

## LYMPHATICS IN THE LUNG OF A PRECOICIAL BIRD BEFORE AND AFTER HATCHING

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### ABSTRACT

*A light and electron microscopic study of pulmonary lymphatics was carried out in quail embryos (embryonic day; ED 13-17), completed with samples of lungs of quail 90 min, 24 h after hatching and two 2-day-old and three adult quail. The aim of the study was to depict the morphology of pulmonary lymphatics by determining the dynamics in ontogeny and to establish the rules of their distribution. The primitive lymphatics appear on ED 13 and 14 as closed thin-walled tubes in abundant interparabronchial mesenchyme. They seemingly differentiate from the mesenchymal cells. Due to the proliferation, growth, and enlargement of the parabronchial compartments, the interparabronchial septa disappear to a large extent, and the external walls of parabronchi appose and join. On ED 16 and 17, the mesenchyme is squeezed to the trigonal fields among the neighboring parabronchi. The lymphatics form broad, voluminous lakes around the arteries; on the other hand, they are also found in close contact with the gas exchange tissue as juxta-air capillary lymphatics. After hatching, the former interparabronchial septa disappear, and the imaginary boundary between parabronchi is demarcated by interparabronchial arteries and veins. The lymphatics are confined to the adventitial connective tissue which conducts the larger arteries and veins of the original trigone of the interparabronchial septa. The richly vascularized parabronchi in mature*

*quail are poor in connective tissue and to a large extent devoid of lymphatics, in comparison to the mammalian lung where the lymphatic capillaries have their roots at the level of the respiratory bronchioles.*

*The avian pulmonary lymphatics serve as an appropriate model for the analysis of principles controlling the origin and distribution of lymphatics in general.*

The system of pulmonary lymphatics in mammals has been investigated under normal and experimental conditions by numerous authors using light, transmission and scanning electron microscopy. Comprehensive information on pulmonary lymphatics in mammals is provided in Leak's review (1). The basic structural plan of the avian lung differs substantially from that of mammals. The question was raised whether this difference would also be reflected in the pattern of distribution of pulmonary lymphatics. The goal of the present study was to investigate the morphology of pulmonary lymphatics in quail, their dynamics in ontogeny and to establish the rules of their occurrence and distribution, based on a light and electron microscopic analysis.

It has generally been established that in mammals the lymphatics form a much larger proportion of all organs in fetal life than at a later age. Embryologically, pulmonary lymphatics in man enter the hilar region (second month of fetal life) as large vessels. As development continues they ramify to

produce plexiform channels which extend peripherally along bronchi, pulmonary arteries, veins and the pleura within the connective tissue areas (2). Lauweryns (3) and Lauweryns and Boussauw (4) described in fetal and neonatal rabbit and human infants numerous and prominent lymphatic capillaries in connective tissue around the pulmonary arteries. Lauweryns (5) also introduced the term juxta-alveolar lymphatic capillaries, which are situated close to the alveolar wall, being separated from the alveolar lumina only by the alveolar epithelium and its continuous connective tissue support which may be very thin and contains capillaries everywhere. Lopez (6) refers to the lymphatic vessels in the peripheral lung tissue of domestic fowl and in intermediary connective tissue septa. He emphasizes that in the relevant literature a pulmonary lymphatic system in avian lung is not mentioned. There exist reviews on the blood vessel system of bird lung (7). However, little attention has been paid to the avian pulmonary lymphatic system and its development. Klika et al. (8) performed a light and electron microscopic study of the lymphatic bed in the heart of embryonic fowl between the 9th and 14th day of development. The mesenchymal cells of the epicardium differentiate into primitive endothelial cells constituting the wall of the primitive lymphatic capillaries. The inception and manner of development of lymphatics was demonstrated to be closely bound to the mesenchymal tissue.

