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Review

The role of the diaphragm in lymphatic absorption from the peritoneal cavity

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ABSTRACT

Lymphatics in the diaphragm form a specialised system draining fluid from the peritoneal cavity and returning it to the vascular system. Fluid enters subperitoneal lymphatic lacunae, between muscle fibres of the diaphragm, the lacunae being separated from the peritoneal cavity by a barrier comprising, successively, lymphatic endothelium, a layer of collagenous fibres, a thin fenestrated layer of elastic tissue, and the peritoneal mesothelium. To reach the lacunae, peritoneal fluid passes through stomata located between cuboidal mesothelial cells of the lacunar roof. Whilst the distribution of mesothelial stomata and subjacent lymphatic lacunae varies in different species, stomata appear to be exclusive to the diaphragm and may serve as the main drainage channels for absorption from the peritoneal cavity. Clinically, they may provide escape for tumour cells, pathogens and toxins from the peritoneal cavity. They could provide access for blood transfusions, for intraperitoneal chemotherapy to treat malignancies, and for peritoneal dialysis in treating chronic renal failure. From the lacunae, fluid traverses the diaphragm via intrinsic lymphatics to reach collecting lymphatics beneath the diaphragmatic pleura. Both intrinsic and collecting lymphatics contain valves. The collecting lymphatics drain principally into retrosternal (parasternal) lymphatic trunks that carry lymph to the great veins after it filters through mediastinal lymph nodes.

Key words: Diaphragmatic peritoneum; diaphragmatic lymphatics; mesothelial stomata; peritoneal absorption.

INTRODUCTION

This review describes the structure and properties of the peritoneal mesothelium, the morphology and distribution of mesothelial stomata and intrinsic lymphatics of the diaphragm, the regional lymphatic drainage from the diaphragm, and highlights the potential of lymphatic absorption from the peritoneal cavity and its clinical significance.

The peritoneal lining comprises flattened mesothelial cells and their basement membrane covering a layer of vascular loose connective tissue containing collagen bundles, fibroblasts, occasional macrophages, mast cells and lymphatics. Although the diaphragmatic contribution to the total serosal surface area lining the abdominal cavity is modest, special stomata between the mesothelial cells overlie a rich plexus of lymphatic lacunae and provide rapid lymphatic drainage from the peritoneal cavity (Leak & Rahil, 1978; Tsilibary & Wissig, 1987, Fukuo et al. 1990; Negrini et al. 1992; Abu-Hijleh et al. 1994).

The lymphatic absorption and drainage routes of the peritoneal cavity have been widely studied in a number of species. Whilst the morphology and distribution of mesothelial stomata and subperitoneal lymphatic lacunae of the diaphragm vary between species (Tsilibary & Wissig, 1977; Leak & Rahil, 1978; Fukuo et al. 1990; Negrini et al. 1991; Abu-Hijleh et al. 1994), the principal extrinsic lymphatic drainage from the diaphragm appears to be via parasternal lymph trunks to the parasternal/mediastinal lymph nodes and, ultimately, to the right lymphatic or upper terminal thoracic duct (Yoffey & Courtice, 1970; Flessner et al. 1983; Abernethy et al. 1991; Abu-Hijleh & Scothorne, 1994).

The absorbing abilities of mesothelial cells have been

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extensively studied to elucidate the mechanism of effusions and dissemination of neoplasms and infections from serous cavities, particularly the peritoneal cavity. The peritoneum is not simply a mechanical covering allowing organs to glide over one another, but is very active functionally having various defensive functions (Gaudio et al. 1990) and a filtering ability making it useful as a dialysing membrane (Bender, 1985).

The diaphragm has a lymphatic drainage system which is particularly effective for rapid absorption from the peritoneal cavity (Tsilibary & Wissig, 1987; Abu-Hijleh & Scothorne, 1994; Abu-Hijleh et al. 1994), a feature of considerable clinical significance. Thus it has proved valuable for continuous ambulatory peritoneal dialysis (CAPD) in chronic renal failure (Raftery et al. 1989), in the investigation of metastatic spread of neoplasm from the peritoneal cavity (Mahon & Libshitz, 1992), in intraperitoneal chemotherapy to combat malignancies (Ward et al. 1987) and in transperitoneal nutrition with triglycerides or lipids (Mahedero et al. 1992).

GENERAL STRUCTURE AND FUNCTION OF PERITONEAL MESOTHELIUM

Mesothelial cells

The peritoneum is the largest of the serous membranes with a surface area equal to that of the skin (Wegner, 1877). It consists of a layer of mesothelium with its basement membrane resting on a layer of connective tissue, the tunica propria. The simple squamous epithelial cells are so flattened that little detail can be identified by light microscopy, the nuclei appearing merely as prominent bulges. 'Hautchen' preparations using silver nitrate define intercellular boundaries, and after appropriate preparation the mesothelial layer can be removed on celloidin film and viewed en face. In normal mesothelium the lines of silver deposition between cells are sharp, regular and continuous, typical of a simple squamous epithelium. Ultrastructural examination of the peritoneum confirms that the mesothelium consists of a single layer of flattened cells resting on a homogenous basal lamina, 40 nm thick (Odor, 1954; Dobbie et al. 1981). The shape and size of the cells vary, particularly over the more mobile and distensible viscera, but they are generally between 0.5 and 2.0 μm in thickness and 30–40 μm in diameter (Gotloib et al. 1983). They have a slightly raised central nuclear region and a thinner margin where they are in contact to form a continuous sheet. The nuclei are irregular discs with a thin peripheral band of electron-dense chromatin. Nucleoli are sometimes evident (Fukata, 1963).

Surface microvilli

Although similar to endothelial cells (Gotloib et al. 1983) and often compared with them functionally (Casley-Smith, 1967), mesothelial cells have a number of distinctive features (Gotloib & Shustack, 1987). They possess long thin microvilli projecting from the peritoneal surface. These are often up to 2.7 μm in length and between 50 and 110 nm in diameter (Odor, 1954; Baradi & Hope, 1964; Gotloib et al. 1983). Their number and density vary according to site and the species, but they are generally more common in cells which cover mobile viscera (Andrews & Porter, 1973; Gaudio et al. 1990). Their length and density may alter according to physiological conditions (Madison et al. 1979). By amplifying the surface plasmalemma, microvilli may be involved in the passage of soluble substances across the cell membrane (Odor, 1954). Andrews & Porter (1973) and Gaudio et al. (1990) proposed that, by trapping a layer of serous exudate, the microvilli may also create a slippery cushion which protects the mesothelium from frictional damage and facilitates movements of abdominal organs.

Intracytoplasmic organisation

The organelle content of the mesothelial cell, consisting of abundant rough-surfaced endoplasmic reticulum in the form of short irregularly arranged cisternae, numerous free ribosomes and a well developed Golgi apparatus, suggests that it is functionally very active. Along with numerous mitochondria these organelles are mainly confined to the perinuclear cytoplasm. Their presence indicates synthetic activity such as production of the glycocalyx, and the complexly folded plasmalemma and plasminogen activators which increase during mesothelial healing (Raftery, 1979). The most characteristic feature of mesothelial cells is the numerous cytoplasmic vesicles, seen especially in the thinner margins of the cells (Odor, 1954; Baradi & Hope, 1964). Rows of spherical vesicles approximately 70 nm in diameter lie along both the peritoneal and basal surfaces of the cells (Fukata, 1963; Gotloib et al. 1983). The existence of transcellular channels may implicate these vesicles in the transfer of solutes across the cell (Digenis et al. 1984), analogous to that demonstrated in endothelia (Simionescu, 1983). However, the distance to be traversed is much greater than in endothelia and the importance of this route in the transfer of solutes has

yet to be established (Digenis et al. 1984; Gotloib & Shustack, 1987). Other vesicles can be seen close to the lateral plasmalemma. They may fuse together to form rosette-shaped cisternae close to the intercellular clefts. In studies on the passage of horseradish peroxidase between the cells, these vesicles take up the marker (Cotran & Karnovsky, 1968). It has been suggested that they may be part of a transmesothelial pathway for the transfer of solutes around tight junctions. However, since vesicles filled with horseradish peroxidase do not appear close to the peritoneal surface, potentially a site of uptake, this suggestion appears unlikely (Cotran & Karnovsky, 1968). Vesicles can also be seen deeper in the cytoplasm, particularly around the Golgi complex. These may be involved in membrane traffic between the cell surface and the interior and therefore might not be involved directly in any transmesothelial transport. Finally, coated vesicles and pits are frequently seen near the borders of the cells. These may be involved in receptor-mediated uptake of macromolecules rather than in the bulk transport of solutes (Raftery et al. 1989). Thus mesothelial cells have several subsets of vesicles, some of which may be involved in the transport of material across the cell.

