ELECTRON-MICROSCOPIC INVESTIGATION OF PENETRATION OF COPPER OXIDE AEROSOL FROM THE LUNGS INTO THE BLOOD AND INTERNAL ORGANS

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Passage of copper oxide particles through the air-blood barrier in the lumen of capillaries and their penetration into the ultrastructures of internal organs were studied in rats after inhalation of a copper oxide aerosol.

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During study of the pathogenesis of the pneumoconiosis resulting from inhalation of ultramicroscopic particles, it became necessary to determine the mechanism concerned in the penetration of particles into tissue ultrastructures and the times required. This is particularly important because no unanimity as yet exists in the literature on this problem. However, there is no doubt whatever about the harmful nature of ultramicroscopic dust particles capable of settling in the alveoli of the lungs [1, 4, 7, 9]. The discovery that dust particles can penetrate into different parts of the air-blood barrier has led experimental workers to seek an explanation of this fact. According to Alksne [6], displacement of colloidal mercury sulfide particles through the endothelial cells takes place by means of vesicles, the process being called cytopemsis, i.e., penetration through the cell. Later, Takahashi [10] observed the movement of ink particles, surrounded by a vacuole, from the endothelium to the lumen of the alveoli in periods of 30 min, 3.5 h, and 24 h. These workers consider that the movement of particles takes place actively, not passively, with the expenditure of energy. The opposite view is held by Heppleston [8], who considers that dust is transported through the alveolar wall by phagocytosis, and subsequently deposited in the ground substance of the connective tissue after destruction of the phagocytes. Salov and Borisenkova [5] regarded the appearance of dust particles up to 0.02 μ in diameter in the ground substance of the connective tissue as a manifestation of the selective function of the basement membrane of the alveolar epithelium.

No information could be found in the literature concerning electron-microscopic investigations of the pathogenesis of pneumoconiosis in welders.

The object of this investigation was to study penetration of finely dispersed industrial dust, namely welding fumes with a particle size below the resolving power of the optical microscope, by the use of the electron microscope.

EXPERIMENTAL METHOD

A condensation aerosol of copper oxide was obtained on FPP filters from wire containing the 100% chemically pure metal. Part of the dust was embedded in a mixture of methacrylates by Palade's method and studied in ultrathin sections. Two groups of albino rats inhaled the welding dust aerosol in a concentration of 50-80 mg/m^3. The duration of inhalation was 15, 30, 45, and 60 min for the rats of group 1 and 3 h for the rats of group 2. The animals of group 1 were sacrificed after 15, 30, 45, and 60 min, and those of group 2 after

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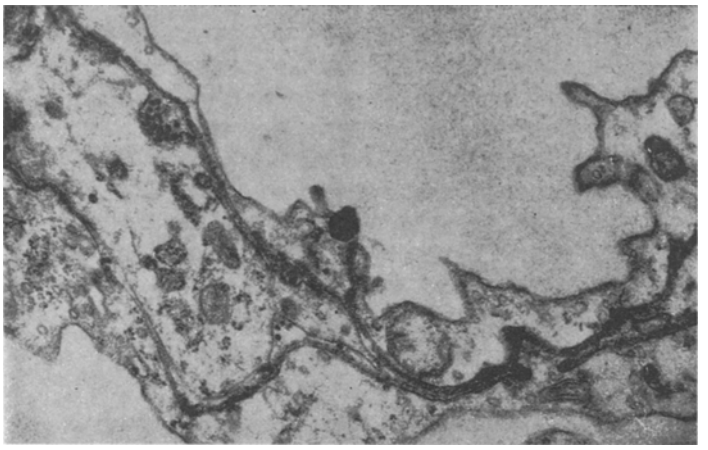

Fig. 1. Penetration of copper oxide crystal into superficial portions of cytoplasmic process of a small alveolar cell. Inhalation 15 min and sacrifice. 0.2 μ – 5 mm, 25,000 ×.

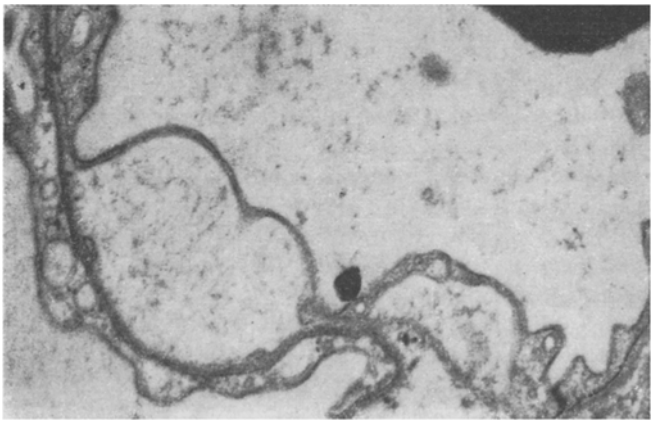

Fig. 2. Liberation of copper oxide crystal into capillary lumen. A pinocytotic vacuole can be seen at the site of penetration of the crystal into the matrix of the cytoplasmic process of an endothelial cell. Inhalation for 3 h and sacrifice. 0.2 μ – 5 mm, 25,000 ×.

