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Intrinsic Lymphatics of the Thymus in the Rat: A Detailed Light and Electron Microscopic Study using Serial Sections

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الأوعية اللمفية الضمنية للغدة الزعترية في الفأر : دراسة تفصيلية بواسطة المجهر الضوئي والإلكتروني باستعمال قطوع مسلسلة

مروان أبو حجلة ، وأوين ريد ، وريموند سكوثورن

خلاصة : تم دراسة شكل وتوزيع الأوعية اللمفية الضمنية في الغدة الزعترية للفأر الأبيض البالغ بواسطة المجهر الضوئي والإليكتروني . وقد تم تتبع القنوات اللمفية في قطوع مسلسلة شبه رقيقة وبتزايد من المنطقة النقرية إلى داخل المنطقة اللبية . وقد أظهرت النتائج وجود أوعية لمفية حقيقية في فواصل الأنسجة الضامة وبالقرب من الأوعية الدموية . وعند تتبع هذه الأوعية اللمفية إلى الداخل تأكد وجودها فقط في الملتقى القشري اللبي ولحد ما أيضا في الطبقة اللبية الخارجية ، ولكن لم يتم ملاحظة وجودها إطلاقا في الطبقة اللبية الداخلية أو المنطقة القشرية . كذلك لم يتم ملاحظة وجود أوعية لمفية واردة إلى الغدة الزعترية . وباستعمال المجهر الإلكتروني الناقل تم ملاحظة تكرار وجود طبقات متمركزة من الخلايا اللمفية الصنغيرة في فسحات الأنسجة الضامة الحول وعائية . وقد لوحظ بأن هذه الخلايا اللمفية الحول وعائية أصغر حجماً وأثخن لونا من مثيلاتها الموجودة في النسيج الحشوي للغدة الزعترية . وقد لوحظ أيضا بأن هذه الخلايا محاطة بنواتىء أرومية ليفية (لحمة متوسطة) طويلة نتج عنها صنع جملة غير منتظمة من قنوات أو فسحات «خارج لمفية» موجودة بالقرب من أوعية لمفية تحتوي على خلايا لمفية صغيرة تشبه إلى حد كبير الخلايا اللمفية الحول وعائية . ومن هنا يمكن استنتاج أن هذه القنوات «الخارج لمفية» قد تكون هي نفسها القنوات 'القبل اللمفية' التي تختص بنقل ومرور الخلايا اللمفية الزعترية والسائل الخلالي من المناطق اللبية العميقة إلى أقرب وعاء لمفي موجود في الأنسجة الضامة الحول وعائية . وقد يكون هذا الطريق لهجرة الخلايا اللمفية من الغدة الزعترية للفأر إضافياً ومكملاً إلى الوريدي .

ABSTRACT: The morphology and distribution of intrinsic lymphatics of the thymus gland of adult Swiss-Albino rats was studied by light and transmission electron microscopy. Lymphatic channels were traced, in serial semithin sections, progressively from the hilar region into the medullary region. The results show the presence of true lymphatic vessels in the connective tissue septa and close to blood vessels. When traced internally, these lymphatics were only identified in the cortico-medullary junction, to a lesser extent in the outer medulla, but never in the inner medulla or cortex. Afferent lymphatics to the thymus were not observed. Transmission electron microscopy, frequently showed concentric layers of small lymphocytes in the perivascular connective tissue spaces. Perivascular lymphocytes were smaller and more pachychromatic than those in the thymic parenchyma. They were enclosed by long mesenchymal/fibroblastic processes which formed a system of irregular "extra-lymphatic" channels or spaces located close to lymphatic vessels which contained many small lymphocytes closely resembling perivascular lymphocytes. These "extra-lymphatic" channels may be *pre-lymphatic* that may be concerned in the passage of thymocytes and interstitial fluid from the deep regions of the medulla to the nearest lymphatic in the perivascular connective tissue. This may provide an additional route for the migration of lymphocytes from the rat thymus gland supplementing the venous route. Further timed injection studies to trace the continuity of these "pre-lymphatic channels" will offer more precise results.

The arrangement of intrinsic lymphatics in the thymus gland has been the subject of considerable research. The consensus is that lymphatics of the mammalian thymus gland, including man, are confined to the interlobular connective tissue septa and capsule, and that the parenchyma itself contains few or no lymphatics (Smith, 1955; Kotani et al., 1966; Harris and Templeton,1968; Omori, 1973; Rosai and Levine, 1976; Weiss, 1988).

Some investigators deny the presence of true

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lymphatics in the thymus and report, instead, the presence of tissue spaces (Seigler, 1964; Bloodworth et al., 1975), or of perivascular lymphatic spaces (Leblond and Sainte-Marie, 1960; Harris and Templeton, 1968). Clark (1963) failed to find any lymphatic endothelium in the perivascular spaces and suggested that they should, therefore, not be regarded as lymphatics. It has been reported that the thymic medulla of man (Singh, 1980; Weiss, 1988) and of rat (Hwang et al., 1974) contains few lymphatic capillaries, and none are in the cortex. However, a more recent study by Kato (1988) on the mouse thymus, reported the presence of lymphatic vessels in both cortex and medulla. He also observed that the perivascular space surrounding the post-capillary venule opened into a terminal lymphatic vessel at the cortico-medullary junction and in the medulla and that this connection may function as a pathway for the migration of lymphocytes into or out of the lymphatic circulation. Ushiki (1986) also demonstrated by scanning electron microscopy a lymphatic vessel in the large perivascular space surrounding the arterioles in the rat thymus, but despite this he suggested that the intracellular route through post-capillary venules is the dominant pathway for migrating lymphocytes.

It is generally accepted that the thymus gland has no afferent lymphatics; efferent lymph vessels leave the connective tissue septa and capsule, and drain to neighbouring mediastinal lymph nodes (Harris and Templeton, 1966; Kotani et al., 1966; Bearman et al., 1975; Abu-Hijleh, 1987; Weiss, 1988). However, an afferent flow of lymph to the thymus is suggested by reports that Percoll particles (Eggli et al., 1986) and tetanus toxoid (Muller et al., 1987) when injected intraperitoneally in mice, later appeared in parathymic lymph nodes and in thymic superficial lymphatics and cortical parenchyma. This was recently supported by Sandberg and Hagelin (1990) who found in the guinea pig afferent lymphatics with valves pointing towards the thymus and containing Kurloff cells resulting from subcutaneous injection of estradiol.

There still appears to be disagreement as to the existence and distribution of lymphatic vessels within the thymic lobules, and whether afferent lymphatics exist. The present study was undertaken to re-investigate in more detail by light and electron microscopy, the morphology and fine structure of intrinsic lymphatics in the rat thymus, with special reference to their origin, distribution and contents, and to the role and significance of the perivascular spaces in drainage of lymph and lymphocytes from the thymus.

