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The Ultrastructure of Pulmonary Lymphatic Capillaries of Newborn Rabbits and of Human Infants

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For a long time morphological studies of the pulmonary lymphatics have erroneously been considered as the simple search for a more precise knowledge of a problem which seemed only a delicate and intriguing anatomical puzzle. This usually involved the disputed presence (1–10) or absence (11–21) of true alveolar lymphatics, the localization and orientation of lymphatic valves and consequently the problem of the direction of lymphatic flow, and the differentiation of tissue clefts or small blood vessels from true lymphatics. Recent physiological (22–24), embryological (25), pathological (26–28, 82) and clinical (23, 24) investigations have revealed however that these problems have a basic and challenging importance in the understanding not only of the structure

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and function of the normal and diseased lung, but even in the regulation of body fluids. Such investigations might well shed some light on many other related problems of the pulmonary lymphatic microcirculation, on which opinions differ or are non-existent, such as the distinction between (pulmonary) lymph and interstitial fluid, and the mechanisms governing their formation and subsequent removal, the way or ways in which fluids and cells enter lymphatics, and the forces which propell them into the larger lymph ducts.

To answer some of these questions, we previously made serial histological (13, 29), morphometric (28) and radiologic studies (23, 30) as well as investigations on corrosion casts after the injection of the pulmonary lymphatics (29–31). The results obtained led to electron microscopical studies (30, 32, 33). Apart from other advantages, a careful electron microscopical investigation avoids some of the difficulties usually encountered in light optical microscopy, (where the distinction between the smallest blood vessels and true lymphatic vessels is often impossible) or in injection or corrosion specimens in which the formation of possible artefacts due to injection of tissue clefts is a potential source of error. Moreover, an electron microscopical investigation seemed warranted as no previous studies are known to us on this subject, except for the studies of *Kato* (34, 35), *Lauweryns* et al. (30, 32, 33) and two low-power micrographs by *Schulz* (36). Neonatal infant and rabbit lungs were more specifically studied, as the pulmonary lymphatics are especially prominent in such lungs (13).

Material and Methods

1. Pulmonary tissue blocks not exceeding 1 mm^3 were taken immediately after death from nineteen rabbit fetuses which were delivered near term by cesarean section. At the time of the section, the umbilical circulation was clamped for 30 to 60 seconds, at intervals of 1 to 2 minutes. The resulting fetal hypoxia produced distinct and immediately respiratory movements of the fetuses, while still in their amniotic sacs. As demonstrated earlier with ordinary optics (13), the primary atelectatic fetal lungs became partially expanded and the lymphatics were more easily identified in the sections. Next, each fetus (still surrounded by its intact membranes) was sacrificed by tightening a noose about its neck.

The tissues were fixed in buffered OsO_4 (37) (7 cases) or in buffered formalin, followed by an OsO_4 postfixation (12 cases) and embedded in Epon (38). Sections were cut at 1 μ with a Porter-Blum MT-2 ultramicrotome and stained with toluidine blue or the *Grimley* (39) technique, using malachite green, toluidine blue, and alcalic fuchsine.

Sections were carefully selected for further trimming of the tissue blocks for ultrathin sectioning. These areas included a) lymphatic capillaries – unmistakeable by plain microscopy, b) "small vessels" which on the basis of their histologic appearance (no red cells in the lumen, walls consisting simply of a sheet of endothelial cell resting upon a basement membrane), could be either lymphatics, small capillaries, or minute blood vessels, and c) numerous interalveolar septa.

The ultrathin sections were stained with uranyl acetate and lead citrate (40) and examined with a Zeiss electron microscope (EM 9A).

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2. From seven infants, who died shortly after birth (1 human fetus of 260 g; 6 with typical idiopathic respiratory distress syndrome) pulmonary biopsies were taken as soon as possible after death (within one hour) and processed in the manner described above (fixation: buffered OsO_4: four cases; buffered formalin, followed by an OsO_4 postfixation: three cases).

3. From 1 and 2, tissue blocks covering the entire cut surface of the lung lobes were fixed in *Bouin's* fluid, embedded in paraffin and serially sectioned for a comparative histological study. The slides were alternately stained with H.E. and *Bielschowsky's* method as modified by *Foot* for reticular fibers (41).

Observations

It is of paramount importance for the clear expression of our views and the understanding of the reader of the observations and discussion to follow, that we explicitly define some terms at the outset, i.e. "air-blood barrier", "interalveolar septum" and "alveolar wall".

The *"air-blood barrier"* (vs. *pathway)* is constituted by the alveolar epithelial cell lining, the alveolar interstitium (mainly the basement membrane[s] and any interposed connective tissue elements) and the blood capillary endothelial cells.

The *"interalveolar septum"* is defined as the septum between two neighboring alveolar lumina; it consists at both sides of an outer alveolar epithelial cell lining, which is supported by a central framework of connective tissue with its blood capillaries.

The *"alveolar wall"* is the boundary of an alveolus which lies in contact with the connective tissue of (a) an interlobular septum, or (b) the pleura, or the connective tissue surrounding (c) the bronchi (bronchioli) or (d) the pulmonary vessels. It consists of an alveolar epithelial lining, its subjacent capillaries and their contiguous connective tissue supports. The latter merge with the other connective tissue elements of a, b, c, d.

Light microscopy

The light optical study of the 6 μ and 1 μ sections confirmed our previous observations (12, 13, 28). The neonatal rabbit and infant lung contained a richly developed plexus of lymphatic vessels in the visceral pleura, as well as numerous lymphatic vessels situated predominantly at the periphery of the peribronchial (fig. 2), periarterial (fig. 1 and 4), perivenous (fig. 3) and septal connective tissues. Lymphatic capillaries were frequently observed in the 6 μ and 1 μ sections to be situated immediately against the "alveolar wall" (as defined above), being separated from the alveolar lumina only by the alveolar epithelium and its contiguous connective tissue support (which may be very thin and does not contain capillaries everywhere). These lymphatic capillaries, situated anatomically between the "alveolar walls" and the interlobular, pleural, peribronchial, or perivascular connective tissue sheets, have been defined in this study as *"juxta-alveolar lymphatic capillaries"* (fig. 3 and 4), because of their close topographical (and probably also functional) relationship to the alveolar lumina without being a part of the interalveolar septa themselves.

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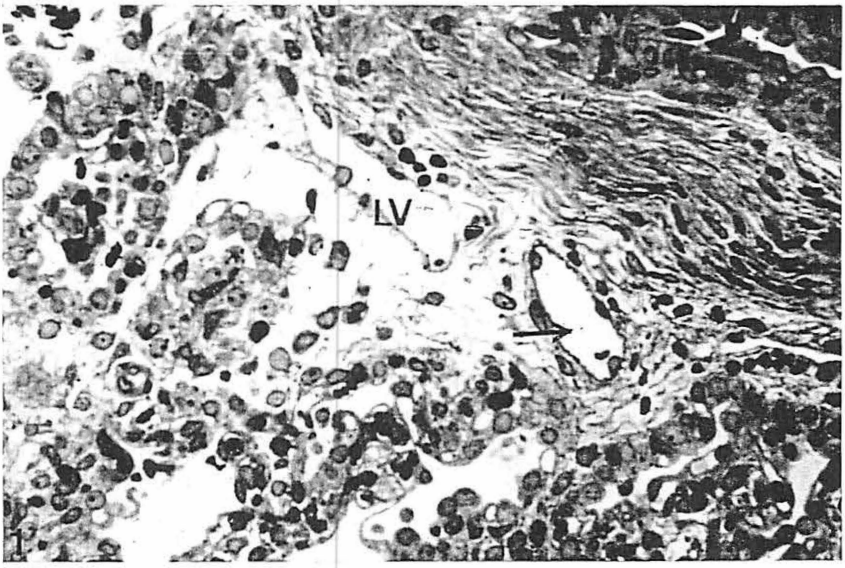

Fig. 1 Photomicrograph of a periarterial lymphatic capillary (group 1) and a light optically undifferentiated small vessel (group 2) (arrow) in the lung of a newborn infant. Both capillaries proved to be lymphatics at electron microscopical examination. (1 μ section; toluidine blue, 415×).

