

Pathways of interstitial fluid and lymph flow in the liver acinus of the sheep and mouse

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(Accepted 13 January 1998)

ABSTRACT

In the acinus of the sheep and mouse liver, lymphatic vessels are restricted to the portal tracts. Vessels less than about 25 µm across form a network around portal venules, and are closely associated with the limiting plate of hepatocytes. The perisinusoidal space of Disse is continuous with the interstitial space of the portal tracts at the origin of the sinusoids. It seems likely that excess interstitial fluid derived from the sinusoids flows along the perisinusoidal space of Disse to enter the portal tracts near the portal venules, and then enters the small lymphatics which lie adjacent to those venules. It then enters the larger vessels, which are adjacent to hepatic arterioles and bile ductules.

Key words: Portal tracts; space of Disse; space of Mall.

INTRODUCTION

The precise origin of lymph within the liver acinus has long been a puzzle (Yoffey & Courtice, 1970). This puzzle has several dimensions: firstly, the protein content of liver lymph is so high—about 80% of the plasma concentration (Yoffey & Courtice, 1970; Courtice et al. 1974)—that it must have originated in a very permeable capillary bed such as the hepatic sinusoids (Gemmell & Heath, 1972; Wright et al. 1983; Wisse et al. 1985). Secondly, lymphatic vessels have not been found in the vicinity of the sinusoids, except near their origin in the portal tracts and, less convincingly, near their end at the hepatic venule (central vein) (Comparini, 1969; Yoffey & Courtice, 1970; Magari, 1990; Trutmann & Sasse, 1994). Thirdly, the volume of lymph produced (0.3–1 ml/kg body weight/h; Yoffey & Courtice, 1970) and the structure of the portal capillaries (Gemmell & Heath, 1972), seem to indicate that the portal tracts themselves are not an important source of liver lymph. And finally, even if it is assumed that most lymph is derived from the sinusoids, there is uncertainty about how that lymph reaches the initial lymphatics in the portal tracts (Yoffey & Courtice, 1970; Trutmann & Sasse, 1994).

Our aim was to provide evidence to help solve this puzzle. Most of the studies were performed on sheep,

as they are excellent subjects for studies on the lymphatic system (Lascelles & Morris, 1961; Heath, 1969; Yoffey & Courtice, 1970), but some tracer studies were done with ferritin in mice. Light and electron microscopy and computer reconstruction techniques were used to provide information on the structure and distribution of lymphatic vessels and related interstitial spaces.

MATERIALS AND METHODS

Nine crossbred lambs aged 4–5 mo and weighing 20–30 kg were housed in covered pens with free access to food and water. They were starved for 24 h, then anaesthetised with intravenous pentobarbitone (Nembutal, Bomac Laboratories, Asquith, NSW). Surgical anaesthesia was maintained throughout with halothane (Fluothane, ICI Pharmaceuticals, Macclesfield, UK) and oxygen in a semiclosed circuit. The lambs did not regain consciousness and were killed either by exsanguination or with an overdose of anaesthetic.

The end of the thoracic duct in the anaesthetised lambs was located just cranial to the first left rib near the junction of the left external jugular and subclavian veins. The duct, or 2–3 of its terminal branches, was ligated and left for 1 h to allow the abdominal lymphatic vessels to distend. The liver was then

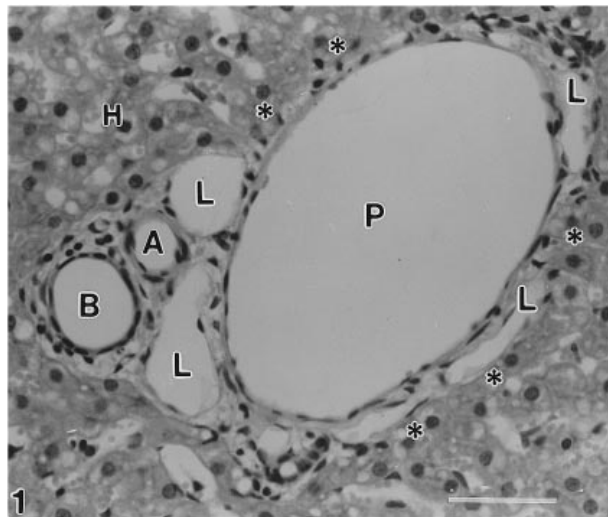


Fig. 1. Light micrograph of a section of sheep liver stained with haematoxylin and eosin, showing lymphatic vessels (L) within a medium-sized portal tract. The larger vessels are adjacent to the hepatic arteriole (A) and bile ductule (B), and the smaller vessels are around a portal venule (P). These are adjacent to a limiting plate of hepatocytes (*) which surrounds the portal tract. H, hepatic parenchyma. Bar, 50 μ m.

