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Abstract

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PHAGOCYTIC PROPERTIES OF LUNG ALVEOLAR WALL CELLS

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Abstract: For the purpose to define the mechanism of heavy metal intoxication by inhalation, morphologic observations were made on rat lungs after nasal instillation of iron colloid particles of positive and negative electric charges. Histochemical observation was also made on the liver and spleen of these animals. The instilled iron colloid particles reach the alveolar cavity easily, as can be seen in the tissue sections stained by Prussian blue reaction. Alveolar macrophages do take up them avidly both of positive and negative charges, though much less the positive particles than negative ones. In contrast, the alveolar epithelial cells take up solely positive particles by phagocytosis but not negative ones. Electron microscope observation revealed that the positive particles are ingested by Type I epithelial cells by pinocytosis and by Type II cells by phagocytosis as well. Then the iron colloid particles are transferred into the basement membrane by exocytosis. Travelling through the basement membrane they are again taken up by capillary endothelial cells by phagocytosis. Some particles were found in the intercellular clefts of capillary endothelial cells but not any iron colloid particles in the intercellular spaces of epithelial cells and in the capillary lumen. However, the liver and spleen tissues of the animals given iron colloid showed a strong positive iron reaction. On the basis of these observations, the mechanism of acute intoxication by inhaling heavy metal dusts like lead fume is discussed from the view point of selective uptake of alveolar epithelial and capillary endothelial cells for the particles of the positive electric charge.

It is well known that the organic or inorganic foreign materials reaching the alveolar spaces are taken up by alveolar macrophages (1-3), and these particles are sent to the oral cavity through bronchi by the aid of ciliary movement or transferred into the interstitial tissues and lymphatics to be sent to the regional bronchial lymph nodes, as is well known in anthracosis by soot dust inhalation.

It is generally accepted, on the other hand, that the inhalation of heavy metal compounds causes acute heavy metal intoxication (4), suggesting that some of these inhaled foreign materials may directly pass through the alveolar walls and transferred into the general blood circulation. OGATA and associates observed the acute intoxication by lead fume inhalation on rats (5).

Observing the lung of these animals by electron microscopy the authors demonstrated that lead particles reach the lung capillary lumen in a mass through alveolar walls. This will explain the mechanism of acute intoxication by lead fume inhalation. SCHNEERBERGER-KEELEY and KARNOVSKY gave horse radish peroxidase to mice by intranasal instillation and found that a small amount of the protein was taken up by alveolar epithelial cells both of Type I and Type II, but no massive transfer of the protein into capillary lumen (6). CORRIN describes vivid uptake of colloidal particles of thorium dioxide by alveolar epithelial cells (7). And later some brief reports supporting his observation have been presented by LADMAN and FINLEY (8) and also by SUZUKI and his associates (9, 10). On the other hand, ESTERLY and FAULKNER reported that the alveolar epithelial cells have no phagocytic activity. The alveolar wall cells of rabbit lungs took up no india ink nor polystyrene spheres instilled into trachea (11). Such inconsistent results will be due to the difference of kinds of substances or particles given to animals, as the lung alveolar epithelia are expected to differ not so much in their function from animal to animal. According to the observations of mouse peritoneal macrophages and ascites tumor cells the phagocytosis of these cells is triggered by the adhesion of the particles to cell surface and the electric charge of the particles will act as an important factor for the adhesion (12, 13). As far as the particles of lead fume (5) and thorium dioxide (7) are concerned, they are of positive charge and the phagocytic ability of the alveolar wall cells for them has been clearly evidenced. Therefore, the positive electric charge of the particles may be related to the phagocytosis of these substances by the alveolar epithelium. From this view point the author observed the uptake of iron colloid particles of positive and negative charges by alveolar epithelial cells of rat. The present paper describes that positive-charged particles are solely taken up by alveolar wall cells and possibly transferred into circulating blood directly but not negative charged ones, suggesting that the inhalation of positive-charged colloid particles may induce acute intoxication if they are of toxic substances.

