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LYMPHATICS AND PRE-LYMPHATICS OF THE RABBIT PERICARDIUM AND EPICARDIUM WITH SPECIAL EMPHASIS ON PARTICULATE ABSORPTION AND MILKY SPOT-LIKE STRUCTURES

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ABSTRACT

The lymphatics and pre-lymphatic connective tissue of rabbit pericardium and epicardium were examined by light and electron microscopy under normal conditions and after the injection of India ink and latex particles into the pericardial cavity. A characteristic lattice structure of connective tissue was present between the small mesothelial cells and the submesothelial lymphatic capillaries in the basal region of the pericardium, but not in the epicardium. Milky spot-like structures bulging toward the pericardial cavity were found in the pericardium, similar to those in the omentum and mediastinal pleura. Within 60 minutes after injection, carbon and latex particles were directly absorbed through the intercellular clefts of the adjacent small mesothelial cells into the submesothelial layer particularly at sites of characteristic lattice structure. Carbon particles were already present in the lumens of lymphatic capillaries at this time. Macrophages in the pericardial cavity and submesothelial layers of the pericardium engulfed both carbon and latex particles. Our results suggest two possible routes of drainage of particulate matter from the pericardial cavity into the lymphatics: direct absorption and indirect absorption after phagocytosis by macrophages. Macrophages probably migrate from the milky spot-like structures described in this study. Epicardial lymphatics, in contrast, drain tissue fluid primarily from the myocardium.

It is generally accepted that tissue fluids and particulate matter in serous cavities are partially absorbed into the lymphatics (1,2). Kihara and his co-workers demonstrated by light microscopy that India ink introduced into a serous cavity was absorbed into the lymphatics freely through the characteristic lattice of connective tissue. Kihara called the pre-lymphatic pathway consisting of collagen bundles and argentophilic fibers "macula cribriformis" (3). The presence of macula cribriformis in the wall of the rabbit pericardium was reported by Kotani (4). To date, however, milky spots have not been reported in the wall of the pericardium, although they have often been noted as aggregates of macrophages in the omentum (5-8) and mediastinum (9-12).

In this study, we examined by light and electron microscopy the macula cribriformis and milky spot-like structures in the wall of the rabbit pericardium under normal conditions and after the injection of India ink and latex particles into the pericardial cavity. The purpose was to elucidate the relationship between the absorption of particulate matter from the pericardial cavity into the lymphatics, in the pericardial wall and the structural differences between the absorbing and non-absorbing areas.

MATERIAL AND METHODS

Control study

The pericardial sacs of two male rabbits were fixed *in situ* by the intravenous injection of 10% formaldehyde-saline into the pericardial cavity under anesthesia with intravenous pentobarbital (30mg/Kg). The pericardial sac was carefully resected and divided into three parts: sternal, diaphragmatic and basal. Each was cut into small pieces for the preparation of fairly flat membranous specimens, which were post-fixed in formaldehyde-saline and treated by a modification of the Bielschowsky silver impregnation method for connective tissue. Five adult rabbits of both sexes weighing 2500-4000g were used for the light and electron microscopic observations of pericardium and epicardium. After general anesthesia with intravenous pentobarbital, the peritoneal cavity was opened by a midventral incision and perfused with 0.85% saline (80~100cmH_2O) through the abdominal aorta. After the blood had been cleared from the blood vessels, a fixative solution of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M sodium cacodylate buffer at pH 7.4 (Karnovsky's fixative) was substituted for the saline. About five minutes later the hearts and pericardial sacs were resected "en bloc," and small pieces of pericardium and epicardium were post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer solution for 90 minutes at 4°C. These specimens were routinely dehydrated with a graded series of ethanol and embedded in epoxy resin (Epon 812) according to the recommendation of Luft (13). Semithin sections (1μm thick) were stained with toluidine blue for light microscopy. Ultrathin sections (60-80nm thick) were cut with a MT2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-300 electron microscope.

