Lymphology 11 (1978) 111–116

Lymph Flow Mechanism of the Subperitoneal Diaphragmatic Lymphatics

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Summary

Inorganic corpuscles like Indian ink and latex particles as well as erythrocytes penetrate via stomata on the peritoneal surface of the diaphragm into the subperitoneal lymphatics when injected intraperitoneally. Electronmicroscopic examinations showed that stomata are formed by intercellular gaps between neighbouring mesothelial and endothelial cells. In these areas there is a lacunar dilatation of the lymphatics, and a basement membrane as well as collagenous fibres are absent.

The back flow of the lymph fluid from the stomata into the peritoneal cavity is prevented by overlapping of mesothelial and endothelial cells during inspiration as well as valve like cell processes of endothelial cells.

The lymph flow is particularly affected by anchoring filaments of the lymphatics and the respiratory movement of the diaphragm.

Introduction

Intraperitoneally injected dyes and blood cells are resorbed via the subperitoneal lymphatics of the diaphragm (7, 13, 26, 37–39). Endogenous erythrocytes enter the lymphatic capillaries via stomata which could be demonstrated by scanning electronmicroscopy in rats (39). By means of transmission electronmicroscopy the intercellular passage of mesothelial and endothelial cells was shown for Indian ink (7, 23, 26). *Allen* et al (1–5) found, that the lymph fluid in the diaphragmatic lymph vessels flows only in one direction. As there are no valves in the subperitoneal lymphatics (19, 20) the question arises how the back flow of the resorbed particles via the stomata is prevented and how a centripetal lymph flow is maintained.

We therefore marked the lymph vessels of the rat diaphragm by intraperitoneal injections of Indian ink, latex particles and avian erythrocytes. We then examined the stomata and lymphatics of the diaphragm by electronmicroscopy. In conjunction with the knowledge of the ultrastructure and animal experiments on the resorptive capacity of the diaphragmatic lymph vessels described in the literature a model for the lymph flow mechanism was to be developed.

Methods

Male and female Wistar rats were injected intraperitoneally with 3 ml of Indian ink, 2 ml of a suspension of latex particles with a diameter of 1.1 μ or 4 ml of a 50% avian erythrocyte suspension. Between 10 minutes and 20 hours the diaphragm was removed and fixed in glutaraldehyd and phosphate buffer (Karnovsky). After dehydration and drying by the critical point dehydration technique the specimen are coated with gold. Subsequently the peritoneal surface of the diaphragm was examined by the scanning electronmicroscope = Stereoscan Mark II A (Cambridge Ltd).

Further 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 EM 9-S2.

Results

The mesothelial cells on the peritoneal surface of the diaphragm showed numerous microvilli on examination by scanning electronmicroscope. Within the mesothelial layer there were stomata into which avian red cells entered (Fig. 1).

In the ultrathin sections only a very thin tissue barrier between the lacuna and the peritoneal

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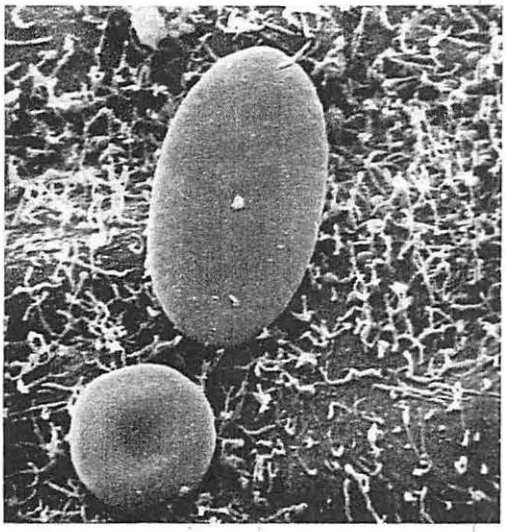


Fig. 1a Avian red cell and rat erythrocyte on mesothelial layer of the diaphragm. Numerous microvilli. X 3,120