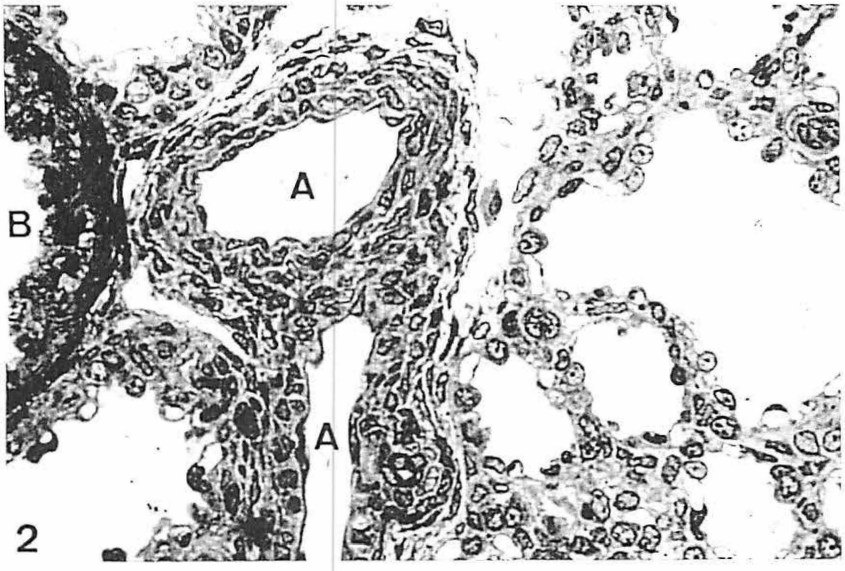

Fig. 2 Photomicrograph of two lymphatic capillaries in the connective tissue between a bronchiole and two sections of a pulmonary arteriole (newborn rabbit, 1 μ section, toluidine blue, 520×).

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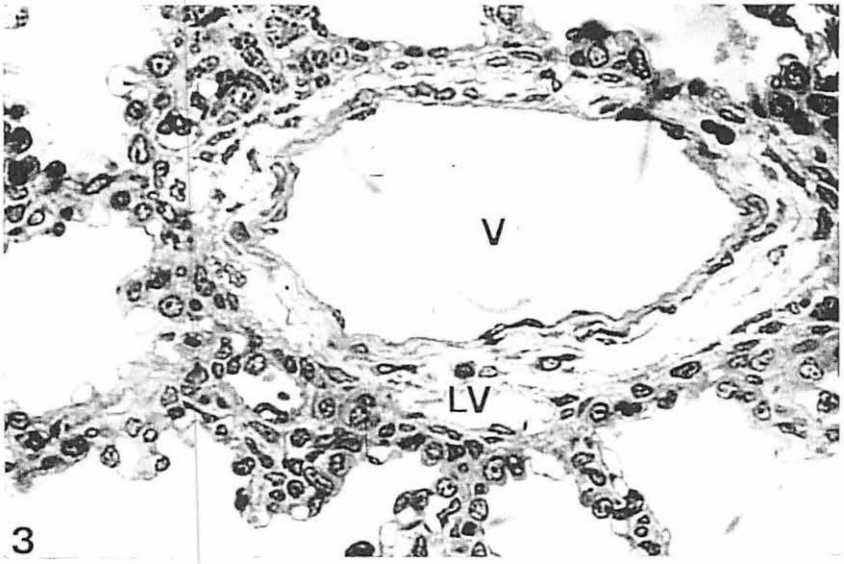

Fig. 3 Photomicrograph of a "juxta-alveolar lymphatic capillary" located between the connective tissue surrounding a pulmonary venule and adjacent "alveolar walls" (newborn rabbit, toluidine blue, 1 μ section, 520×).

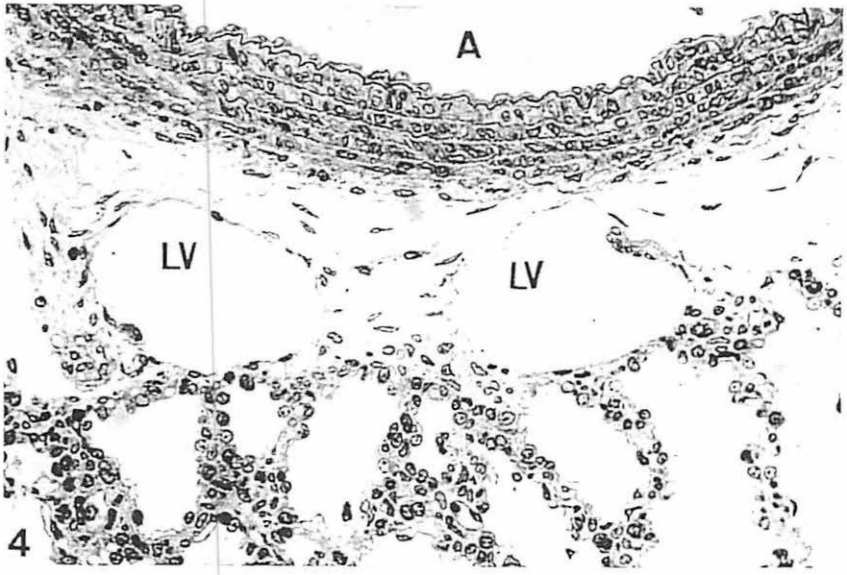

Fig. 4 Photomicrograph of two larger "juxta-alveolar lymphatics" between the adventitial connective tissue of a large pulmonary artery and adjacent "alveolar walls" in a newborn rabbit (1 μ section, toluidine blue, 292×).

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In typical instances they were easily recognized: they appeared relatively large, more or less spherical or oval, sometimes elongated, revealing a distended lumen, devoid of blood cells. Their walls consisted of endothelial cells and a tenuous connective tissue layer, which contained numerous reticular fibers (group 1).

Many minute vessels were observed in various localizations which on the basis of the light optical structure of their walls, could be either lymphatic or blood capillaries, or even terminal ramifications of precapillary arterioles or origins of postcapillary venules. We found it completely impossible to classify them with the light microscope (group 2).

The interalveolar septa appeared to be devoid of true lymphatic capillaries as we had observed before. Nevertheless, in view of the conflicting opinions of other investigators, we felt obliged to scrutinize many tissue samples at the ultrastructural level (group 3).

Electron microscopy

Group 1: *Unmistakeable lymphatic capillaries*

No major differences in ultrastructure have been observed between the lymphatics of human newborn infants and newborn rabbits.

Lymphatic capillaries are lined by large, flattened endothelial cells. Thick portions, containing the nucleus and most of the cell organelles alternate with thin lateral cytoplasmic extensions (170 Å minimal thickness). The endothelial cells show irregular or wavy outlines, owing to many irregular cytoplasmic projections which extend into the lumen of the vessel and into the perilymphatic connective tissue spaces (fig. 6, 9 and 18).