Experimental study

The hearts of 16 rabbits were exposed by left thoracotomy under general anesthesia using artificial ventilation through an endotracheal tube. Eleven rabbits (group 1) were injected with 0.5-1.0ml of India ink, and five (group 2) were injected with 0.5-1.0ml of a suspension of latex particles (0.2μm in diameter) into the pericardial cavity. About 40-60 minutes later, the rabbits were sacrificed with an overdose of pentobarbital. The pericardium of three group 1 rabbits was fixed in 10% neutral formalin for 24 hours. Membranous specimens of pericardial sacs were prepared by the method used in the control study and stained with hematoxylin-eosin for light microscopy. Eight group 1 rabbits and five group 2 rabbits were perfused with Karnovsky's fixative. Small pieces of pericardium and epicardium were post-fixed, dehydrated and embedded in Epon 812 by the method used in the control study.

RESULTS

Pericardium

The pericardium consisted of mesothelium and submesothelial connective tissue which contained blood vessels, lymphatics, and nerves. Fat cells were seen in the deeper layer. The structure of the mesothelial layer and submesothelial connective tissue differed somewhat from region to region of the pericardium. The apical region was composed mainly of dense collagen bundles, and only a few blood vessels and lymphatics were present in the submesothelial connective tissue. The basal region, on the other hand, consisted of relatively loosely interwoven collagen bundles and fibers among which many blood vessels and lymphatics were observed. Lymphatic capillaries were characterized by an irregularly-shaped lumen and abluminal cytoplasmic projections of endothelial cells. The endothelial cells were thinner than those of the blood capillaries except in the perinuclear region. The intercellular junctions of adjacent lymphatic endothelial cells showed simple overlapping or interdigitation. Lysosomes were occasionally seen in the endothelial cells of the lymphatics. Microfilaments

about 10nm in diameter, reported by Leak and Burke as anchoring filaments (14), were detected just beneath the abluminal plasma membrane of the lymphatic endothelium. The basal lamina was absent or discontinuous. These fine structures of lymphatics in the pericardium were similar to those seen elsewhere in the body. Lymphatics in the deeper layer of the pericardium were large in caliber and possessed valves. The basal lamina was discontinuous and no smooth muscle cells or nerves were observed around the endothelial cells of the lymphatics.

Membranous specimens of pericardium treated by the Bielschowsky method occasionally showed a characteristic lattice of coarse collagen bundles. Argentophile fibers were detected in the meshes of the lattice (*Fig. 1*). The distribution of the lattice structure was prominent in the basal and diaphragmatic regions of the pericardium. These structures were similar to those of the macula cribriformis in the diaphragm, parietal pleura and pericardium reported by Kihara and his coworkers (3,4,15) but they were small and scattered. Except in the macula cribriformis areas, the connective tissue was composed of densely interwoven collagen bundles and fibers.

Light and electron microscopic observations of the macula cribriformis sections revealed that the coarse collagen bundles forming the lattice were covered with small mesothelial cells, 50-100μm in diameter, and often in contact with the endothelial cells of the submesothelial lymphatic capillaries (*Fig. 2*). The adjacent mesothelial cells were loosely apposed, but no openings were found in the meshes of the lattice. The mesothelial cells seen in the other areas were somewhat larger (100-200μm in diameter) and at a considerable distance from the lymphatic capillaries. A basal lamina was faintly observed in both areas. The junctions of the large mesothelial cells were interdigitations or overlappings. Tight junctions were detected at the border of the adjacent mesothelial cells. Fibroblasts and macrophages were present in the connective tissue between the large mesothelial cells and lymphatic capillaries (*Fig. 3*).

The elastic fibers in the pericardium were composed of extremely large amorphous portions (0.5-1.0m in diameter) surrounded by conspicuous microfibrils. They were distributed widely beneath the mesothelial layer (*Figs. 4, 5*) and among the bundles of collagen fibers in the connective tissue throughout the pericardium. Fibrillar elements of elastic fibers were occasionally in contact with the abluminal surface of the lymphatic endothelial cells where intracellular microfilaments were concentrated to form an electron-dense spot. Anchoring filaments of lymphatic capillaries were also seen in these electron dense spots (*Fig. 4*).