MATERIALS AND METHODS

Thirty-six Wistar strain albino rats weighing about 200–250 g, males and females, were used. They were divided into two groups, 24 animals in experiment and 12 animals as control. Animals for experiment were lightly anesthetized by inhaling ether and several droplets of iron colloid solution of positively or negatively charged particles, about 0.5 ml as total, were instilled intranasally. Five, 15 and 30 minutes later, animals, 8 animals in each observation, were sacrificed and small pieces of left lung, liver and spleen in each animal were fixed with 1.25% glutaraldehyde in Millonig's phosphate buffer

solution (pH 7.4) at 4°C for 3 hrs. For light microscopy these pieces of lung, liver and spleen were dehydrated through ethanol, embedded in paraffin, sectioned and stained by Prussian blue reaction with or without post-staining by KERNECHTROT (14). For electron microscopy of lung tissues the fixed tissues were postfixed with 1% OsO_4 for 2 hrs. (15, 16), dehydrated in graded series of ethanol and embedded in Epon by the conventional method (17). Thin sections were made with Porter-Blum MT-1 microtome and stained with lead citrate (18) and observed under electron microscope, Hitachi HU-11A. As controls, lung, liver and spleen of untreated healthy rats were fixed in the similar way and observed under the same conditions.

For preparation of iron colloid particles of positive charge, 5 ml of 1 M $FeCl_3$, was perfused into 60 ml of boiling distilled water. After cooling, the pH of the solution was adjusted to 6.5 with sodium cacodylate and made isotonic by adding glucose. The solutions were prepared just before use. As the negative charged iron colloid particles, the iron chondroitin sulphate for medical use, Dainippon Seiyaku, Osaka, was used. Before use the iron chondroitin sulphate was suspended in cacodylate buffer solution, pH 6.5, containing glucose at isotonic level. Final concentration of iron was 2 mg Fe per ml in all the solutions. The electric charges of the iron colloid particles in these solutions were determined by electrophoresis on cellulose acetate membrane, 0.6 mA/cm for 15 min., and observed by staining with Prussian blue reaction. Absorption tests of iron colloid particles to ion exchange resin of negative (IR-120B) and positive charges (IRA-401) were also made. The charges of the colloid particles are determined by staining the resin particles by Prussian blue reaction after exposing them to the iron colloid solution for 10 min. at room temperature and washing with distilled water.

RESULTS

The iron hydroxide colloid particles prepared with $FeCl_3$ solution were adsorbed on the IR-120B resin particles of negative charge after simply mixing them, as can be seen by staining with Prussian blue reaction but not adsorbed on IRA-401, positive charged particles, while chondroitin sulphate iron colloid particles were adsorbed on IRA-401 particles but not on IR-120B. Electrophoretic pictures of the iron colloid particles proved that the iron hydroxide particles moved toward cathode and the chondroitin sulphate iron colloid particles to anode (Fig. 1). The tests clearly showed that iron hydroxide particles prepared with $FeCl_3$ and suspended in cacodylate buffer solution of pH 6.5 were of positive charge, though they turned to negative charge in alkaline solution. The iron chondroitin sulphate colloid particles were originally of negative charge and the charge did not change in the buffer solution of pH 6.5.

In the animals sacrificed 30 min. after intranasal instillation of the negatively charged iron colloid particles the lung tissues were stained blue by

