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STUDIES OF CHRONIC INFLAMMATION IN A RED TATTOO BY ELECTRON MICROSCOPY AND HISTOCHEMISTRY

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Abstract. Findings by electron microscopy and histochemistry are described in three red (mercury-containing) tattoos of which two were uncomplicated and the other complicated by chronic inflammation. In the former, the mercurial had the structure of cinnabar and was found in dermal mononuclear cells and free in the dermis. In the latter, some tattoo material with the structure of cinnabar was present, but in addition, by histochemical processing, mercury was found in sites other than that in the uncomplicated red tattoo. Such mercury was associated with histochemically demonstrable densities found in perinuclear spaces, in the cisternae of rough and smooth endoplasmic reticulum, within membrane-bound inclusions, and associated with mitochondria of both epidermal cells and mononuclear cells in the dermis.

Mercuric sulfide, mixtures of mercuric sulfide and cadmium sulfide, cadmium selenide, carmine, and occasionally red ochre, and dyes derived from madder, sandalwood, or brazilwood are used to make red tattoos (1, 3, 7, 15). The purpose of this paper is to describe findings by electron microscopy and histochemistry in a red tattoo complicated by a chronic inflammatory reaction.

MATERIALS AND METHODS

Tissue was obtained from the red portions of three tattoos from three different individuals. Two of the tattoos were clinically normal and asymptomatic, one having been placed 10 years ago and the other 12 years ago. The third tattoo had been placed 10 years ago, and for the last 5 years was marked by pruritus and inflammation in the form of swelling, erythema, and scales (Fig. 1). This subject was not sensitive by conventional and photopatch tests to several mercury and cadmium compounds, and carmine. The mercury compounds used included mercuric sulfide (cinnabar) (0.1%), mercuric chloride 0.05%), phenylmercuric acetate (0.1%), ammoniated mercury ointment (10%), and yellow mercury oxide ointment. Examination under the light microscope of this inflamed tattoo was interpreted by Dr Arthur B. Hyman as follows: "The epidermis is irregularly acanthotic. No spongiosis is seen. The upper cutis shows an infiltrate of lymphocytes and histiocytes. In the mid and deep cutis, there are perisvascular infiltrates of lymphocytes and pyknotic elements, mixed with scattered, fine, almost black pigment granules (Fig. 2), some apparently intracellular. With toluidine blue there are 10–12 mast cells per high power field. PAS stain only shows glycogen in the epidermis, but no other changes. The histologic reaction is reasonably attributable to metallic tattoo."

Excised specimens of the ordinary, asymptomatic red tattoo and the inflamed red tattoo were processed for electron microscopy as described previously (16, 17). Specimens were fixed in glutaraldehyde (4%) in 0.1 M cacodylate buffer (pH 7.4) for 2 hours at 4°C. Blocks were then washed in two changes of cold isotonic saline for 5 min and postfixed for 1 hour in cold osmium tetroxide (1%) in 0.1 M phosphate buffer (pH 7.4). Some blocks of both tattoo specimens were immersed in an aqueous solution of ammonium sulfide (1%) for 10 min and washed in three changes of cold isotonic saline after fixation with glutaraldehyde and before postfixation in osmium tetroxide. All specimens were finally washed again in three changes of cold saline solution, dehydrated in graded alcohols, and embedded in epoxy resin (Maraglas). Silver to gold sections were cut from blocks treated in the above ways and placed on 200 mesh, Formvar-coated or uncoated stainless steel grids. Some sections were stained with uranyl acetate, lead citrate, or both. Other specimens were stained by the gold chloride technique as first described by Hand and co-workers to detect mercury by light microscopy (8), and modified by us for electron microscopy (18, 19). A stock solution of 1% chlorauric acid in distilled water, stored in a dark bottle, was used in a concentration of two parts of stock solution to eight parts of distilled water for staining thin sections. The latter solution was prepared fresh on the day the sections were to be stained. One drop of the diluted chlorauric acid solution (0.2%) was placed on sections on Formvar-coated or uncoated stainless steel grids for about 20 seconds. The sections when then rinsed in doubly-distilled water and dried.

