

UPTAKE AND TRANSFER OF PARTICULATE MATTER FROM THE PERITONEAL CAVITY OF THE RAT*

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PLATE 39

INTRODUCTION

I wish to report some preliminary observations on the uptake by mesothelial cells of colloidal particles of mercuric sulfide or thorium dioxide from the peritoneal cavity. The general question of the manner by which such particles may enter the cytoplasm of any cell type is of basic interest. There is also the specific question of the pathway of absorption of granules from the peritoneal cavity through the mesothelial layer of the serosa. Divergent ideas concerning the latter point exist in the literature. Some investigators have reported the presence of preformed stomata between the mesothelial cells, through which particles of varying sizes, such as India ink, red blood cells, and leucocytes, might travel (1, 2, 14). Others have described the movement of particles through the intercellular spaces between adjacent mesothelial cells, particularly those of the diaphragm (3, 10, 11, 12, 16). Evidence for an intracellular localization of transferred particles (7, 9, 11, 15), or of localization and transport of particles into and through the mesothelial cytoplasm (5, 6) has also been presented. Information about absorption from the peritoneal cavity has been reviewed in considerable detail recently by Courtice and Simmonds (4).

Materials and Methods

Adult female rats of the Wistar strain were injected intraperitoneally with 0.2 cc. of a commercially prepared 2 per cent colloidal mercuric sulfide solution or 24 to 25 per cent colloidal thorium dioxide solution. The animals were perfused with a 1 per cent solution of buffered osmic acid at intervals between 15 minutes to 12½ hours after the injection. Pieces of diaphragm and of the mesentery were fixed by immersion in either 1 per cent or 0.75 per cent buffered osmic acid, with potassium chloride added, for 2 to 4 hours. They were dehydrated in methyl alcohol and embedded in a mixture of 9 parts *n*-butyl methacrylate and 1 part methyl methacrylate. Thin sections of the tissues were examined with a RCA EMU-2C electron microscope.

OBSERVATIONS

At intervals between 15 minutes and 5 hours after the injection it was noted that some of the particles of the administered material were adherent to the

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surfaces of the microvilli and plasma membrane of the mesothelium (Figs. 1 and 2). Occasionally at 3 hours, or later, macroscopically visible masses, consisting of clumps of particles and phagocytic cells, were attached to the cell surface. At 12½ hours after the injection particles were no longer aligned on the mesothelial microvilli and plasma membrane (Figs. 3 and 4).

Fifteen to 30 minutes after injecting the material some of the granular matter lay within the cytoplasm of the mesothelium. Often it was situated in small vacuoles near the plasma membrane (Figs. 1 and 2). It was also present in larger vacuoles, apparently distributed at random in the cytoplasm. Occasionally particles appeared attached to minute invaginations of the plasma membrane (Fig. 1). After ½ hour macrophages of the subjacent connective tissue likewise contained some particulate matter segregated in cytoplasmic vacuoles. As the time between injection and killing of the animals was increased greater numbers of granules became localized within the mesothelial cytoplasm. These particles were situated either in clear vacuoles (Fig. 2), of widely varying sizes, or in bodies having a relatively dense matrix (Figs. 3 and 4). The intracellular mercuric sulfide particles measured from 50 to 220 A. The upper limit of size is difficult to determine since some particles may be so closely aggregated as to give the appearance of singleness. In cases where the limiting membrane of the vacuole or dense body containing the particles could be adequately visualized it appeared smooth, having no particles such as those associated with ergastoplasmic membranes. No evidence of mitochondrial structure has been noted in the particle-containing bodies.

Particulate matter has not yet been noted within the intercellular spaces of the mesothelium of either the mesentery or diaphragm. However, due to the proportionally small area of such spaces the probability of detecting such a passage in thin sections is considerably less than that of observing particles within the cytoplasm. At the time intervals studied, the particles were found more abundantly within the mesothelial cells of the diaphragm than within those of the mesentery.

DISCUSSION

The preliminary observations reported here indicate that an appreciable quantity of particulate matter injected into the peritoneal cavity passes into, and presumably, through the cytoplasm of the mesothelium, rather than between the cells and hence directly into the subjacent connective tissue. The fact that there is a rapid transport of granules through the diaphragmatic mesothelium and into the lymphatics has been established by other investigators. Some particles may appear in the lymph of the diaphragm as soon as 2 to 3 minutes after an intraperitoneal injection (8). The question of the pathway followed by the particles across the mesothelial layer has not been conclusively answered by past workers or by the present study. The results reported here indicate that much of the material enters the mesothelial cytoplasm. A

tentative working hypothesis of one manner by which such absorption may occur can be postulated from these observations. The particles, possibly coated with protein, become attached to the plasma membrane. Minute invaginations of the membrane, with the adherent granules, develop. The invaginated membrane is pinched off, resulting in the formation of an intracellular vacuole. Possibly a type of micropinocytosis takes place. At first the vacuole may lie near the cell surface, but later may assume any position in the cytoplasm and may coalesce with other vacuoles. Some of the granules come to lie within bodies having a matrix of relatively greater density than that of the vacuoles. This matrix may be deposited in the clear type of vacuole or these dense bodies may be of a different nature. The individual particles may be transferred then from the basal layer of the cytoplasm into the underlying connective tissue by means of minute vesicles or vacuoles similar to those reported in the endothelium (13).

SUMMARY

1. Colloidal mercuric sulfide or thorium dioxide injected intraperitoneally passes into the cytoplasm of the mesothelium of the mesentery and of the diaphragm as early as 15 to 30 minutes after the injection.
2. Between 15 minutes and 12½ hours the number of particles within the mesothelial cells increases as the time between injection and termination of the experiment is lengthened.
3. The particulate matter is usually localized in the cytoplasm within clear vacuoles or bodies having a relatively dense matrix.
4. A greater quantity of the absorbed material is commonly observed within the cytoplasm of the diaphragmatic than of the mesenteric mesothelium.