#### MATERIALS AND METHODS

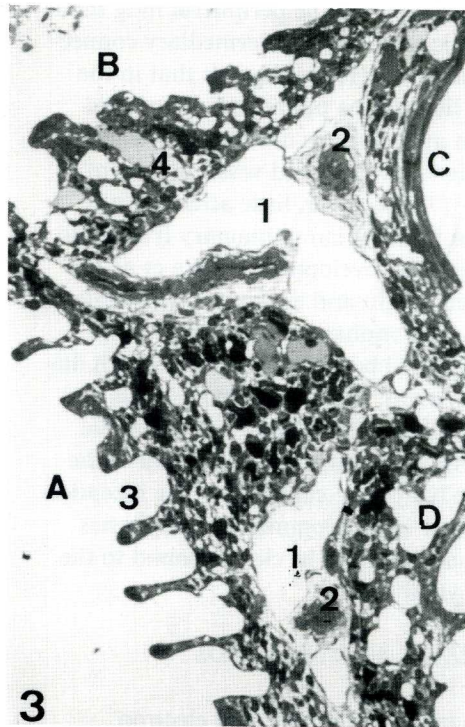
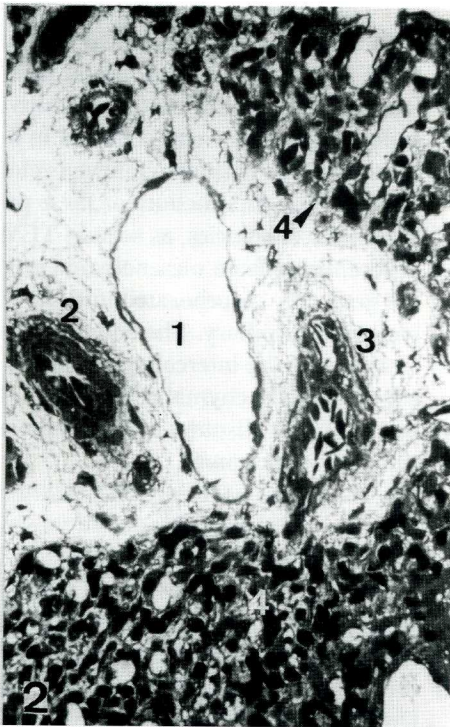
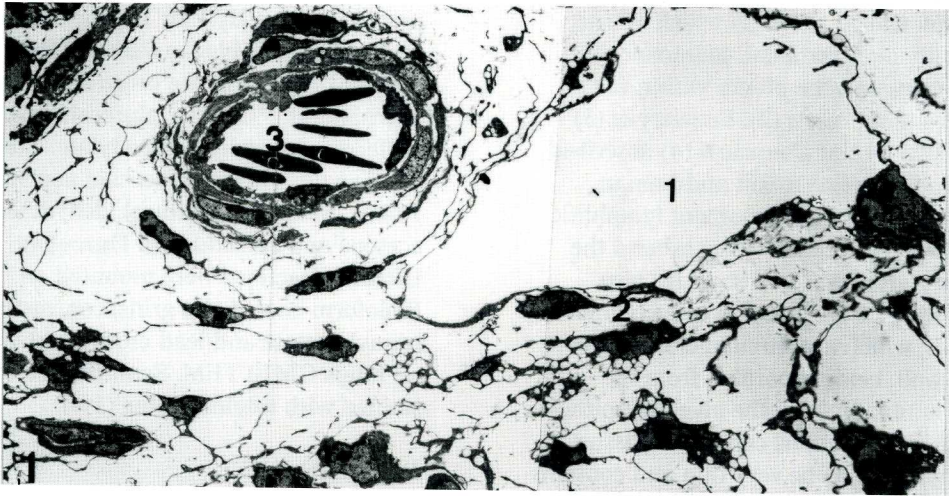
The material for light and electron microscopic analysis was collected from embryonic quail (ED 13, 14, 15, 16, 17; two for each stage), two newly hatched quail taken 90 min after hatching, two 24 h, 2 and 10-day-old quail and three adult quail. After the intraperitoneal injection of a lethal dose of Nembutal, the lungs were instilled via the trachea with cooled 3% glutaraldehyde buffered with sodium cacodylate (4°C,

pressure 20 cm H<sub>2</sub>O for 15 min). After removal, 1 mm<sup>3</sup> blocks were cut from different regions of the lungs and immersed overnight at 4°C in 3% glutaraldehyde buffered with sodium cacodylate. They were postfixed in 2% OsO<sub>4</sub> (veronal acetate buffer, 1 hour, room temperature), dehydrated in acetone and embedded in Durcupan. Ultrathin sections were mounted on Pioloform-coated slot grids, contrasted with uranyl acetate and lead citrate and viewed in a Philips CM10 TEM. Semithin sections were stained with toluidine blue (1%).

#### RESULTS

The lymphatics in abundant interparabronchial mesenchyme appear first on ED13 and ED14 as thin-walled, broad tubes with an irregular lumen lined with a primitive endothelium. They can be found in a network of stellate-like mesenchymal cells close to the arteries. The endothelium of these primitive lymphatic capillaries, as seen in TEM, differentiates from mesenchymal cells which are gradually incorporated into the wall of a lymphatic capillary. The endothelium retains temporarily the intercellular contacts with adjacent mesenchymal cells (*Fig. 1*). The parabronchus displays on ED14 the atrial stage of development.