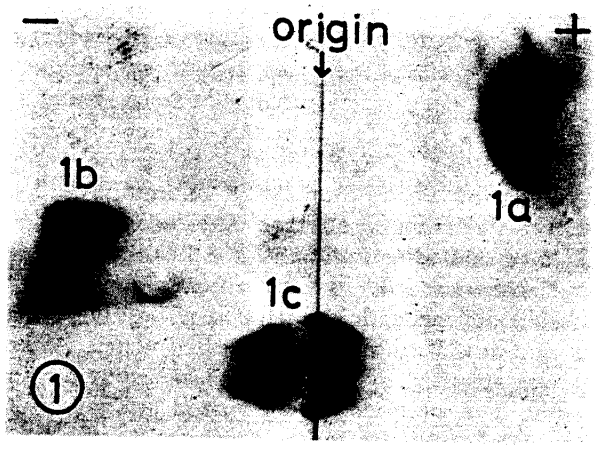


Fig. 1. Picture of electrophoresis of two kinds of iron colloid particles. The electrophoresis was performed with a cellulose acetate membrane (Separax, Jo Ko Sangyo Co., Ltd.) using 0.05 M cacodylate buffer (pH 6.5) under the condition of 0.6 mA/cm for 15 minutes. 1a; chondroitin sulphate iron colloid particles, negative charge, suspended in cacodylate buffer. 1b; iron colloid particles prepared by dispersing $FeCl_3$ solution in boiling water and suspended in cacodylate buffer, positive charge. 1c; rat serum for a reference. Staining: Prussian blue reaction for iron colloid particles and Ponsou 3R staining for protein.

Prussian blue reaction, while the lung of the control healthy animals gave no positive iron reaction. The light microscope observations of the tissues taken 30 min. after instillation of iron colloid particles of negative charge revealed the alveolar macrophages swollen and stained deep blue by Prussian blue reaction (Fig. 2), but no iron reaction in the alveolar wall cells, showing that the macrophages had phagocytic activity on negatively charged iron colloid particles but the alveolar epithelial cells had no such activity. The iron particles were trapped by alveolar macrophages as quickly as 5 minutes after the instillation.

The lung tissues of rats instilled the iron colloid particles of positive charge were also stained deep blue by Prussian blue reaction but light microscope observation revealed that the cells taking up colloid particles were essentially different from those reacting to the negative-charged iron colloid particles (Fig. 3). In the cases treated with positive charged colloidal iron the alveolar wall cells were stained deep blue by iron reaction, while alveolar macrophages appeared normal in size and gave only a slight Prussian blue reaction. The findings were nearly identical with the tissues obtained from the upper and lower lobes of the lung. Observations suggested that the alveolar epithelial cells take up positively charged colloidal particles but not

