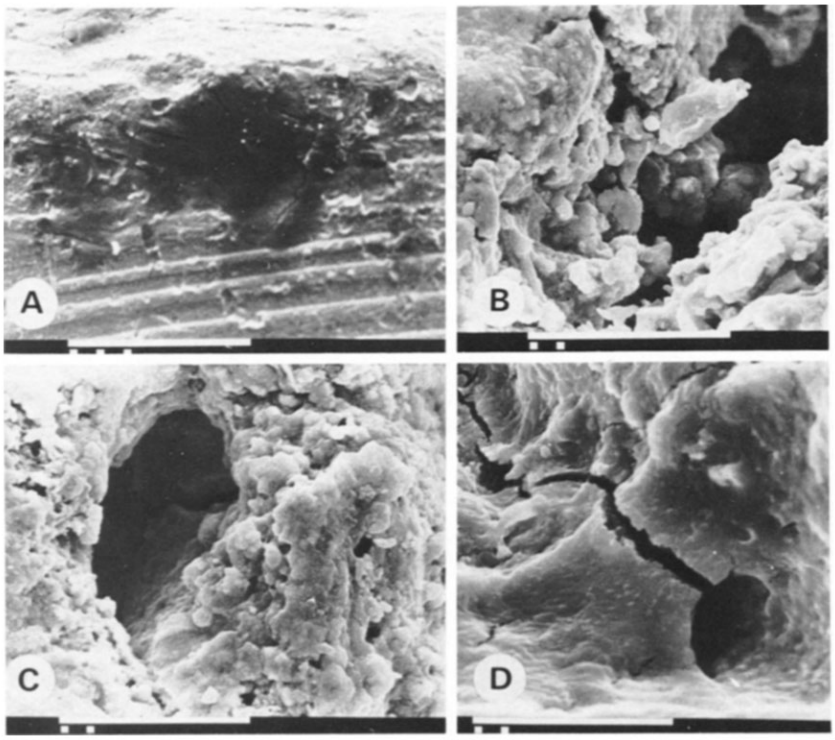


FIG 9. Different corrosive formations on the surface of silver cones removed 160 days after implantation. *A*, Scanning micrograph of a dark-pitted area. Note that normal striations had been erased. *Bar*, 100 μm (original magnification ×450). *B* and *C*, Scanning micrographs of two cratered areas containing numerous globular particles and accretions. *Bar*, 10 μm (original magnifications ×4,500). *D*, Scanning micrograph of a large corroded area showing a deep pitting formation. Note the presence of an extensive microfracture on the surface. *Bar*, 10 μm (original magnification ×7,000).

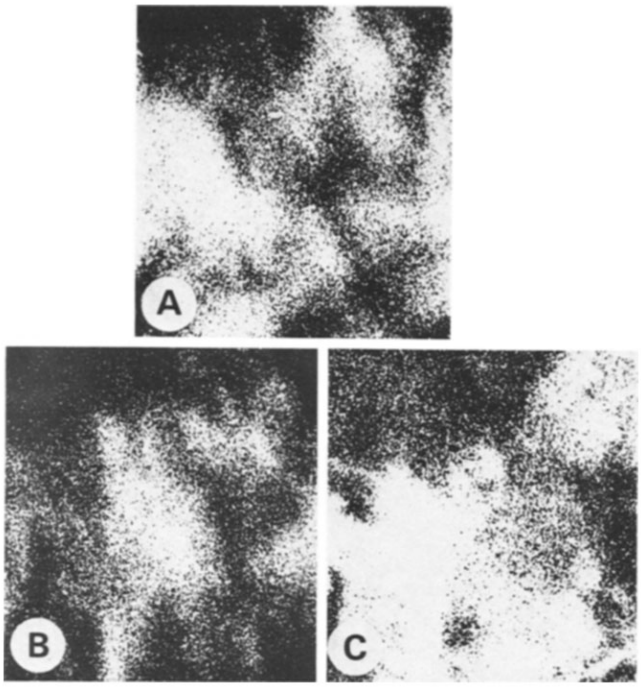


FIG 10. The EMP analysis of a silver cone removed 160 days after implantation. *A*, Elemental mapping of silver showing less concentration of this element in dark areas (original magnification ×750). *B*, Elemental mapping of chlorine (*white masses*) in the same area of *A* (original magnification ×750). *C*, Elemental mapping of sulfur (*white masses*) in the same area (original magnification ×750).

vealed low concentrations of sulfur and chlorine on the surface.