The mitochondria of the endothelial cells are usually small, round to oval, with thin cristae. The rough endoplasmic reticulum appears to be poorly developed. Occasionally, a Golgi apparatus, a centriole, multivesicular bodies, or lipid droplets are observed (fig. 1).

Round or oval dense bodies (fig. 19), measuring 70–300 mμ in diameter, tend to occur in small groups. They are lined by a unit membrane, separated from the dense core by a narrow rim of lesser electron density. The core is homogeneous or can display more or less parallel or concentric lines; these may be lysosomes.

The endothelial cytoplasm also contains small (70–100 mμ) and round coated vesicles (fig. 9 and 18). Their lining membranes are covered on the outside with short spiked projections. They could also be described as small invaginations of specialized sites in the cell membrane (fig. 9), like micropinocytotic vesicles: also present (though in a smaller number than in the endothelium of blood capillaries).

The endothelial cytoplasm of lymphatic capillaries contains also a few large vacuoles of irregular shape with a clear content and smooth surface. Some of these may be extracellular spaces taken in cross section (fig. 18).

A few intracellular filaments are present throughout the cytoplasm (fig. 15) but especially along the abluminal cell wall opposite from the lumen where they tend to occur in bundles, oriented parallel to (fig. 6) or more or less perpendicular to the cell surface.

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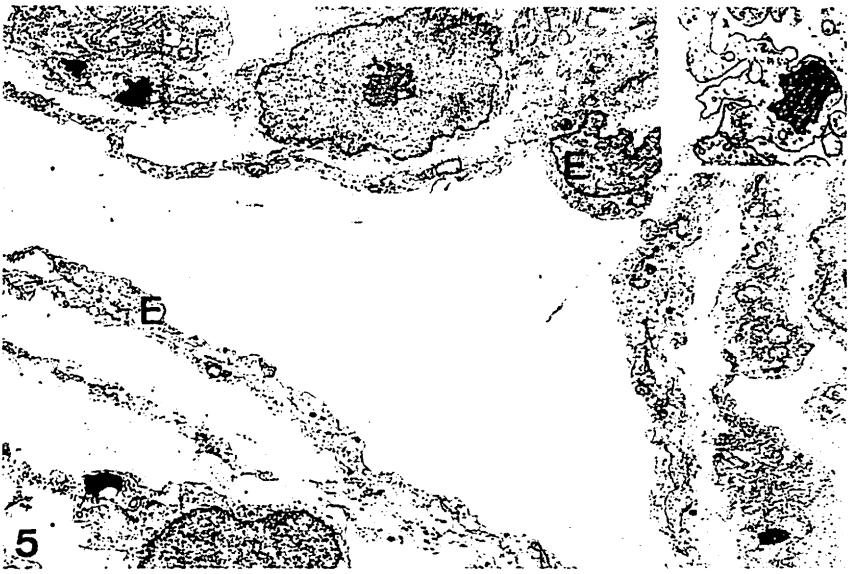

Fig. 5 Low power electron micrograph of a peribronchial lymphatic capillary in the lung of a newborn rabbit. The lumen is lined with large flattened endothelial cells (4.750×).
Insert: A lipid droplet in the cytoplasm of an endothelial cell, lining an interlobular lymphatic capillary (newborn infant, 28.500×).

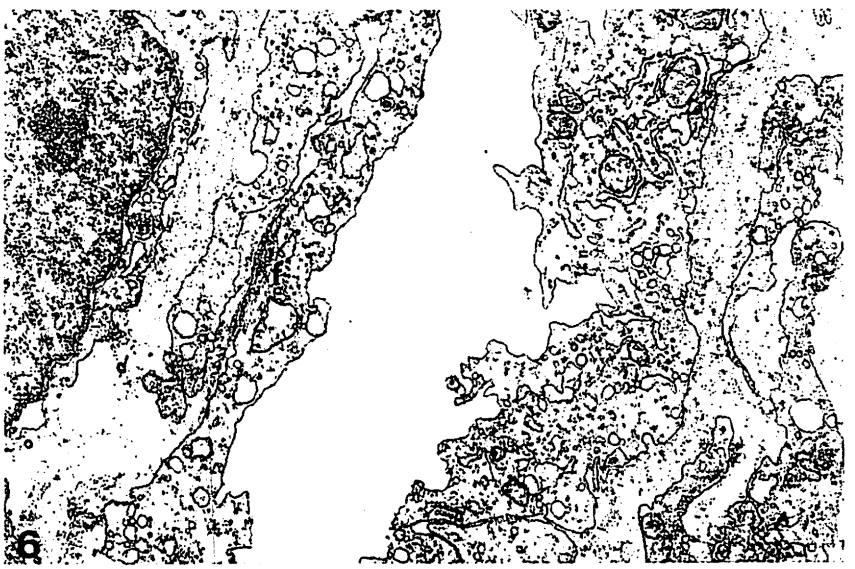

Fig. 6 Part of a peribronchial lymphatic capillary in the lung of a newborn rabbit. The endothelial cells show small and irregular cytoplasmic projections into the lumen. A few cytoplasmic filaments (f), lying parallel to the cell membrane are also present (15.000×).

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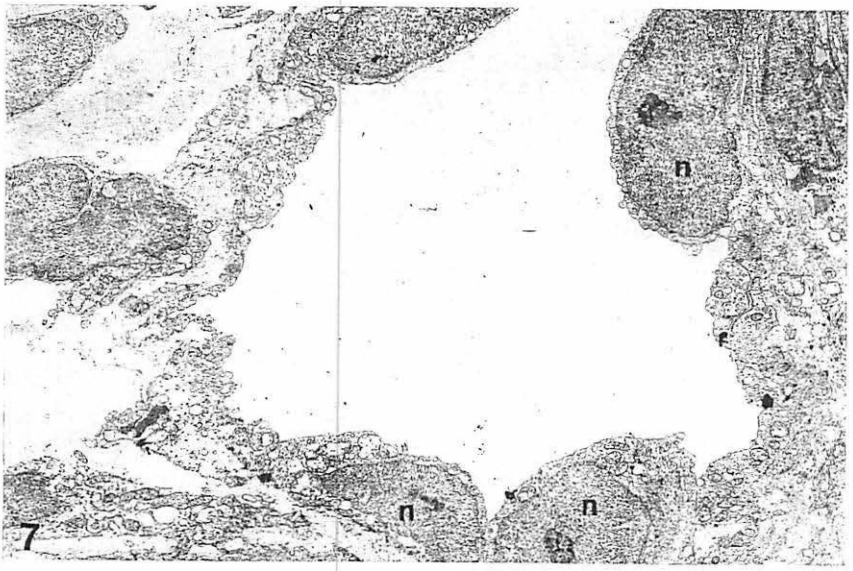

Fig. 7 Survey picture of a periarterial lymphatic capillary in the lung of a newborn rabbit. The nuclear portions of the endothelial cells bulge into the lumen (4.860×).