Intercellular spaces and junctional systems

Under pathological conditions the mesothelial cells may separate, but normally they are in close contact (Odor, 1954; Baradi & Hope, 1964; Gotloib et al. 1983). At the points of contact the mesothelium may be no more than 150–200 nm thick, but the intercellular cleft is much longer due to cell overlap, and the adjoining membranes are oblique and tortuously folded. At the end of the cleft nearest to the peritoneal cavity, the cells are joined by tight junctions (Baradi & Rayns, 1976; Gotloib et al. 1983). Freeze-fracture studies have shown that these tight junctions are poorly developed and may not be very permanent (Simionescu & Simionescu, 1977). Minimal gaps of 4 to 7 nm between the cells (Casley-Smith, 1967; Alavi et al. 1982), sufficient to transmit various ions and molecules up to the size of horseradish peroxidase (Cotran & Karnovsky, 1968) but too small to allow the passage of iron dextran (Digenis et al. 1984) have also been described. Von Recklinghausen (1863) first described the presence of pores or openings between mesothelial cells. Recent ultrastructural studies have confirmed the presence of these 'stomata' which are restricted to the mesothelium overlying the lymphatic lacunae of the diaphragm and provide a direct route between the peritoneal cavity and lymphatics (Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Fukuo et al. 1990; Negrini et al. 1992; Abu-Hijleh et al. 1994). These stomata will be discussed later in this review.

Metabolic properties

Although solutes may pass through the peritoneal membrane through intercellular spaces by simple diffusion (Aune, 1970), histochemistry suggests a more active transport. A variety of enzymes such as acid and alkaline phosphatase are present in the peritoneal mesothelial cells of rabbits and cats (see Raftery et al. 1989). ATPase activity is involved in transport mechanisms, and may be concerned with the active transport of materials across the mesothelium (North, 1966; Raftery, 1973). Cascarano et al. (1964) have shown that mesothelium exerts an appreciable control over the passage of certain solutes across it, and their findings suggest that oxidative metabolism and ATP formation are intimately linked with mesothelial membrane permeability.

Mesothelial cells have fibrinolytic activity (Gervin et al. 1973; Raftery, 1979). Cultures of isolated mesothelium induce fibrinolysis (Whittaker et al. 1982), suggesting mesothelium itself is a source of plasminogen activator. This fibrinolytic activity increases during wound healing, and may be a major factor in preventing fibrin adhesions from being converted to permanent fibrous adhesions, with their attendant damage (Ellis, 1971; Raftery, 1979). Mesothelial cells also produce prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation (Herman et al. 1979). These properties have led to the use of mesothelium as an alternative to endothelium as a lining for prosthetic vascular grafts (Bull et al. 1988). Peritoneal tube grafts, created from the parietal peritoneum of animals, have also been used to replace veins, utilising the antithrombotic properties of mesothelium as a substitute for venous endothelium (Price-Thomas et al. 1976; Ribbe et al. 1988).

STRUCTURE OF DIAPHRAGMATIC PERITONEUM

Studies of the pathways of peritoneal fluid absorption indicate that the peritoneal surface of the diaphragm is a particularly important site of drainage (Courtice & Steinbeck, 1951*b*; Bettendorf, 1979; Flessner et al. 1983). Lill et al. (1979) showed a significant reduction in peritoneal absorption of saline solutions and Krebs-Ringer solutions containing 9% bovine serum albumin (BSA) after sealing the diaphragm by fibrous tissue following abrasion.

Several electron microscopic studies of the per-

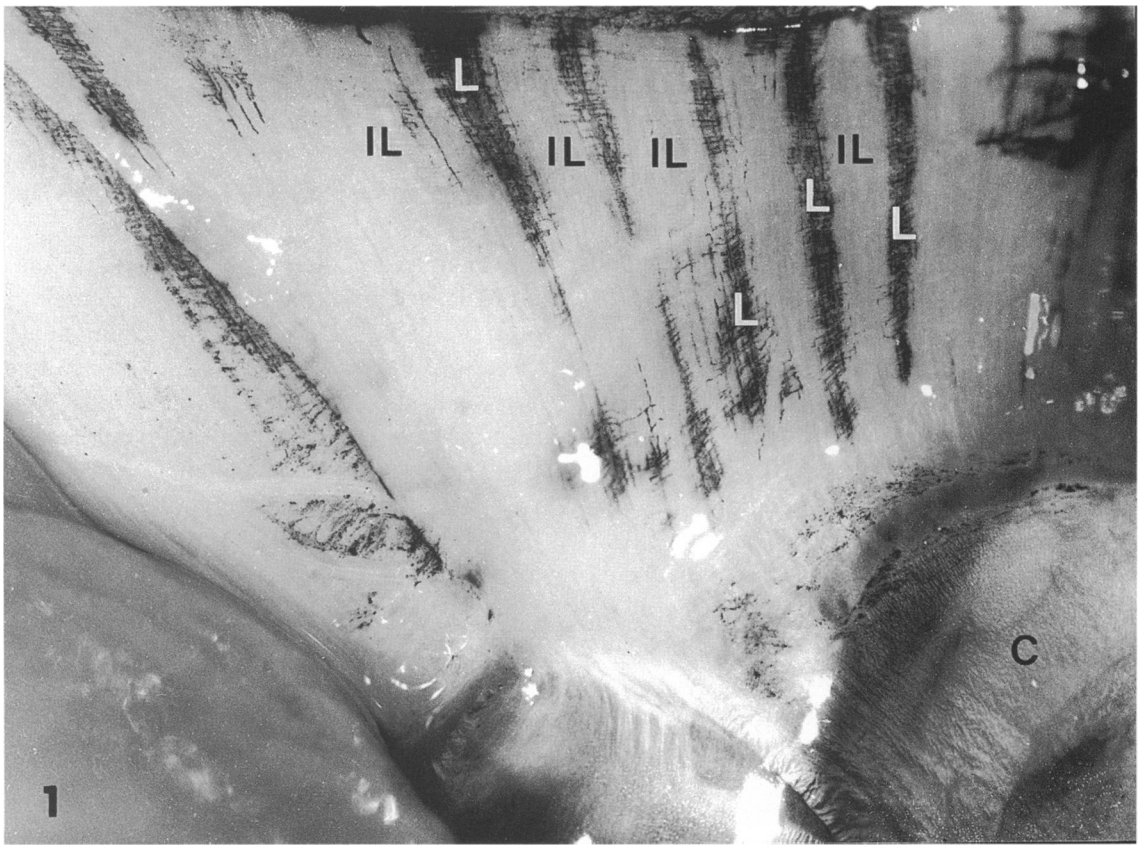

Fig. 1. Low power view of peritoneal surface of the rat hemidiaphragm after intraperitoneal injection of India ink. Ink-filled subperitoneal lymphatic lacunae (L) run parallel to muscle fibres. They are sharply demarcated from interlacunar areas (IL). C, central tendon. ×8.