exposed through an incision just distal to the last right rib. A polyethylene cannula (Dural Plastics and Engineering, Auburn, NSW) was introduced into the common bile duct, threaded into the hepatic duct at the porta, and tied in place. The caudate lobe of the liver was clamped with uterine forceps and the lobe

excised immediately. Tissue for transmission electron microscopy was removed from this lobe then placed in a cold solution of 2.5% glutaraldehyde and 4% paraformaldehyde and processed as described by Lowden & Heath (1992).

The rest of the liver was fixed by perfusing 120–150 ml of cold 4% paraformaldehyde into the biliary cannula over a 5 min period. Portions of left, right and quadrate lobes were removed and placed in the same fixative. Six blocks were selected from each liver; they were dehydrated in alcohol, cleared in xylol and mounted in wax.

Histological sections (5 μ m) were cut from these blocks, stained with haematoxylin and eosin, and then examined and photographed using an Olympus PM-10AD photographic system attached to an Olympus BH-2 binocular microscope.

A further 225 sections (5 μ m thick and 10 μ m apart) were cut from each of 2 blocks and were used to create 3-dimensional images of the vessels in selected portal tracts. This was done by scanning images of the vessels using Ofoto for MacIntosh Version 1.1 (Light Sources Computer Images, California) and processing these images with a Micro Voxel Version 2.1 (Indec Systems, California) and a MacIntosh Quadra 950 computer (Apple Computer, California).

Tissue for scanning electron microscopy was dehydrated in alcohol, cleared in xylol and mounted in wax, then a face was prepared with a microtome.

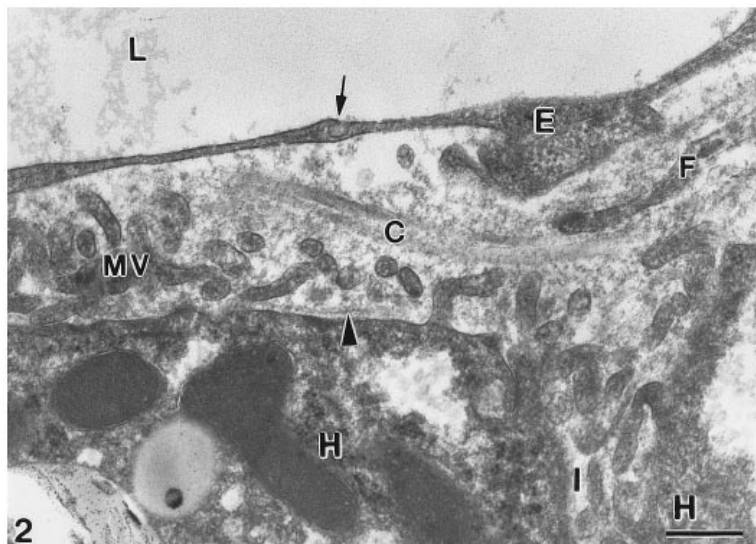


Fig. 2. Transmission electron micrograph from a sheep liver showing hepatocytes of the limiting plate (H) closely adjacent to a portal lymphatic vessel (L). Microvilli (MV) are present on the surface facing the lymphatic vessel and that facing an adjacent hepatocyte. A discontinuous basement membrane (arrowhead) is visible at the base of the microvilli. Collagen (C) and fibroblastic cell processes (F) separate the hepatocytes from the lymphatic endothelium (E), which is attenuated. I, interstitial space filled with matrix; arrow, interdigitating endothelial cell junction. Bar, 0.4 μ m.

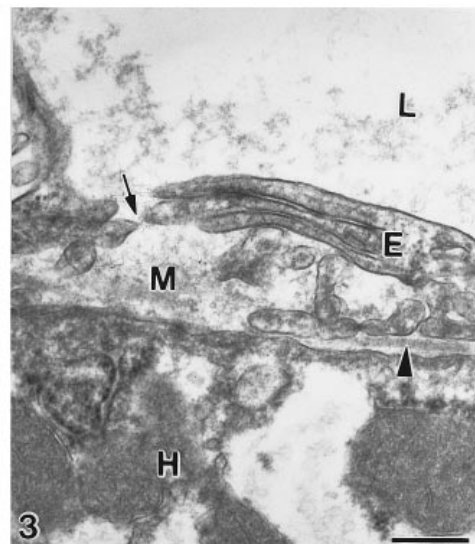


Fig. 3. Transmission electron micrograph from a sheep liver showing a gap opening (arrow) in the endothelial lining (E) of a small portal lymphatic vessel (L). H, hepatocyte of the limiting plate; M, interstitial matrix; arrowhead, basement membrane. Bar, 0.4 μ m.