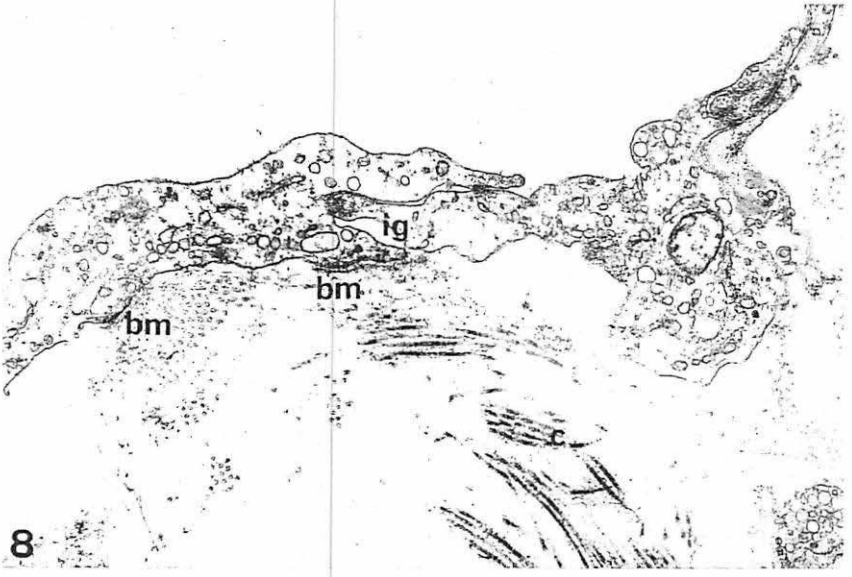

Fig. 8 Detail of the wall of a lymphatic capillary showing a distinct intercellular gap at the cell junction. Two small fragments of basement membrane (bm) are also present. The underlying connective tissue contains collagen fibers and a few fine filaments (newborn rabbit, periarterial lymphatic capillary, 16.600×).

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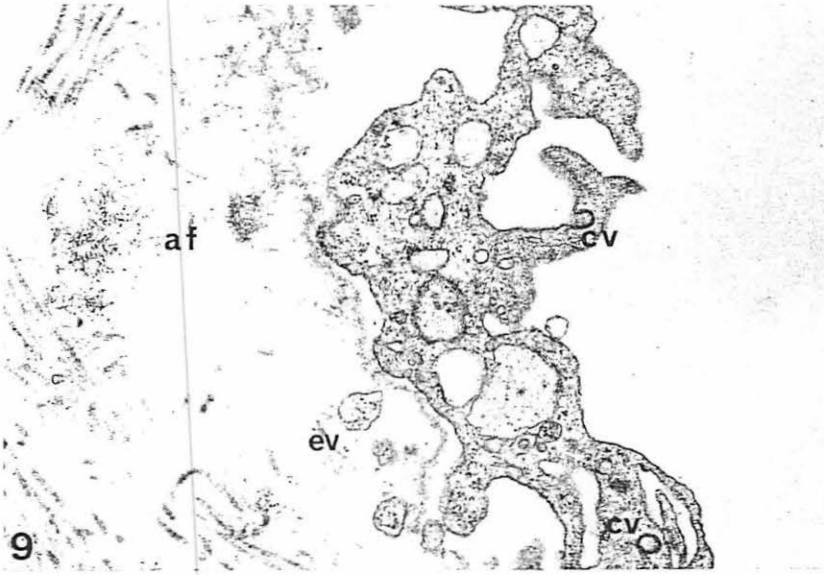

Fig. 9 Detail of a pleural lymphatic capillary of a newborn infant showing the irregularity of the endothelium, owing to many luminal and abluminal cytoplasmic projections. Two coated vesicles (cv) are seen, the left one is probably in a stage of formation. A fragment of basement membrane, collagen fibers and fine filaments (af) are present in the underlying connective tissue (21.600×).

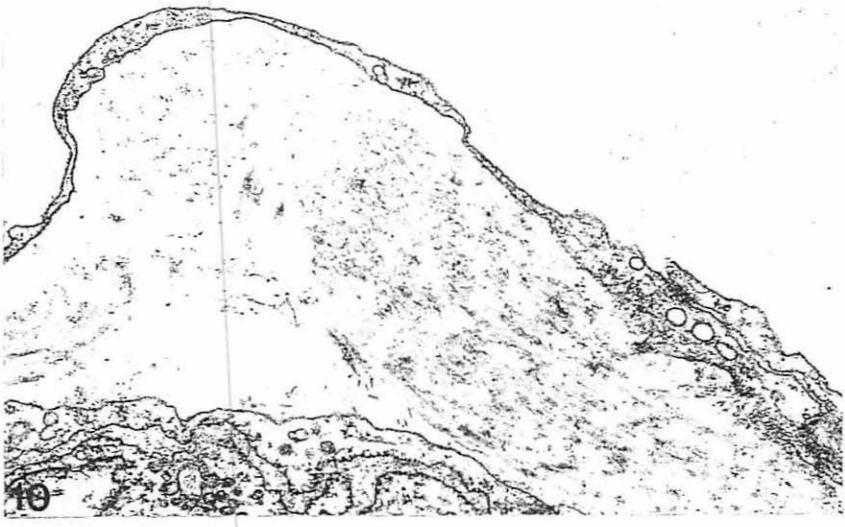

Fig. 10 Portion of a pleural lymphatic capillary in a newborn infant, demonstrating extreme attenuation of the endothelial lining (29.400×).

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Contact between endothelial cells is sometimes realized by simple end to end junctions, but mostly by overlapping of the endothelial cells (fig. 16 and 17) or by a series of interdigitations (fig. 12) reinforced with various types of attachment devices (zonulae adherentes, zonulae occludentes, maculae adherentes (42–44), between which intercellular gaps may occur (fig. 8 and 12). Occasionally the endothelial cell lining is interrupted (fig. 13).

The endothelium rests on a thin interrupted basement membrane (fig. 8, 9 and 16), which may be absent over long distances, especially along the thinnest parts of the endothelial cells (fig. 10).

The perilymphatic connective tissue contains collagenous fibers, fine filaments, a few vesicles of variable dimensions and shape with clear contents, and connective tissue cells (fig. 5 and 7). In favorable sections, the finer filaments exhibit a distinct periodicity. Frequently there is a close contact between the collagenous fibers and the fine filaments on the one hand, and the endothelial cells (fig. 14) or the basement membrane along them on the other hand suggesting "anchoring mechanisms" and which resemble the fine elastic fibrils described by *Greenlee* et al. (45) and *Kobayasi* (46). The extracellular vesicles may represent pinched off endothelial projections (fig. 9) as observed by *Friederici* (47) around the endothelium of blood capillaries in edematous skin of the rat.

Group 2: *Small vessels of questionable nature*

Once familiarized with the ultrastructure of typical pulmonary lymphatic capillaries (as detailed under group 1), it is quite easy to differentiate them by electron microscopy from blood capillaries and terminal ramifications of precapillary arterioles or beginnings of postcapillary venules. This observation concurs with those of other authors (44, 48–50) concerning the ultrastructure of lymphatics in other organs of the body.

Investigated in this way, the many small vessels of light optically undefinable nature, did not reveal additional data on the ultrastructure or the topography of the pulmonary lymphatics as detailed in group 1 and group 3.

Group 3: *Alveolar lymphatics*

Although many tissue blocks containing only "interalveolar septa" were carefully investigated (especially, when vessels were unidentifiable by light microscopy in 1 μ sections), we have never observed the occurrence of a true alveolar lymphatic capillary in any of them. At the ultrastructural level, all of the capillaries which were of questionable identity with light microscopy displayed all of the characteristics of blood capillaries.

On favorable ultrathin sections which included "alveolar walls" (as defined above), the "juxta-alveolar" occurrence of lymphatic capillaries, as detailed already light-optically, was frequently confirmed on the electron micrographs also (fig. 11).