In order to determine if gold staining was truly attrib-

Fig. 1. The figure in this tattoo is that of "Woody Woodpecker" who has red head feathers. Scaly, erythematous, raised patches of dermatitis are seen in the red portion of the tattoo (*arrow*).

utable to the presence of a heavy metal, a blocking reaction against amalgamation of mercury and gold with penicillamine was done first. A 6% aqueous solution of penicillamine (Cuprimine; Merck Sharp and Dohme) was allowed to remain in contact with the sections for 4 hours at room temperature and then the sections were washed thoroughly in doubly-distilled water overnight and dried. After this treatment the sections were stained with chlorauric acid as above.

In order to check for the presence of mercury or cadmium, sections of the inflamed tattoo and the extract of the penicillamine-treated sections were submitted for neutron activation analysis.[1]

For the purpose of chemical identification of mercury and cadmium in the uncomplicated tattoo specimens, sections were flooded with a mixture of nitric and hydrochloric acids (aqua regia). Control specimens of normal skin were treated in the same manner. This mixture of acids was then tested for mercury with diphenylcarbazone (11) and cuprous iodide (6), and for cadmium with di-*p*-nitrophenylcarbazide (11). Neutron activation analysis was not performed on these specimens because of the expense involved.

[1] Neutron activation analysis was done by Mr Harold Nass at Union Carbide, Sterling Forest, New York.

RESULTS

Findings in two uncomplicated red tattoos

Chemical analysis of the tissue sections from the tattoos revealed that mercury was present but cadmium was absent. Control specimens of normal skin gave negative results for mercury and cadmium. The positive cuprous iodide tests for mercury in specimens from the two uncomplicated red tattoos indicated the presence of at least 0.20 mg of mercury which is the smallest amount of mercury detectable by this method (9). Ultrastructural findings in these cases were similar to those of our previous study in which electron-dense material was proved to be cinnabar (15). Dermal cells contained large amounts of tattoo pigment in their cytoplasm (Fig. 3). Some tattoo material was also found free in the dermis. An occasional mast cell was present. Epidermal cells did not show significant changes. Histochemical processing with ammonium sulfide did not reveal

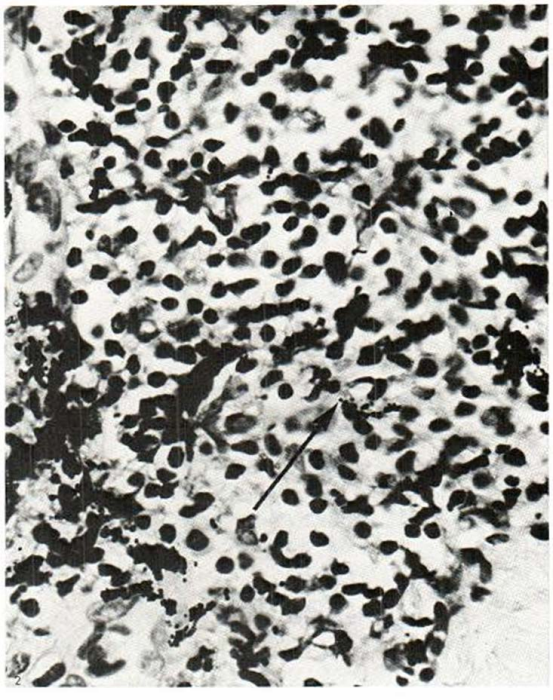

Fig. 2. Photomicrograph of a thick section of the chronically inflamed tattoo processed for light microscopy. The inflammatory infiltrate and tattoo material (*arrow*) in the midepidermis are shown. × 570.

changes markedly different from those seen with routine fixation.