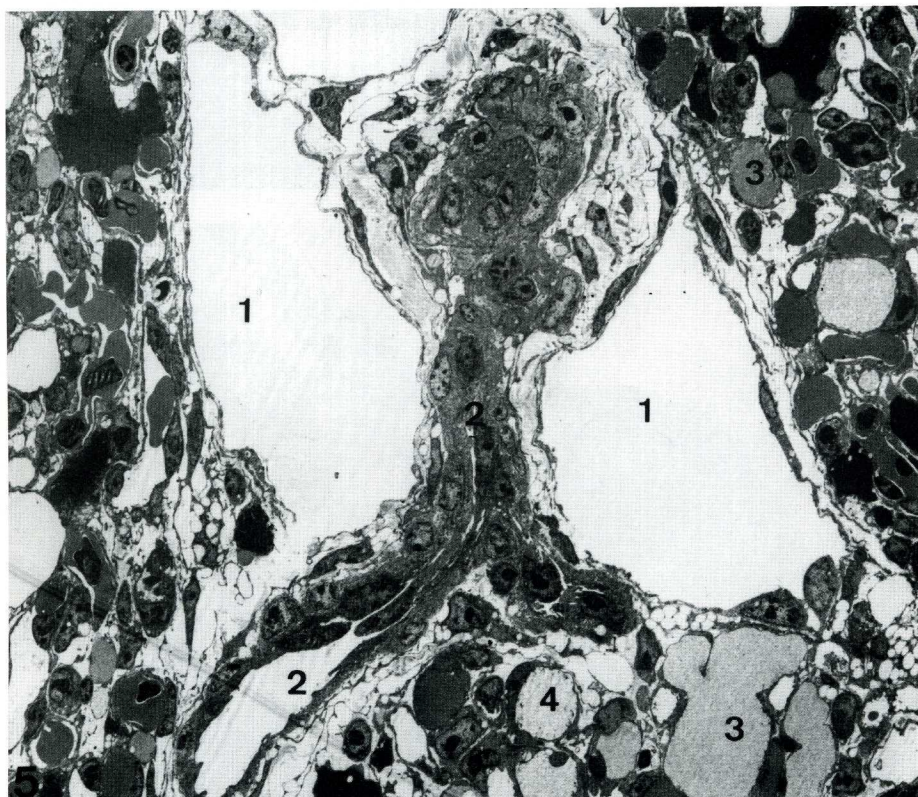
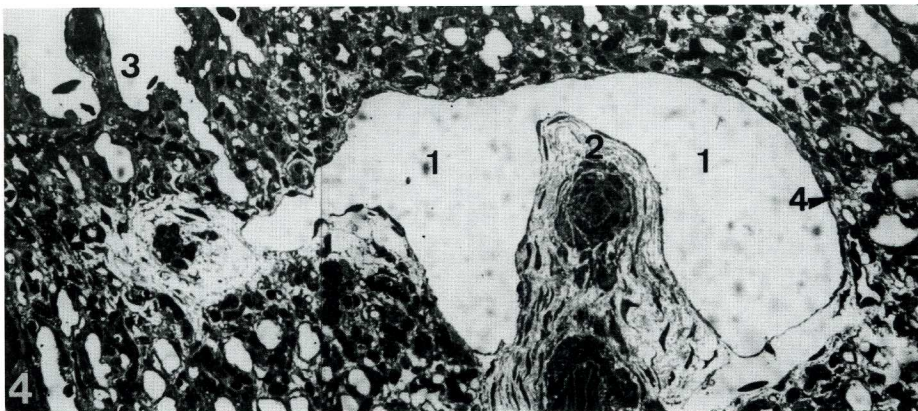
ED15 and ED16 are characterized by a decrease of interparabronchial mesenchymal septa (IPBS), due to the growth and enlargement of parabronchial units. From the very beginning the lymphatics distinctly differ from veins (*Fig. 2*). The reduced IPBS are formed by a very thin layer of mesenchyme. Only in places of three or more neighboring parabronchial units are trigonal fields with mesenchyme present. Here, broad lymphatics encircle primarily the arterial tributaries (*Fig. 4*). The primitive lymphatics are to a large extent orientated to the gas exchange tissue (*Figs. 3-5*). On ED17 even large lakes formed by lymphatics are present in mesenchymal tissue of interparabronchial trigona (*Fig. 4*). Mesenchymal cells that do



*Fig. 1 (above). Parabranchial unit of quail, ED14; 1 - primitive lymphatic capillary lined with endothelium; 2 - mesenchymal cells in contact with endothelium; 3 - arteriole. x1500.*

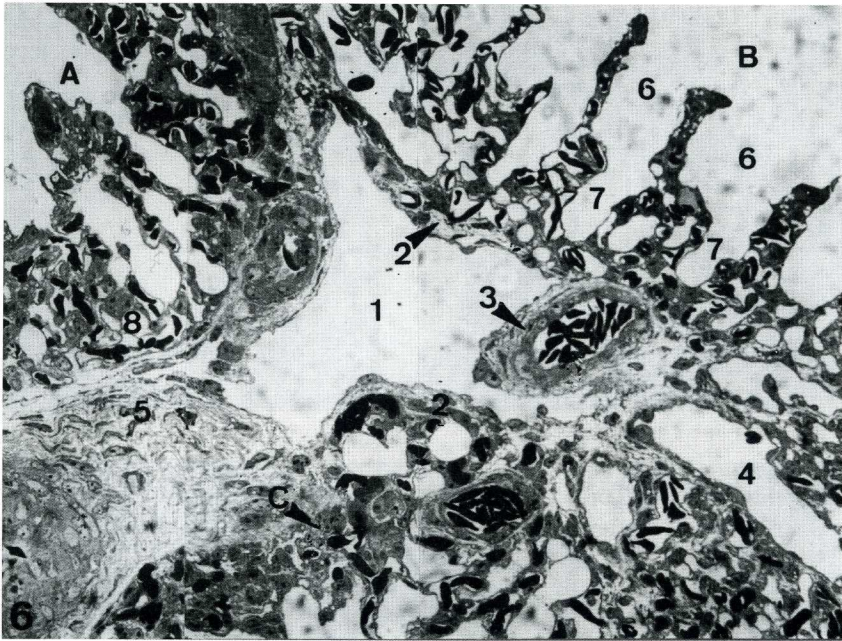
*Fig. 2 (lower left). Parabranchial unit of quail, ED15; 1 - primitive lymphatic vessel in interparabranchial mesenchyme; 2 - arteriole; 3 - venule; 4 - gas exchange tissue. Semithin Durcupan section, toluidine blue. x320.*

*Fig. 3 (lower right). Parabranchial unit (PBU) of quail, ED16; 1 - large primitive lymphatics in mesenchymal trigona of four neighboring PBU (A, B, C, D); 2 - arteriole; 3 - atria; 4 - infundibulum. Semithin Durcupan section, toluidine blue. x280.*