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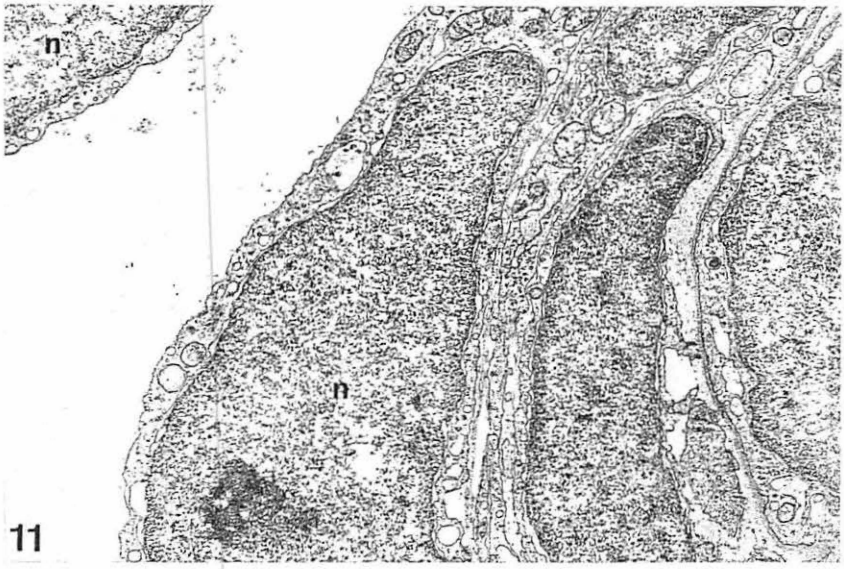

Fig. 11 Detail of two lymphatic endothelial cells, each containing a nucleus and a few cell organelles. The juxta-alveolar localization of this lymphatic capillary is evident (at the right of the figure is a portion of an alveolar epithelial cell) (newborn rabbit, 12.250×).

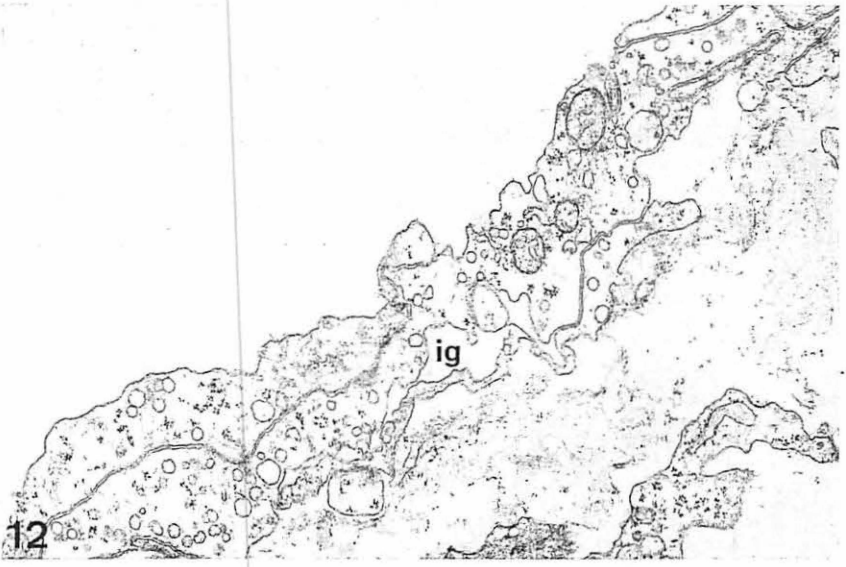

Fig. 12 Complex cell junction showing intercellular gaps (ig) between contiguous endothelial cells in a peribronchial lymphatic capillary of a newborn rabbit (21.000×).

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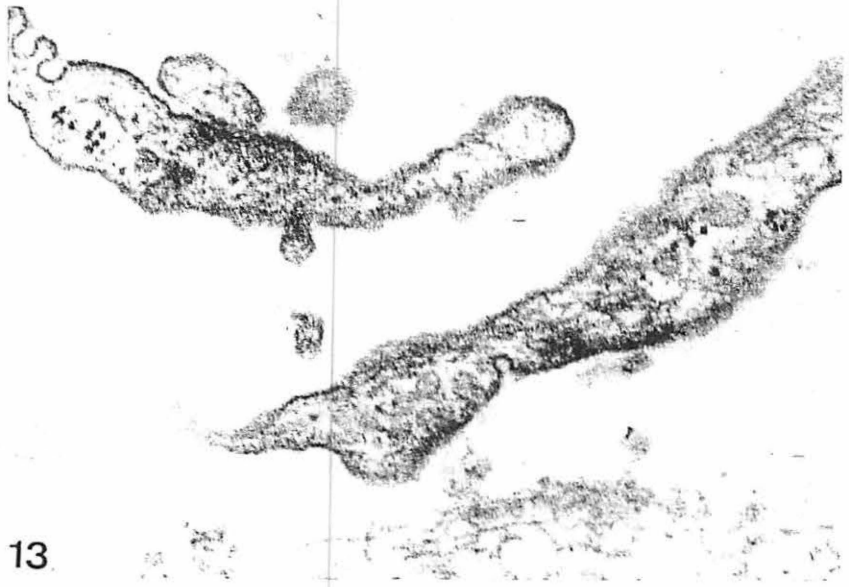

Fig. 13 Open cell junction in a pleural lymphatic capillary of a newborn infant. Some fine filaments and collagen fibers are seen in the underlying connective tissue (66.500×).

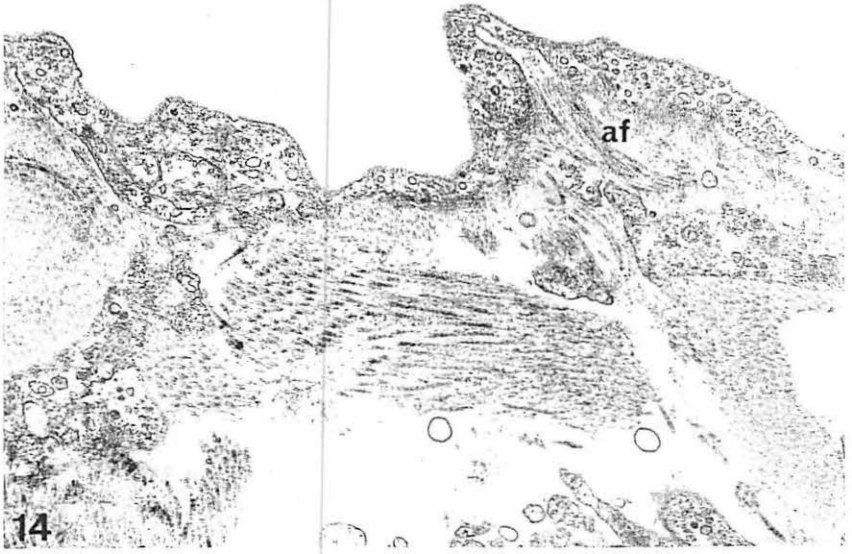

Fig. 14 "Anchoring filaments" (af) in the connective tissue surrounding the endothelium of a periarterial lymphatic capillary of a newborn infant lung; they are intimately associated with the endothelium and the subjacent collagen fibers (18.000×).

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Fig. 15 Higher magnification of endothelial cell cytoplasm of a pleural lymphatic capillary, showing many cytoplasmic filaments and several cisternae of the rough endoplasmic reticulum (human infant, 68.000×).