Findings in the inflamed red tattoo

Neutron activation analysis of the tattoo material revealed that mercury was present in a concentration of 238 parts mercury per million, but that cadmium was absent. Three control specimens of normal skin were found to contain less than 0.001 parts mercury per million, by the same method. The following changes were seen when routine processing of the specimen for electron microscopy was performed. Dermal cells contained tattoo pigment (Fig. 4). Types of cells were present in the dermis which were absent in the ordinary red tattoos. Some had prominent endoplasmic reticulum and resembled plasma cells (Fig. 5); others had scanty cytoplasm and resembled lymphocytes; and still others resembled macrophages. Many mast cells were present. Epidermal cells contained the following in their cytoplasm: glycogen, clumped tonofilaments, lysosomes (acid phosphatase positive), and membrane-bound inclusions with lipid-like material. Desmosomes were disrupted and the intercellular spaces contained much particulate material of unknown nature. Exocytic cells such as neutrophils, eosinophils, and mononuclear cells were found. The extent of changes found in epidermal cells varied in different parts of the specimen.

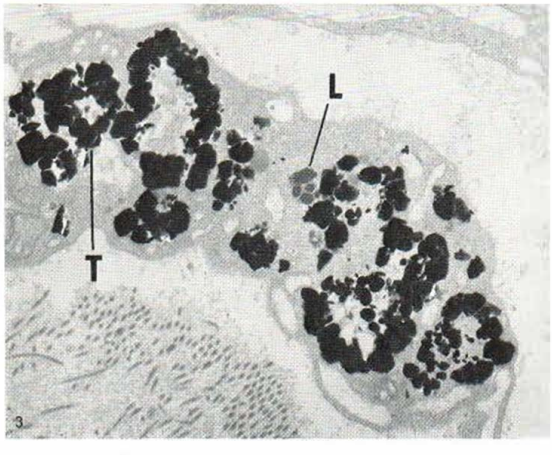

Fig. 3. Electron microscopic appearance of material in an ordinary red tattoo stained with uranyl acetate and lead citrate. Part of a dermal cell is shown with electron-dense bodies resembling cinnabar (*T*), and some bodies of lesser opacity (*L*). × 19 500.

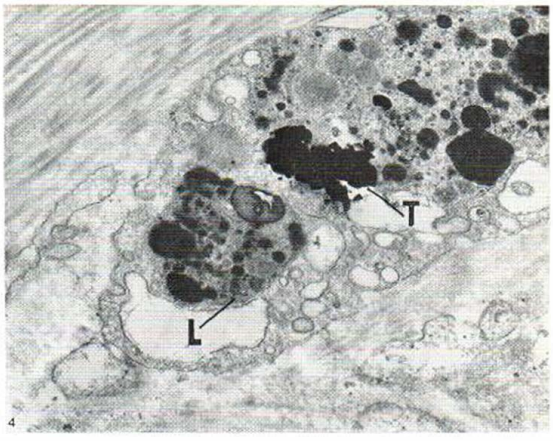

Fig. 4. Electron microscopic appearance of material in the inflamed red tattoo stained with uranyl acetate and lead citrate. Part of a dermal cell is shown with electron-dense bodies (*T*) resembling those seen in Fig. 3, and bodies of lesser opacity (*L*), which are seen in the cytoplasm. × 19 700.

The following changes occurred when processing was carried out with glutaraldehyde, ammonium sulfide and osmium tetroxide. Histochemically demonstrable densities were then seen which were not found in tissue when processed with routine fixation. Fig. 6 shows a cell similar to that seen in Fig. 5. Note the presence of densities in perinuclear positions, in channels of rough endoplasmic reticulum and in smooth-surfaced membrane-bound inclusions. Other examples of the densities are shown in Figs. 7 and 8. Fig. 7 shows parts of two cells, one with scanty cytoplasm resembling a lymphocyte, the other, which has engulfed part of another cell, resembling a macrophage. Densities are also visible in some epidermal cells as seen in Fig. 8. These densities are found in perinuclear spaces, associated with mitochondria, and with cisternae of endoplasmic reticulum.

The histochemically demonstrable densities in epidermis and dermis are stainable with gold chloride. Some gold staining was also seen, unassociated with densities. These gold particles were located in nuclei, among glycogen deposits, and within mitochondria (Fig. 8). Prior treatment of sections with penicillamine resulted in blocking of the gold staining. Neutron activation analysis of the penicillamine-extracted material revealed that mercury was present, but that cadmium was absent.