After the injection of India ink into the pericardial cavity, carbon particles, singly and in clusters, were seen not only on the luminal surface of the mesothelial cells, but also in the lumens of the submesothelial lymphatic capillaries lying just beneath the small mesothelial cells (*Fig. 5*). Macrophages located on both the luminal and abluminal sides of the mesothelial cells had engulfed many carbon particles (*Fig. 6*). Sometimes, the small mesothelial cells also took up particles into the lysosome-like dense bodies in their cytoplasm.

In contrast to India ink, latex particles injected into the pericardial cavity were not found in the lumens of the submesothelial lymphatic capillaries within 60 minutes. However, latex clusters composed of 6-15 particles were present in the submesothelial connective tissue where the endothelial cells of the submesothelial lymphatic capillaries almost came into contact with the small mesothelial cells (*Fig. 7*). Latex particles were occasionally seen in the enlarged intercellular clefts of adjacent mesothelial cells.

Macrophages in the pericardial cavity and in the submesothelial connective tissue engulfed particles singly or in clusters of 20-30 particles (*Fig. 8*). Some mesothelial cells took up one to three particles in their cytoplasm.

Fig. 1. Light micrograph of a membranous specimen of the pericardium impregnated by Bielschowsky method. Argyrophile fibrils (arrow) are observed in the meshes of the lattice of a macula cribriformis formed by coarse bundles of collagen fibers (arrowhead) (x550).

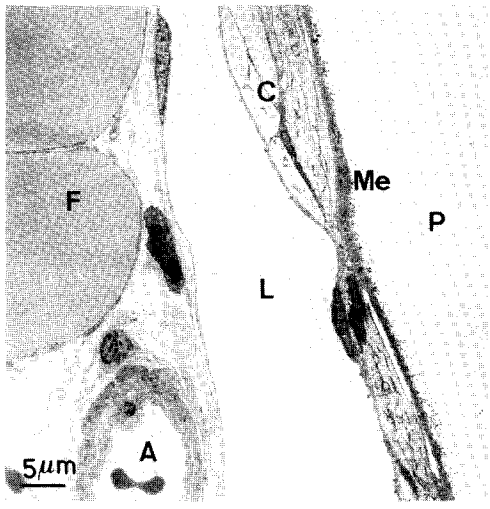

Fig. 2. Electron micrograph of a lymphatic capillary (L) seen just beneath the small-sized mesothelial cells (Me) covering a macula cribriformis in the pericardium. Pericardial cavity (P), collagen fibers (C), fat cells (F), artery (A) (x1100).

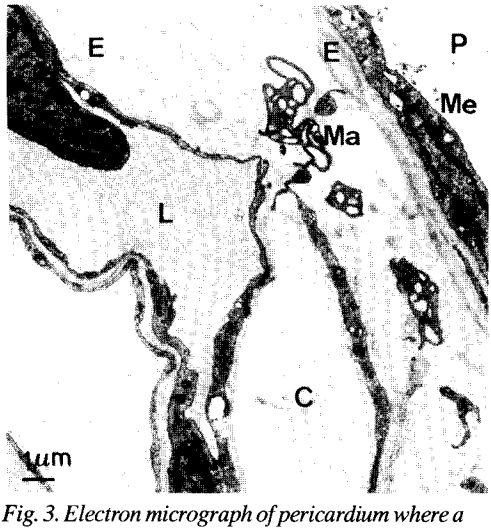

Fig. 3. Electron micrograph of pericardium where a lymphatic capillary (L) is at a distance from the mesothelial cells (Me). Collagen fibers (C), elastic fibers (E), and macrophages (Ma) are present between the mesothelial cells and the lymphatic capillary. Pericardial cavity (P) (x4200).