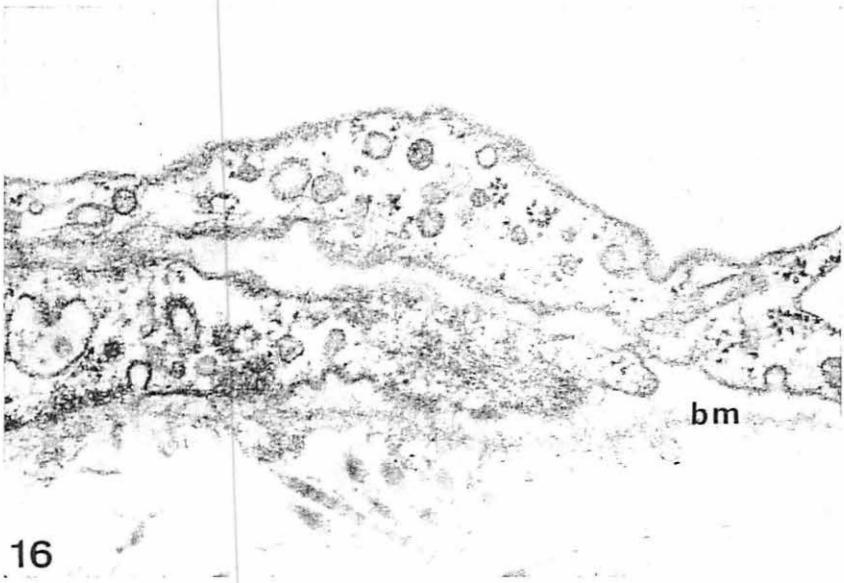

Fig. 16 Junction between two endothelial cells. Their cytoplasm contains a few micropinocytotic vesicles and free ribosomes. A fragment of basement membrane (bm) is visible. (Pleural lymphatic capillary, human infant, 57.000×.)

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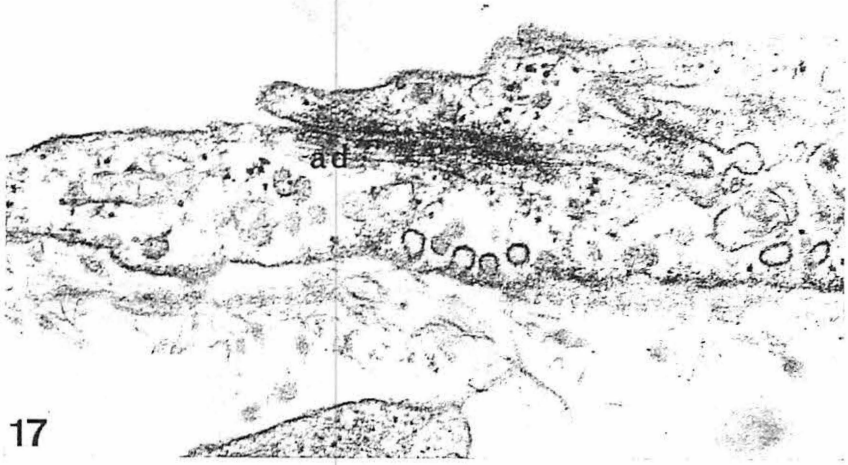

Fig. 17 A cell junction with attachment devices (ad). Note fine filaments in the underlying connective tissue. (Pleural lymphatic capillary, human infant, 56.000×.)

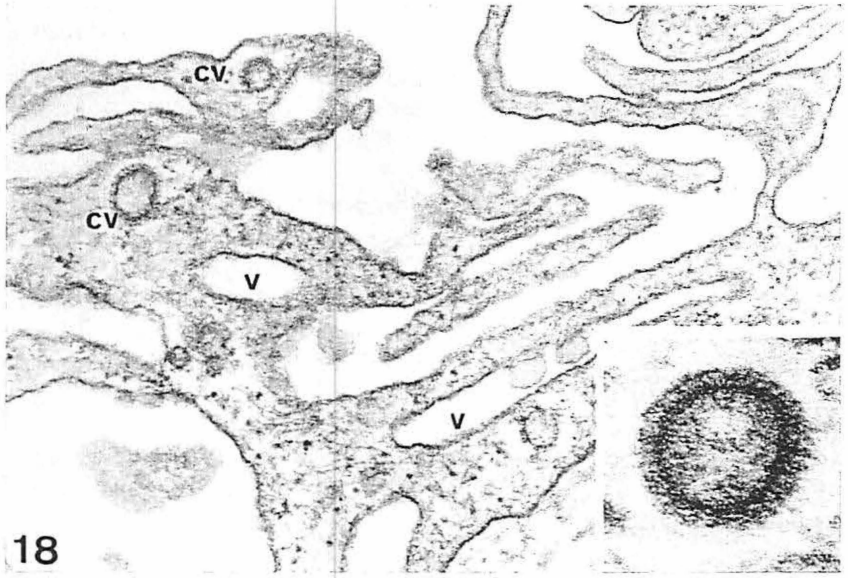

Fig. 18 Portion of the wall of a pleural lymphatic capillary in a human infant, showing an extremely irregular endothelial cell having many cytoplasmic projections; two coated vesicles (cv) are seen. Enclosures which appear to be vacuoles (v) are probably extracellular spaces, taken in cross section (56.000). Insert: highly magnified coated vesicle (170.400×).

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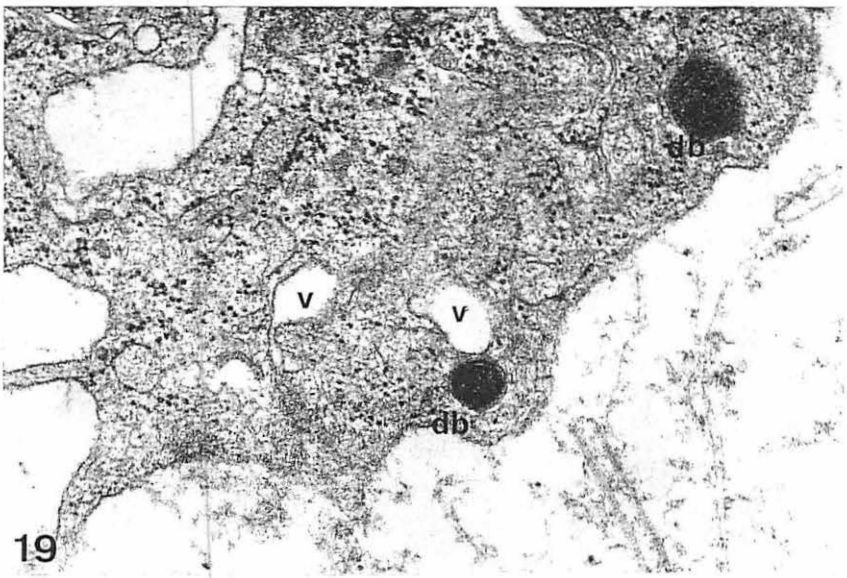

Fig. 19 Detail of a pleural lymphatic endothelial cell, containing two dense bodies and some vacuoles. Fine filaments and collagen fibers are seen in the adjacent connective tissue (newborn rabbit, 56.000×).

Discussion

1. This study is concerned with the ultrastructure of pulmonary lymphatic capillaries and does not include larger lymphatic vessels and lymphatic trunks. The material consisted of premature and mature lungs of newborn rabbits and human infants which are different from adult lungs in many respects.

Difficulties in obtaining human material immediately after death have prompted us to use formalin fixation in some instances, although osmium fixation was used as a rule. Moreover, since fetal tissues are generally difficult to fix, even with OsO_4, and since lymphatic capillaries are very delicate, thinwalled structures which are easily torn or otherwise mechanically disrupted, the results must always be interpreted with the utmost care to avoid considering possible artifacts as real findings. A comparison of our results with reports describing the ultrastructure of damaged lymphatic (51, 52) or blood capillary endothelium (53) suggests that the generally clear cytoplasmic matrix of the endothelial cells, and the clear and irregular vesicles in the perilymphatic connective tissue space could be artifacts. Perhaps also the large vacuoles with smooth surface and irregular shape in the endothelial cells are signs of endothelial damage.