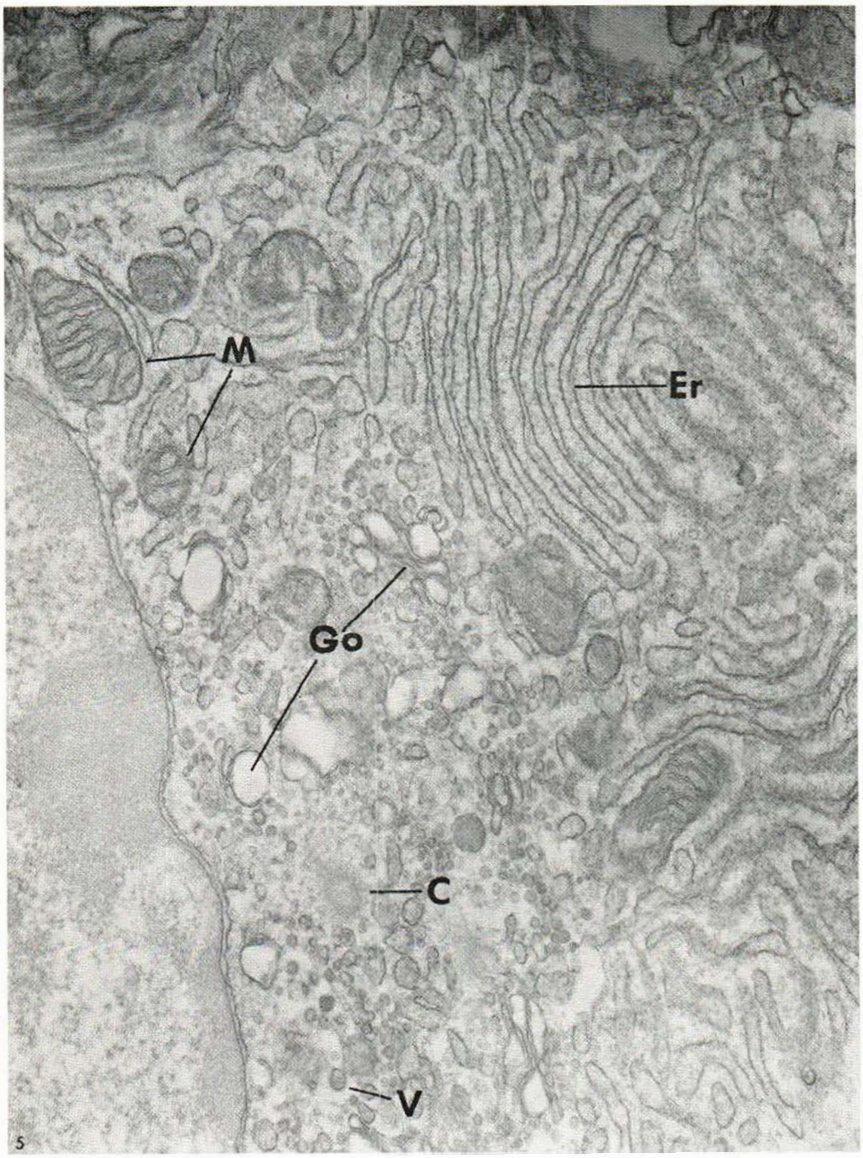

Fig. 5. Ultrastructural appearance of a mononuclear cell in the dermis of the inflamed red tattoo. The cytoplasm contains several channels of endoplasmic reticulum (*Er*), the surface of which is partially rough and partially smooth. The Golgi area is prominent (*Go*), and multiple smooth-surfaced vesicles are seen. Coated vesicles (*V*), mitochondria (*M*), and a centriole (*C*) are also present. Tissue was processed with glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate. × 26 300.