*Fig. 4 (above). Mesenchymal trigonum of parabronchial unit of quail on ED17; 1 - large lymphatic lake; 2 - arteriole; 3 - atrium; 4 - gas exchange tissue of parabronchus. Semithin Durcupan section, toluidine blue. x300.*

*Fig. 5 (below). Mesenchymal trigonum of parabronchial unit of quail on ED16; 1 - lymphatics closely apposed to the gas exchange tissue encircle 2 - a longitudinally cut arteriole; 3 - blood capillaries; 4 - air capillaries of gas exchange tissue. x1500.*



*Fig. 6 (above). Mesenchymal trigonum of parabronchial unit (PBU) of quail 90 min after hatching; 1 lymphatic vessel largely apposed to the gas exchange tissue (2); 3 - arteriole; 4 venule; A, B, C - PBUs bordering the mesenchymal trigonum (5); 6 - atria; 7 - infundibula; 8- air and blood capillaries. Semithin Durcupan section, toluidine blue. x400.*

*Fig. 7 (below). Detail of mesenchymal trigonum of parabronchial unit of quail 90 min after hatching; 1 - endothelial cell of lymphatic capillary; 2 - overlapping processes of endothelial cells; 3- young fibroblasts; 4 - wall of arteriole. x15000.*

not differ from the other cells of the mesenchyme directly circumscribe the lumen of the primitive lymphatics and form an integral part of its wall. The inner and outer surface of the lymph capillary is uneven, the primitive endothelium being extremely thin (*Fig. 1*). Endothelial cell processes project outwards where they come into multiple contact with the mesenchymal cells of the pericapillary space. The endoplasmic reticulum is somewhat more developed in the mesenchymal cells than in lymphatic endothelium. The intercellular contacts consist mostly of endothelial processes with a region of overlap provided with punctate specializations for attachment. The outer surface of the blood vessel endothelium is smooth with a distinct basement membrane while there is no trace of a basement membrane on the outer surface of embryonic lymphatic capillaries.

In quail, 90 min after hatching, the volume of lymphatics gradually decreases, lying in cellular young connective tissue (*Fig. 6*). The lymphatic endothelium retains a primitive form and is not yet fully differentiated. The interstitium is composed predominantly of young fibroblasts. Scarcely dispersed collagen fibrils are seen among the cells (*Fig. 7*).

Further reduction of interparabronchial connective tissue as well as advanced differentiation of tissue compounds can be demonstrated in quail, 2 days after hatching. The described trigonal fields of mesenchyme transform into the virtual interparabronchial septa ensheathing the interparabronchial arteries and veins. The arteries in particular are accompanied by large collecting lymphatic vessels encircling a substantial part of the arterial wall. The lymphatics largely enter into close contact with the gas exchange tissue, representing the juxta-air capillary-lymphatics (*Figs. 8-10*). The imaginary borderline between joined parabronchi is demarcated merely by tributaries of pulmonary vessels.

The lymphatic collecting vessels are

continuously lined by a thin endothelium. The valves are formed by a duplication of endothelial cells with an interposed layer of collagen fibrils (*Fig. 11*). A basement membrane of the lymphatic endothelium is lacking. The wall consists of a very thin layer of condensed collagen connective tissue; smooth muscle cells are not seen in collecting vessels of this size. The lymphatic collecting vessels and three arterial profiles render a sufficient comparison of their ultrastructure (*Figs. 9,10*). The lymphatic vessel forms an integral part of adventitia of arterial tributaries (*Fig. 9*). Also in the capsule of intraparenchymatous autonomic ganglia the lymphatic capillaries and vessels are visualized (*Fig. 11*). Lymphatic capillaries are found in periadventitial connective tissue and in connective tissue capsule, often in juxtaposition to the gas exchange mantle (*Figs. 10, 11*). In 2-day-old (*Figs. 12,13*), as well as in mature quail, the lymphatic spaces are seen (9). They are lined by discontinuous endotheliiform cells which may gradually become continuous when they are confluent with the lumen of a typical lymphatic capillary (*Fig. 13*).

The pulmonary lymphatics, capillaries in particular, are characterized by the presence of numerous overlapping endothelial processes which form simple or complex intercellular junctions (*Figs. 14-16*). Closely apposed areas formed by maculae adherentes, occasionally maculae occludentes, can be visualized. Punctate specializations for attachment are also often seen (*Figs. 14,16*). There are focal areas of close approximation, while widths of 0.5-1  $\mu\text{m}$  may extend for varying lengths of the cleft, to provide a patent junction. The cytoplasm of the lymphatic endothelium is characterized by a paucity of smooth or granular endoplasmic reticulum in comparison to adjacent fibroblasts (*Fig. 15*) albeit with clusters of ribosomes already present. Mitochondria are found throughout the perinuclear area and are randomly distributed in the attenuated cytoplasm. Plasmalemmal vesicles may be seen but not in such an amount as to speak of