2. A comparison of the ultramicroscopical characteristics of the pulmonary lymphatics of newborn rabbits and human infants with those in other organs i.e. in the intestinal villi of man (54–57) and other mammals (49, 50, 59–61), in the skin of rats (48, 51, 62), guinea pigs (44, 51) and frogs (63), in the diaphragm (52, 64) and in mesenteric lymph nodes of mice (65), reveals only minor points of difference.

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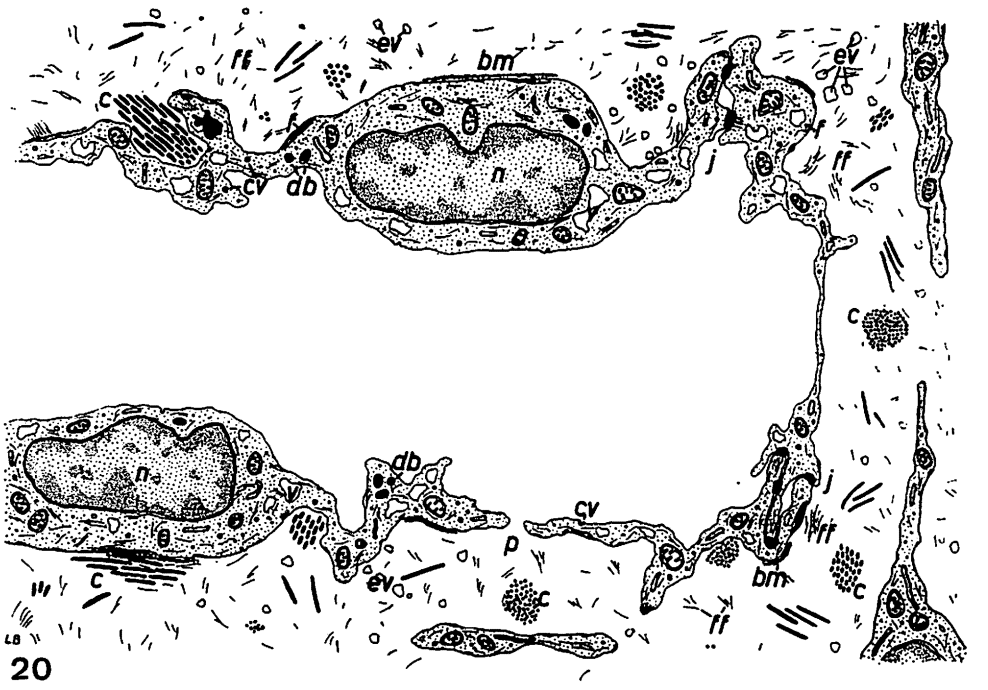

Fig. 20 Schematic drawing of a pulmonary lymphatic capillary combining its most prominent ultrastructural characteristics.

Key to figures 1 — 20

A	= artery, arteriole	db	= dense body
B	= bronchus, bronchiole	ev	= extracellular vesicle
E	= endothelium	f	= cytoplasmic filaments
LV	= lymphatic capillary	ff	= fine filaments
V	= vein, venule	ig	= intercellular gap
ad	= attachment device	j	= cell junction
af	= anchoring filaments	n	= nucleus
bm	= basement membrane	p	= open junction
c	= collagen fibers	v	= vacuole
cv	= coated vesicle		

N.B.: Fig. 1–8, 10–12 and 14 show osmium-fixed tissues, fig. 9, 13 and 15–19 show aldehyde-fixed tissues.

As in other organs, the lymphatic capillaries of the lung exhibit an irregular lumen, which is generally larger than the blood capillary lumen. The basement membrane is either discontinuous, poorly defined or absent. The endothelium is thin and irregular, owing to many cytoplasmic projections into the lumen of the vessel and the surrounding connective tissue space; it contains the usual cell organelles, micropinocytotic vesicles, vacuoles, dense bodies, multivesicular bodies, coated vesicles, and occasionally lipid droplets. The nucleus bulges into the lumen. Pericytes are absent (for references, see table 1).

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Table 1 Ultrastructural characteristics of lymphatic capillaries in different organs according to various authors. Only characteristics explicitly mentionned to be present (+) or absent (–) are indicated.

Localization	Intestinal villus (lacteal)											Cutaneous						Lymph node	Diaphragm	
Species	man	man	man	man	rat	rat	rat	guinea pig	mouse	mouse	cat	rat	rat	guinea pig	guinea pig	mouse	frog	mouse	rabbit	mouse
Reference	54	55	56	57	58	49	60	60	59	61	50	48	62	44	51	51	63	65	64	52
Lumen																				
– irregular			+				+				+	+	+							
– larger than BC											+			+		+				+
Basement membrane																				
– interrupted	+		+			+				+			+	+			+			
– absent				+			+		+		+	+							+	
– poorly developed					+					+				+		+				+
Endothelium																				
– thin	+	+				+	+			+	+	+		+						
– thicker than BC						+										+				
– clear cytoplasm							+													+
– luminal projections	+					+					+	+		+						
– abluminal projections	+	+				+					+	+	+							
– open junctions or pores	+	–		+	+	+	–			+?	–	–	+	+		+	+	±	+	
– attachment devices	+	+			–		+					–		+		-				-
Endothelial organelles																				
– nucleus bulges into lumen										+		+		+						
– micropinocytosis	+						+	+			+	+	+	+		+	+		+	+
– vacuoles	+	+		+			+				+			+			+			+
– dense bodies	+	+									+									
– multivesicular bodies	+	+																		
– poorly developed end. ret.	+	+					+					+		+		+				
– free ribosomes	+	+					+				+	+		+						
– coated vesicles														+						
– hemidesmosomes																	+			
– intracellular filaments	+	+										–		+			+			
Pericytes																				
– absent	+											+								

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Minor differences include the occurrence in pulmonary lymphatics of cytoplasmic filaments, many attachment devices, and endothelial discontinuities. Cytoplasmic filaments have been observed also in human lacteal vessels and in the cutaneous lymphatics of guinea pig ears; they were absent in the penile skin of the rat. Attachment devices between the neighboring endothelial cells have been noted in human lacteals and in the cutaneous lymphatics of the guinea pig ear, in the frog interdigital webs, and in the marginal sinus of mesenteric lymph nodes of mice; they were absent in cat lacteals, in the rat penile skin, as well as in the intermediate of mice mesenteric lymph nodes (for references, see table 1).

According to *Casley-Smith* (51, 52) these seemingly minor topographical differences could, in fact, be related to a different lymphatic permeability and and muscle activity; many open cell junctions and only a few attachment devices occur in lymphatics in the vicinity of actively contracting muscle, while the converse is observed in relatively motionless areas (where lymph flow is also slow).

Since respiratory movements occur only occasionally in utero (66, 67) the lymphatics of lungs of rabbit fetuses sacrified immediately before birth and of human infants who died shortly after birth, should be placed in the second category. In fact, and corroborating *Casley-Smith's* hypothesis (51, 52), we observed many attachment devices and only a few open cell junctions or pores. His hypothesis requires comparative investigation of pulmonary lymphatic ultrastructure and of actual lymph flow in the fetus, newborn (25, 68) and the adult as active respiratory movements occur continuously in the latter. It has also been demonstrated that at the onset of ventilation at birth there is a transient "large increase in lymph flow which is probably due to the clearance of liquid from the alveoli of the lung through lymphatics" (25, 68, 69). Its relationship to the statistically significant histometric increase in diameter of lymphatic vessels (pulmonary lymphangiectasis) in the "idiopathic respiratory distress syndrome" of the newborn infant is also of paramount importance in understanding the lymphatic microcirculation (26–28, 31).