COMMENTS

Studies by electron microscopy and histochemistry revealed strikingly different findings in two ordinary red tattoos and in a tattoo complicated by a chronic inflammatory reaction. Histochemical processing of the inflamed red tattoo revealed mercury-containing densities similar in properties and location to those described after the topical application of mercuric chloride in individuals not having a severe clinical reaction to mercury (16, 18). The presence of mercury in the epidermis in the inflamed portion of the tattoo may represent extrusion of the material from the dermis by the inflammatory process. This correlates well with

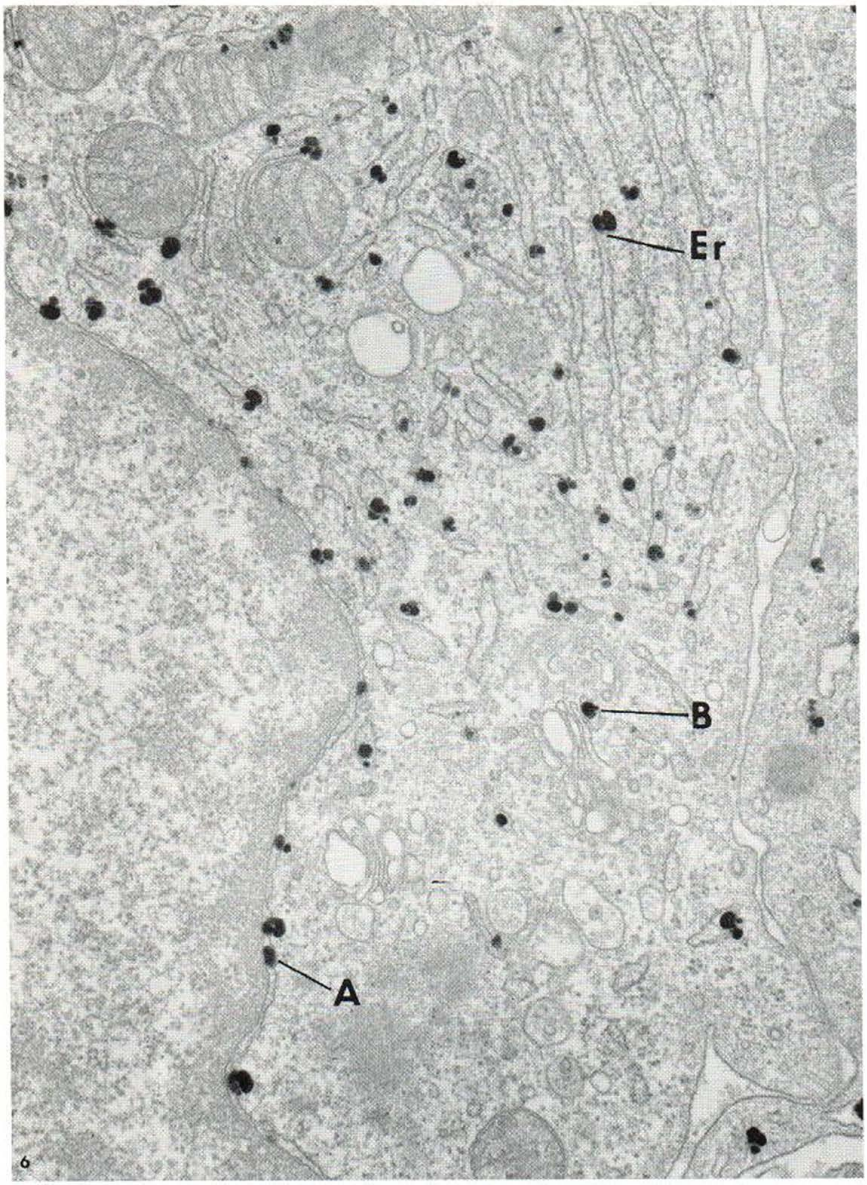

Fig. 6. Electron microscopic appearance of a mononuclear cell in the dermis of the inflamed red tattoo. This cell is similar to that seen in Fig. 5 except that this specimen was processed with glutaraldehyde, ammonium sulfide and osmium tetroxide. Note the striking change that is now seen. Histochemically demonstrable densities are present in the perinuclear space (*A*), in cisternae of endoplasmic reticulum (*Er*), and in smooth-surfaced membrane-bound inclusions (*B*). × 26 800.

the clinical history of gradual fading of the red portion of the tattoo over a period of years.