itoneal surface of the diaphragm (Tsilibary & Wissig, 1977; Leak & Rahil, 1978; Ji-Chang & Shou-Min, 1991; Abu-Hijleh et al. 1994) have confirmed a nonuniform distribution of mesothelial cells, two distinct populations being described. Areas in which the cell contours appear rounded or cuboidal can be clearly distinguished from areas populated by very flat cells. According to whether they directly overlie submesothelial lymphatic lacunae or cover remaining areas of the diaphragm these two areas are classified as lacunar and interlacunar, respectively. In lacunar areas boundaries between individual mesothelial cells are prominent and the central region of each cell, containing the nucleus, protrudes towards the peritoneal cavity. Numerous microvilli occur on their cell surfaces. They also display elaborate cytoplasmic processes extending from their lateral borders, processes from adjacent cuboidal cells interlacing to form a very fine anastomosing network with numerous intercellular channels between them. Many areas of the mesothelial lacunar roof possess stomata located at the junction of several mesothelial cells (Figs 5, 6). These are oval or circular openings, a few micrometres in diameter. However, mesothelial cells overlying interlacunar regions form a continuous flat surface with ill defined outlines of individual cells which are covered by many microvilli. Stomata have never been observed in these areas (see Fig. 7) (Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994).

On the upper surface of the diaphragm, the pleural mesothelium is also not entirely continuous, the subpleural connective tissue being exposed to the pleural cavity through stomata (Wang, 1975; Mariassy & Wheeldon, 1983; Abu-Hijleh, 1987; Negrini et al. 1991; Abu-Hijleh et al. 1994). Using scanning electron microscopy (SEM) the pleural surface of the diaphragm is seen to differ significantly from the peritoneal. Pleural mesothelial cells are of one type only, being flattened and elongated with well defined boundaries, microvilli and lateral cytoplasmic processes similar to those on the peritoneal aspect. Stomata on the pleural surface are lentiform rather than circular in outline and their average density and size is less than on the peritoneal surface (Negrini et al. 1991; Abu-Hijleh et al. 1994).

INTRINSIC LYMPHATICS OF THE DIAPHRAGM

Studies of the normal reabsorption of fluid from the peritoneal cavity indicate that most is absorbed by lymphatics of the diaphragm, diffusion into peritoneal blood vessels, playing only a minor role (Allen & Vogt, 1937; Courtice & Steinbeck, 1951*b*; Bettendorf, 1979; Miserocchi et al. 1982; Gotloib & Shustack, 1987). Recent electron microscopic studies (Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Negrini et al. 1991; Abu-Hijleh et al. 1994) have shown the existence of open stomata between mesothelial cells of the diaphragm, providing an effective mechanism for absorption from the peritoneal cavity. These stomata connect the peritoneal cavity directly to an underlying rich plexus of flattened terminal lymphatics, the so-called lacunae, located within the submesothelial connective tissue. In animals injected intraperitoneally with India ink, these lacunae become rapidly and intensely blackened (Fig. 1). Under the dissecting microscope, they appear as large, separate, elongated spaces with sharply demarcated borders running radially and parallel to muscle fibres of the diaphragm (Tsilibary & Wissig, 1977; Leak & Rahil, 1978; Abu-Hijleh & Scothorne, 1994). The ink-filled lacunar areas can easily be distinguished from interlacunar areas (Fig. 1). They intercommunicate at regular intervals by transverse anastomoses at a slightly deeper level and empty at a still deeper level into a rich plexus of valved connecting vessels, which according to their position may be designated as intermuscular and perivascular collecting lymphatics. The location and general arrangement of the diaphragmatic subperitoneal lymphatic lacunae vary in different species. They are particularly abundant in the central tendon of dogs and rabbits (MacCallum, 1903; Negrini et al. 1991). In the rodent they are present exclusively in the muscular portion (Allen, 1936; Tsilibary & Wissig, 1977; Abu-Hijleh & Scothorne, 1994), whereas in the golden hamster they are found throughout (Fukuo et al. 1990).

Species differences also occur concerning the rate of removal of fluid and substances such as plasma proteins (Courtice & Steinbeck 1950*a*, *b*, 1951*b*) and erythrocytes (Courtice et al. 1953), absorption being most rapid in rats, intermediate in rabbits and slowest

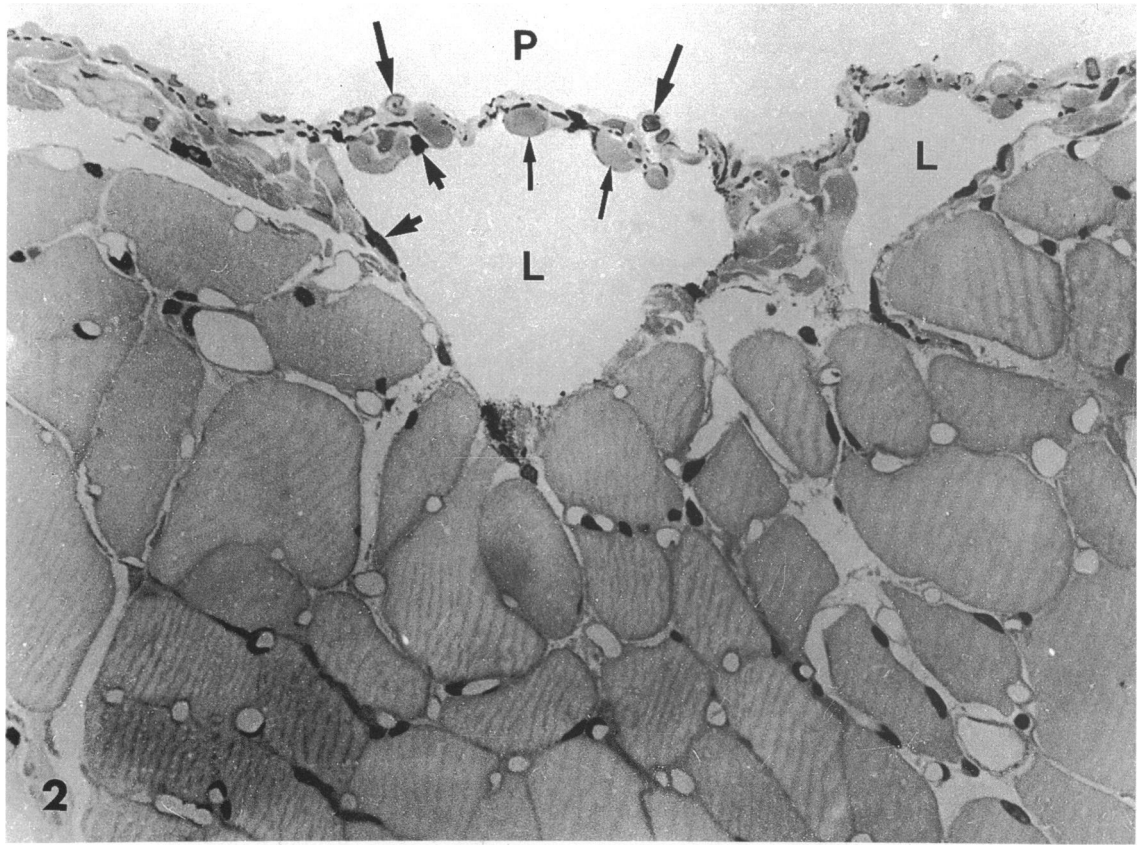

Fig. 2. High power view of a subperitoneal lymphatic lacuna (L) of the diaphragm. The lacunar lumen is separated from the peritoneal cavity (P) by a very thin wall, the roof of the lacuna. This consists of a cuboidal mesothelium (large arrows), a fenestrated elastic membrane represented by a darkly stained interrupted line, a layer of collagenous bundles (small arrows), and the lymphatic endothelium (arrowheads). Semithin resin section stained Azar II. ×400.