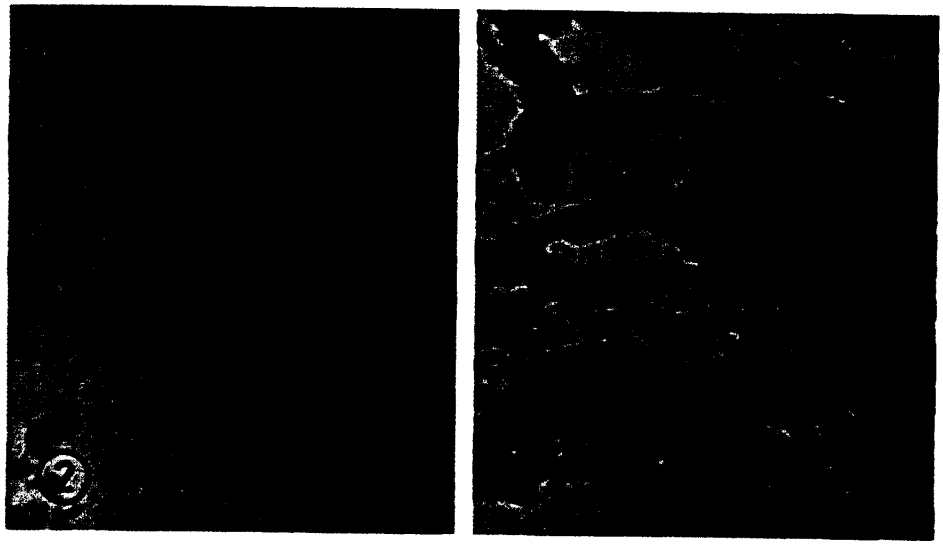


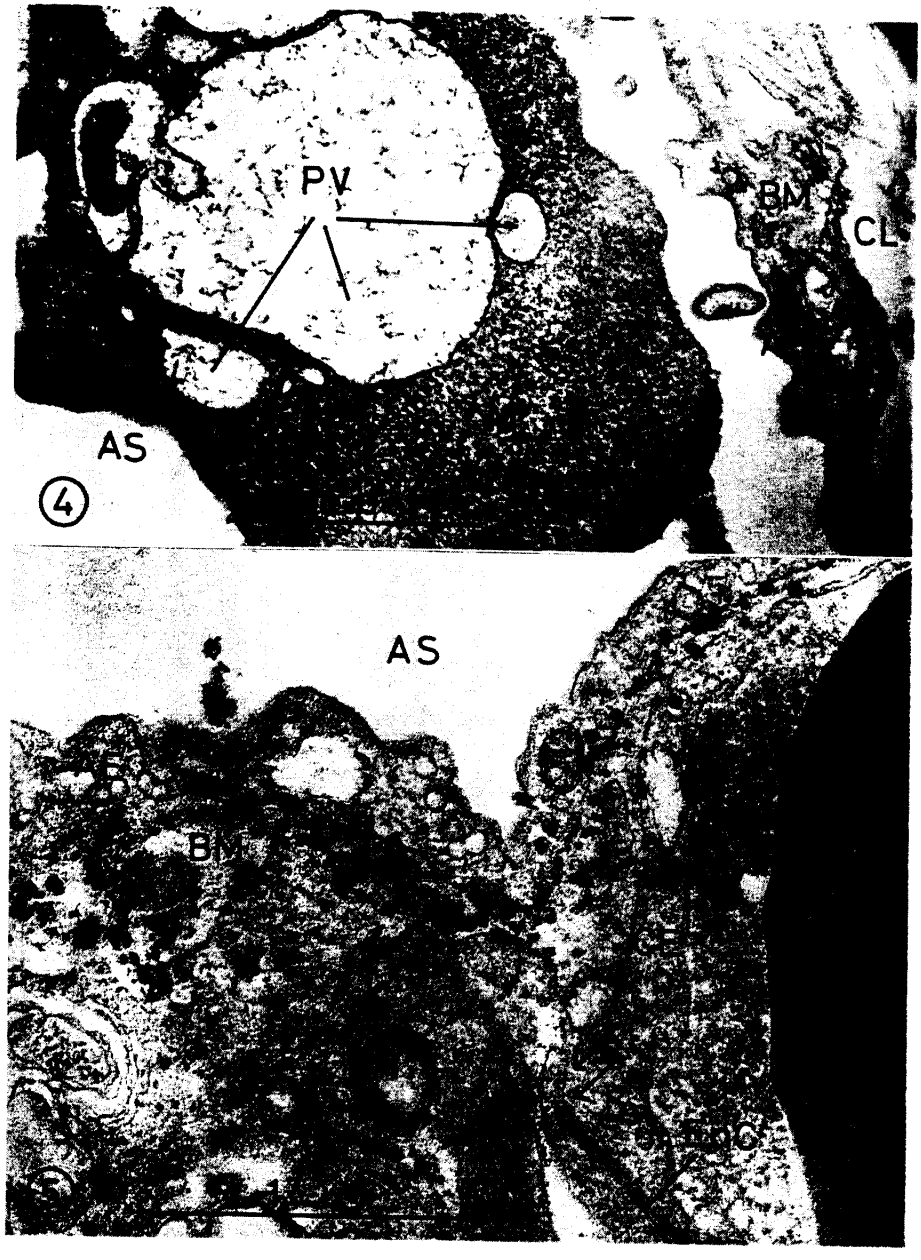
Fig. 2. A light micrograph of rat lung. The tissue was taken 30 min. after intranasal instillation of negative-charged iron colloid particles. The section was stained with Prussian blue reaction. Note that alveolar macrophages are strongly stained with the iron reaction but alveolar walls are hardly stained.

Fig. 3. A light micrograph of rat lung. The tissue was taken 30 min. after intranasal instillation of iron colloid particles of positive charge suspended in cacodylate buffer. The tissue sections were stained with Prussian blue reaction. Note that alveolar walls are strongly stained with the iron reaction, but only slightly that of alveolar macrophages.

negative ones, while alveolar macrophages phagocytise the particles of negative charge mainly, though they take up the positive charged ones to some extent.