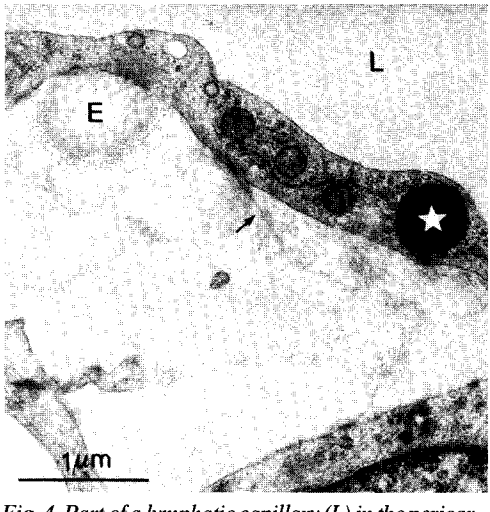

Fig. 4. Part of a lymphatic capillary (L) in the pericardium. An elastic fiber (E) consisting of an amorphous portion and a surrounding fibrillar portion is seen just beneath the endothelium of the lymphatic capillary. Anchoring filaments (arrow), lysosome (star) (x16,400).

It was noteworthy that milky spot-like structures were seen by light microscopy in the basal region of the pericardium where they "bulged" toward the pericardial cavity (*Fig. 9*). Many free cells were present in these bulges. Electron microscopy revealed that the

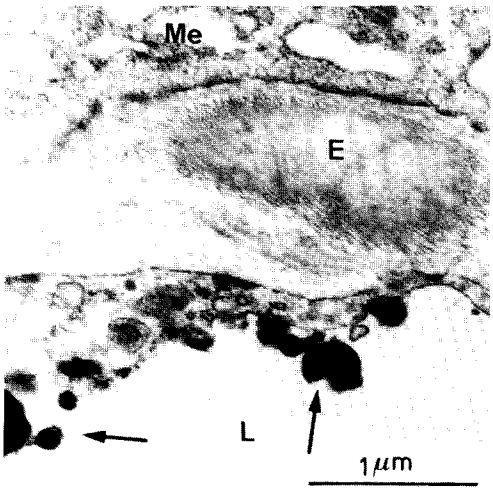

Fig. 5. Part of a lymphatic capillary (L) in pericardium 60 minutes after injection of India ink into pericardial cavity. Carbon particles (arrow) are present in the lumen of the lymphatic capillary. Elastic fiber (E), mesothelial cell (Me) (x21,600).

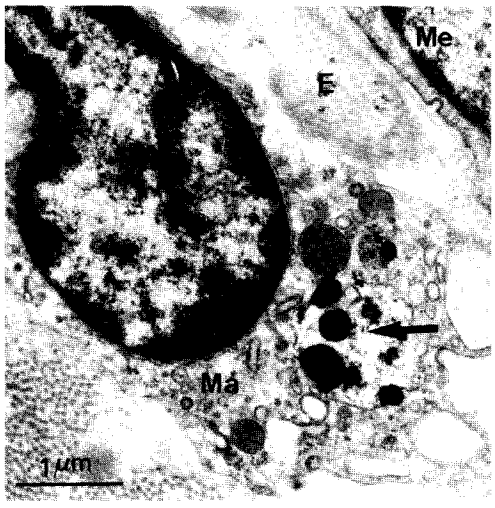

Fig. 6. Part of a macrophage (Ma) in submesothelial connective tissue of pericardium 60 min after injection of India ink into pericardial cavity. Carbon particles (arrow) are seen in phagosomes of the macrophage. Mesothelial cells (Me), elastic fibers (E) (x13,600).