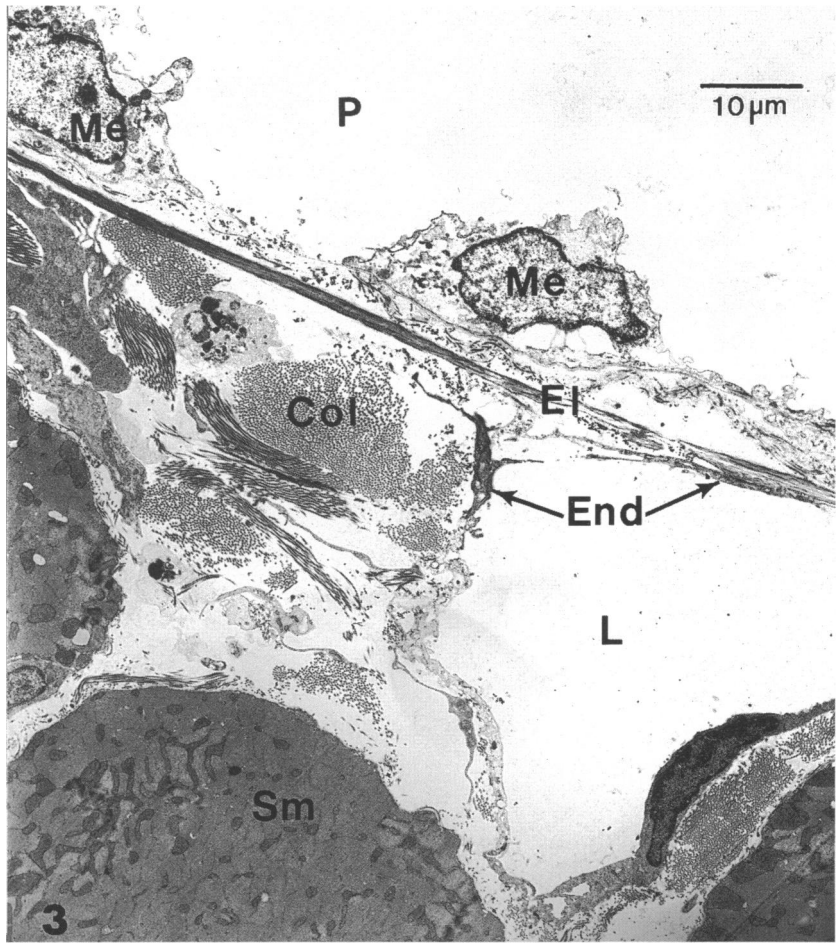

Fig. 3. A TEM micrograph demonstrating the ultrastructure of the lacunar roof. L, lymphatic lacuna; P, peritoneal cavity; Me, cuboidal mesothelial cells; El, fenestrated elastic membrane; Col, collagenous bundles; End, lymphatic endothelial cells; Sm, skeletal muscle. Bar, 10 µm.

in guinea pigs. Other factors which may affect the mechanism and rate of lymphatic absorption from the peritoneal cavity include rhythmic stretching of the diaphragm during normal respiratory movements (Allen & Vogt, 1937; Wang, 1975; Bettendorf, 1978), variations in respiratory pattern, as in different types of anaesthesia (Mengle, 1937; Morris, 1953), changes in intraperitoneal pressure (Higgins & Graham, 1929; Yoffey & Courtice, 1970; Tsilibary & Wissig, 1983), and the effect of posture (Dandy & Rowntree, 1914; Courtice & Steinbeck, 1951*a*; Bangham et al. 1953). It was found that Fowler's position (low pelvis and trunk at 45° to the horizontal) impaired lymphatic absorption in rabbits (Dandy & Rowntree, 1914; Courtice & Steinbeck, 1951*a*). Bangham et al. (1953) showed that radioactive glass particles were more slowly absorbed in rats in the head-up than in the head-down position, while absorption rates were intermediate in animals moving about normally. To delay the absorption of toxins from intraperitoneal infections it is common practice to nurse patients sitting in the Fowler position, the infected peritoneal fluid gravitating downwards into the pelvic cavity where the rate of toxin absorption is slow (Snell, 1992).

The submesothelial lymphatic lacuna of the diaphragm is unique for its large size and its multiple direct links to the peritoneal cavity via the stomata and associated channels. The lumina of lymphatic lacunae are separated from the peritoneal cavity by a barrier consisting of cuboidal mesothelial cells with underlying thin basement lamina, bundles of collagenous fibres of variable thickness, and a thin endothelial cell layer. These three basic layers form the so-called roof of the lymphatic lacuna (MacCallum, 1903; Allen, 1936; Tsilibary & Wissig, 1977; Leak & Rahil, 1978). An additional fourth layer of fenestrated elastic tissue separating the peritoneal mesothelium from the collagenous layer has been recently described in the rodent diaphragm (see Figs 2, 3, 7) (Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994). It may serve to keep the lymphatic

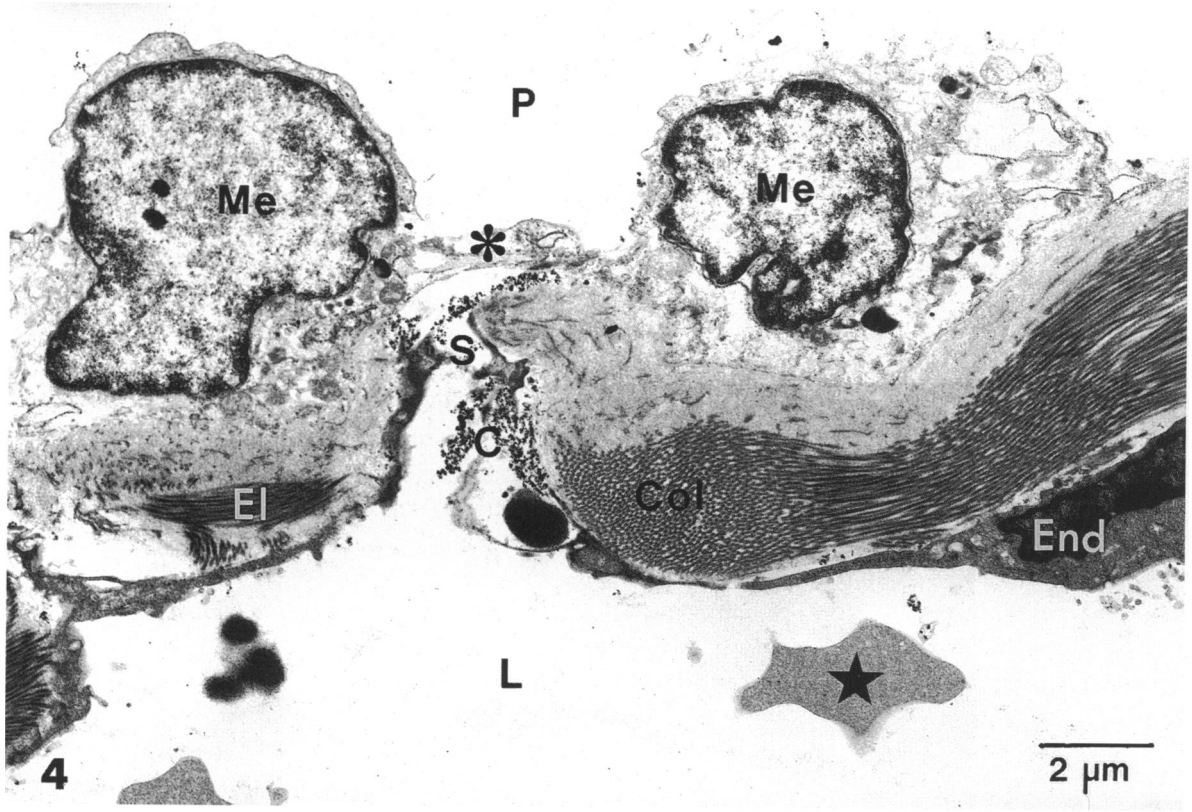

Fig. 4. A stomal channel (S) and underlying lacuna (L). Endothelial cells (End) extend from the lacunar lumen towards the peritoneal cavity (P) between bundles of collagen fibres (Col) and meet the mesothelial cells (Me). This provides a direct passage way through which carbon particles (C) and red blood cells (star) pass freely and rapidly from the peritoneal cavity into the lymphatic lumen. A bridge-like filamentous process (asterisk) is seen connecting opposing mesothelial cells. TEM. Bar, 2 μm.