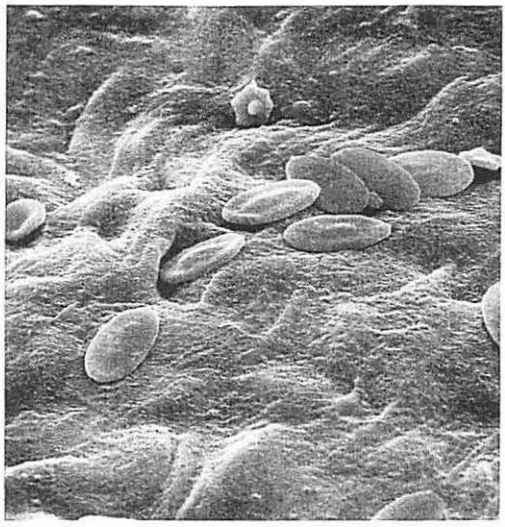


Fig. 1b Mesothelial layer of the peritoneal diaphragmatic surface with stomata for the entrance of avian red cells. X 910

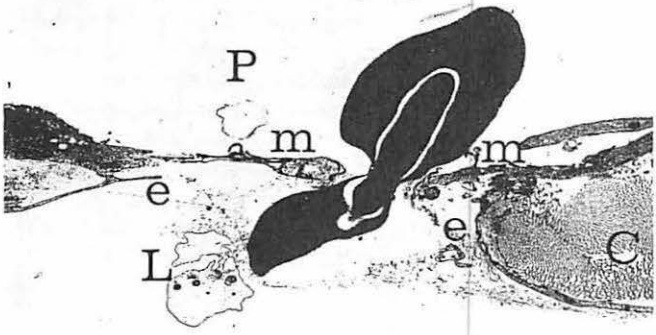


Fig. 2 The tissue barrier between peritoneal cavity (P) and lacunar dilatation of subperitoneal diaphragmatic lymphatic capillary (L) consists only of thin mesothelial (M) and endothelial (E) cells. Intercellular gaps between neighbouring mesothelial cells with a free communication between peritoneal cavity and lacuna for the entrance of an avian erythrocyte. X 8,775.
C = collagenous fibres

cavity was visible in the area of the lacunar dilatations with wide gaps between neighbouring mesothelial and endothelial cells (Fig. 2).

As a basement membrane and collagenous fibres were missing only in these areas (see also Fig. 4) the entrance of erythrocytes into the lymph vessel is easily possible.

Sometimes the stomata are closed by overlapping processes of neighbouring mesothelial cells, while in wide intercellular gaps latex particles were still present (Fig. 3).

In the immediate vicinity of the stomata the endothelial cells formed valve-like cell processes (Fig. 4).

The lymph vessels of the diaphragm showed microfilaments between their endothelial cells and the surrounding collagenous fibres (Fig. 5).

Discussion

The respiratory movement of the diaphragm is of crucial importance for the resorptive capacity of its lymphatics.

From animal experiments it is known that anaesthetics with a respiratory depressing effect reduce the resorption via the diaphragmatic lymph vessels (14, 27, 35, 36, 42). On the other hand resorption is increased with more intense respiratory movement (16, 17, 35, 36).

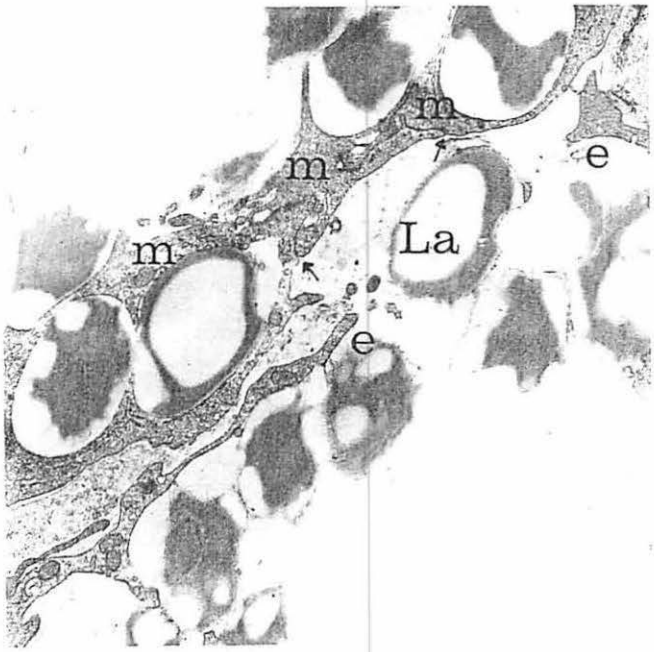


Fig. 3 Stoma closed by overlapping mesothelial cells (M). Wide intercellular gaps between neighbouring endothelial cells (E) filled with latex particles (La). Note the absence of a basement membrane (bm) and of collagenous fibres (c) in this area. X 14,200. IS = Intercellular space (↑)