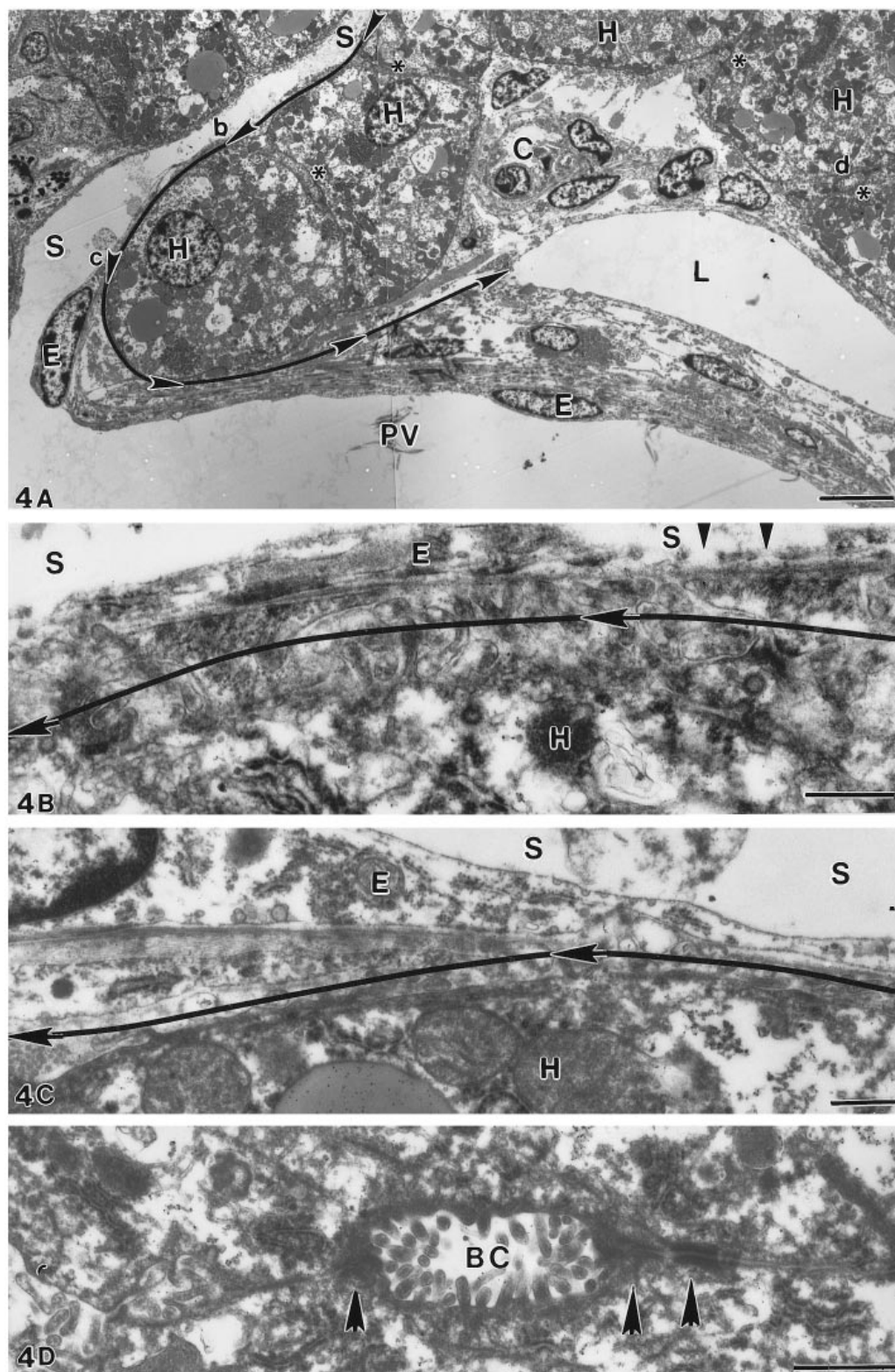


Fig. 4. Transmission electron micrographs of part of a portal tract from a sheep liver showing a sinusoid (S) emerging from a portal venule (PV) and penetrating through the limiting plate of hepatocytes (H). These hepatocytes are joined to one another by junctional complexes associated with bile canaliculi (*). The lines with arrows represent a potential lymph pathway through the perisinusoidal space of Disse to the interstitial space between the portal venule and the limiting plate, and to a small lymphatic vessel (L). Segments of this pathway at b and c in A appear in higher magnification in B and C. Small arrowheads indicate gaps in the sinusoidal wall. (d) Higher magnification of the region marked d in A, and shows the fusion of the cell membranes (arrowheads) on either side of a canaliculus (BC). C, blood capillary. E, endothelial cell. Bars: A, 4 μ m; B–D, 0.5 μ m.