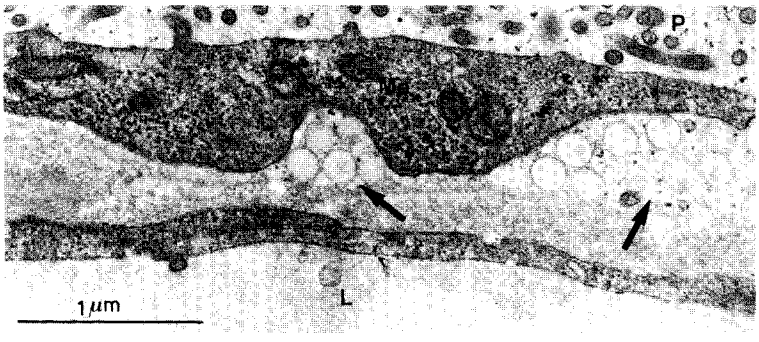

Fig. 7. Part of mesothelial cell (Me) and lymphatic capillary (L) 60 min after injection of latex particles into pericardial cavity. Latex particles (arrows) are seen in the submesothelial connective tissue. Pericardial cavity (P) (x29,000).

bulges were covered by characteristic plump mesothelial cells with microvilli and blunt folds and sometimes cilia (*Fig. 10*). Their cytoplasm contained many free ribosomes, mitochondria, a well-developed Golgi apparatus and intracellular filaments. Numerous pinocytic vesicles and several lysosomes were also noted. The adjacent mesothelial cells were loosely apposed and there were often openings between them (*Fig. 10*). Macrophages occasionally migrated through these openings into the pericardial cavity. They often engulfed latex particles into their cytoplasm (*Fig. 11*). The free cells in the bulges were mainly macrophages. Some lymphocytes, plasma cells and fibroblast-like cells were also present.

Epicardium

Lymphatics were more abundant in the epicardium than in the pericardium. The number of lymphatics was greatest in the left ventricle, followed by the right ventricle, then

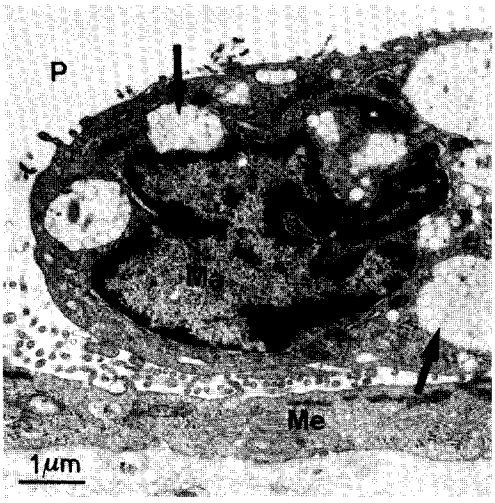

Fig. 8. Part of a macrophage (Ma) in pericardial cavity (P) 60 min after injection of latex particles into pericardial cavity. Latex particles (arrows). Mesothelial cell (Me) of the pericardium. (x8200).

both atria. Lymphatics in the epicardium were usually at a distance from the mesothelial layer because of the presence of dense collagen bundles and fibers. The mesothelial cells covering the bundles were large and possessed numerous microvilli. Elastic fibers in the epicardium were smaller in diameter and fewer than in the pericardium. Some lymphatics in the epicardium were connected with the lymphatics in the myocardial layer (*Fig. 12*). Ultrastructural characteristics of lymphatics in the epicardium were similar to those in the pericardium. No carbon or latex particles were observed in the mesothelial cells, macrophages or lymphatics in the epicardium within 60 minutes after their injection into the pericardial cavity (*Fig. 13*).

DISCUSSION

There have been many studies on the absorption and drainage pathways of the pericardial cavity (16-23). Most agree that low molecular weight components leave the pericardial cavity probably by way of the subepicardial blood capillaries. Less agreement exists, however, about the drainage pathway of high molecular weight compounds or particulate substances from the pericardial cavity. Drinker and Field demonstrated that serum is absorbed slowly from the pericardial cavity via lymphatic vessels, but graphite particles enter lymphatics only after phagocytosis by macrophages in rabbits (16). Stewart reported that high molecular weight compounds, such as vital red, are unable to leave the human pericardial cavity (17). These differences in drainage pathways from the pericardial cavity may be explained by the different species or substances used.