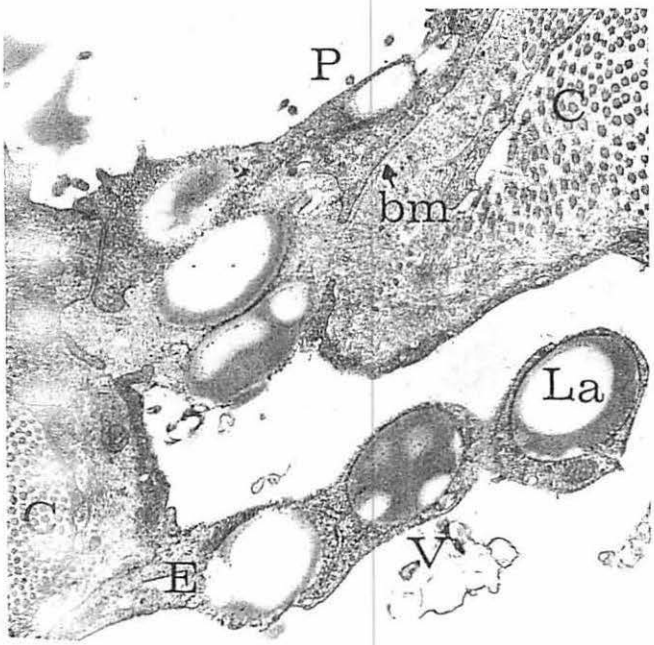


Fig. 4 Valve like cell process (V) of an endothelial cell (E) in the vicinity of a stoma. Latex particles (La) between neighbouring endothelial cells. X 14,200. P = peritoneal cavitiy, C = Collagenous fibres, bm = basement membrane

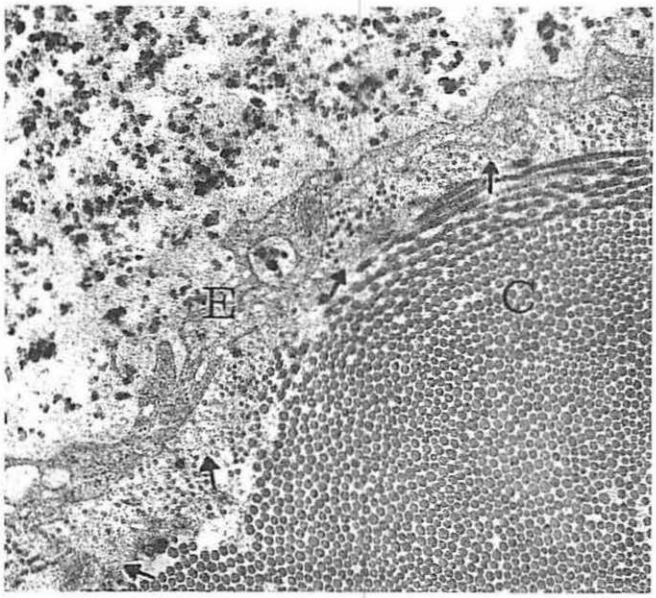
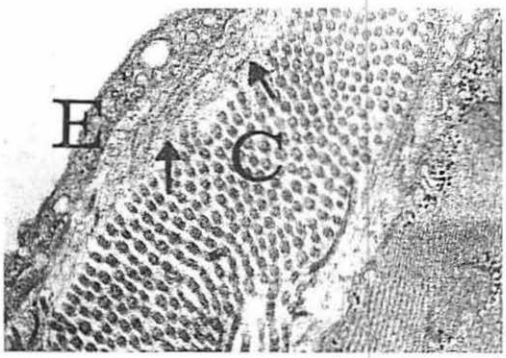


Fig. 5 Lymphatic capillary filled with Indian ink. Microfilaments (↑) between endothelial cells (E) and surrounding collagenous fibres (C). X 14,200.



A uni- or bilateral phrenectomy causes a delayed and quantitatively reduced resorption (6, 14, 15, 18, 25, 33, 36).