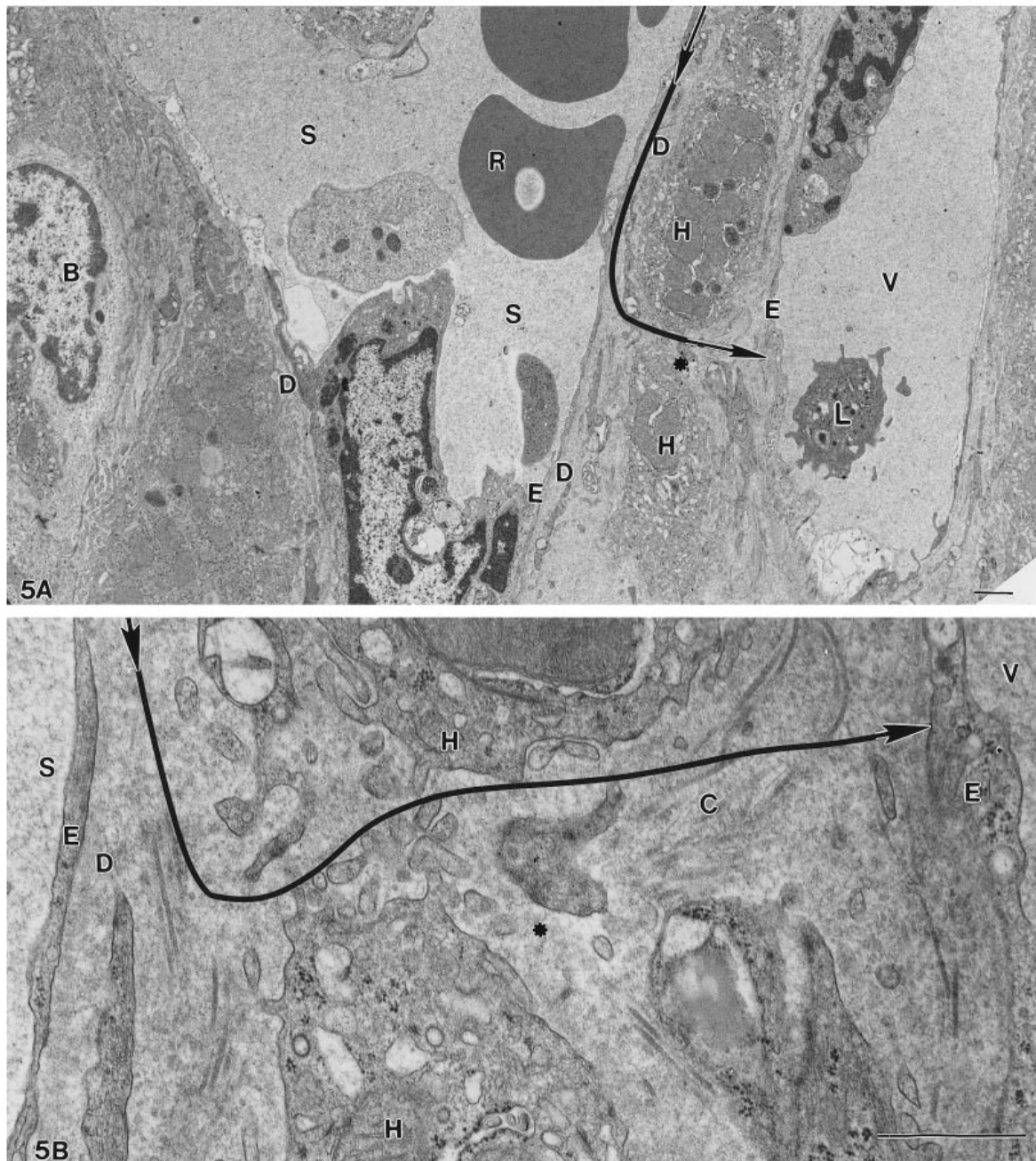


Fig. 5. Transmission electron micrographs from a portal tract of a mouse liver, showing a lymph vessel (V) and the beginning of a sinusoid (S). At the top of the figure this sinusoid is bifurcating as it extends into the acinus. The interstitial fluid space, shown at * in *A* and enlarged in *B*, extends from the perisinusoidal space of Disse (D), between hepatocytes (H) of the limiting plate, to the vicinity of the lymph vessel. The lines represent a possible lymph pathway from the acinus to the lymph vessel. R, erythrocyte; L, lymphocyte; E, endothelial cells; B, bile ductule epithelium. Bars, 1 μ m.