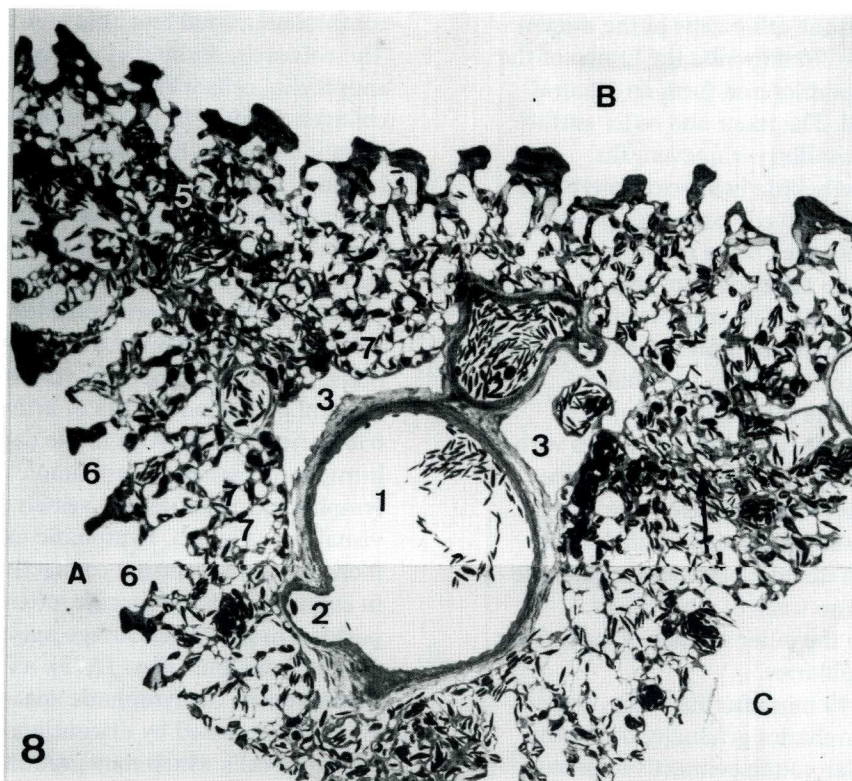


Fig. 8 (above). Interparabronchial septum (IPBS) of 2-day-old quail, A, B, C, - parabronchial units constituting the IPBS; 1 - interparabronchial artery with tributaries (2); 3 - lymphatic collecting vessels encircle partly the arterial wall; 4 - imaginary borderline between fused external parabronchial walls; 5 - interparabronchial vein; 6 - atria; 7 - gas exchange tissue. Semithin Durcupan section, toluidine blue. x300.

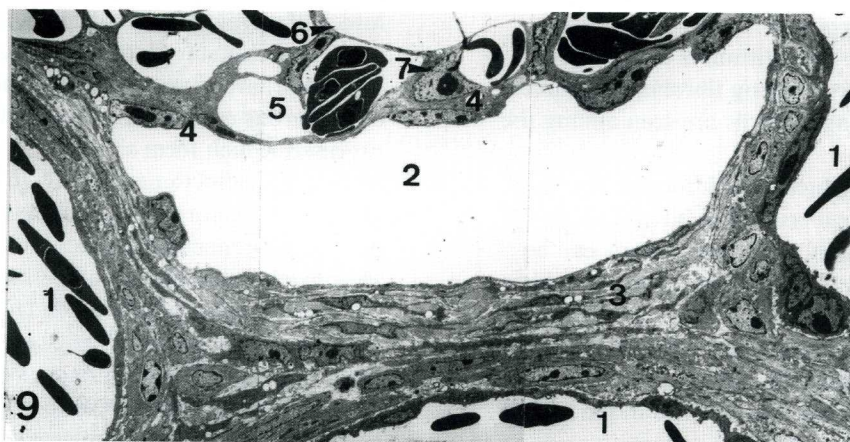
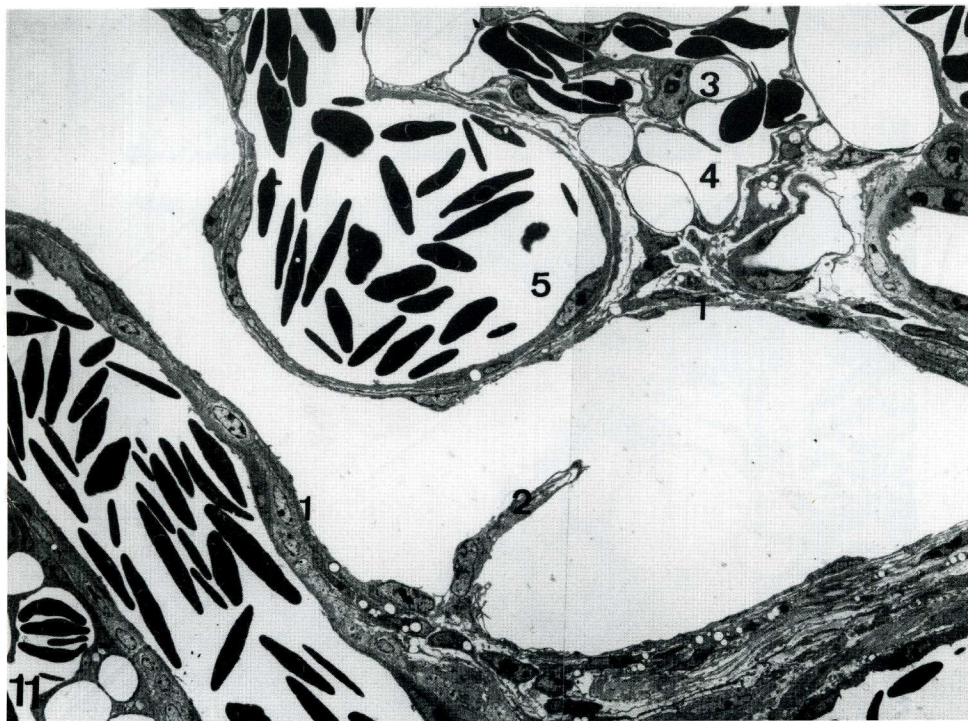