The importance of the diaphragm movement for the resorption is seen in the fact that simply through rhythmical movements of the diaphragm in animal cadavers resorption is achieved (4, 12, 34, 40).

Apart from the respiratory movement the intraperitoneal pressure conditions play a role.

Decreased intraperitoneal pressure reduces resorption (42), while increased pressure promotes resorption (21, 22, 24, 35, 41).

The microfilaments in the vicinity of the lymph endothelial cells are equivalent to the anchoring filaments which have been described outside the diaphragm in the literature (8–11, 28, 29–32). According to these authors their function is to link the endothelial cells with the surrounding interstitial tissue in order to prevent the collaps of the lymph vessels in case of inflammatory oedema. Similarly their function in the diaphragm would be to keep the lymph vessels open inspite of continous deformation during inspiration and expiration.

As the surface of the diaphragm is increased during expiration it can be assumed that neighbouring mesothelial and endothelial cells move apart in the area of the stomata. During inspiration stomata are closed by overlapping cells (Fig. 3).

The following model can be derived from the findings (Fig. 6): During expiration the peritoneal surface of the diaphragm enlarges. Thereby intermesothelial and interendothelial gaps are formed. In the absence of a basement membrane and collagenous fibres in these areas a free communication between the peritoneal cavity and the interior of the lacuna results. As the muscle bundles decrease in diameter during relaxation (expiration) the lymph vessels between them can be dilated with the aid of their anchoring filaments. Thus the dia-

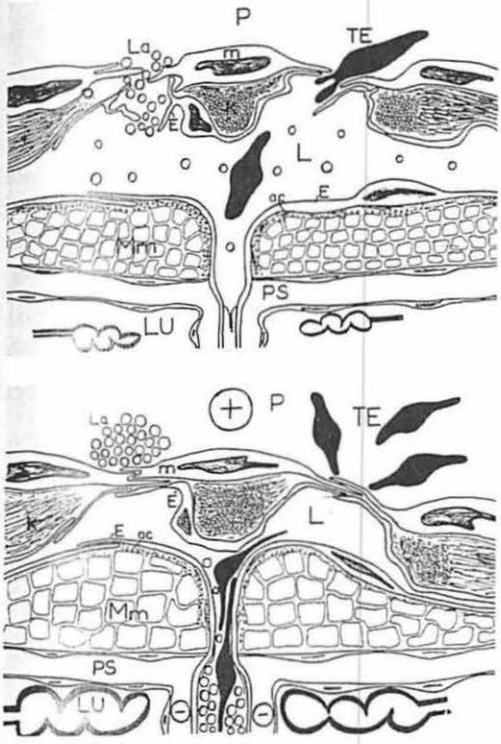


Fig. 6 Scheme for lymph flow mechanism during expiration (above) and inspiration (below). Intercellular gaps od mesothelial (m) and endothelial (E) cells filled with latex particles (la) and avion red cells (TE) during expiration. Extended lacuna (L). Thin muscles fibres (Mn), dilated lymph vessel between them.
Increased pressure in the peritoneal cavity during inspiration (+). Compression of lacuna and lymph vessel. Lymph flow into the subpleural diaphragmatic lymph vessel.
(−) = negativ pressure in the pleural cavity (PS), Lu = lungs; ac = anchoring filaments, k = Collagenous fibres

meter of the lymph vessels is enlarged and a suction effect on the lacunae results. Intraperitoneal pressure reduction during expiration leads also to a suction effect on the lacunae, which are extended in all directions.

In this way lacunae and lymphatics are filled.

At the beginning of the inspiration the surface of the diaphragm decreases again and neighbouring mesothelial and endothelial cells overlap. The intraabdominal pressure increases during inspiration and the lacunae are compressed and emptied. The valve like cell processes of the endothelial cells in the neighbourhood of the stomata may help to seal the stomata.

Through the contraction of the muscle bundles during inspiration the intermuscular lymphatics are also compressed and emptied ("muscle pump").

The lymph flow into the subpleural diaphragmatic and retrosternal lymphatics is favoured by negative pressure in the pleural cavity. Due to the valves in this area lymph back flow is impossible.

The lymph flow is further enhanced by the rhythmic movement of thoracic organs and the suction effect of the heart on the junction between the ductus lymphaticus dexter and the angulus venosus.

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