The reasons for assuming that mercury-containing densities are present in the inflamed tattoo are as follows: 1) Neutron activation analysis revealed mercury present in high amounts; 2) Positive staining was seen with gold chloride, which is a known stain for mercury at the light microscopic level, and has recently been modified by us for use at the electron microscopic level (18, 19); 3) Penicillamine is a known chelating agent for metals such as mercury. Penicillamine treatment

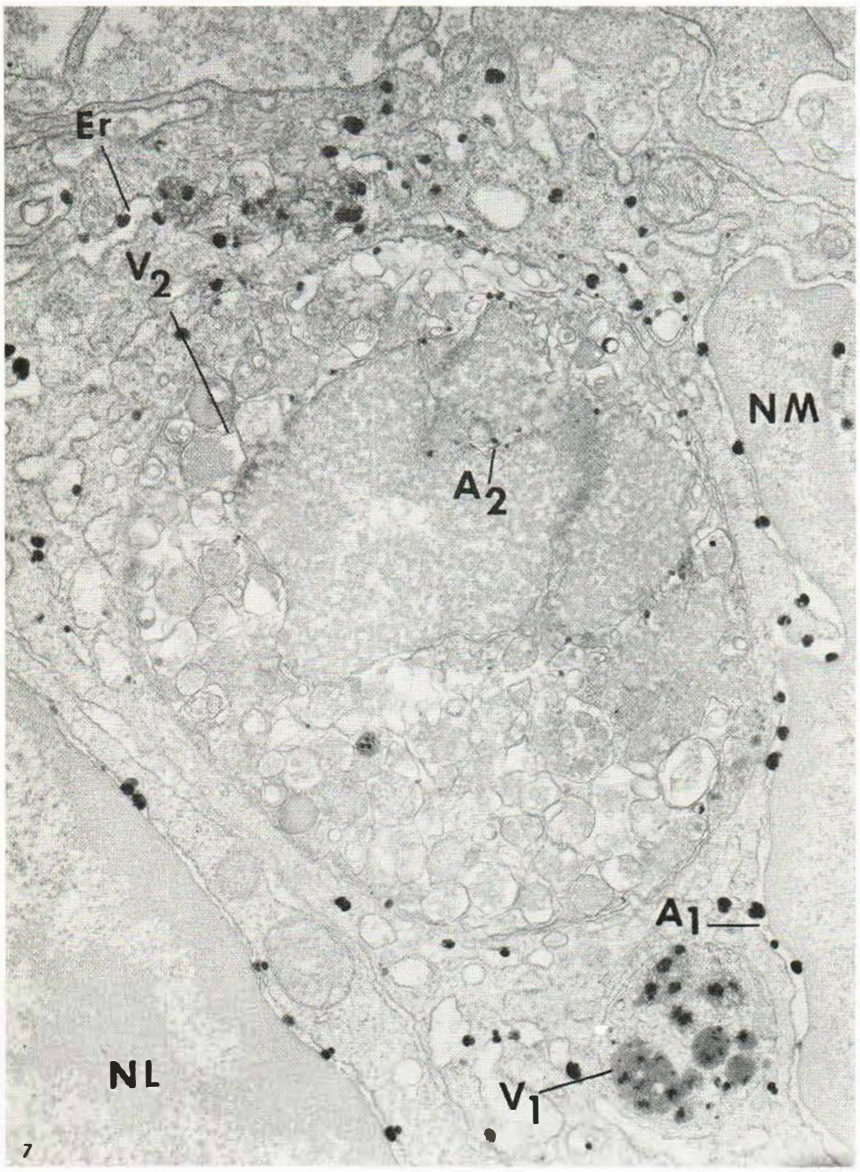

Fig. 7. Electron microscopic appearance of parts of two cells in the dermis of the inflamed red tattoo. The cell on the right resembling a macrophage (*NM*) has two phagocytic vacuoles in its cytoplasm (V_1V_2). V_1 contains amorphous debris, and V_2 contains part of a cell. Note the presence of densities in the prenuclear space (A_1) of the cell on the right, as well as the remainder of the perinuclear space (A_2) in the phagocytosed cell in V_2. These densities are also in channels of endoplasmic reticulum (*Er*) and associated with the amorphus material in V_2. The cell on the left (*NL*) has scant cytoplasm and resembles a lymphocyte. It also has histochemically demonstrable densities in the perinuclear space. × 21 200.

of sections prior to gold chloride staining resulted in blocking of this staining 4) Mercury was revealed by neutron activation analysis of the penicillamine extract used in blocking.

