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Electronmicroscopic Studies on the Peritoneal Resorption of Intraperitoneally Injected Latex Particles via the Diaphragmatic Lymphatics

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Summary

Intraperitoneally injected latex particles with a diameter of 1,1 μ are resorbed via the subperitoneal diaphragmatic lymphatics.The latex particles enter the lymphatics by stomata. These are formed by simultaneous intracellular gaps between neighbouring mesothelial and endothelial cells in the tissue barrier, between the lumen of the lymph vessels and the peritoneal cavity. A transcellular passage (cytopempsis) does not occur. Stomata occur where collagenous fibres and a submesothelial basement membrane are absent. Due to the complete absence of collagenous fibres in the area of the stromata particles smaller than the diameter of the stomata may be resorbed easily.

Introduction

Intraperitoneally injected dyes, Indian ink, and avian red cells are resorbed via the subperitoneal diaphragmatic lymphatics 1–3, 5, 7–12, 13–16). The resorption takes place in the tissue barrier between the lumina of dilated lymphatics (lacunae) and the peritoneal cavity (1), described as "roofs of the lacunae" (7).
Electronmicroscopic studies following intraperitoneal injection of Indian ink (3, 4, 7, 17) have shown that the roof of the lacunae consists of a layer of mesothelial cells, a sebmesothelial basement membrane, collagenous fibres and an endothelial cell layer of the lymphatics.
Carbon particles pass the mesothelial and endothelial cell barrier through intercellular gaps, but they are also phagocytized and transported by means of pinocytosis (transcellular passage) (10)..
According to several authors (4, 7, 17) the layer of collagenous fibres is incomplete, with holes in the area of the stomata.
This submesothelial network of fibres is considered to limit the size of the particles which can be resorbed after intraperitoneal injection (7).
The literature contains different opinion about the existence of a basement membrane in the roof of the lacunae. Casley-Smith (4) considered the basement to be "poorly defined", French et al (7) found it to be incomplete; and Kotani et al (10) stated that in the area of the stomata usually no basement membrane is found.
The present investigations with latex particles (diameter 1,1 μ) were determined to elucidate the following questions:

1. Is the resorption of latex particles due to transcellular and/or intercellular mechanisms, although they are much larger than carbon particles?
2. Can a principal structure of the roof of the lacune be found, especially in view of the existence of a basement membrane and of the distribution of the submesothelial collagenous fibres?
3. Which is the limiting factor for the size of the stomata?

Material and Methods

Two male and two female Wistar rats were injected intraperitoneally with 2 ml of a suspension of latex particles with a diameter of 1,1 μ. Twenty minutes to four hours following the injection the diaphragm was removed and fixed in phosphate buffered glutaraldehyd. Tissue specimens were post-fixed in OsO_4, dehydrated and embedded in araldite. Ultrathin sections, obtained by means of an Ultrotome III LKB, were stained with uranyl acetate and lead citrate and examined with the Zeiss Elmiskope E M 9- S2.

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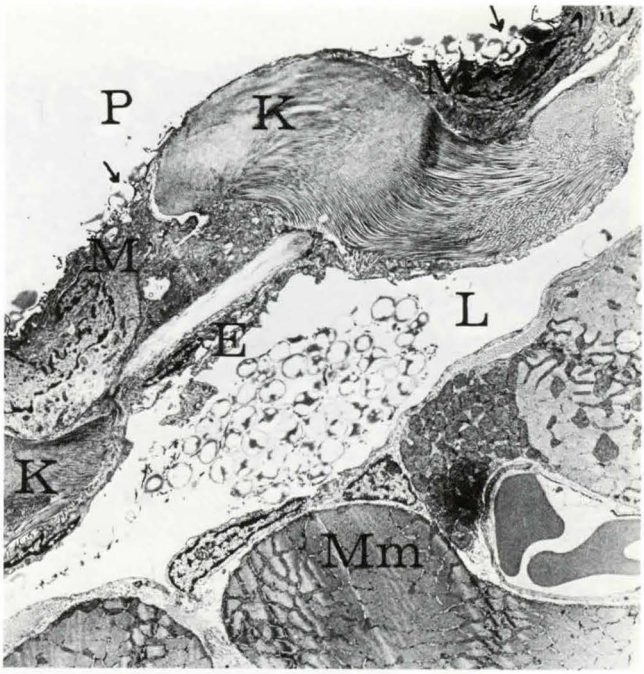