Kotani demonstrated by light microscopy that carbon particles injected into the rabbit pericardial cavity are absorbed directly into the submesothelial lymphatics of the pericar-

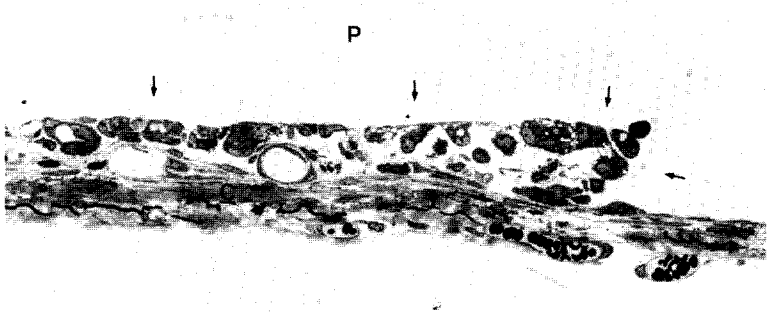

Fig. 9. Light micrograph of a milky spot-like structure (arrow) bulging toward pericardial cavity (P). Collagen fibers (C). (x350).

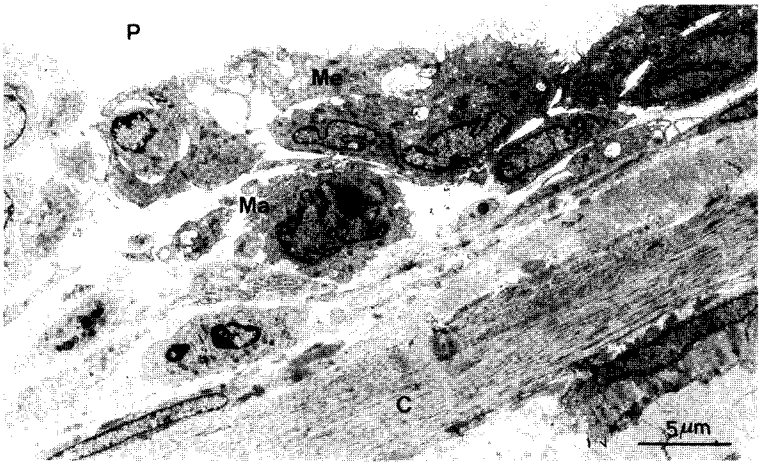

Fig. 10. Electron micrograph of a milky spot-like structure showing specific mesothelial cells (Me) and macrophages (Ma). Pericardial cavity (P). Collagen fibers (C). (x2900).

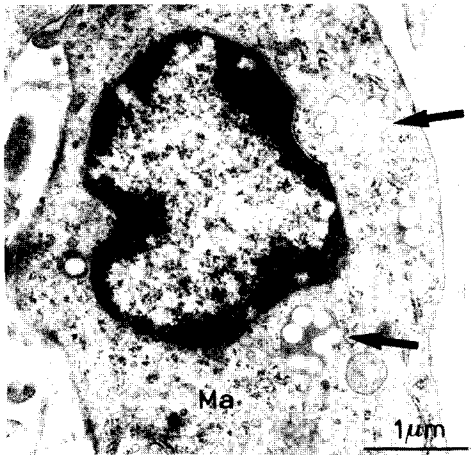

Fig. 11. Macrophage (Ma) in a milky spot-like structure 60 min after injection of latex particles into pericardial cavity. Latex particles are seen in the cytoplasm (arrow). (x12,900).

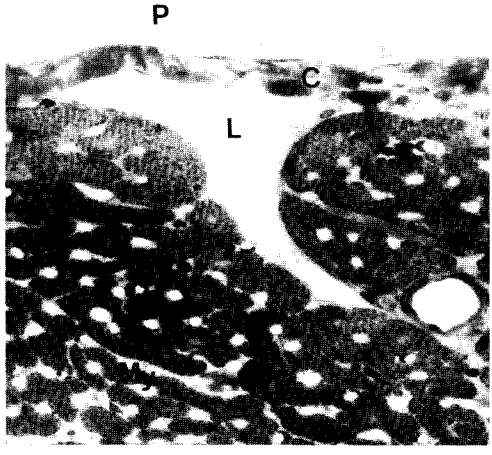

Fig. 12. Light micrograph of a lymphatic (L) in epicardium showing connection with a lymphatic capillary in myocardium (My). Collagen fiber (C), pericardial cavity (P) (x270).