Materials and Methods

Twelve adult Swiss-Albino rats of either sex, from a closed colony, age range from 2-7 months and body weight from 150-350g, were used in this study. were killed by anaesthetic ether. The heart and vessels were exposed, and the vascular system for 2 minutes with Ringer's solution containi lignocain chloride as a vasodilator, through a inserted into the left ventricle, with outflow estab incising the right atrium. Perfusion was then c for 30-40 minutes with the fixative; 5% sol glutaraldehyde in Millonig's buffer at pH7. perfusion also contained dextran, 40.000 M.W. a concentration of 3-5% W/V, since pre experiments showed this reduced the interstitia which often occurs during vascular perfusion (B and Maunsbach, 1970) and also made thymic ly easier to identify. The intact thymus was remo immersed overnight in the same fixative as that perfusion. The tissues were rinsed in 2-3 cha phosphate buffer over a 2-3 hour period. With t a binocular microscope, 2-4mm slices of the thym cut with a razor blade. These slices were then po in 1% osmium tetroxide, dehydrated, and embe Spurr's resin (Spurr, 1969). Interrupted series of s sections, 1-1.5µm in thickness, were cut on a Port microtome MT-2 through 5 blocks from each giving a total of about 40 blocks. Continuous s semithin sections were cut in 6 of these blocks. A sections were stained with Azur II and with Haem and Eosin and studied by light microscopy. areas of 15 blocks, from 5 thymus glands, were cu sections, 60-80nm in thickness, picked up on u 200 mesh copper grids and double stained with acetate (Stempak and Ward, 1964) and with lead (Reynolds, 1963). Specimens were then examin a JEOL 100S transmission electron microscope. way lymphatic channels were traced progressive the interior of the thymus starting at the hilar Identification, by light microscopy, of intrinsic lymphatics in perfusion-fixed material was based following criteria (Leak and Burke, 1966; Fawcett 1969; Abu-Hijleh, 1987): (i) the lymphatic w irregular contour of sectional profiles, bounded endothelial lining (ii) the luminal contents of a stained precipitate of lymphoprotein, whilst blood were empty (iii) continuity between hilar, sept cortico-medullary lymphatics when traced in semithin sections.

Results

In the rat, the thymus gland is composed closely related lobes completely surrounded connective tissue capsule broken only by neurova bundles (Figure 3). Each thymic lobe is subdivi septa that extend from the surrounding connective capsule. Every lobule, 0.5-2.0 mm in diameter, dis

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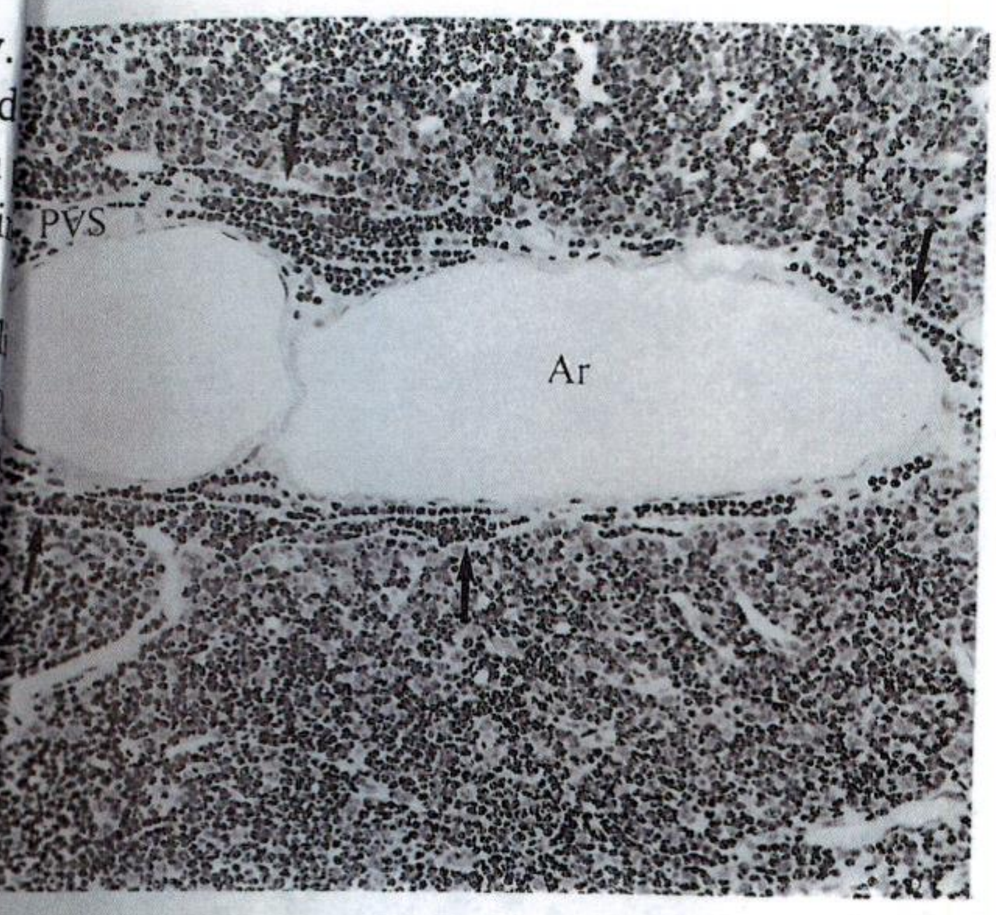


ire 1. A secondary septal (intralobular) arteriole (Ar) ounded by a perivascular space (PVS) packed with several s of cells, predominantly small lymphocytes which appear ller and more deeply stained than parenchymatous phocytes. Note the prominent epithelial boundary (arrows) ch separates the space from the rest of the thymic enchyma. Semithin resin section stained H&E. x250.

arker peripheral cortex rich in lymphocytes, and a tral pale medulla rich in epithelial-reticular cells, gether with Hassall's corpuscles. The thymic cells sisted mainly of epithelial-reticular and lymphocytes, gether with other cells (interdigitating-reticular cells, crophages, plasma cells, mast cells, eosinophils and her granulocytes).The disposition of the intraparen-ymal blood vessels is linked with the lobulation of the gan.

Examination, by light microscope, of H & E sections, vealed spaces bounded by an eosinophilic basal lamina parating the thymic blood vessels, particularly the large es, from the thymic parenchyma (Figure 1). The erivascular space could be easily distinguished from the rrounding parenchyma. This was confirmed by TEM Figure 2). Perivascular spaces appeared to be completely rrounded by elongated epithelial-reticular cell processes nked by desmosomes and the associated basal lamina, elineating several well defined layers. These were rmed by: endothelial cell cytoplasm, with a muscular oat in arterioles and veins, bounding the vascular lumen; ndothelial cell basal lamina; a perivascular space, of arying width, containing collagen fibres and cells; pithelial-reticular basal lamina; and epithelial-reticular ell cytoplasm.

The width of the perivascular space was in general proportional to the size of thymic blood vessels. While perivascular spaces around arterioles and venules contained only collagen and some cells, those around septal blood vessels were packed with a range of cells mainly small lymphocytes (Figures 1,2). Constantly, perivascular lymphocytes were noted to be smaller and