roof taut and prevent it expanding excessively when the diaphragm contracts during inspiration. Also any elastic recoil of the roof would contribute to emptying of lymph in the lacuna by providing compression. At the site of a stoma the underlying lymphatic endothelium extends up to the peritoneal surface of the diaphragm to become directly continuous with the surface mesothelium (Fig. 4). The mesothelial basal lamina and collagen fibres are absent. In such areas, intraperitoneally injected carbon particles have direct access to the lymphatic lacunar lumen (Bettendorf, 1978; Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994). Examples of direct contact between epithelial cells and endothelium are rare and are restricted to the lymphatic drainage units of the peritoneal (Leka & Rahil, 1978; Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994) and pleural cavities (Wang, 1975; Mariassy & Wheeldon, 1983) and ovarian bursae (Shinohara et al. 1985), and possibly the pericardial cavity (Nakatani et al. 1988), although their ultrastructure has yet to be examined satisfactorily.

Excluding the diaphragm, absorption from the peritoneal cavity by parietal and visceral peritoneum does not appear to be very significant quantitatively. Courtice & Steinbeck (1951*b*), working on rabbits, observed extraperitoneal collections of dyed fluid in the greater omentum and some areas of the mesentery. Simer (1948) and Abu-Hijleh (1987), after injecting India ink into the peritoneal cavity of rats, observed that most peritoneal surfaces retained the ink to a variable extent. This macroscopic retention of the ink was particularly prominent in the omentum, mesentery and peritoneal fat. Although microscopic examination of semithin sections revealed the presence of many lymphatics in both omentum and mesentery, very few particles of carbon were observed within their lumina (Abu-Hijleh, 1987), most being adherent to mesothelial cell surfaces, or phagocytosed by interstitial connective tissue macrophages and macrophages of the omental milky spots. The macrophages present throughout these tissues, particularly the omentum, form an efficient defence mechanism against particulate matter and bacterial invasion which may be more important than removal by the omental lymphatics.

Some authors report involvement of the liver and spleen in the absorption mechanism of materials

injected into the peritoneal cavity. Olin & Saldeen (1964) found large amounts of thorotrast in the liver and spleen of the rat 10 h after intraperitoneal injection. Whaley et al. (1972) noted in the mouse that Kupffer cells of the liver and perifollicular zones of the spleen were laden with carbon deposits 30 min after its intraperitoneal injection. Roser (1970) followed the fate of peritoneal macrophages labelled with radioactive colloidal gold in mice and found that, although 12 h after cell transfer, labelled cells were confined to the subcapsular sinus of parathymic lymph nodes, by 24 h labelled cells were present within the parenchyma of the liver and spleen. Abu-Hijleh (1987) observed that a few sinusoidal Kupffer cells of the rat liver were packed with fine particles of carbon 20 min after its intraperitoneal injection. No trace of carbon particles, however, was found before this time. This suggests that a certain period is required for the tracer substance to migrate through the diaphragmatic lymphatics to reach the regional lymph nodes and then enter the venous circulation before it reaches the liver sinusoids.

MORPHOLOGY AND DISTRIBUTION OF MESOTHELIAL STOMATA

Peritoneal fluid enters the lymphatic lacunae via openings between mesothelial cells covering the diaphragm. Von Recklinghausen (1863) described these openings as stomata, visualised for the first time by using silver nitrate. He considered stomata as pores, opening onto the peritoneal surface on one side and communicating directly with the underlying lymphatics within the diaphragm. Although the concept of open channels between the peritoneal cavity and the lymphatics was supported by some workers (Allen, 1936; Simer, 1948) others disputed the concept of stomata and regarded the peritoneal cavity as being completely enclosed by an intact layer of cells (e.g. MacCallum, 1903). These workers suggested that stomata were artifacts resulting from silver salts reacting with serous fluids to form deposits that adhered to the surfaces and boundaries of mesothelial cells to give the impression of pores. Following this controversy, Tsilibary & Wissig (1977) and Leak & Rahil (1978) observed stomata in mice diaphragmatic peritoneum for the first time by electron microscopy. More recent ultrastructural studies have confirmed their presence in other animal species (Fukuo et al. 1990; Negrini et al. 1991; Abu-Hijleh et al. 1994) and also in the human fetus (Ji-Chang & Shou-Min, 1991).

The distribution of stomata in the serous membranes is not uniform. They have been described on the parietal pleura but not on the visceral (Wang, 1975; Pinchon et al. 1980; Mariassy & Wheeldon, 1983; Abu-Hijleh et al. 1994), whereas in the peritoneum they are described only on the diaphragmatic surface (Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Fukuo et al. 1990; Negrini et al. 1991; Abu-Hijleh et al. 1994). Whilst Nakatani et al. (1988) described circular fenestrations or pores in the pericardium of rodents, others have maintained it is a continuous serous membrane (Kluge & Hovig, 1967). Similar fenestrations have also been described in the mesothelium of the ovarian bursa (Shinohara et al. 1985) and in the diaphragmatic pleura of the golden hamster by Fukuo et al. (1990), but they insisted that fenestrations differ fundamentally from stomata since they are surrounded solely by mesothelial cells, without any junctions with the endothelium of underlying lymphatics. It is possible that species differences and variations in techniques may account for the discrepancies in the morphology and distribution of stomata in serous membranes. However, the general consensus is that the criterion for the SEM confirmation of a true stoma is that it consists of an outer mesothelial margin and inner lymphatic endothelial orifice (see Figs 5, 6). This suggests that true mesothelial stomata are not found all over the peritoneal and pleural cavities, but are confined to the serosal surfaces of the diaphragm (Abu-Hijleh, 1987; Abu-Hijleh et al. 1994).

There is some disagreement regarding the distribution of mesothelial stomata on the peritoneal surface of the diaphragm in different species and even within species. In rabbits, stomata are abundant on the central tendinous part and sparse on the muscular part (Allen, 1967; Negrini et al. 1991), whereas in mice (Tsilibary & Wissig, 1977; Leak & Rahil, 1978) and in rats (Abu-Hijleh et al. 1994) they occur exclusively in the muscular portion of the diaphragm. In contrast, in the golden hamster they are found throughout both parts of the diaphragm, with higher density in the tendinous part (Fukuo et al. 1990). However, contradictory results describing the virtual absence of stomata in the rabbit diaphragm (Gaudio et al. 1990) and their uniform distribution throughout the human fetus and mouse diaphragms (Ji-Chang & Shou-Min, 1991) have also been reported. Whilst it seems there is a species difference in the distribution of mesothelial stomata on the peritoneal surface of the diaphragm, it would seem unlikely to find stomata in regions where there is a flat mesothelium with continuous basement membrane, tight intercellular junctions, and a thick submesothelial collagenous layer (Bettendorf, 1979); conversely, stomata can easily be found in areas with