Fig. 1. General view of a subperitoneal diaphragmatic lymph vessel (L) The tissue between its lumen and the peritoneal cavity (P) consists of mesothelial cells (M), submesothelial collagenous fibres (K) and endothelial cells (E). There ist no possibility of passage for latex particles through this compact barrier. Phagocytosis of latex particles by mesothelial cells is shown (→). (Mm = diaphragmatic muscle bundles). x 2350

Results

The lumina of the subperitoneal diaphragmatic lymphatics and the peritoneal cavity (Fig. 1) are separated by a tissue barrier consisting of mesothelial cells, submesothelial collagenous fibres and a rather thin endothelial cell layer.

The cytoplasma of the mesothelial cells, abundant in the area of the nucleus, is very thin at the margins. These cells have many microvilli on the surface. The mesothelial cells phagocytize the injected latex particles. In areas with a complete and thick layer of collagenous fibres, however, latex particles are not able to pass the tissue barrier because of its compactness.

Although the mesothelial cells are able to phagocytize many latex particles the entry of these particles into the submesothelial space could never be seen (Fig. 2). Outside the stomata (Fig. 4) latex particles were never found in the submesothelial region as would be the case if there were transcellular transport (cytopempsis).

Latex particles are also phagocytized by endothelial cells. But in these cells they are also situated near the lumen, and the uptake of latex particles from the submesothelial space was never seen. Therefore it may be concluded that latex particles which have been resorbed via the stomata into the lymphatics, are only phagocytized by the endothelial cells secondarily. Thus the phagocytosis of the latex particles by the mesothelial and endothelial cells is no evidence of transcellular transport.

In other areas of the lacunae (Fig. 3) collagenous fibres are confirmed in bundles. Between them the tissue barrier is especially thin. Only in such areas and only beneath junctions between neighbouring mesothelial cells are an accumulation of latex particles observed as a result of a transport. Wide intercellular gaps between neighbouring endothelial cells are passed by latex particles. The lumen of the lacune is separated from the peritoneal cavity only by thin cytoplasm processes of neighbouring mesothelial cells because in these areas a basement membrane is also absent. On the other hand, outside the stomata a continous basement membrane may be identified.

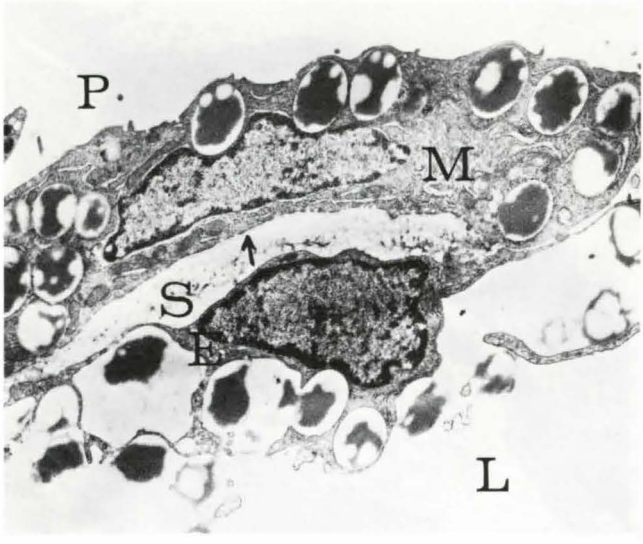

Fig. 2. Latex particles are phagocytized by mesothelial (M) and endothelial (E) cells. The submesothelial space (S) is free of latex particles. The basement membrane (↗) is just visible. (P = peritoneal cavity, L = Lacune). x 7350