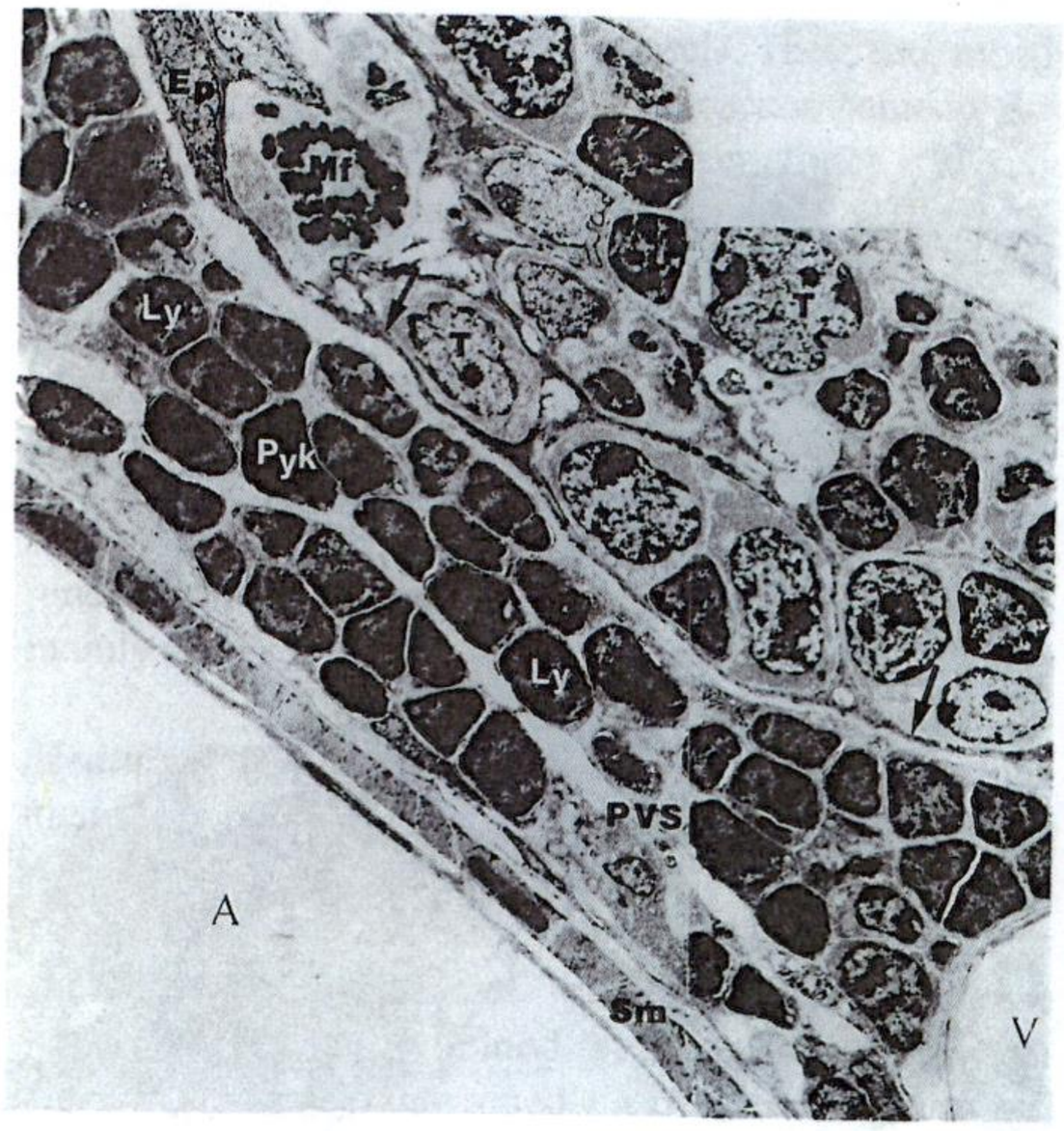
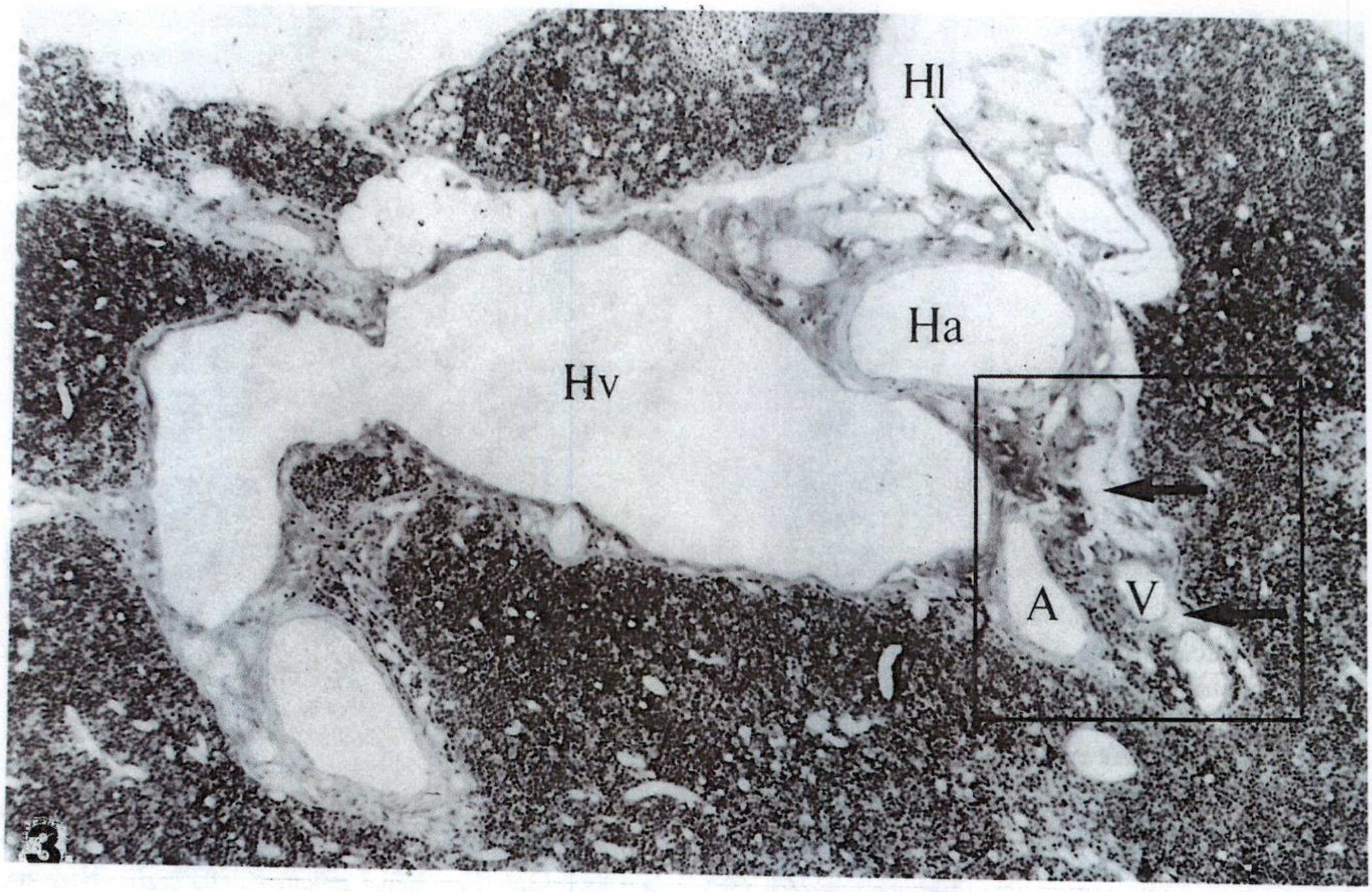