The specimens were dewaxed, dried by the critical point method, and examined in a JSM 6300F SEM.

Studies were also performed on 4 young adult mice. They received ferritin (Sigma Chemical Co, St Louis, MO) at 0.5 mg in 0.1 ml saline intraperitoneally, and were killed by cervical dislocation at 4 h (2 mice) or 25 h (2 mice). Blocks of liver were fixed, prepared and examined by transmission electron microscopy (Lowden & Heath, 1992).

RESULTS

Lymphatic vessels were found only in the portal tracts within the acini (Fig. 1); none were seen amongst the hepatocytes, or near the hepatic venules.

In sheep, although less clearly in mice, each portal tract was surrounded by a limiting plate of hepatocytes, which were joined together by junctional complexes on either side of bile canaliculi. Most of the

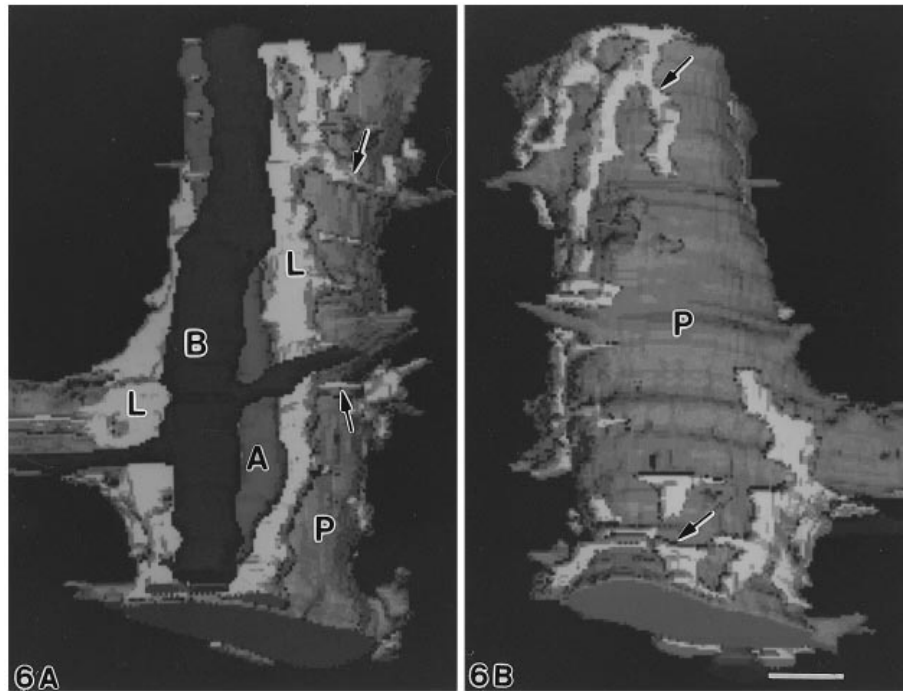


Fig. 6. Opposite planes of a computer-generated 3-dimensional reconstruction of a medium-sized portal tract in a sheep liver. In *A*, the largest lymphatics (L) lie adjacent to the hepatic arteriole (A) and bile ductule (B). In *A* and *B* it is evident that these vessels are continuous with a lymphatic network (arrows) around the portal venule (P). Bar, 60 μ m.