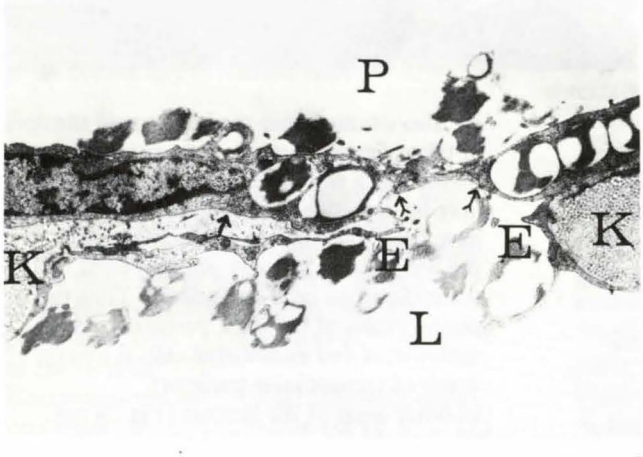

Fig. 3. Roof of a lacuna (L). The collagenous fibres (K) are in compact bundles. There is a wide intercellular gap between neighbouring endothelial cells (E), with an accumulation of latex particles. Due to the absence of a basement membrane in this area the roof consists only of thin mesothelial cells (↕ = intracellular junctions). Outside the stomata a continous basement membrane is found (↗). (P = pertioneal cavity). x 7000

Complete stomata only develop when the mesothelial cells also form wide intercellular gaps (Fig. 4). Here a free communication between the peritoneal cavity and the lumen of the lacune is produced. Although this passes a massive accumulation of latex particles.

In many ultrathin sections of the roofs of the lacunae the following principal structure could be found:

The roof of the lacune consists of a mesothelial cell layer, an underlying basement membrane, a layer of collagenous fibres of different thickness, and an endothelial cell layer. Outside the lacunae the collagenous fibres form a compact tissue barrier. They are almost periodically arranged, however, in the roofs of the lacunae. Where the bundles of collagenous fibres are situated directly beneath the mesothelial intercellular spaces, they consolidate the roof, and stomata cannot occur. In areas where no collagenous fibres are found beneath the intercellular spaces, wide intercellular gaps between neighbouring mesothelial and endothelial cells are formed producing a free communication between the peritoneal cavity and the lymphatics (stomata).

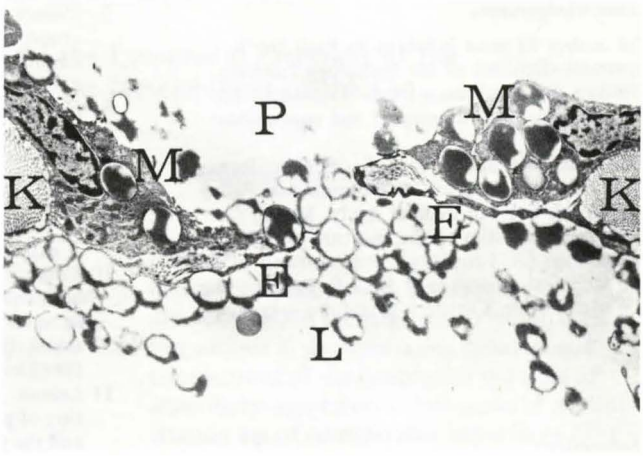

Fig. 4. Stoma between neighbouring mesothelial (M) and endothelial (E) cells, without a basement membrane and collagenous fibres. Compact bundles of collagenous fibres (K) lie around the stoma. There is a free communication between peritoneal cavity (P) and the lacune (L).
x 5750

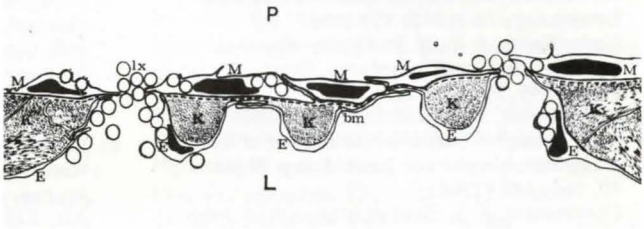

Fig. 5. Principal structure of the roof of the lacune (L): Compact bundles of collagenous fibres (K). Stomata, filled with latex particles (1x) formed by separated mesothelial (M) and endothelial (E) cells, in areas where collagenous fibres and a basement membrane (- - -) are absent. Phagocytosis of latex particles by mesothelial and endothelial cells. No transcellular transport.