Our findings raise questions of the nature and significance of these densities. Not all the material within the densities is mercury, as evidenced by the speckled pattern of gold staining (Fig. 8). The chemical nature of the main portion of the densities is unknown, and we have defined them in this, as in previous studies, on the basis of their fixation requirements (17). In other studies of

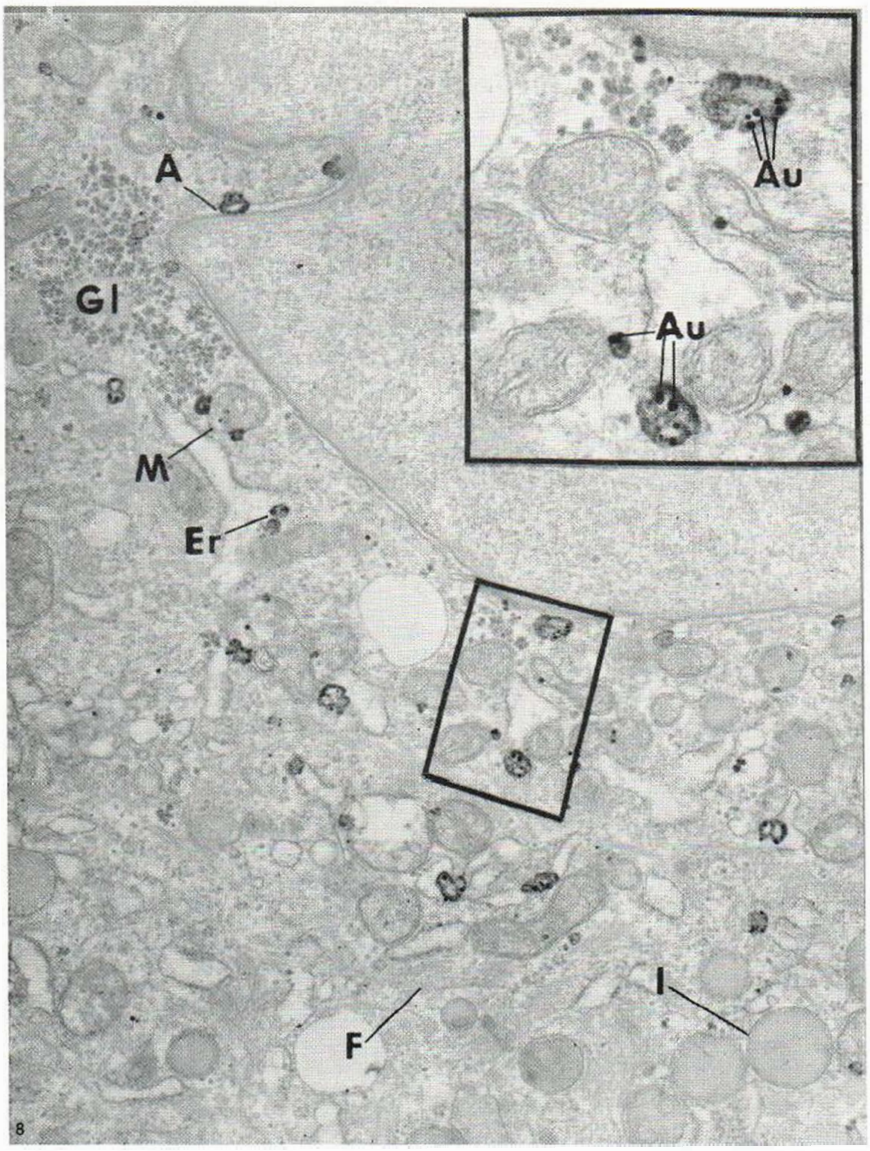

Fig. 8. Ultrastructural appearance of part of a keratinocyte in the epidermis of the inflamed red tattoo. The specimen was processed with glutaraldehyde, ammonium sulfide and osmium tetroxide. The section was then stained with gold chloride and counterstained with uranyl acetate and lead citrate. This cell differs from a keratinocyte in a normal red tattoo because of the following components: 1) accumulations of glycogen in the cytoplasm (Gl), 2) numerous membrane-bound inclusions with grayish deposits which resemble lipid (*I*), 3) numerous mitochondria (*M*), 4) scanty tonofilaments (*F*), and 5) histochemically demonstrable deposits which are stained with gold chloride particles (*Au*), best seen in the inset. These densities are present in the perinuclear space (*A*), associated with mitochondria (*M*), and with cisternae of endoplasmic reticulum (*Er*). × 26 400.

Inset: a higher power magnification of area demarcated in the rectangle showing the particulate, electron-dense deposits of gold (*Au*) upon the densities. × 59 500.

mercury in serum and tissues, close association with proteins or liproproteins has been shown. A substance called metallothionein has been isolated from the kidney and liver of humans and rats. This substance is produced by cells and is said to bind mercury (20). The location of the densities demonstrated in our study suggested they were also cell products. The densities were present in

perinuclear spaces and in cisternae of rough and smooth endoplasmic reticulum, which are known sites of synthesis and segregation of proteins (10, 5). Whether or not the densities demonstrated in our study are similar to metallothionein remains to be investigated.

The localization of the reaction to the red area of the tattoo suggested a possible etiologic association with mercury. The first mechanism considered was contact allergy. Others have proposed that certain pigment granules in tattoos, such as cinnabar, may dissociate into more soluble compounds which then act as haptens to induce an allergic response (3, 12, 13). Investigators have used patch tests