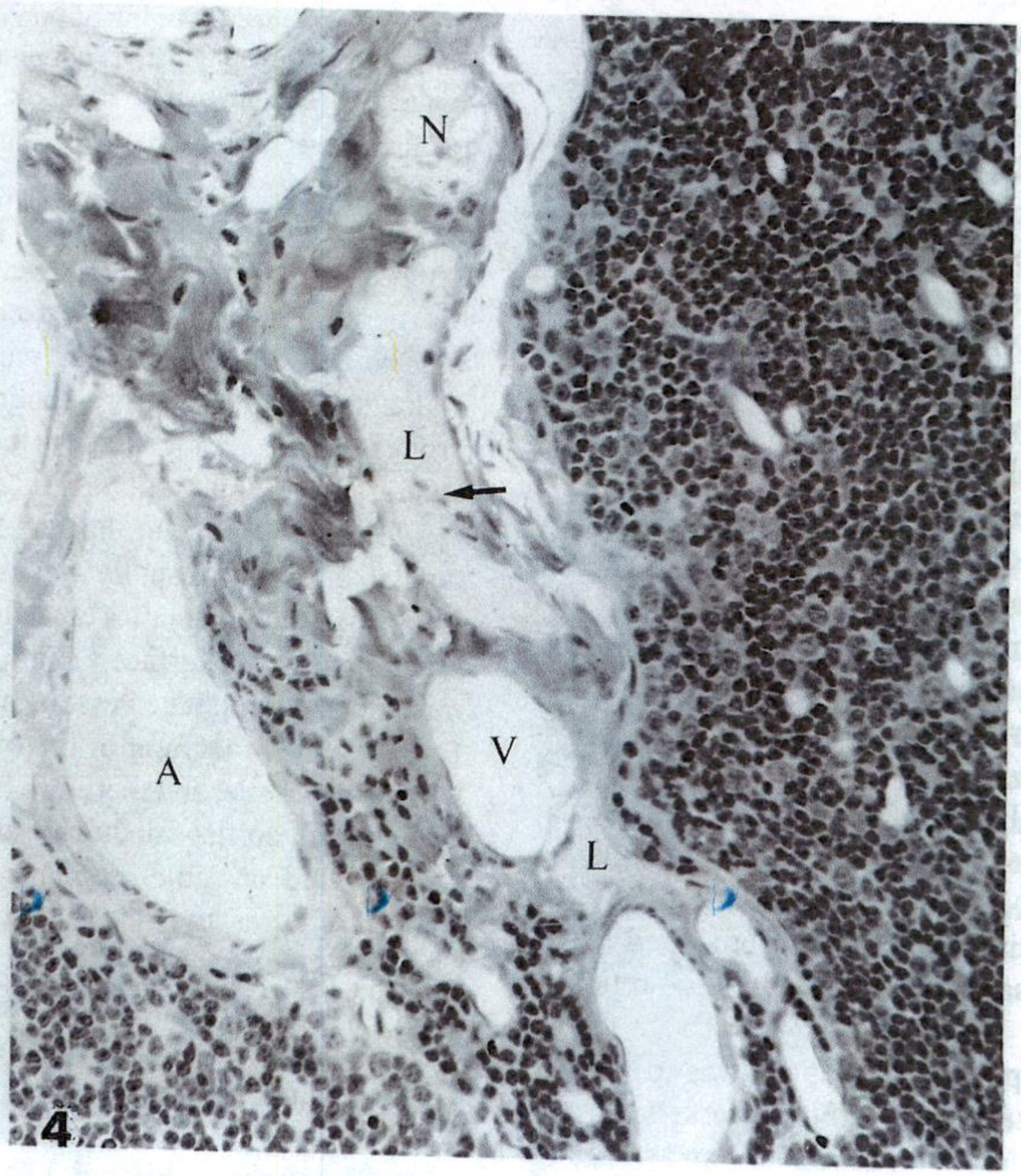
Figure 2. Montage of TEM of a perivascular space (PVS). It is separated from the thymic parenchyma by a clearly visible process (arrows) of epithelial-reticular cells (Ep). Note the small size and deep staining of perivascular lymphocytes (Ly) compared to parenchymatous lymphocytes (T). A, arteriole; V, venule; Sm, smooth muscle cell; Pyk, pyknotic lymphocyte; Mf, mitotic figure. Bar, 5µm.

more pachychromatic than those in the thymic parenchyma (Figure 2). They were arranged in concentric rows, ranging from one to several, around the blood vessels (Figures 1,2). They were often surrounded by collagen fibres and fibroblastic or mesenchymal-like processes; all embedded in connective tissue matrix and/or possibly interstitial fluid (Figures 2, 10).

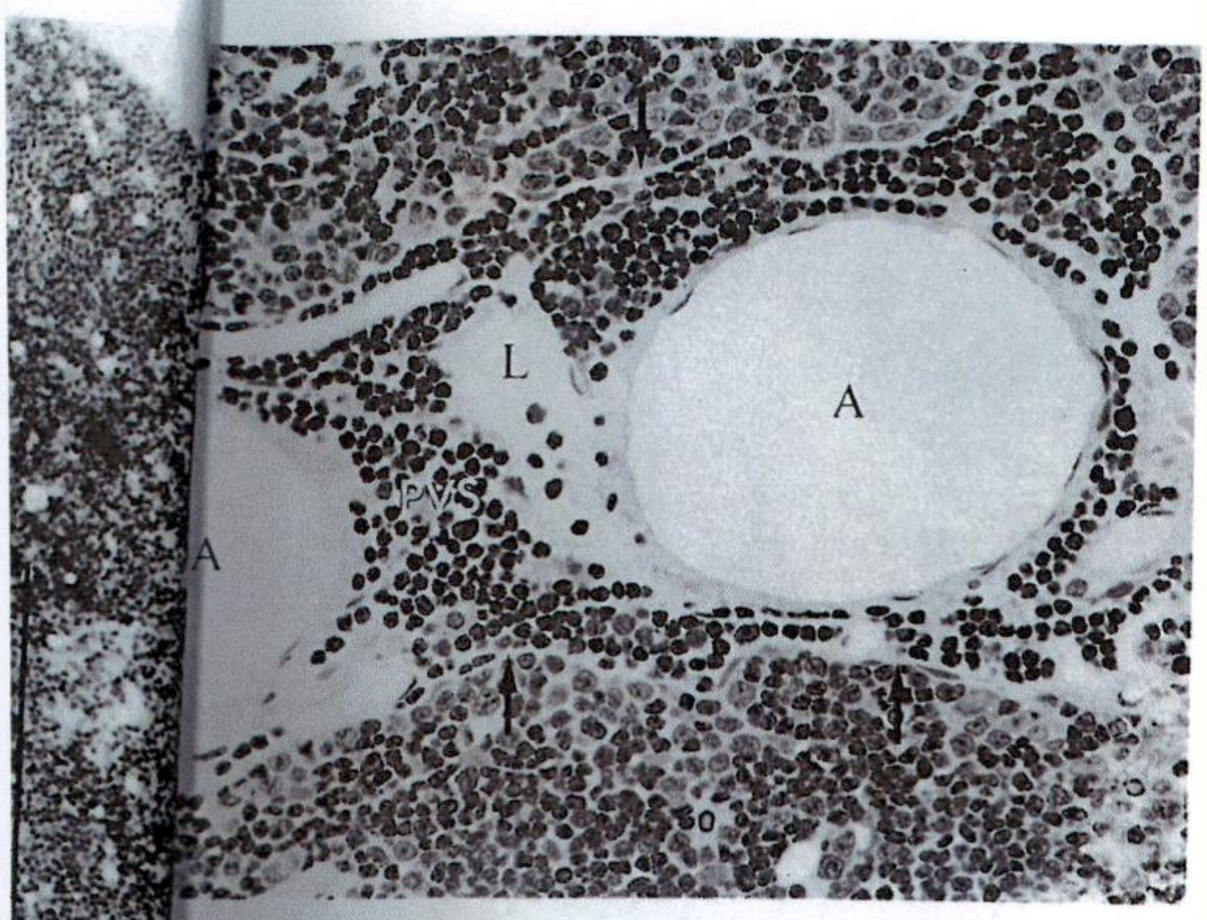
Each thymic lobe had at least one neurovascular bundle or hilus, usually on its postero-lateral aspect. Large efferent lymphatics contribute to the hilum as they reach the thymic surface (Figures 3,4). Under the light microscope, lymphatic vessels, when traced retrogradely, followed the branching of the blood vessels in the loose connective tissue, and therefore, were named accordingly: hilar, septal, cortico-medullary and medullary. The most peripheral identifiable elements of the lymphatic system were capillaries and "extra-lymphatic channels" located in perivascular spaces. Afferent lymphatic vessels to the thymus were never observed. In the following account, lymphatics are illustrated and described in sequence from hilum to inner medulla :