Discussion

Whereas carbon particles may cross the mesothelial cell layer through intercellular gaps and by means of cytopempsis (transcellular transport) (10), latex particles with a diameter of 1,1 μ enter the subperitoneal diaphragmatic lymphatics exclusively via the stomata, although many of them are phagocytized – especially in the vicinity of the stomata. Considering how easily a large number of carbon particles (with a diameter of 0,05–0,065 μ) passes from the stomata into the lacunae, it is obvious that the transcellular transport even for carbon particles is quantitatively not important.
In the literature there are differing opinions concerning the existence of a basement membrane in the area of the lacunae (4, 7, 10). From our studies it can be concluded, however, that in the area of a stoma collagenous fibres and a basement membrane which might impede the process of resorption are completely absent. Therefore the particles, passing through the stomata, do not have to cross a submesothelial network of collagenous fibres. Thus the limiting factor for the size of the particles which are able to be resorbed, is the distance between the separated mesothelial and endothelial cells. It may be possible that the stomata can be stretched during the passage of some bigger particles.
The lacunae stomata can only occur in areas where collagenous fibres and a basement membrane are absent. Thus the location of the stomata is structurally determined and, therefore, they always occur at the same place. Tactile stimuli may play a role in their formation (6).

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References

1 *Allen, L.:* The peritoneal stomata. Anat. Rec. 67, 89–99 (1936)
2 *Bettendorf, U.:* Lymph flow mechanism of the subperitoneal diaphgragmatic lymphatics. Lymphology 11 (1978) 111–116
3 *Casley-Smith, J. R., H. W. Florey:* The structure of normal small lymphatics. Quart. J. exp. Physiol. 46, 101–106 (1961)
4 *Casley-Smith, J. R.:* Endothelial permeability – the passage of particles into and out of diaphragmatic lymphatics. Quart. J. exp. Physiol. 49, 365–383 (1964)
5 *Cunningham, R. S.:* Studies in absorption from serous cavities. IV. On the passage of blood cells and granules of different sizes through the walls of the lymphatics in the diaphragm. Amer. J. Physiol. 62, 448–452 (1922)
6 *Forteza-Vila, J., W. Mohr, T. Ricker, G. Beneke:* Raster-elektronenmikroskopische Untersuchungen am Mesothel bei einer experimentellen Peritonitis. Virchows Arch. Abt. B 8, 225–229 (1971)
7 *French, J. E., H. W. Florey, B. Morris:* The absorption of particles by the lymphatics of the diaphragm. Quart. J. exp. Physiol. 45, 88–103 (1960)
8 *Habu, K.:* Studies on the distribution of lymphatic vessels in the diaphragm and absorption of carbon particles from the peritoneal cavity into these vessels. J. Kumamoto Med. Soc. 45, 964–984 (1971)
9 *Hahn, P. F., L. L. Miller, F. S. Robscheit-Robbins, W. F. Bale, G. H. Whipple:* Peritoneal Absorption. J. exp. Med. 80, 77–82 (1944)
10 *Kotani, M., M. R. Nakao, S. Nakao:* Observations with the electron microscope on the absorption of india ink from the peritoneal cavity of the rabbit. Okajimas Folia anat. jap. 38, 149–173 (1962)
11 *Lemon, W. S., G. M. Higgins:* Lymphatic absorption of particulate matter through the normal and the paralyzed diaphragm: an experimental study. Amer. J. med. Sc. 178, 536–547 (1929)
12 *MacCallum, W. G.:* On the mechanism of absorption of granular materials from the peritoneum. Bull. Johns Hopk. Hosp. 14, 105–115 (1903)
13 *Morris, B.:* The effect of diaphragmatic movement on the absorption of protein and of red cells from the peritoneal cavitiy. Aust. J. exp. Biol. med. Sci. 31, 239–246 (1953)
14 *Recklingshausen, F. v.:* Zur Fettresorption. Virchows Arch. Path. Anat. 26, 172–208 (1863)
15 *Remmele, W., H. Chr. Brodersen:* Umfang und Wege der Resorption homologer Erythrozyten aus dem Bauchraum. Z. ges. exp. Med. 141, 201–218 (1966)
16 *Wegner, G.:* Chirurgische Bemerkungen über die Peritonealhöhle, mit besonderer Berücksichtigung der Ovarietomie. Langenbecks Arch. klin. Chir. 20, 51–145 (1877)
17 *Yamagishi, T.:* Fine structure of lypmh vessels in the abdominal wall of the diaphragm of dogs and their attidude following absorption of china ink. Nagoya med. J. 7, 1–6 (1961)

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