Electron microscopy of the lung taken 30 min. after instillation of iron colloid particles of negative charge revealed electron dense granular and fibrous particles of 50 to 200 mμ in width accumulate in the phagocytic vesicles of alveolar macrophages but not in epithelial cells nor in capillary endothelial cells. Such electron dense substances were not observed in the lungs of untreated controls, consequently these electron dense substances had to be the instilled iron colloid particles (Fig. 4).

In the lungs taken 30 min. after instillation of positively charged iron particles, electron dense gross particles of 200 to 5, 000 mμ were found being distributed in the alveolar epithelial cells, basement membrane and capillary endothelial cells (Fig. 5). The similar electron dense particles were not seen in the lungs of untreated controls, therefore, these should be instilled iron colloid particles. The particles were found most dense in distribution in the


PV
BM
CL
AS
4
AS
BM

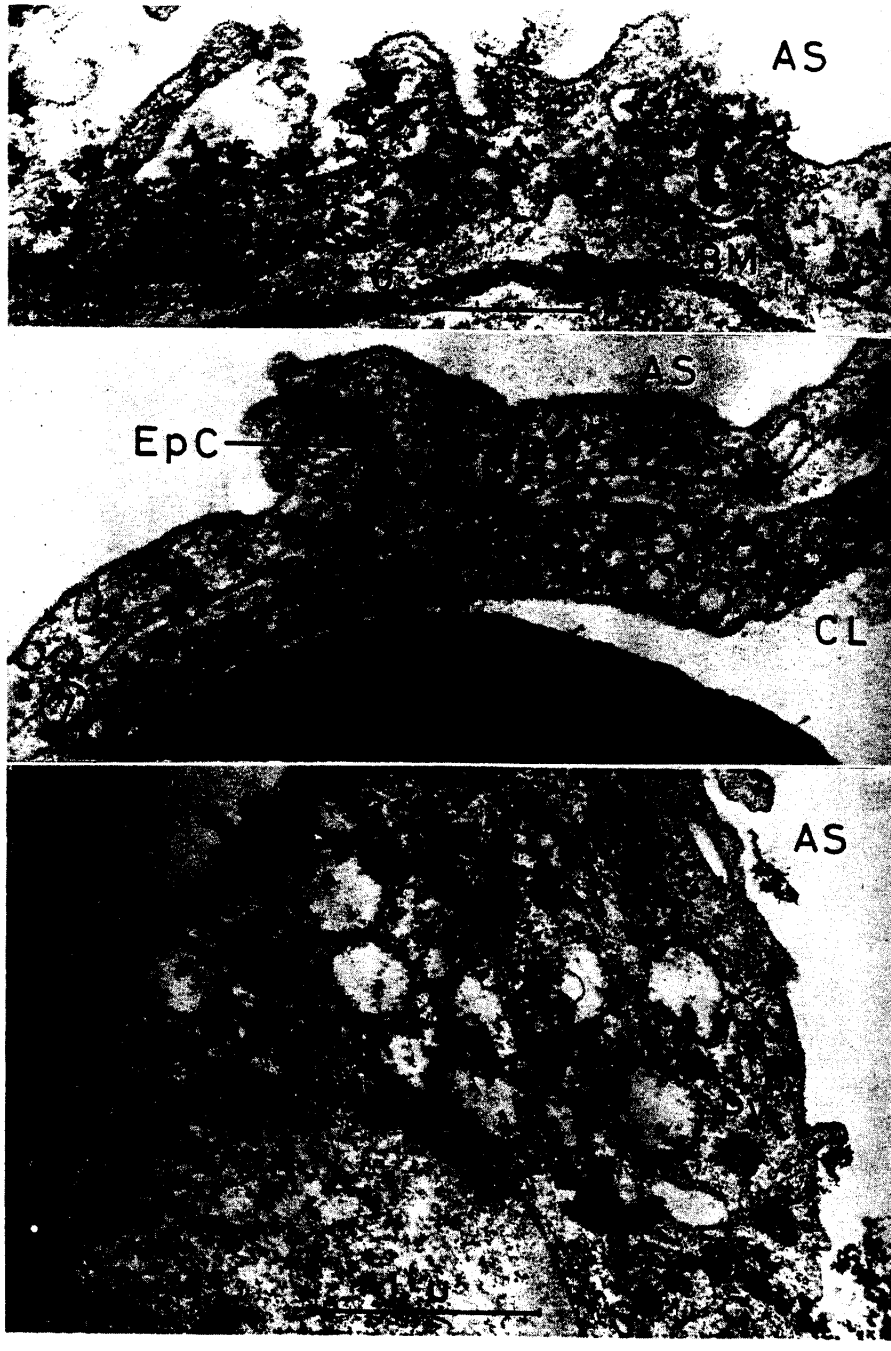
basement membrane (Figs. 5, 7), some in pinocytic vesicles of Type I alveolar epithelium and of capillary endothelial cells. A few granules were found in alveolar cavity but not in capillary lumen. In some epithelial cells the colloidal iron particles were found in their pinocytic vesicles (Figs. 5, 6) and also in exocytic vesicles toward basement membrane (Figs. 5-7). In the capillary endothelium the particles were also found in pinocytic vesicles toward the basement membrane, but not in the vesicles opening to capillary lumen. The findings suggested that the positively charged particles are taken up by Type I alveolar epithelial cells and then they are transferred to the basement membrane and taken by capillary endothelial cells. No pictures showing the iron colloid particles translocated into the capillary lumen by the exocytosis of the endothelial cells were encountered.