Hilar lymphatics: (Figures 3,4). Multiple large irregular lymph vessels accompanied hilar blood vessels, mainly arteries. Nerve fibres were frequently observed in association with these lymphatics (Figure 4). These lymph vessels were lined by a single layer of endothelium,





Figures 3,4. Semithin resin sections of the thymus gland showing an artery (Ha), a vein (Hv) and a lymphatic (Hl) in the hilar An artery (A), a vein (V) and a lymphatic (large arrows) are also present in the outer end of a septum. Figure 4 is a high powe of the area outlined in Figure 3. It shows a sectional profile of lymphatic (L) which contains a valve (small arrow). N, nerve H&E. Fig. 3 x80, Fig. 4 x320.



ure 5. Two profiles of a secondary septal (intralobular) ery (A) with a lymphatic vessel (L) in between. The ivascular space (PVS) is clearly outlined by epithelial ement membrane (arrows). The lymphatic contains a few all lymphocytes being of the same size and with the same sity of staining as perivascular lymphocytes. Semithin resin tion stained H&E. x400.

sting on connective tissue. Endothelial nuclei bulged to the lumen. Typical valves were found in them igure 4).

ptal lymphatics: (Figure 5). Examination of serial ctions showed that hilar lymphatics were continuous ith similar vessels in the interlobular (primary) and tralobular (secondary) connective tissue septa. The ptal lymphatics were usually smaller and less numerous than the hilar lymph vessels. They, too, mostly accompanied arteries, although some accompanied veins. A medium sized lymph vessel sometimes suddenly disappeared, only to re-appear after an interval of several sections. When there were more than one lymphatic vessel they were linked via a bridging channel.

Cortico-medullary lymphatics: (Figure 6). As septal lymphatics were traced towards the medulla, they became progressively smaller at the cortico-medullary junction but were still associated with blood vessels, mainly arteries.

Medullary lymphatics: (Figure 7). From the cortico-medullary junction lymphatics extended locally into the adjacent medulla but never deeply.

The lumina of thymic lymphatic vessels were filled with a grey precipitate of protein, in contrast to the accompanying empty blood vessels due to vascular perfusion; and also contained a variable number of cell profiles (Figures 5,7). The cells were identified as predominantly small lymphocytes, closely resembling perivascular small lymphocytes, and also few macrophages and eosinophils.

Under the transmission electron microscope, two types of lymphatic vessels were identified; lymphatic capillaries , usually located in the cortico-medullary and medullary regions, and collecting lymphatics, **mostly in** the capsule and interlobular septa . The fine structure of thymic lymphatics (Figure 8) was similar to that of general lymphatics elsewhere in the body.

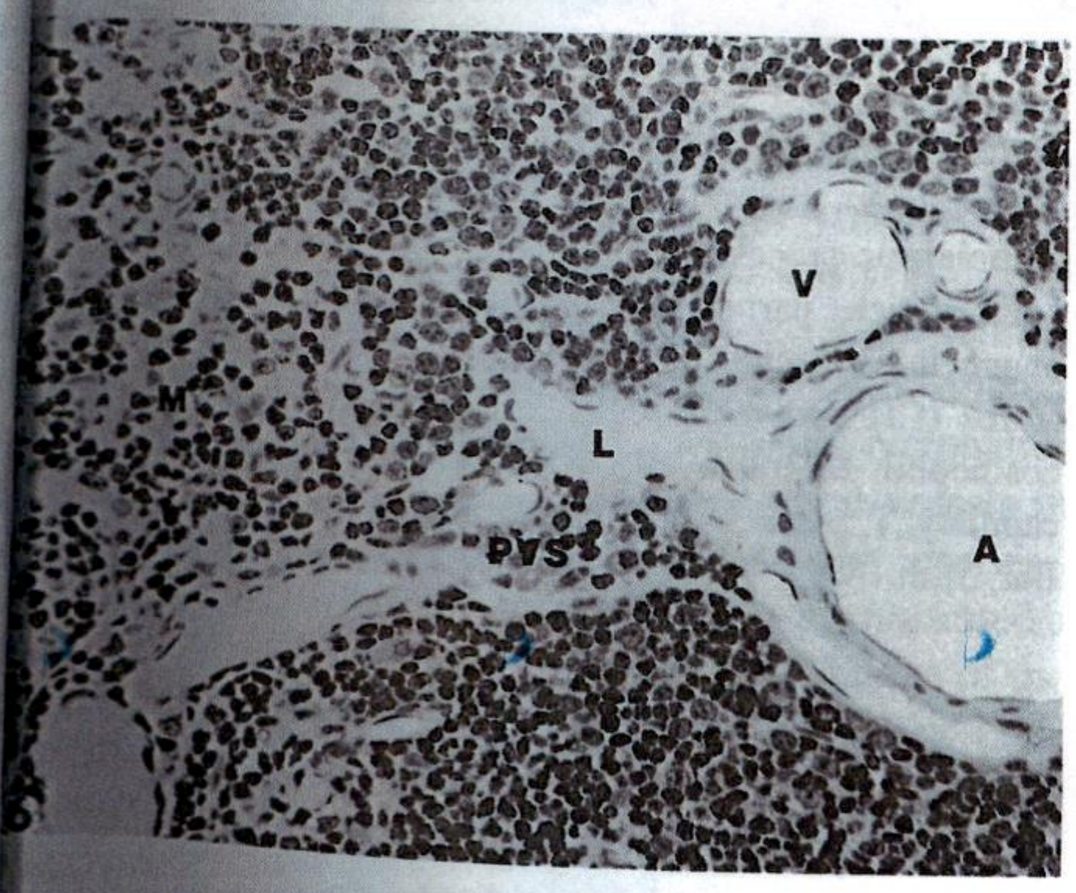


Figure 6. The artery (A) , accompanied by a vein (V) and lymph vessel (L) are located at the cortico-medullary junction. Following vascular perfusion, blood vessels appear empty and distended and have a well defined wall. The lymphatic vessel appears grey and with an irregular outline. The nuclei of its lining endothelial cells are visible. M, medulla; PVS, perivascular space. Semithin section stained H&E. x400.