Fig. 9 (below). Interparabronchial septum of 2-day-old quail; 1 - arterial tributaries; 2 - lymphatic collecting vessel formed by endothelium only; 3 - common adventitial connective tissue of artery and lymphatic; 4 - lymphatic wall in juxtaposition to the gas exchange tissue, 5 - blood capillary; 6 - blood-air barrier; 7 - squamous respiratory cell. x2800.

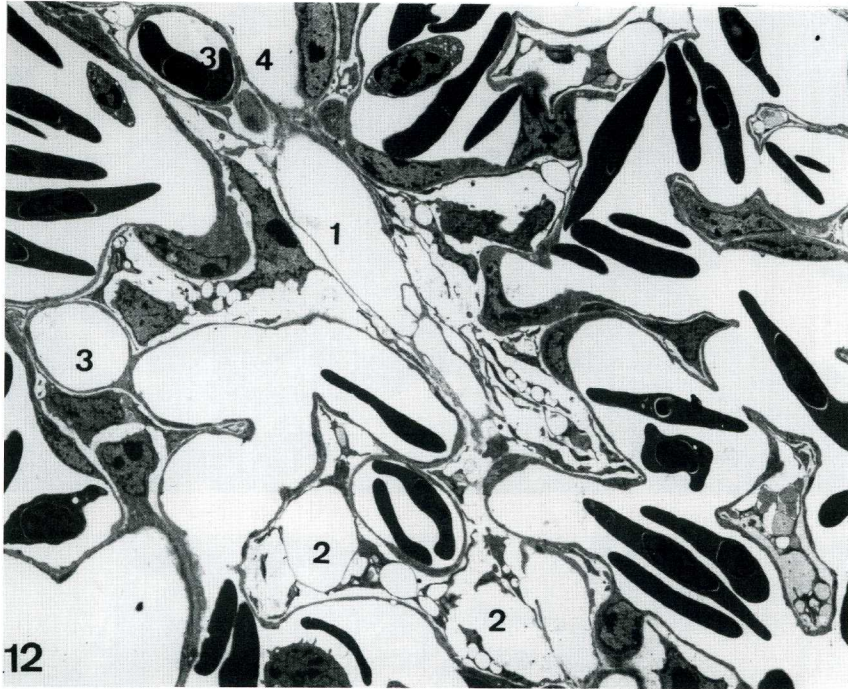


*Fig. 10 (above). Interparabronchial septum of 2-day-old quail; 1 - ganglionic cell; 2 - its connective tissue capsule; 3 - lymphatic capillary; 4 - lymphatic collecting vessel in juxtaposition to the gas exchange tissue (5); 6 - artery. x2800.*



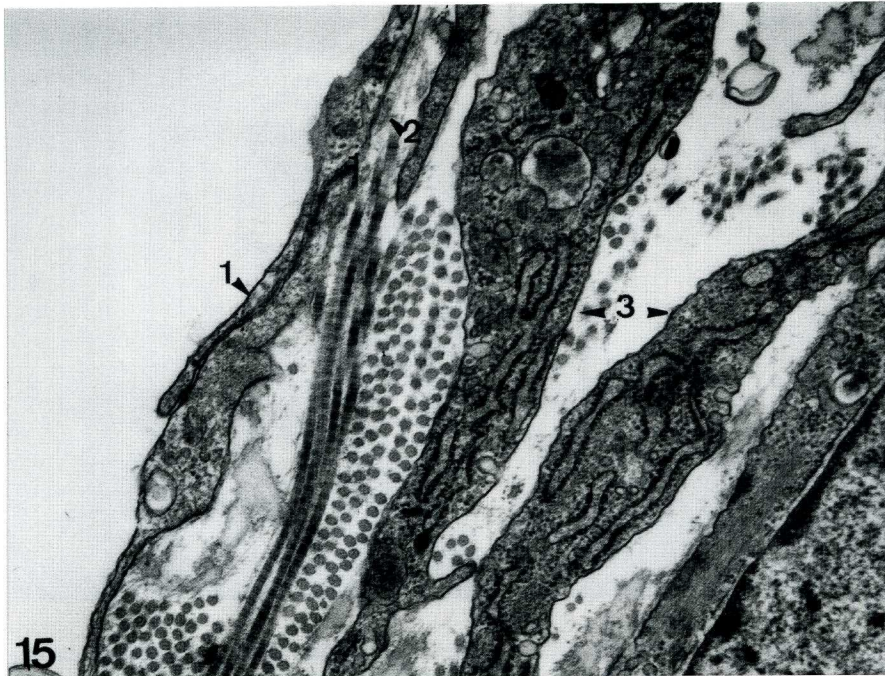
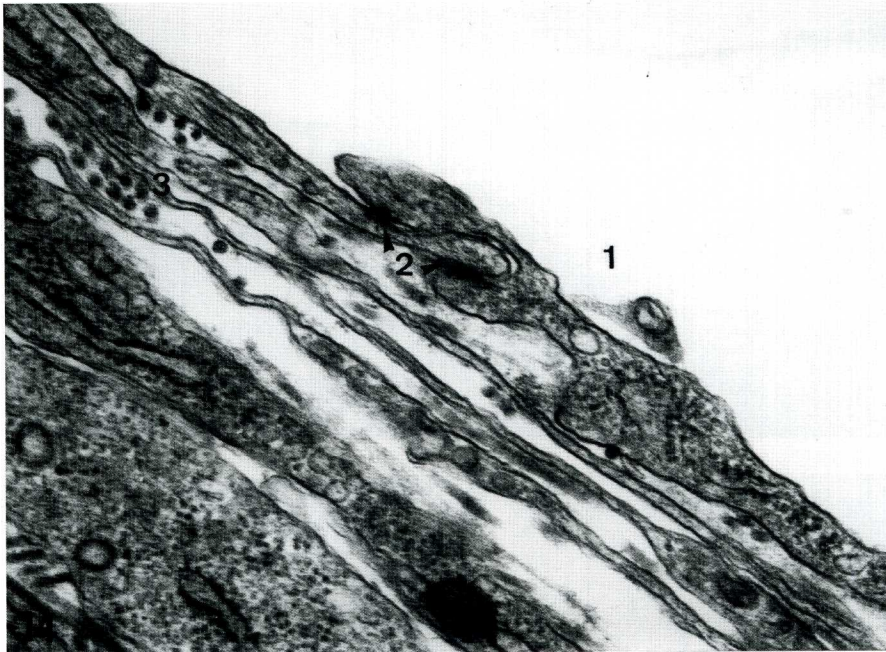
*Fig. 11 (below). Interparabronchial septum of 2-day-old quail; 1 - the wall of a lymphatic collecting vessel; 2 - lymphatic valve formed by two leaflets of endothelial cells; 3 - air capillary; 4 - blood capillary; 5 - arteriole. x1600.*