3. As reported for other regions of the body, the ultrastructure of the pulmonary lymphatics is compatible with a great permeability and active transport. The discontinuity of the basement membrane and the presence of open junctions or pores establish a direct communication between the perilymphatic intercellular space and the lumen of the lymphatic vessel. Also, the large gaps between adjacent endothelial cells can be considered as tortuous thoroughfare channels through the lymphatic endothelial wall. They could correspond to a part of the funnel-shaped passages reported by Allen (70) . . . "looking down on the (peritoneal) spread, one could see hundreds of frog erythrocytes in the intercellular position like fish caught in a gill net, one end in the peritoneal cavity and one end in the lymphatic, the intermediate portion being pinched by the fenestration in the basement membrane".

The endothelial cytoplasm also reveals a number of structures, which are involved most probably in the transport of materials across the cell. Micropinocytotic vesicles have been interpreted as the morphological expression of the phenomenon of cytopempsis (71), while coated vesicles seem to be involved in the transport of proteins (42, 72, 75). Also, the large smooth surfaced vacuoles are most probably related to the

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transport of fluid through the endothelium. Finally, the discontinuous basement membrane eliminates from consideration a selective filter between the lymphatic capillary wall and the connective tissue.

4. A few criteria exist to distinguish lymphatic capillaries from the smallest blood vessels (capillaries, precapillary arterioles, postcapillary venules) with ordinary light optics; viz.: lymphatic capillaries have a larger lumen, a thinner endothelial lining, larger endothelial cells, a more irregular shape than blood vessels, and are generally devoid of red blood cells, but none of these criteria permits an easy and absolute distinction between the two categories of vessels. Electron microscopically, however, pulmonary lymphatic capillaries are quite different from pulmonary blood capillaries. Lymphatic capillaries have a poorly developed and interrupted basement membrane. Their endothelial cells are more irregular by the presence of many luminal and abluminal cytoplasmic projections. They contain fewer micropinocytotic vesicles, but do contain coated vesicles, dense bodies and intracellular filaments. Pericytes are absent, and the endothelium is rendered discontinuous by the open cell junctions and intercellular gaps. Pulmonary blood capillaries, in contrast, have a continuous basement membrane. The endothelial cells are more regular with only a few and exclusively luminal cytoplasmic extensions and the endothelial cytoplasm contains more micropinocytotic vesicles, but no coated vesicles, dense bodies, or intracellular filaments. Their wall contains pericytes and the endothelium is continuous without open junctions or intercellular gaps. This observation is in agreement with those of other authors (44, 48–50) concerning the ultrastructural distinction between blood and lymphatic capillaries in other organs of the body. Hence an electron microscopical investigation allows one to settle the disputed presence of "alveolar" lymphatic capillaries. "Alveolar" lymphatics have been described by *Wywodtzoff* (10), *Sikorski* (8), *Traska-Chrzomsczewski* (9), *Kutsuna* (6), *Ivano* (8), *Parfenowa* (7), *Engel* (4), *D'Arrigo* (3), *Barroso-Moguel* (1), and *Costero* (2). Their existence was doubted by *Tobin* (76) and denied by *Miller* (14–17), *von Hayek* (11), *Tamaska* (19), *Tobin* (20), *Renyi-Vamos* and *Papp* (18), *Van Waasbergen* (21) and *Lauweryns* (12, 13, 30).

The disagreement on this subject is probably attributable to the close resemblance of lymphatic capillaries to small blood vessels, when studied with the light microscope, to the ease with which artifacts arise when lymphatics are injected with coloured or plastic substances, to the fact that some of the earlier reports considered connective tissue clefts as part of the lymphatic system (5), and perhaps also by the use of inadequately defined terms.

If "alveolar" lymphatics are considered as lymphatic capillaries situated within the "interalveolar septa" (as defined above), then we have not observed "alveolar" lymphatics. Hence arises the problem of the removal of intra-alveolar fluid. Water, instilled in the trachea of living horses is very rapidly absorbed, probably by the alveolar blood vessels (77). Protein rich fluid, on the contrary, cannot be absorbed by blood vessels but has to be removed via the lymphatic vessels (77, 78). The slowness of its resorption (79, 80) hence has been attributed to the absence of alveolar lymphatics (79, 80). It appears thus, that the resorption of alveolar fluid is a very complex problem in which the lymphatics are only one facet and which deserves further investigation. Actually,

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alveolar septal lymphatics may not be required at all. Indeed lymphatic capillaries were frequently observed in the 6 μ, 1 μ and ultrathin sections being situated immediately against the "alveolar wall" (as defined above) being separated from the alveolar lumina only by the alveolar epithelium and its contiguous connective tissue support (which may be very thin and does not contain capillaries everywhere). These lymphatic capillaries, situated anatomically between the alveolar walls and the periarterial, peribronchial, perivenous or septal connective tissue sheets, but functionally probably intimately connected with the alveoli have been defined in this study as *"juxta-alveolar lymphatic capillaries"*. This juxta-alveolar occurrence was already suggested by *Tobin* (20, 76), who stated (20) from the study of serial sections of alveoli, or casts of them "... it is apparent that an artery and a vein with their accompanying lymphatics make contact with some part of every alveolus ..." This could perhaps explain the recent findings of *Dominguez* et al. (81) which have revealed – in sharp contrast with the conclusions of the above mentioned authors – that albumin introduced into the lung of dogs and guinea pigs was quite rapidly absorbed, intact.

Also alveolar lumina do not really seem to be primary sites of fluid accumulation. It has been demonstrated recently in studies of rapidly frozen lungs that, in experimentally induced and fulminating pulmonary edema "fluid appears first in the interstitial connective tissue compartment around the large blood vessels and airways" (22) i.e. in the locations where lymphatics are present, and well developed.

Summary

Pulmonary lymphatic capillaries of newborn rabbits and babies have been studied with the electron microscope. They were situated either in the pleura or in the periarterial, peribronchial, and perivenous connective tissue. As regards the disputed occurrence of "alveolar lymphatics", a sharp distinction has been made in this study between the "air-blood barrier", the "interalveolar septum" and the "alveolar wall". We have not observed lymphatic capillaries at the level of the air-blood barrier or the interalveolar septum. On the other hand, lymphatic capillaries are present between the alveolar walls and the interlobular, pleural, peribronchial or perivascular connective tissue sheets; these have been defined in this study as *"juxta-alveolar"* lymphatic capillaries, because of their close topographical (and probably also functional) relationship to the alveolar lumina without being a part of the interalveolar septa themselves.

Lymphatic endothelial cells form many irregular projections into the lumen of the vessel, and into the surrounding connective tissue. Occasionally, the endothelium is extremely thin. Contact between adjacent endothelial cells varies from a simple end-to-end junction to complex ones, with many interdigitations and various types of attachment devices. Sometimes open junctions are observed. The cytoplasm of endothelial cells contains dense bodies, coated vesicles and a few irregular smooth surfaced vacuoles and micropinocytotic vesicles. A variable amount of collagen fibers, finer filaments and a number of vesicles and connective tissue cells are present in the perivascular connective tissue. The basement membrane is discontinuous.

These salient ultrastructural features distinguish lymphatic capillaries from blood capillaries and suggest an intense transport of fluid across their wall.

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