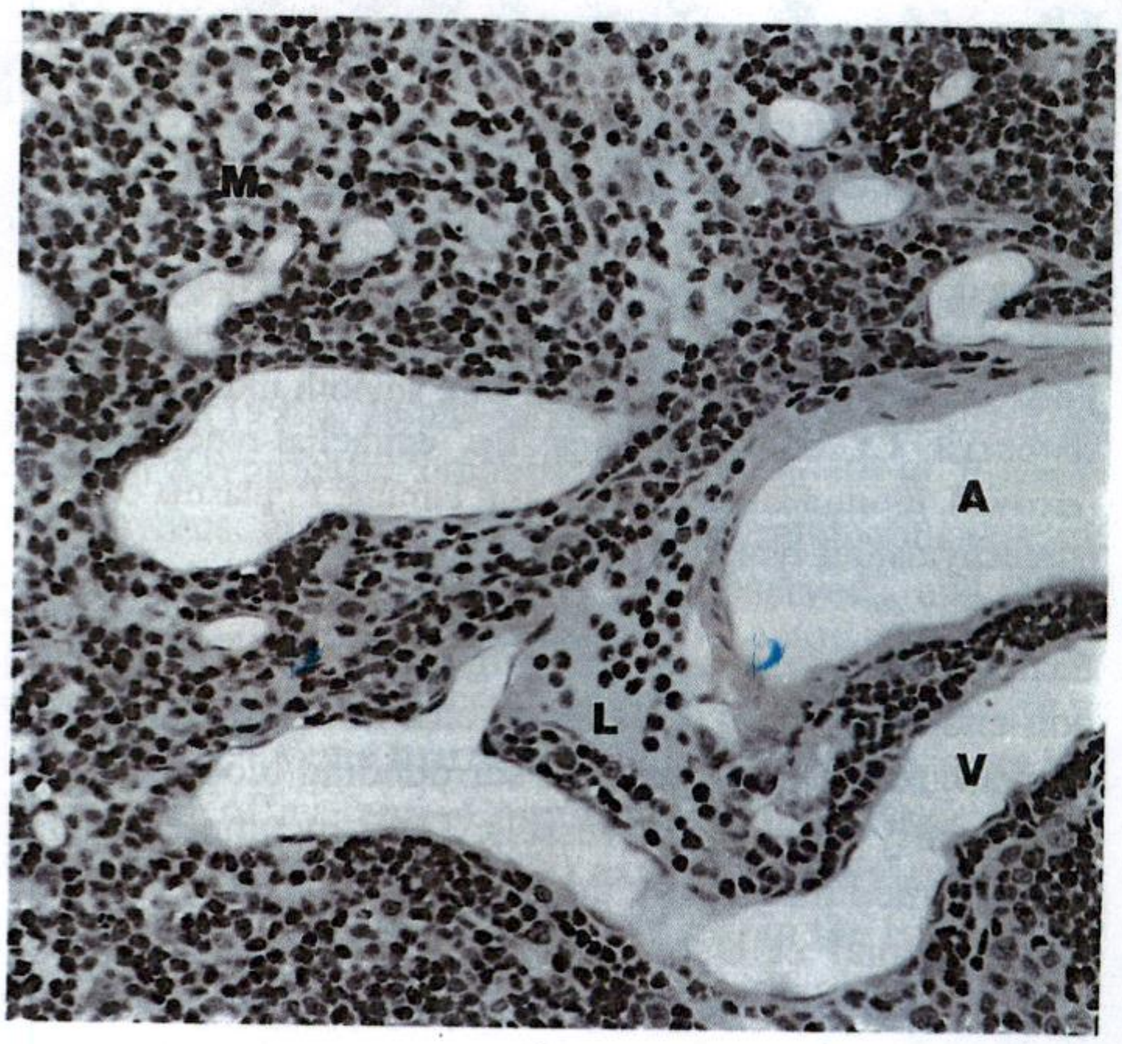


Figure 7. A lymphatic vessel (L) located in the outer medulla (M). It contains many small lymphocytes. A, artery; V, vein. Semithin section stained H&E. x320.

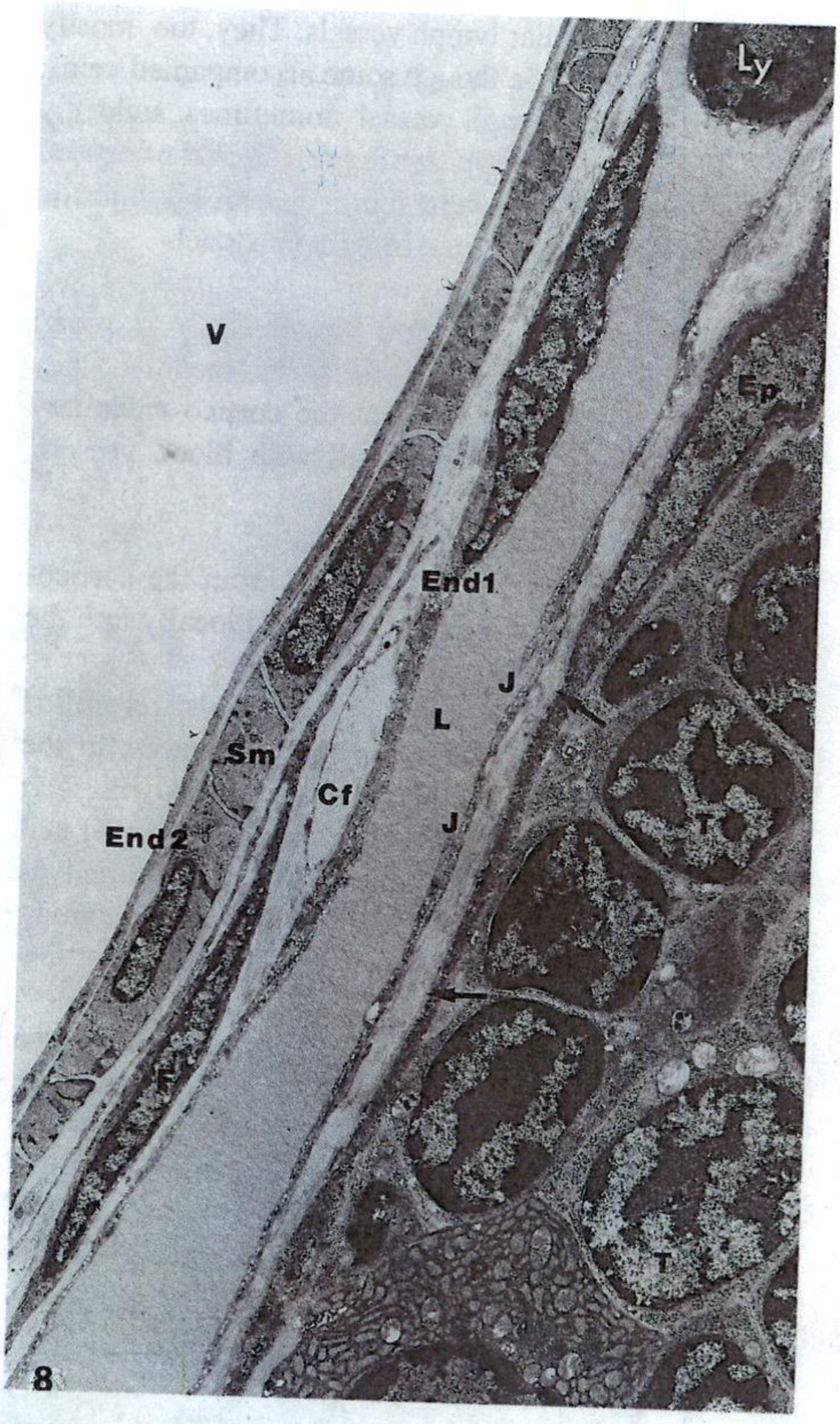


Figure 8. TEM of a primary septal (interlobular) lymphatic vessel (L) located close to an empty vein (V). It contains gray precipitate of lymph protein and one small lymphocyte (Ly). It is lined by endothelial wall (End1) which shows many pinocytotic vesicles and overlapping intercellular junctions (J). End2, endothelial lining of vein; Sm, smooth muscle cell; F, fibroblast; Cf, collagen fibres; Ep, epithelial-reticular cell; (arrows), prominent epithelial basal lamina; P, plasma cell; T, parenchymatous lymphocyte. Bar, 1 μm.