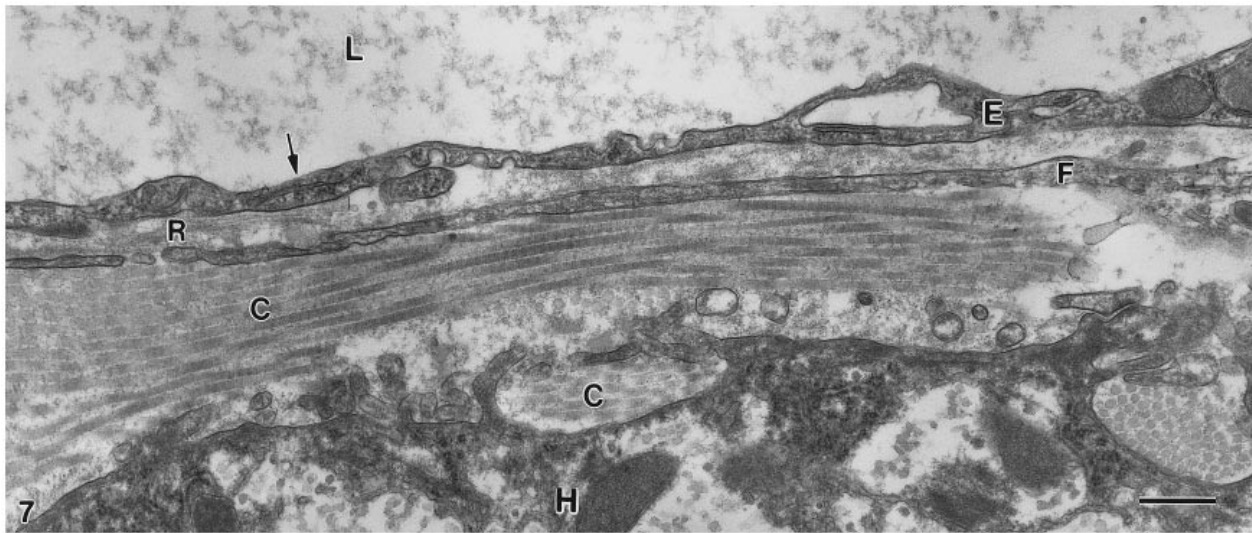


Fig. 7. Transmission electron micrograph from a sheep liver showing a lymphatic vessel (L) which forms part of the network around the portal venule. Attenuated lymphatic endothelium (E) is separated from the limiting plate of hepatocytes (H) by collagen bundles (C) and fibroblastic cell processes (F). Reticular fibres (R) lie adjacent to the abluminal endothelial surface. Arrow, overlapping endothelial cell junction. Bar, 0.5 μ m.

hepatocytes of the limiting plate had microvilli on their periportal surfaces, and in some cases these extended into the space between adjacent hepatocytes (Figs 1–5).

The limiting plate was penetrated by sinusoids extending outwards from the portal tract. Each sinusoid was surrounded by the space of Disse and, where the sinusoid emerged from its origin at the portal venule, this 'space' was continuous with the

interstitial tissue of the portal tract (Figs 1, 4, 5). In tracer studies, ferritin was present in the space of Disse and along the interstitial fluid space to the vicinity of the lymphatic vessel. Although it is possible that some sinusoids arose from hepatic arterioles, none were seen.

Lymphatic vessels were readily identified in medium-sized portal tracts (200–600 μ m across), as thin walled vessels, 4–60 μ m across, with an irregular