At the 90-day observation, none of the silver cones were free of morphological changes on their surfaces. Higher magnifications revealed numerous irregular pitted areas (Fig. 8, *A* to *C*) and microfractures which appeared to be randomly dispersed. The EMP analysis disclosed surface concentrations of sulfur and chlorine. In addition, numerous areas with low concentrations of silver were detected (Fig. 8*D*).

At the 160-day observation, all of the silver cones revelaed important signs of corrosion. They showed numerous dark-pitted and cratered areas (Fig. 9) in which the normal surface striations had been erased. The EMP analysis of these areas disclosed minor concentrations of silver and chlorine with a high concentration of sulfur (Fig. 10).

## DISCUSSION

Our observations showed that silver cones rapidly corrode in the subcutaneous connective tissue of the rat and this confirmed the results obtained by other investigators (1–4, 7). Despite this corrosion, the silver cones appeared to be well tolerated by the tissues, although the occurrence of sulfur and silver accumulations in the tissues surrounding the implants may have important implications in the interpretation of the results. The release of corrosive by-products from the cones could be the main cause of the foreign body reactions observed at different localized areas. At these sites several dark particles, which appeared to be phagocytized by macrophages and multinucleated giant cells, were frequently observed and recognized as sulfur and silver accumulations when the EMP was used. However, the silver cones were irregularly corroded and the damaging concentrations of such products in the tissues is unknown. Consequently, possible correlations between the presence of granulomatous areas in the fibrous capsule surrounding the 90- and 160-day implants and the corrosive by-products which appeared to be released from the cones was not determined and still remains unclear from this study.

Moreover, our findings suggest that the implantation of noncorroded silver cones is not a reliable procedure to study any deleterious action of the corrosive process on the surrounding tissues after different observation periods. It is our opinion that since silver cones have been successfully used for many years in clinical endodontics, more extensive assays are quite necessary before one can extrapolate the observations described above to the clinical situation. However, we feel that this experiment may provide the basis for further studies on tissue responses to the corrosive by-products released from silver cones. Such studies are now in progress.

## SUMMARY

Thirty-six size 140 silver cones were implanted in the subcutaneous connective tissue of white male Wistar rats in order to analyze their corrosive patterns after different observation periods. This was accomplished by the use of the SEM and the EMP. Teflon rods and unused silver cones of the same size were used as controls. In addition, histological sections of the surrounding tissues were obtained. The SEM and EMP examinations showed that the silver cones rapidly and progressively corroded as the observation period increased. Different elements such as sulfur, chlorine, and silver accumulations were detected in the tissues surrounding the implants, whereas low concentrations of silver in the presence of sulfur and chlorine were found on the silver cone surfaces. Histological examination showed localized areas of foreign body reaction with many dark particles which appeared to be silver accumulations. These particles were frequently observed to be phagocytosed by macrophages and multinucleated giant cells, but in general the implants were well tolerated by the tissues. In fact, the methods used in this study do not allow any correlations to be made between the corrosion of the silver cones and the tissue responses observed. However, we believe that this preliminary experiment may provide the basis for further studies of the nature of the tissue response to the corroded silver cone.

This study was conducted at the Scanning Electron Microscopy Department, Faculty of Odontology, University of Buenos Aires, and the Metallurgy Department of the National Council of Atomic Energy, Buenos Aires, Argentina.

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