*Fig. 12 (above). Interparabronchial septum of 2-day-old quail; 1 - lymphatic capillary; 2 - lymphatic spaces not continuously lined by endothelioform cells; 3 - blood capillary; 4 - air capillary. x2800.*

*Fig. 13 (below). Interparabronchial septum of 2-day-old quail; 1 - lymphatic collecting vessel; 2 - closely linked to the lymphatic space; 3 - artery; 4 - blood capillary; 5 - air capillary. x1400.*



*Fig. 14 (above). Interparabronchial septum of 2-day-old quail; 1 - lumen of lymphatic capillary; 2 - intercellular contact formed by overlapping endothelial processes with punctate specialization for attachment; 3 - interstitium. x47500.*

*Fig. 15 (below). The same material as in Fig. 14; 1 - overlapping processes of lymphatic endothelium; 2 - fibroblasts with numerous profiles of granular endoplasmic reticulum. x28000.*

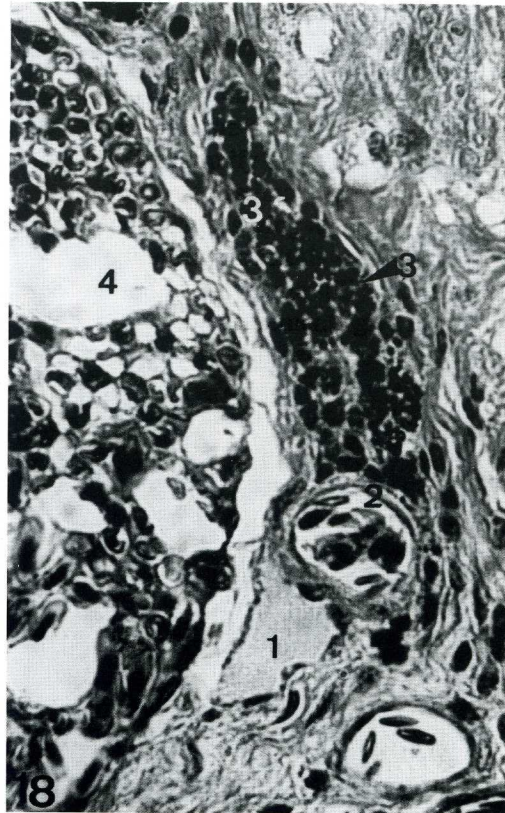
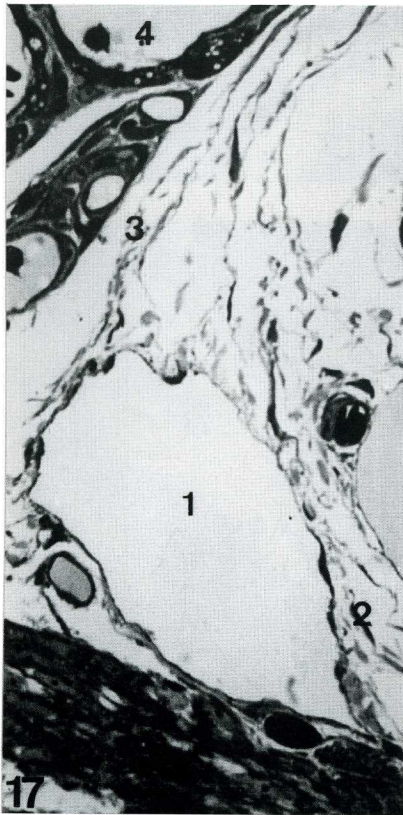
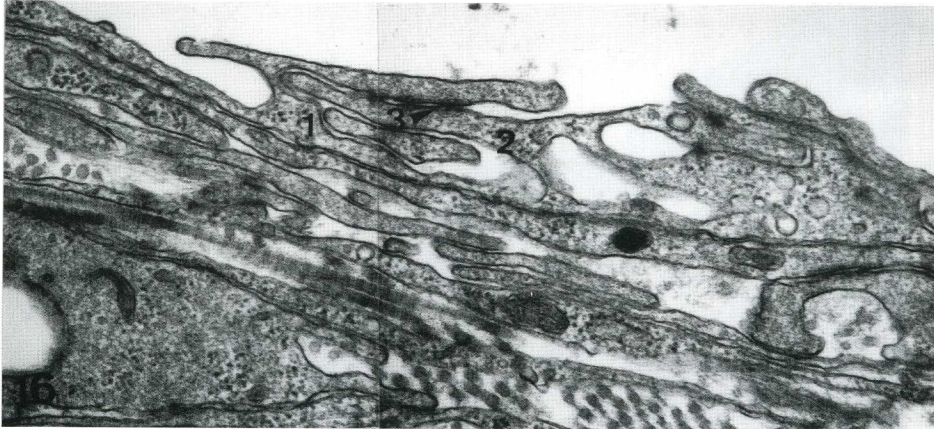