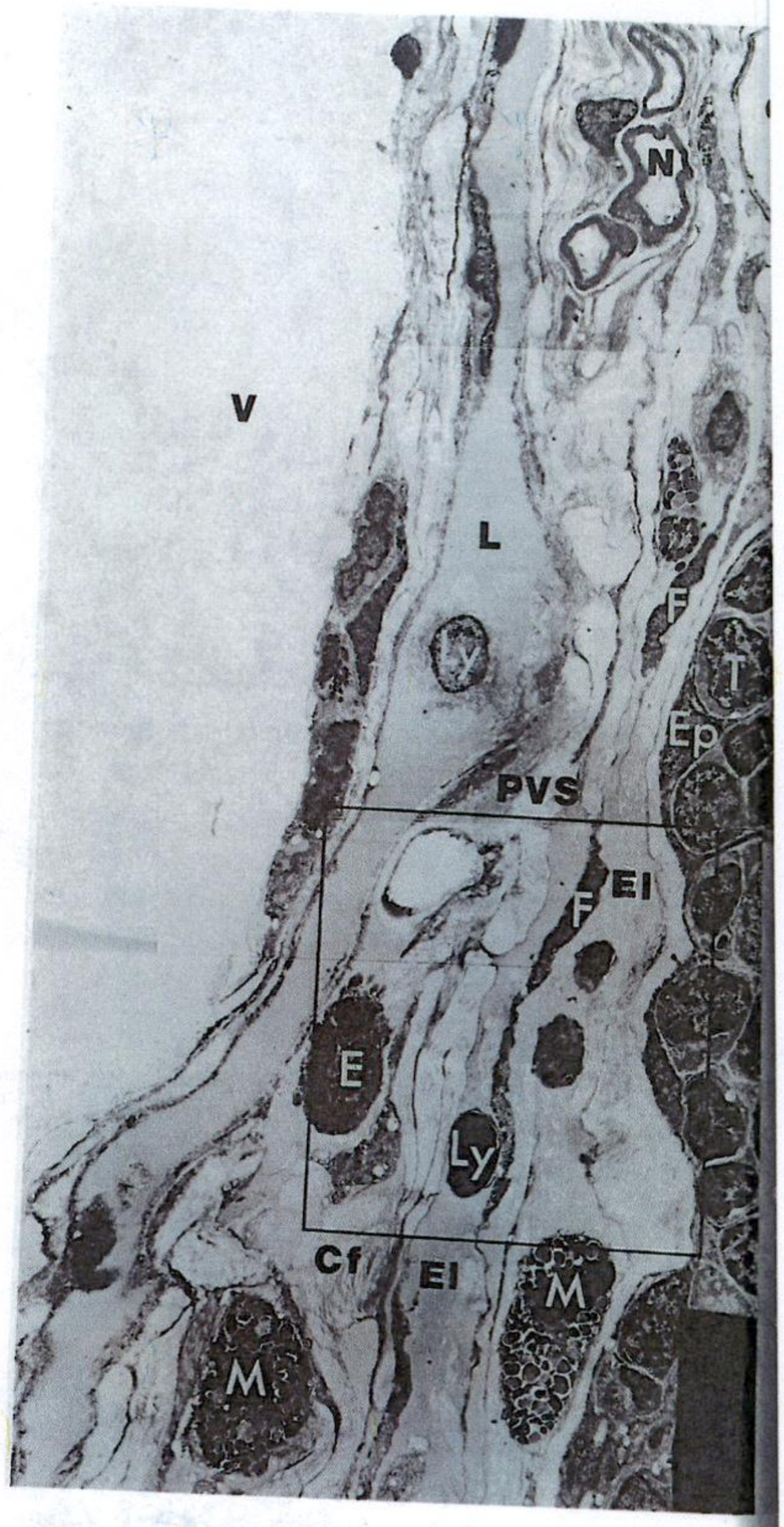


Figure 9. TEM montage of a perivascular space (
bordering a primary (interlobular) vein (V). The space co
a lymphatic vessel (L), a nerve bundle (N) and
cytoplasmic processes of fibroblasts (F) or mesenchyma
cells which partially enclose irregular (extra-lymph
channels (EI). The channels contain interstitial fluid, col
fibres (Cf) and many cells. Ep, epithelial-reticular ce
eosinophil; M, mast cell; Ly, small lymphocytes; Cap,
capillary; T, parenchymatous lymphocyte. Inset: see Figu
Bar, 5μm.

Extra-lymphatic channels: (Figures 9,10). These were non-endothelialized spaces or channels, found in the perivascular connective tissue surrounding blood vessels, particularly large septal vessels. Their incomplete walls were formed mainly by thin cytoplasmic extensions of mesenchymal cells or fibroblasts and surrounded by bundles of collagen fibres. They contained grey homogenous material and numerous small lymphocytes. These lymphocytes were similar in shape and staining to those present in the septal lymphatics. Other cells found in these spaces included macrophages, mast cells and eosinophils.

Discussion

The objective of this paper was to study morphology and distribution of intrinsic lymph channels in the rat thymus gland by tracing them in de from the hilar region internally into the medulla progressive serial sections. Also, to investigate significance of the perivascular spaces in relation lymphocytic emigration from the thymus.

Typical lymphatics were identified in the connect tissue of the capsule, interlobular and intralobular sep