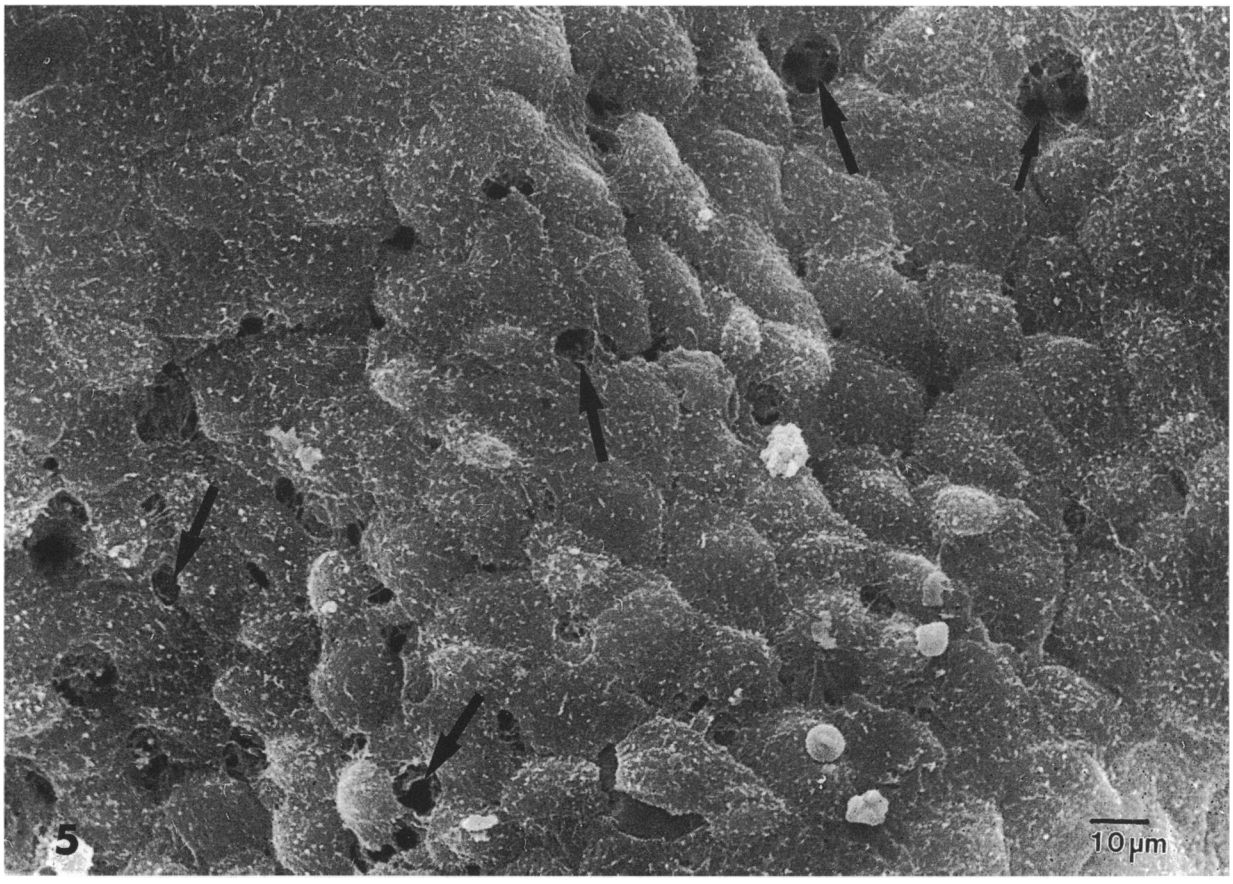

Fig. 5. SEM micrograph demonstrating a lacunar zone of the rat diaphragmatic peritoneum. Circular stomata (arrows) are present only among cuboidal mesothelial cells overlying the lymphatic lacuna. Bar, 10 µm.

cuboidal cells covering submesothelial lymphatic lacunae (Leak & Rahil, 1978; Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994). In fact, correlation exists between the distribution of mesothelial stomata and submesothelial lymphatic lacunae (Negrini et al. 1991). In the rabbit diaphragm, for example, it has been reported that the density of lymphatic lacunae is about 8-fold higher on the peritoneal than on the pleural side and consequently the average density of mesothelial stomata is about 4 times higher on the peritoneal than on the pleural surface (Negrini et al. 1991). Similar correlation has been described in the rat diaphragm (Fig. 7) (Abu-Hijleh, 1987).

From evidence obtained by SEM of the peritoneal surface of the diaphragm of several small mammals (rat, mouse, rabbit, and golden hamster) and of the human fetus, stomata appear as round or oval holes measuring 4–10 µm in diameter and bordered by mesothelial cells (Figs 5, 6). Their size and shape vary, possibly depending on the contractile state of the diaphragm at the time of fixation. Some of the stomata appear as shallow pits that expose parts of the submesothelial connective tissues, whereas others are quite deep (Fig. 5). Cell processes crossing stomal openings have also been described. These include mesothelial processes bridging adjacent cells (Fig. 4) and/or endothelial flaps partially occluding the stomata (Fig. 6). The function of these cell processes is unclear. They may act as valves ensuring unidirectional lymph flow into the submesothelial lymphatics (Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994).

Normally the lymphatic drainage system of the diaphragm functions continuously in the turnover of fluid within the peritoneal cavity (Yoffey & Courtice, 1970). To determine whether the system can alter flow rates as conditions within the peritoneal cavity change, we need to know about the plasticity of stomata and associated channels and about the patency of stomata under different conditions in the peritoneal cavity. With regard to the first point, the existence of a stoma and associated channel is based upon specialised local differentiation of the lacunar mesothelium, submesothelial connective tissue, and lacunar endothelium, resulting in the formation of structurally differentiated units (Leak & Rahil, 1978, Tsilibary & Wissig, 1987; Abu-Hijleh et al. 1994) (see Fig. 4). Thus (1) along the