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EXPLANATION OF PLATE 39

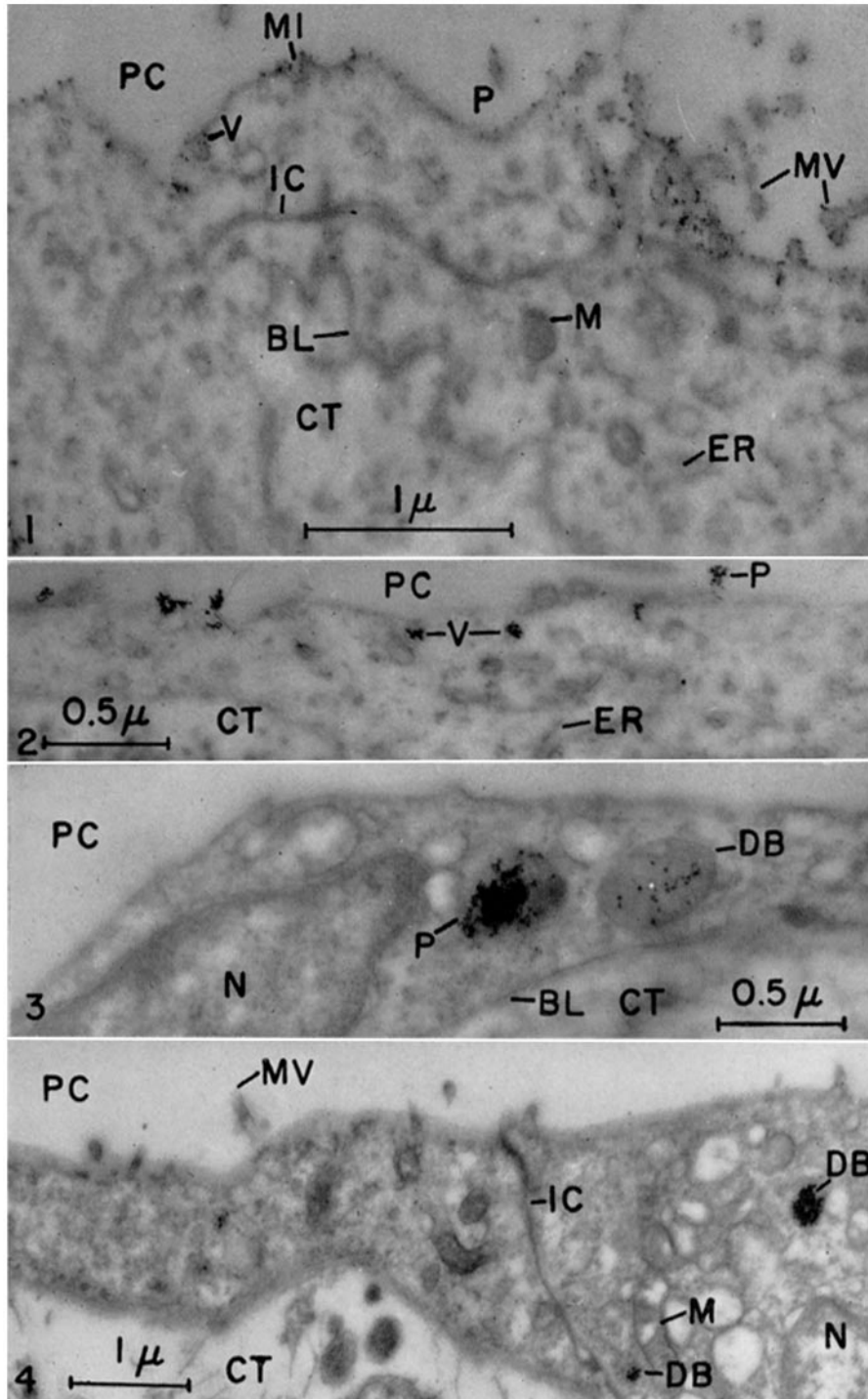
<i>BL</i> , basal lamina.	<i>MI</i> , membrane invagination.
<i>CT</i> , connective tissue.	<i>MV</i> , microvillus.
<i>DB</i> , dense bodies containing particles.	<i>N</i> , nucleus.
<i>ER</i> , endoplasmic reticulum.	<i>P</i> , colloidal particles.
<i>IC</i> , intercellular boundary.	<i>PC</i> , peritoneal cavity.
<i>M</i> , mitochondrion.	<i>V</i> , vacuole.

FIG. 1. Rat killed 15 minutes after intraperitoneal injection of thorium dioxide. The colloidal particles (*P*) are aligned on the plasma membrane and surfaces of the microvilli (*MV*). Some of the granules lie within intracellular vacuoles (*V*) which either abut upon, or, are closely adjacent to the cell surface; others are within membrane invaginations (*MI*). $\times 27,670$.

FIG. 2. Animal killed $1\frac{3}{4}$ hours after intraperitoneal injection of thorium dioxide. Note the granules situated in small vacuoles (*V*) lying near the surface membrane. Some of the particles (*P*) are clumped on the surface of the cell. $\times 33,900$.

FIG. 3. Animal killed $12\frac{1}{2}$ hours after intraperitoneal injection of a colloidal mercuric sulfide solution. The injected particles are localized within bodies (*DB*) having a dense matrix. $\times 33,900$.

FIG. 4. Tissue from same rat as that in Fig. 3. The mercuric sulfide particles are located within bodies (*DB*) having a relatively dense matrix, and surrounded by a membrane. $\times 15,910$.



(Odor: Particulate matter from peritoneal cavity of rat)