In the intercellular clefts between two epithelial cells no electron dense particles were observed (Fig. 7), indicating that the iron colloid particles could not reach the basement membrane through the clefts but the minute iron colloid particles lying in the intercellular spaces between capillary endothelial cells were frequently encountered (Fig. 5). This may indicate that iron particles reach the capillary lumen passing through the intercellular spaces of the capillary endothelial cells.

None of iron colloid particles was found in the capillary lumen but the liver and spleen sections showed strong, positive Prussian blue reaction. The findings seem to indicate that the iron of positive charge instilled was transferred into blood through alveolar walls, as the liver and spleen of control animals and those instilled negatively charged iron colloid particles gave only a slight or negative iron reaction. Iron colloid particles found in the phagocytic and pinocytic vesicles of alveolar epithelial, and capillary endothelial cells were rather fewer in number than those found in the basement membrane of alveolar walls (Figs. 5, 7). The pictures suggest that the positive-charged iron colloid particles pass through the cells fairly rapidly.

Fig. 4. An electron micrograph of the alveolar macrophage cytoplasm. Lung was taken 30 min. after instillation of iron colloid particles of negative charge. Fine fibrous electron dense particles are seen in the phagocytic vesicles of the alveolar macrophage but not in alveolar wall cells. AS; alveolar space, M; macrophage, PV; phagocytic vesicle, BM; basement membrane, AE; alveolar epithelial cell, CL; capillary lumen.

Fig. 5. An electron micrograph of the rat alveolar wall. Tissue was taken 30 min. after intranasal instillation of iron colloid particles of positive charge. Some electron dense iron colloid particles are seen in alveolar space (AS), and pinocytic vesicles of Type I alveolar epithelial cell (AE). Basement membrane (BM) has a mass of the particles. The capillary endothelial cells (CE) have also some particles and small granular particles are seen in the intercellular cleft (EnC) of the capillary endothelium (arrows). RBC; red blood cell, CL; capillary lumen.


AS
BM
AS
EpC
CL
AS

In the lungs taken 15 min. after instillation of positively charged iron particles, alveolar macrophages also took up the iron particles in their phagocytic vesicles (Fig. 8).