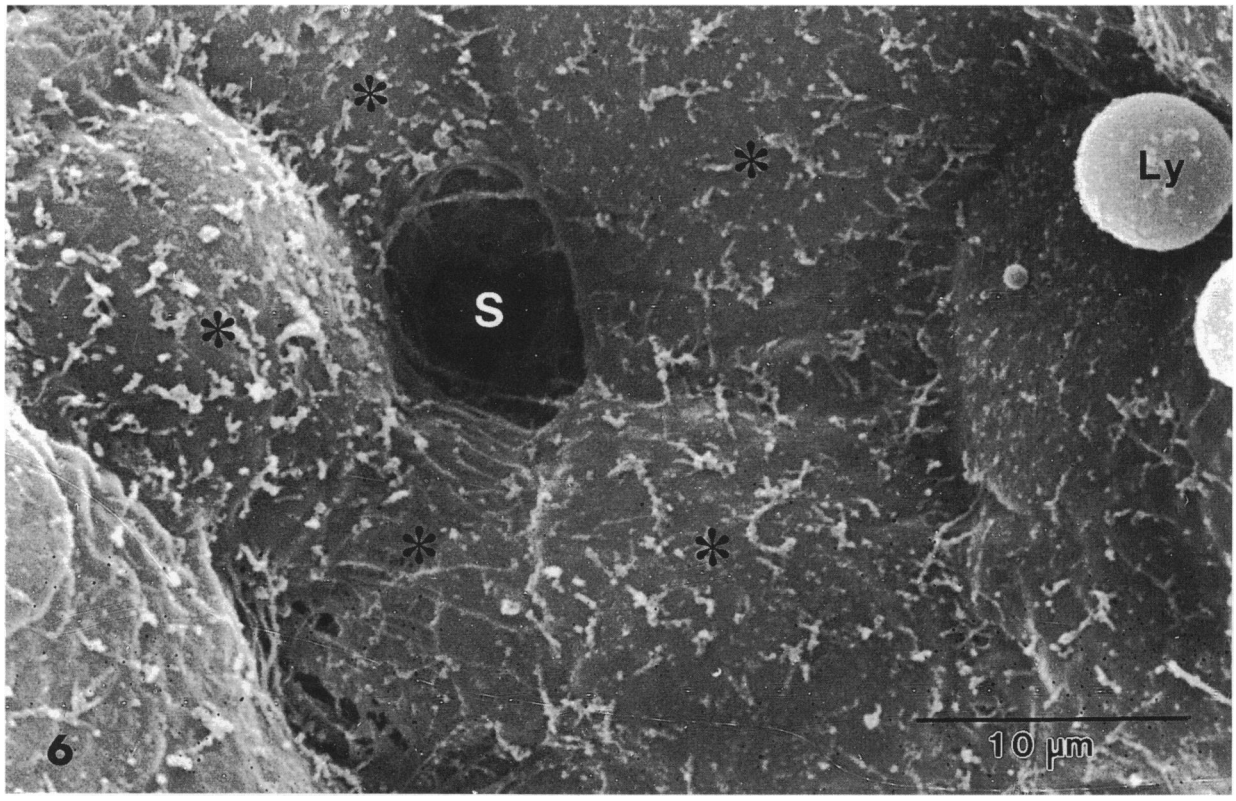

Fig. 6. A stoma (S) located at the junction of 5 cuboidal mesothelial cells (asterisks). An endothelial cell process crosses the space below the stomal orifice. Cells of the size of lymphocytes (Ly) can pass easily through the stoma. SEM. Bar, 10 μm.

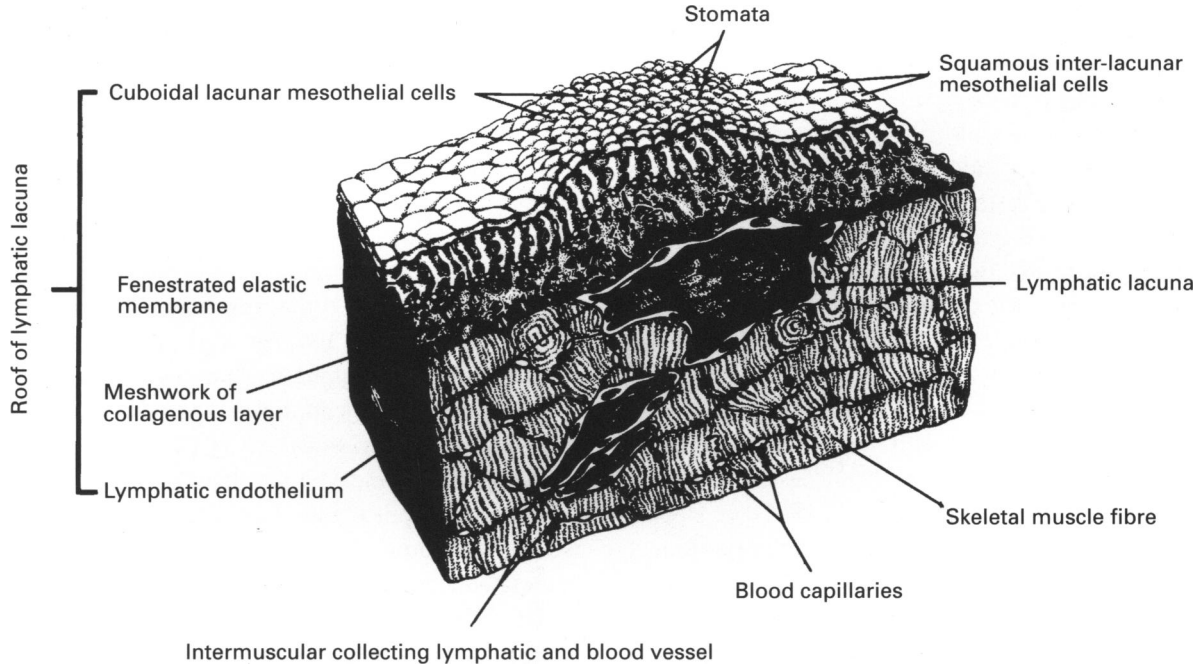

Fig. 7. Composite 3-dimensional diagram of the rat diaphragm showing different components of the lymphatic lacunar roof. The peritoneal surface is uppermost.

margin of a stoma, mesothelial and lacunar endothelial cells are joined to one another so they line a channel that leads from the peritoneal cavity into the lumen of the lymphatic lacuna; (2) both the mesothelium and lymphatic endothelium lack a basement membrane at the site of the channel; and (3) the

submesothelial connective tissue at the site of a stoma is interrupted and structurally modified to accommodate the channel. Given the ultrastructural complexity of the stomata and associated channels it appears more likely they would form over the longer rather than shorter term and that they are relatively stable structures. With regard to regulation of stomal patency, it has been suggested it is modified in response to functional conditions, both passive (stretch-related) (Allen, 1956; Bettendorf, 1978; Miserocchi et al. 1989) and active (induced experimentally) (Tsilibary & Wissig, 1983; Miserocchi, 1989). The latter may result from the contractile state of actin-like filaments in the mesothelial and endothelial cytoplasm responsible for the conformation of stomata and initial lymphatic channels (Bettendorf, 1979; Tsilibary & Wissig, 1983). In the rabbit diaphragm after injecting carbacol choline intraperitoneally, Miserocchi (1989) claimed stomal conductance increased abut 30 times in the change from relaxed to contracted state, and diaphragmatic contraction also caused recruitment of new stomata (about 40% of their total). Whether the patency of stomata changes during the normal respiratory cycle is difficult to establish, because fixation of the diaphragm is usually slow and results in major changes in peritoneal structure. Fixation itself and any experimental manipulations like those used by Tsilibary & Wissig (1983) and Miserocchi (1989) may result in artificial and false changes in patency of the stomata. Examining the rat diaphragm by SEM and using two different methods of fixation (vascular perfusion and immersion) Abu-Hijleh et al. (1994) observed patent stomata consistently irrespective of the method of fixation or the contractile state of the diaphragm. This suggests that the number and location of mesothelial stomata of the diaphragm are relatively permanent.

EXTRINSIC LYMPHATIC DRAINAGE FROM THE DIAPHRAGM AND PERITONEAL CAVITY

Lymphatic drainage routes from the peritoneal cavity have been studied in a number of species. In the rat, it has been suggested that lymph enters blood via the thoracic duct after passing through mesenteric and retroperitoneal channels and through associated lymph nodes, including the cisternal node (Abdou et al. 1952). However, radiographic studies suggest that lymph originating from diaphragmatic lymphatics enters large collecting ducts associated with the internal thoracic blood vessels on either side of the sternum. Then after passing through the anterior mediastinal lymph nodes close to the thymus, lymph enters the blood stream through the right lymphatic duct (Menville & Ane, 1932; Olin & Saldeen, 1964). In most other species, such as the dog (Higgins & Graham, 1929), cat, rabbit, and guinea pig (Courtice & Steinbeck, 1950*a*, *b*) and sheep (Abernethy et al. 1991), the internal thoracic pathway (parasternal lymph trunks) is considered the principal lymphatic route transporting materials from the peritoneal cavity to mediastinal and/or parasternal lymph nodes and, thence, to the right lymphatic or thoracic duct (reviewed by Yoffey & Courtice, 1970, and Abu-Hijleh, 1987). In a recent detailed study of rats injected intraperitoneally with India ink Abu-Hijleh & Scothorne (1994) described other subsidiary lymph pathways running from the diaphragm both cranially and caudally. Four pathways were noted, and on the basis of their size, frequency, and intensity of colouration with ink, their order of importance appears to be: (1) retrosternal (parasternal), draining the anterior diaphragm cranially to parathymic lymph nodes; (2) retroperitoneal, draining the posterior diaphragm caudally to the cisternal and renal nodes; (3) intercostovertebral, draining the costal part of the diaphragm cranially to the posterior mediastinal nodes; and (4) mediastinal, draining the peritendinous area of the diaphragm cranially to parathymic nodes (see Fig. 8). All 4 routes involved one or more lymph nodes before entering the great veins or cisterna chyli. The cranial drainage of lymph from the peritoneal cavity, mainly by way of the retrosternal trunks, predominates over the caudal route and lower thoracic duct; the intercostovertebral, mediastinal, and retroperitoneal lymphatic channels are only secondary pathways. The embryological development of the diaphragm from 4 distinct parts (Sadler, 1990); pleuroperitoneal membranes, mesentery of the foregut, mesoderm of the abdominal wall and septum transversum, may explain the different routes of lymphatic drainage from the diaphragm and peritoneal cavity.