Fig. 16 (above). The same material as in Fig. 14; 1 - complex intercellular contacts of lymphatic endothelium formed by 2 - overlapping processes and 3 - punctate specializations for attachment. x29000.

Fig. 17 (lower left). Adult quail; 1 - lymphatic capillary in 2 - peri- and 3 - parabronchial connective tissue; 4 - lumen of parabronchus. Semithin Durcupan section stained with toluidine blue. x550.

Fig. 18 (lower right). Adult quail; 1 - lymphatic capillary; 2 - arteriole; 3 - lymphoid tissue; 4 - parabronchus. Paraffin section, Azan stain. x550.

transendothelial channels. Intimate association of the lymphatic capillary to the adjacent interstitium is provided by anchoring filaments (*Figs. 14,15*). The pulmonary lymphatic capillaries and collecting vessels in general do not differ ultrastructurally from those of mammals. The rich intrapulmonary lymphatic bed in embryonic quail and in young individuals is in sharp contrast with the poor one in adult quail where the lymphatics paralleling the interparabronchial vessels are very scarce. Also in adventitial tissue of secondary bronchi the lymphatics represent only small and scarcely distributed profiles (*Figs. 17,18*). Scarce lymphatics are observed in the pleural and air sac connective tissue, too.

#### DISCUSSION

The basic structural plan of the parabronchial unit and its gas exchange mantle differs in principle from that of mammalian terminal airways (1,5). The difference is reflected also in the pattern of lymphatic bed organization which greatly depends on the participation of loose connective tissue in respiratory units. In mammals, the loose connective tissue can be traced up to the level of respiratory bronchioles, and, consequently, the most peripheral lymphatic capillaries can be visualized here. On the other hand, the parabronchial units in adult bird lung are extremely poor in connective tissue that encircles infundibula and intercapillary spaces of the gas exchange mantle. A small amount of connective tissue accompanies the intraprabronchial vessels and nerves. Only interatrial septa possess a representative amount of connective tissue, the main seat of the avian respiratory macrophage system (10). The minimal critical mass of loose connective tissue must be present to establish the initial roots of lymphatic capillaries. In general, the enormously vascularized parabronchial unit in mature birds reveals an extremely poor lymphatic bed when compared with that of mammalian terminal airways.

The hypothesis of the critical mass of connective tissue needed for the lymphatic bed was seemingly clarified in the example of the development of the lymphatic bed shown in embryonic and neonatal lung (3,4). The same was experienced in the lungs of embryonic and young quail. In the same way as in mammals the lymphatic bed in interparabronchial mesenchyme develops a rich network of lymphatics. It must be emphasized that during the last third of the embryonic period lymphatics represent a conspicuous compound of interparabronchial mesenchyme accompanying the blood vessels, arteries in particular. The mesenchymal trigona of squeezed mesenchyme along the contacting adjoining parabronchial units are in fact the future interparabronchial septa of young and adult parabronchus conducting the larger tributaries of interparabronchial arteries and veins. The critical mass of connective tissue is present in young quail along the interparabronchial septa, and the lymphatics form a relatively rich network here. In adults, on the other hand, the lack of connective tissue is obvious and the lymphatics are hardly seen to accompany the interparabronchial vessels. The layout of such a rich lymphatic bed is obscure considering that its destiny is to be almost abolished. It also remains open to speculation how tissue fluid is drained from connective tissue of the parabronchial luminal framework (representing on sections interatrial septa, the prevailing seat of avian respiratory macrophages and of a sphincter-like system of interwoven clusters of smooth muscle cells). No lymphatics were visualized in this very active compartment of the parabronchial unit. On the whole, the avian pulmonary lymphatics serve as an appropriate model for the analysis of principles controlling the origin and distribution of lymphatics in general.

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