0, 3, 6, 12, 18, and 24 h. Ultrathin sections from pieces of the lungs of the experimental animals, treated by Palade's method, were examined in the UÉMB-100 A electron microscope. Crystals of the condensation aerosol of copper oxide vary in diameter from 30 to 3500 Å, and their shape is that of a tetragonal prism with a square horizontal section. Particles from 30-250 Å are difficult to distinguish in sections, while crystals measuring more than 200 Å are fragments which are square or polygonal in shape.

EXPERIMENTAL METHOD

Inhalation of the condensation aerosol of copper oxide welding dust led ultimately to penetration of the particles through all sections of the air-blood barrier and to their introduction into the ultrastructure of the alveolar epithelial cells. After 15-60 min, single crystals and groups of crystals were found only in the ultrastructures of the air-blood barrier (Fig. 1). Whereas after 15 min dust particles had penetrated into the cytoplasm of the alveolar process, by 60 min they had penetrated through the general basement membrane and were found in the matrix of the endothelial cell process. The ground substance of the connective tissue, together with depolymerized basement membranes, merged in these areas with the matrix of the alveolar and endothelial cytoplasmic processes, containing numerous pinocytotic vesicles measuring 400-

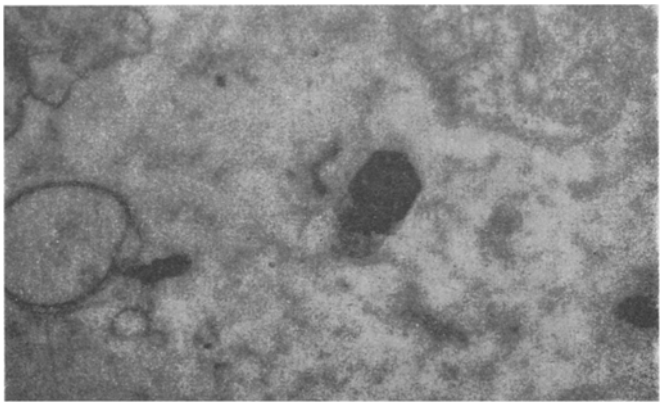

Fig. 3. Copper oxide crystals in blood plasma. Circular structures can be seen in the crystal matrix. Inhalation for 3 h, sacrifice 6 h after beginning of inhalation. 0.2–7 mm, 35,000 ×.

500 Å. In individual electron micrographs, contact between the crystal and pinocytotic vacuole could be clearly seen, but in others it could not, and the crystals were separated from the surrounding medium by a thin, loose membrane.

After 3 h, partial liberation of the crystals into the capillary lumen of the alveolar wall was observed (Fig. 2). At the site of penetration of the crystals, on the internal membrane of the endothelium, defects were frequently found, measuring from 500 to 1000 Å. The surface of the crystal buried in the cytoplasm of the endothelial process still carried a membrane, but the crystal fragment facing the capillary lumen now had clean edges.

Crystals of copper oxide were found after 6 h in the blood plasma (Fig. 3). Against the background of the amorphous matrix of the crystal, circular areas of translucency, up to 160 Å in diameter, with an electron-dense rim 40 Å in thickness, were seen. These structures were evidently the result of saturation of the intermolecular bonds of the crystal lattice with blood plasma. Investigation of the kidneys, for instance, revealed the presence of welding aerosol dust in the ultrastructures of the proximal division of the convoluted tubules. The crystals observed here were typical in shape, with circular areas of translucency in the matrix.

In inhalation experiments the crystals penetrated not only through the air-blood barrier into the lumen of the blood vessel, but also into ultrastructures of the alveolar cells and into the ground substance of the connective tissue of the alveolar septa. For example, copper oxide crystals and crystal complexes were found in the saccular dilatations of the endoplasmic reticulum of large alveolar cells, alongside or within the mitochondria. Well marked changes were found not only in the external membrane, at the sites of penetration of the particles, but also in the matrix and cristae.

After inhalation of a condensation aerosol of copper oxide dust by experimental animals for 3 h, crystals were thus observed to penetrate through all components of air-blood barrier. In addition, it was found that from 60 min to 24 h after inhalation, crystals are present in the ultrastructures of the alveolar cells and ground substance of the connective tissue of the alveolar septa. Migration of crystals of copper oxide aerosol from the alveoli into the lumen of capillaries in most cases takes place by cytopemsis. The possibility of other mechanisms of penetration of dust particles cannot be ruled out. In the course of the experiment changes take place due to disturbance of capillary permeability, leading to edema and swelling of the ultrastructures of the lung cells.

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