The role of the thoracic duct in absorption from the peritoneal cavity differs between species and for different substances (Abdou et al. 1952; Yoffey & Courtice, 1970; Abu-Hijleh & Scothorne, 1994). Courtice & Steinbeck (1950*a*, *b*, 1951*b*) showed that only 20–30% of the lymphatic drainage of the peritoneal cavity in the cat, rabbit, and guinea pig passes into the thoracic duct, and Flessner et al. (1983) found a similar figure for the rat. Olin & Saldeen (1964) failed to visualise the thoracic duct in rats using radiographs to trace intraperitoneally injected

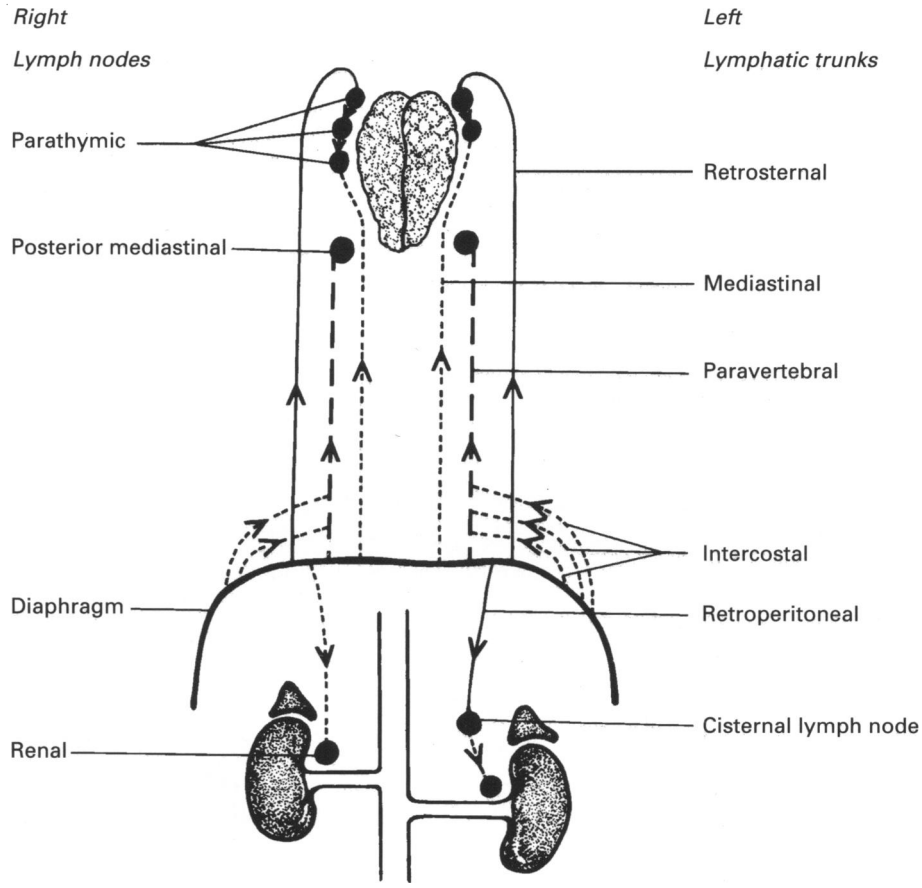

Fig. 8. Diagram showing an anterior view of the lymphatic drainage routes of the diaphragm and their relative importance. The latter is judged on the basis of size, frequency, and intensity of colouration with ink: ——, constant; — —, usual; – – – –, occasional.

thorotrast and concluded that most of the lymph in the thoracic duct originated from the intestine and not from the diaphragm. In the golden hamster, Fukuo et al. (1990) found drainage of intraperitoneally injected ink to primary lymph nodes of the diaphragm (located at the level of the 1st lumbar vertebra), efferent lymphatics from which contributed to the formation of the thoracic duct. No reference was made to retrosternal (parasternal) drainage, which has been shown to be the principal one in most other species, but species differences may exist in the lymphatic pathways from the peritoneum. Certainly, the volumes and properties of the solutions instilled into the peritoneal cavity has varied greatly in different studies (Higgins & Graham, 1929; Courtice & Steinbeck, 1950*a*, *b*, 1951*b*; Abdou et al. 1952; Olin & Saldeen, 1964; Flessner et al. 1983; Abernethy et al. 1991; Abu-Hijleh & Scothorne, 1994). Nevertheless, the general consensus is that the bulk of fluid and particles leaving the peritoneal cavity passes via the retrosternal route to the parathymic (mediastinal) nodes and thence to the upper terminal thoracic duct or right lymphatic duct.

CLINICAL SIGNIFICANCE OF THE PERITONEUM AND STOMATA

The ultrastructure of the peritoneum and its role in absorption from the peritoneal cavity has important clinical implications. (1) Continuous ambulatory peritoneal dialysis (CAPD) has become a widely accepted alternative to haemodialysis for the management of patients with chronic renal failure (see Raftery et al. 1989). (2) It helps to explain neoplastic metastatis from the peritoneal cavity (Mahon & Libshitz, 1992). (3) The mechanism of peritoneal lymph drainage is important in following intraperitoneal inoculation and lymphatic targeting of pathogens such as tetanus toxoid (Mueller et al. 1987) and Listeria monocytogenes (Marco et al. 1992). (4) Whole blood is absorbed from the peritoneal cavity by diaphragmatic lymphatics in man and animals (reviewed by Hedenstedt, 1947) and provides a possible route for blood transfusion in humans, especially in infants (Clausen, 1940) and the fetus (Liley, 1963). (5) Intraperitoneal nutrition with triglycerides or lipids is feasible and when supplemented

by glucose and amino acid solutions can provide a balanced nutritional intake (Mahedero et al. 1992). (6) Intraperitoneal dialysis is used in chemotherapy to combat ovarian, colorectal and other intra-abdominal malignancies (Dedrick, 1985). Most recently radioactive monoclonal antibodies have been injected intraperitoneally to image or treat malignant tissue selectively (Ward et al. 1987). (7) The fibrinolytic properties shared by vascular endothelium and peritoneal mesothelium have led to mesothelium being used as a substitute for endothelium, both for coating prosthetic vascular grafts (Bull et al. 1988), and also in peritoneal tube grafts for replacing segments of vein (Price-Thomas et al. 1976; Ribbe et al. 1988).

CONCLUSIONS

The rapid and profound toxaemia associated with general peritonitis, the risk of metastatic spread of neoplasms from the peritoneal cavity and the advent of peritoneal dialysis all emphasise the absorptive efficiency of the peritoneum. This review focuses attention on the major role of the diaphragmatic lymphatic system in draining fluids from the peritoneal cavity. Despite the modest diaphragmatic contribution to the total peritoneal surface area, mesothelial cells covering the diaphragm overlie a rich plexus of lymphatic lacunae, separated from the peritoneal cavity by only a thin barrier, the roof of the lacuna. The presence of special stomata between the mesothelial cells provides rapid direct drainage from the peritoneal cavity into the subjacent lymphatic lacunae. The stomata are confined strictly to the peritoneal surface of the diaphragm. The regional lymph drainage from the diaphragm is predominantly to mediastinal lymph nodes by way of retrosternal (parasternal) lymphatic trunks, the thoracic duct and other smaller lymph channels being only secondary pathways.

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