DISCUSSION

The present experiment on rats shows that iron colloid particles, both of negative and positive charges, reach in the alveolar cavity readily when they are given by nasal instillation. The iron colloid of negative charge is taken up solely by alveolar macrophages by phagocytosis but that of positive charge is mainly taken up by alveolar epithelial cells and some by macrophages. The particles of negative charge are not taken up by alveolar epithelial cells and do not reach the capillary endothelium, while the positively charged iron colloid particles are taken up by epithelial cells, transferred into the basement membrane by exocytosis of the epithelial cells and then taken up by capillary endothelial cells. They will be further transferred into circulating blood through intercellular spaces of capillary endothelium. Though the exocytic process of iron colloid particles by capillary endothelial cells was not observed, they will probably be translocated into capillary lumen. As reported in the previous paper, the inhaled lead fume particles are taken by epithelial cells, translocated into basement membrane and reach capillary lumen passing through the capillary endothelial cells by the exocytic mechanism (5).

The electrophoretic test indicated that the lead fume particles were of positive electric charge and probably lead oxide particles. Lead hydroxide colloid particles of negative charge instilled intranasally were solely taken by macrophages and did not enter into the capillary lumen (19). The phenomena were nearly the same as in the cases instilled with iron colloid particles of positive and negative charges. The difference observed is that in the case of lead fume inhalation the lead particles transferred into blood vessels were

Fig. 6. An electron micrograph of rat lung alveolar wall. The tissue was taken 30 min. after intranasal instillation of iron colloid particles of positive charge. Iron particles are adhered to Type I alveolar epithelial cell surface and some of these particles are also present in the pinocytic vesicles. Basement membrane (BM) has only a few particles.

Fig. 7. An electron micrograph of rat lung alveolar wall. The tissue is from the same rat as in Fig. 6. Some iron colloid particles of positive charge are present in pinocytic vesicles of alveolar epithelial cells, basement membrane and capillary endothelial cells. Note that particles are not present in epithelial intercellular cleft (EpC) and in capillary lumen.

Fig. 8. An electron micrograph of a lung alveolar macrophage. Taken 15 min. after intranasal instillation of positively charged iron colloid particles. Some electron dense iron colloid particles are present in alveolar space (AS), and also within phagocytic vacuoles (PV). N; nucleus, Mt; mitochondria.

found being adsorbed on the red cell surface which is negative in charge, but in the instillation of iron colloid particles of positive charge no particles were found on red cell surface. The difference may be due to the fact that the positive iron colloid particles undergo the change to negative in the alkaline solution higher than pH 7.0, while the lead particles do not undergo any change of the charge in a slight, alkaline solution like blood plasma.

SCHNEERBERGER-KEELEY and KARNOVSKY state that the intranasally instilled horse radish peroxidase is phagocytised by alveolar epithelial cells (5), and their observation may be explained by the positive charge of this basic protein. It remains still obscure why peroxidase does not reach basement membrane, differing from the cases of instilled iron colloid or inhaled lead particles of positive charge. But this might be due to the decomposition of the protein in phagocytic vesicles of the epithelial cells. The fact that thorium dioxide particles, which are of positive charge, are taken up by epithelial cells and transferred into the basement membrane (7), may be explained in a similar way and be understood as the general pathway of the positive charge particles. India ink (20, 21), carbon particles (7), and polystyrene spheres (11), which are of negative charge, are trapped by alveolar macrophages but not ingested by epithelial cells. Thus, it can be reasonably deduced that the positively charged materials are phagocytised mainly by alveolar wall cells and some of them reach capillary endothelial cells and then are transferred into circulating blood, but the negatively charged substances are trapped solely by macrophages and do not penetrate alveolar walls.

As just described, according to SENO and others (12) and YOKOMURA (13), the adhesion of some particles to cell surface, the initial step of phagocytosis, is largely concerned with the electric charge of the particles. Mouse peritoneal macrophages adsorb the particles of negative charge very actively and also ingest positive charged particles to some extent, while Ehrlich ascites tumor cells do take up selectively those of positive charge but never negative ones. Such a difference in phagocytic activity between these two kinds of cells may be attributable to the difference between general somatic cells and macrophages, the latter of which take up selectively the foreign bodies that are generally of negative charge. Acute intoxication by inhaling the heavy metal colloid particles like lead fume particles will be due to their positive charge by which they reach the general circulation passing through alveolar walls.

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