dium and epicardium through the lattice of coarse collagen bundles called "macula cribriformis" (4). Our study shows that the macula cribriformis are present mainly in the basal and diaphragmatic regions of the pericardium, and that they are smaller and fewer than those described in the diaphragm and parietal pleura (24-27). In the diaphragm and parietal pleura electron microscopic studies reveal many openings between adjacent small mesothelial cells covering the macula cribriformis and endothelial cells of the underlying lymphatic capillaries, through which particulate matter is absorbed directly into the lymphatics. We do not see openings between the mesothelial cells in the pericardium. Carbon particles injected into the pericardial space are, however, seen not only on the luminal surface of the mesothelial cells of the pericardium, but also in the lumens of the submesothelial lymphatic capillaries within 60 minutes after the injection. Latex particles are seen both on the luminal side of the mesothelial cells and in the submesothelial connective tissue of the peri-

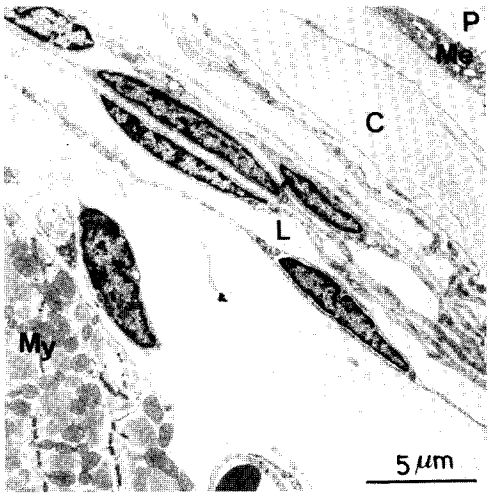

Fig. 13. Electron micrograph of epicardium. Collagen fibers (C) are distributed densely between the mesothelium and a lymphatic (L) with a valve. Myocardium (My), pericardial cavity (P) (x2800).

cardium, but not in the lymphatic lumens. Our morphological evidence suggests that direct absorption occurs to some extent at the sites of macula cribriformis of the pericardium, but less than in the diaphragm and parietal pleura. Macrophages in the pericardial space and in the pericardium may act as the first line of defense since they actively engulf both carbon and latex particles injected into the pericardial cavity. It may be a secondary occurrence that particles enter lymphatics after phagocytosis by macrophages. Small mesothelial cells also took up the particles, but their activity was less prominent.

It is a conspicuous feature of the pericardium that large elastic fibers are in close contact with the endothelial cells of the submesothelial lymphatics. These elastic fibers may be involved in the absorption of serous fluid into the lymphatics and in the propulsion of lymph (28).

Our most important finding was the milky spot-like structures in the basal parts of the pericardium, which protruded toward the pericardial space. There fine structural features were similar to those reported in the omentum and mediastinal pleura (5-12). They were covered by characteristic plump mesothelial cells and contained numerous macrophages, some of which migrated through openings in the mesothelium. Latex particles injected into the pericardial space were actively engulfed by these macrophages. A few were also seen in the cytoplasm of the mesothelial cells. These findings indicate that the cells in the milky spots constitute an important mobile reserve to combat infections in the pericardial cavity.

Kotani described macula cribriformis and the absorption of India ink from the pericardial cavity into the submesothelial lymphatic capillaries in the epicardium. However, we were unable to find a lattice structure in the submesothelial layer. Neither carbon nor latex particles were seen in the submesothelial connective tissue or in the lumens of lymphatics in the epicardium within 60 minutes after the injection. Lymphatics in the epicardium were of large caliber and occasionally joined with the lymphatics in the myocardial layer. These observations suggest that lymphatics in the epicardium drain tissue fluid from the myocardium rather than from the pericardial cavity.

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