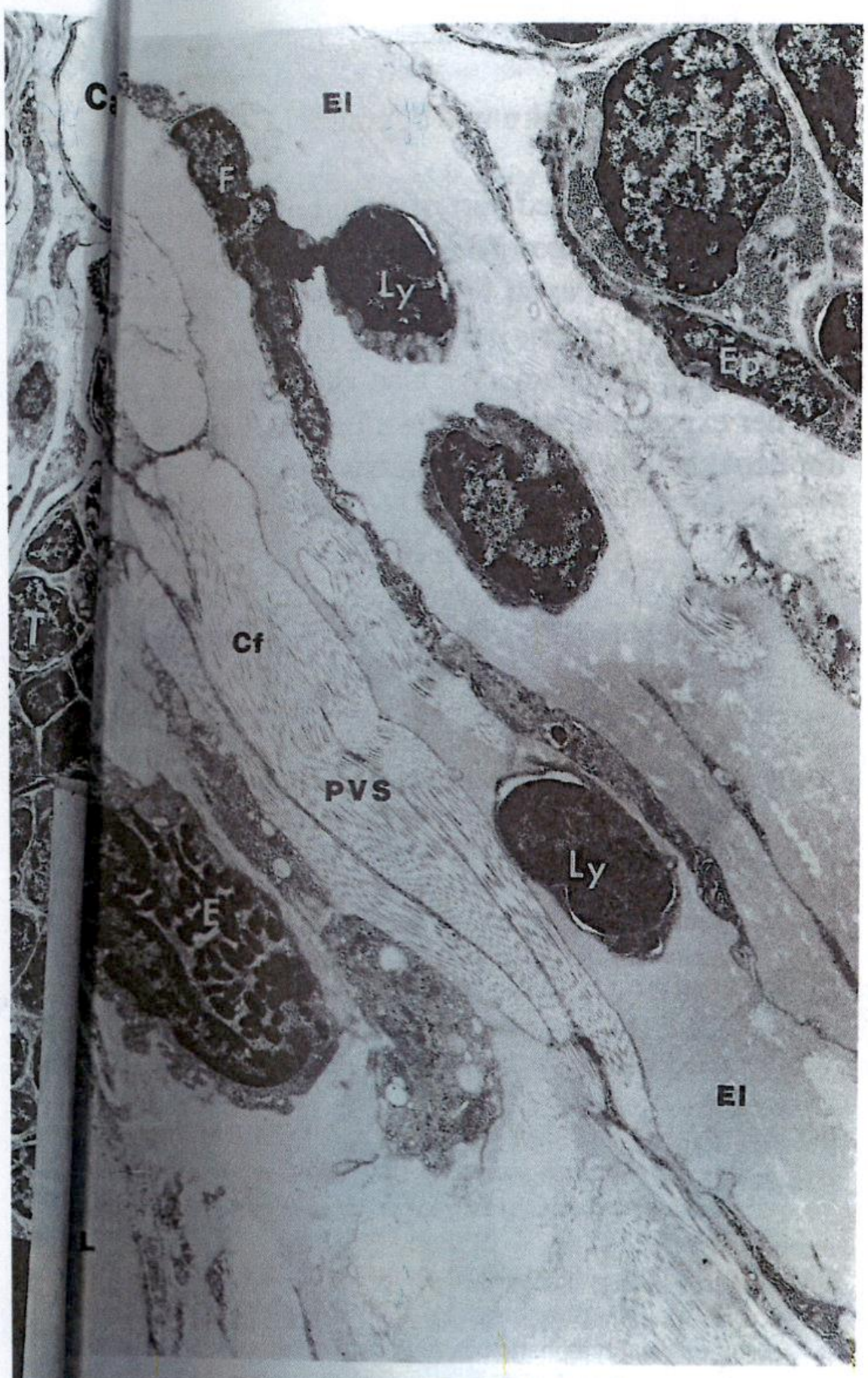


Figure 10. TEM enlargement of area outlined in Figure 9 with same abbreviations. Bar, 1 μm.

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cortico-medullary junction and in the outer medulla. The cortex, as well as the inner medulla, contained no lymphatics. Lymph capillaries, characterised by attenuated endothelium and discontinuous basal lamina were demonstrated by the TEM in the cortico-medullary junction and in the outer medulla. Collecting lymphatics constantly accompanied the branching blood vessels in the connective tissue septa, particularly large arteries.

Some light-microscopic studies have demonstrated a perivascular space in silver-impregnated sections of the thymus (Sainte-Marie and Leblond, 1964). In their TEM study of the human thymus, Bearman et al. (1975) visualised a perivascular space in the cortico-medullary region. The present study clearly demonstrated these spaces, which are limited by a distinct lining of reticulo-epithelial cells. In their SEM observations of the rat thymus, Irino et al. (1981) reported that the perivascular spaces were present only around postcapillary venules. The present study, demonstrated the existence of these spaces around arterioles as well as venules in the cortico-medullary region and in the medulla. Furthermore, as demonstrated by Pereira and Clermont (1971) in their histochemical study of the reticulo-epithelial cells, continuity between the perivascular spaces and extrathymic connective tissues was present, with lymphocytes concentrated in tissue spaces.

The thymus produces large numbers of lymphocytes that migrate to the thymus-dependent areas of the spleen and lymph nodes (Sainte-Marie and Leblond, 1964; Weissman, 1967; Yoffey and Courtice, 1970; Abu-Hijleh, 1987; Weiss, 1988). The most obvious sites for thymocyte emigration are the medullary venules (Clark, 1963; Toro and Olah, 1967; Sainte-Marie and Peng, 1971; Ushiki, 1986). However, when considering total thymic lymphocyte output, the lymphatic drainage of the thymus must also be involved (Miyasaka et al., 1990). The perivascular spaces have been regarded by some authors as true lymphatics through which both lymphocytes and, unusually, erythrocytes leave the thymus (Smith, 1955; Harris and Templeton, 1968). Leblond and Sainte-Marie (1960) using light microscopy found that true lymph vessels were sparse in the thymus; however, numerous "lymphatic spaces" were found associated with blood vessels in the medulla and in the septa. These spaces were seen to envelope blood vessels in cross-section, hence, they were called "perivascular lymphatic channels." However, electron microscopy of these spaces (Clark, 1963; Bearman et al., 1975) has shown that they do not have the morphology of typical lymphatic vessels.

An afferent flow of lymph into the thymus has been recently suggested by Eggli et al. (1986), Muller et al. (1987) and Sandberg and Hagelin (1990). Two of these studies arrived at this conclusion indirectly. After they injected two different stimulants into the peritoneal cavity of mice it resulted in an immune reaction in the parathymic lymph nodes and the adjacent thymic cortex (Eggli et al., 1986; Muller et al., 1987). After injecting guinea pigs subcutaneously with estradiol Sandberg and Hagelin (1990), found large numbers of Kurloff cells within a lymphatic containing valves pointing towards the thymus. In the present study of the rat thymus afferent lymphatic vessels were never observed, and their existence still remains to be clearly established.

It is generally accepted that in some tissues (e.g. brain, retina, bone marrow, etc.) true lymphatics are not found (Yoffey and Courtice, 1970; Weiss, 1988). However, Foldi et al. (1968) expressed the view that, in spite of the absence of lymph vessels in the brain itself, the cervical lymphatics play a significant role in drainage of cerebral interstitial fluid, since perivascular spaces have been shown to be long "pre-lymphatic tissue channels" connecting the deep cerebral tissues with the cervical lymphatic system. Later, this view was confirmed when the lymph flow in the collecting

lymphatics in the neck was obstructed (Casley-Smith et al., 1976). These "pre-lymphatic pathways" are believed to serve as vessels emptying into the true lymphatics (see Abu-Hijleh, 1987).

In the present study we demonstrated by the TEM the presence of a similar "extra-lymphatic system" of tissue spaces situated in the perivascular connective tissue around thymic blood vessels. These formed irregular, randomly arranged, and interconnected channels frequently associated with collagenous bundles and other connective tissue elements. The walls of these special "extra-lymphatic spaces" were formed by long flattened processes of fibroblastic or mesenchymal cells which joined or overlapped each other as they travelled peripherally forming, what appears to be, a continuous system of spaces. They contained grey homogenous material, similar to interstitital fluid, and numerous small lymphocytes similar in shape, size and intensity of staining to circulating lymphocytes in the adjacent septal lymphatics. This suggests that small lymphocytes found in the lymphatic vessels may gain access from "extra-lymphatic" channels or spaces and that a communication between these fibroblastic spaces and the lymphatic vessels may exist. In fact, the number of small lymphocytes in the lumina of lymphatics appeared to gradually increase as the lymphatics were traced towards the hilum, so that many lymphocytes were present in septal and hilar lymphatics. Furthermore, collagens and other connective tissue elements, associated with the perivascular "extra-lymphatic spaces," may offer a morphological pathway for the transport of interstitial fluid and lymphocytes towards the lymphatics, similar to what has been suggested in the intrinsic lymphatics of the liver (reviewed by Trutmann and Sasse, 1994). Thus, it is possible that distinct channels begin to become defined as they get nearer and nearer to the lymphatics by the sheer number of the thymocytes "marching" towards the lymphatics.

The findings of the present study suggest that the mesenchymal/ fibroblastic "extra-lymphatic channels" frequently observed within the perivascular spaces of the thymus may be regarded as *pre-lymphatic* spaces from which small lymphocytes gain access to the adjacent lymphatics. This may explain the difficulty of some workers (Ito and Hoshino, 1966; Bearman et al., 1975) in showing diapedesis of lymphocytes through blood vessel walls as a major route for lymphocyte migration from the gland. Recently, similar "pre-lymphatic" fibroblastic channels have been demonstrated in the portal tracts of the liver (Al-Jomard, 1987). He found these channels to drain lymph, and intravenously injected tracers from the space of Disse to the adjacent portal lymphatics. Additional evidence will be gained by timed studies to follow labelled substances/tracers injected into the thymus and their progression through the parenchyma to reach the efferent lymphatics.

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