to confirm the diagnosis of mercury sensitivity in tattoos. In most cases, patch tests to the more soluble and highly dissociated mercurials have been positive, but have been negative to cinnabar itself (1). There was no evidence for a contact allergic reaction in our patient, as all the patch tests to mercury compounds were negative. A dermal delayed (tuberculin-type) sensitivity was another possibility. Light microscopic findings of prominent dermal perivascular infiltrates composed largely of small lymphocytes with only minor epidermal changes is consistent with a cell-mediated type of allergic reaction (4). However, a dermal sensitivity could not be confirmed, as the patient refused intradermal skin testing.

The next mechanism considered was an indirect effect of mercury on cells through action on lymphocytes not involving an immune response. It has been shown that some forms of mercury act like phytohemagglutinin by stimulating lymphocytes in vitro to undergo blast transformation and mitosis, with the liberation of cytotoxic substances (14, 2). Perhaps in our patient the mercury present in the inflamed portion of the tattoo had stimulated cells to liberate cytotoxic substances such as could result in low-grade inflammation. In Fig. 7, cells or parts of cells containing mercury appear to have been phagocytosed. Such phagocytosis could result in protracted presence of mercury in the skin, which would thus prolong the chronic inflammatory reaction.

Other etiologies of the chronic inflammation in this patient's tattoo were investigated. A sarcoidal reaction and foreign body granuloma were considered clinically; however, light microscopic findings did not confirm these diagnoses.

In summary, we demonstrated the localization of mercury in three tattoos. In our case of a chronically inflamed red tattoo, mercury was present in locations other than those in the two uncomplicated red tattoos. The special histochemical techniques involving ammonium sulfide precipitation and gold chloride staining enabled us to visualize mercury-containing densities which would have been missed with routine processing of the tissue. We have thus far been limited in studying further cases of inflamed mercury-containing red tattoos because of the relative rarity of this condition. Further interpretation of the significance of our findings is dependent on the study of additional cases, including those with positive intracutaneous and/or patch tests to mercury compounds.

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