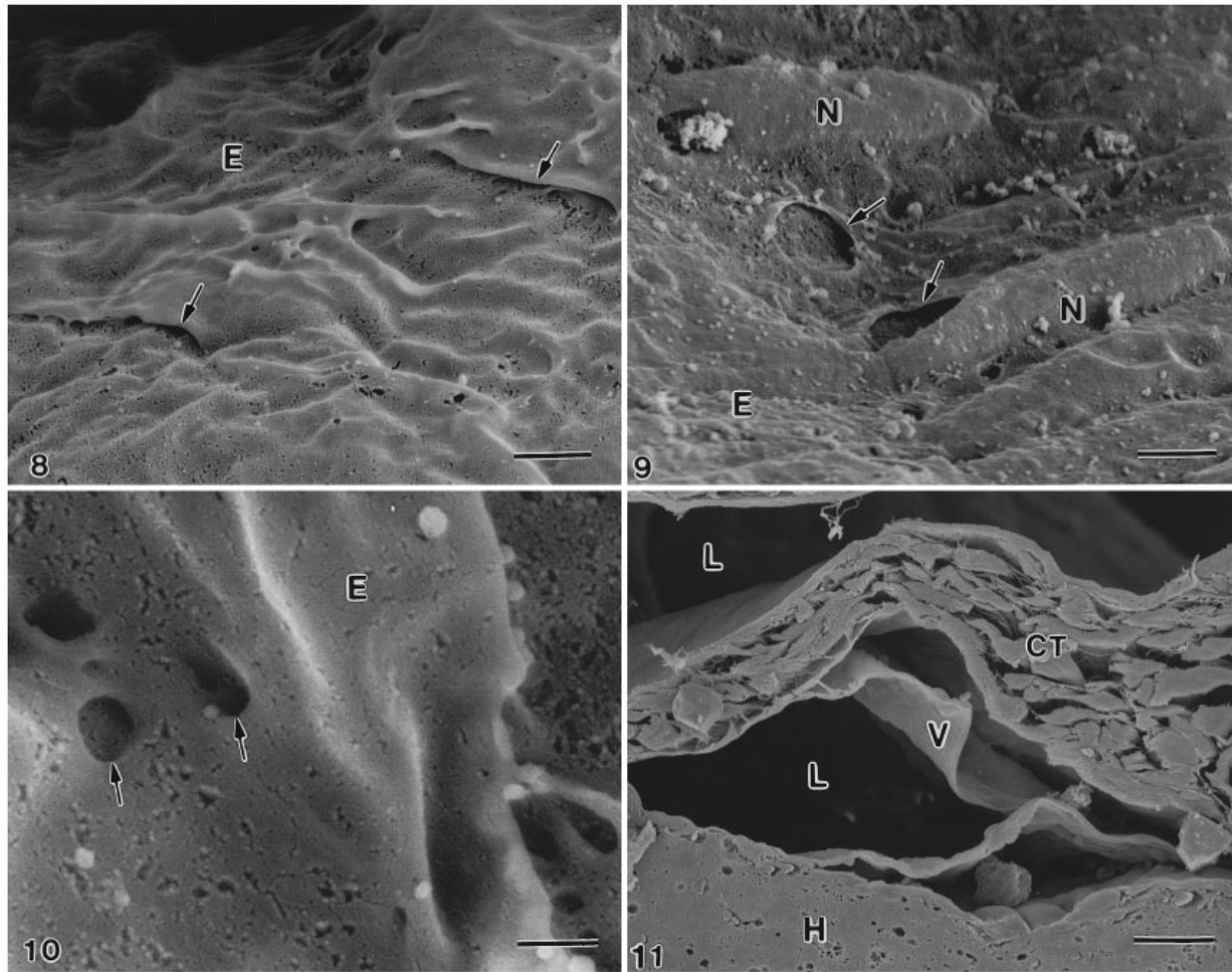


Fig. 8. Scanning electron micrograph from a sheep liver showing adjacent lymphatic endothelial cells (E) overlapping one another to form intercellular clefts (arrows). Bar, 2.0 μ m.

Fig. 9. Scanning electron micrograph from a sheep liver showing lymphatic endothelial cells (E) which contain rounded holes (arrows), but appear separated from interstitial tissue by a second layer of endothelial cells. N, endothelial cell nuclei. Bar, 2.0 μ m.

Fig. 10. Scanning electron micrograph from a sheep liver showing small rounded holes ($< 0.5 \mu$ m across; arrows) in lymphatic endothelium (E). Bar, 0.5 μ m.

Fig. 11. Scanning electron micrograph from a sheep liver showing a valve (V) in a portal lymphatic (L). CT, connective tissue; H, hepatocytes. Bar, 23 μ m.

shape (Figs 1, 4, 5; Courtice, 1981; Weber et al., 1991). The largest lymphatics were adjacent to the hepatic arterioles and bile ductules (Fig. 6). They were continuous with a network of smaller ($< 25 \mu$ m across) vessels which were present around portal venules (Fig. 6). These vessels were in a discontinuous rim of connective tissue adjacent to the hepatocytes of the limiting plate (Figs 1–5). They were lined by attenuated endothelial cells joined mainly by overlapping or interdigitating junctions (Figs 2, 3, 5, 7).

Scanning electron microscopy revealed several types of opening in this endothelial lining. In some areas for example, adjacent endothelial cells overlapped one another to form intercellular clefts up to 6 μ m long (Figs 7, 8). In other areas, endothelial cells contained rounded holes up to 5 μ m across which seemed

separated from the interstitial tissue by a second layer of endothelial cells (Fig. 9). Gaps were evident in these holes when viewed obliquely. Smaller rounded holes, less than 0.5 μ m across, were also seen in the endothelium (Fig. 10). It could not be determined if these holes formed a direct connection to the underlying tissue. Transmission electron microscopy revealed that the smaller vessels had occasional gap openings, which appeared to allow direct communication between the interstitial matrix and the vessel lumen (Fig. 3).

In vessels more than 25 μ m across, the endothelium was reflected over the surface of valves (Fig. 11).

There was some evidence of a basement membrane around the lymphatic wall (Figs 2–5, 7). Reticular fibres also occurred near the abluminal endothelial

surface, particularly in larger vessels (Fig. 7). These vessels had outer layers of collagen and fibroblasts with prominent processes, and in some places these separated the endothelium from hepatocytes of the limiting plate (Fig. 7). The smaller vessels had little or no connective tissue, and they approached the hepatocytes of the limiting plate so closely that they were separated only by a thin layer of interstitial matrix (Fig. 4).

None of the lymphatic vessels had smooth muscle cells in their walls.

DISCUSSION

The only lymphatic vessels in the hepatic acinus are in the portal tracts. The structure of these vessels is similar to that of blood vessels of similar size and in the absence of specific markers it could be difficult to distinguish them from empty blood vessels. However, in most cases morphological features including location, structure and content (lymphocytes but no erythrocytes), together with the distribution of ferritin where this was used, provide a clear basis for identification. The lymphatic vessels in the portal tracts drain fluid from the portal interstitial tissue, which is continuous with the perisinusoidal space of Disse where the sinusoids emerge from the portal tract.

The formation of lymph would seem to involve lymph constituents emerging from the hepatic sinusoids into the space of Disse, passing back along the outer surface of the sinusoids to their origin in the portal tracts, and entering the network of lymphatic vessels which is associated with the portal venules (Al-Jombard et al. 1985; MacSween & Scothorne, 1994). This conclusion is consistent with those of Magari (1990) and Trutmann & Sasse (1994) that the perisinusoidal space of Disse can be regarded as a prelymphatic space from which hepatic lymph could originate.

Some authors have also considered that the space of Mall, which is described as being between the outer limiting plate of hepatic parenchyma and the portal interstitial tissue (Trutmann & Sasse, 1994), is a potential prelymphatic space. These conclusions have generally been based on light microscopy, and are not easy to reconcile with the ultrastructural view of the portal tracts. When viewed with the electron microscope, it is evident that there is no true space between the hepatocytes and the portal interstitial tissue; the matrix of the portal interstitial tissue is in contact with the hepatocytes. The smaller portal lymphatics lie within the matrix adjacent to the hepatocytes.

It may be argued that the 'space of Mall' can communicate with the 'space' of Disse—which is also filled with interstitial matrix (Reid et al. 1992)—along the intercellular spaces between adjacent hepatocytes (Schatzki, 1978). However, the hepatocytes are joined together by junctional complexes, most of which are on either side of bile canaliculi and which are of limited permeability (Fawcett, 1994). It therefore seems unlikely that the so-called space of Mall plays any special role in lymph formation, at least in sheep.

The space of Disse, being in direct communication with blood plasma through the highly permeable walls of the sinusoids, seems the only possible source of high-protein interstitial fluid in the acinus. If that is the case, then a mechanism must be proposed whereby the fluid in this space is able to flow towards the portal tract, when that in the adjacent sinusoids is flowing in the opposite direction. Concentration gradients in the extracellular matrix are likely to be important (Reid et al. 1992; Trutmann & Sasse, 1994) though their specific role is still unclear. Pressure gradients produced by the massaging effects of leucocytes passing along the sinusoids, may also be important (Wisse et al. 1985). Magari (1990), in a review of the hepatic lymphatic system, proposed that fluid flows along the fine fibrils of collagen in the perisinusoidal spaces.

More recently, Trutmann & Sasse (1994), referring to an unpublished dissertation by Trutmann, claimed that 'it can be demonstrated that the fluid leaves this (perisinusoidal) space in both directions with and against the current in the inner tube'. If the fluid leaves the perisinusoidal space in both directions, one would expect to find lymphatic vessels around the hepatic venules. These were not evident in our sheep, but we cannot exclude the possibility that some lymph leaves the liver in vessels accompanying hepatic veins, as occurs in some other species (Comparini, 1969; Magari, 1990; Yoffey & Courtice, 1970; Trutmann & Sasse, 1994).

The excess interstitial fluid which enters the portal tracts along the perisinusoidal space of Disse enters the small lymphatic vessels which lie around the portal venules. The endothelium of these vessels contains overlapping cell edges which form clefts and holes of various sizes. Many features of the vessel walls, including the intercellular junctions, are similar to those in rat and human portal lymphatics (Schatzki, 1978; Niir & O'Morchoe, 1986; Magari, 1990). However reticular fibres on the abluminal surface appear to be less common. Those fibres which are present resemble anchoring filaments described by Leak & Burke (1968) and are believed to bind the

lymphatic wall to adjoining connective tissue, allowing vessel dilation and aiding fluid movement into the lumen (Leak & Burke, 1968; Courtice, 1981; Castenholz, 1988).

ACKNOWLEDGEMENTS

We are grateful to Gary Godbold and Tina Chua for their help, and the Australian Research Council for financial support.

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