STUDIES ON THE LYMPHATIC SYSTEM

(i) The intrinsic lymphatics of the thymus gland;

(ii) The thymic haemolymph nodes;

(iii) Lymphatics of the diaphragm and the uptake of particles from the peritoneal cavity.

By

© DR. MARWAN F.M. ABU-HIJLEH, MB.B.ch.

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Department of Anatomy,
University of Glasgow.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

DEDICATED

To my parents,

whose example, help and encouragement

have contributed more to my life

than I can express.

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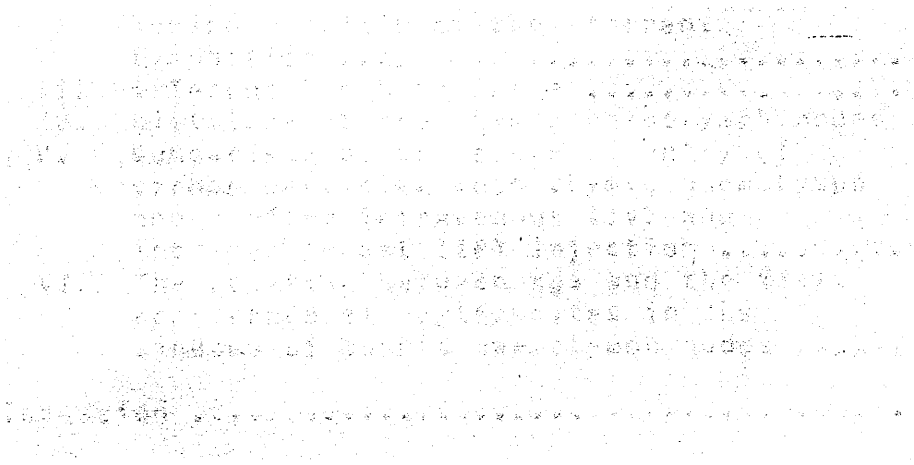


CONTENTS

PREAMBLE

This thesis reports the results of a study which was originally concerned with the thymic haemolymph nodes of rats; as the work progressed it extended to two sources of lymph draining to these nodes, namely the thymus and the diaphragm, and to the wider problems of the intrinsic lymphatics of the thymus and the uptake of particulate material from the peritoneal cavity.

The aims of each part of the project will be briefly outlined:

I. THE THYMIC HAEMOLYMPH NODES

In the rat, the thymic lymph nodes, along with the renal and splenic nodes, are of the haemolymph variety (Drummond, 1900; MacMillan, 1928; Turner, 1969); they differ from typical nodes in that their sinuses contain large numbers of erythrocytes, sufficient to give them a red colour when viewed with the unaided eye. The nature of haemolymph nodes has been much debated. Several previous studies from this Department have shown that, in the case of the renal and splenic nodes, these erythrocytes are of extrinsic origin, and enter the node via its afferent lymphatics, from the kidney and from the spleen, respectively (Kazeem & Scothorne, 1982; Hogg, Reid & Scothorne, 1982; Abbas & Scothorne, 1983). Another view is that the intrinsic venules of haemolymph nodes allow the escape of erythrocytes directly into the node (Nopajaroonsri et al., 1974). In an attempt to resolve

this problem, as it applies to the thymic haemolymph nodes, the first part of the thesis examines their structure, with particular reference to the following points:-

i) their topography and histological structure.

ii) their regional lymph drainage.

iii) the source of their sinusoidal erythrocytes.

iv) the route of entry of erythrocytes, into the nodes.

v) a comparison of the site and rate of entry into the thymic haemolymph node, of carbon particles, injected either intravenously (IV) or intraperitoneally (IP).

vi) the age at which erythrocytes first appear in their nodal sinuses, and the significance of this.

II. THE LYMPHATICS OF THE THYMUS

The distribution and extent of intrinsic lymphatics of the thymus are still controversial. Early workers claimed that the thymus gland contains only a few or no lymph vessels (Jolly, 1923; Policard, 1950); while others denied the presence of typical (true) lymphatics and reported, instead, the presence of tissue spaces (Bloodworth et al. 1975; Seigler, 1964), or of perivascular lymphatic spaces (Blanc et al. 1973; Harris & Templeton, 1968; Leblond & Sainte-Marie, 1960). Clark (1963) failed to find any lymphatic endothelium in the perivascular spaces and suggested that they should, therefore, not be regarded as lymphatics. Other investigators have demonstrated the presence of a "few" true lymphatics in the thymus, but only located in the interlobular septa and the capsule

(Goldstein & Mackay, 1969; Kotani et al., 1967; Omori, 1973; Smith, 1955). Recently, some workers went a step further and reported, in addition, the existence of lymphatics in the medulla of the thymus of man (Singh, 1980) and of rat (Hwang et al., 1974). No one has demonstrated lymphatics in the cortex of the thymus (Weiss, 1983). In the present study, the intrinsic lymphatic vessels of the rat thymus gland have been reinvestigated by optical and electron microscopy, with special reference to the following features:-

i) their origin, distribution and contents.

ii) their relation to the thymic blood vasculature, with reference to the concept of a blood-thymus barrier.

iii) the role, if any, of the perivascular spaces in lymph drainage from the thymus.

iv) the lymph nodes which receive lymph from the thymus.

III. THE LYMPHATICS OF THE DIAPHRAGM AND THE UPTAKE OF PARTICULATE MATERIAL FROM THE PERITONEAL CAVITY.

The third part of the thesis deals with the lymphatics of the diaphragm.

It is well known that fluid, particulate material and various cells (including erythrocytes) introduced into the peritoneal cavity are rapidly absorbed (Allen, 1936; Cunningham, 1922; 1926; Courtice & Steinbeck, 1950; Courtice & Simmonds, 1954; Leak & Rahil, 1978; MacCallum, 1903; Yoffey & Courtice, 1970). It is also well known that this rapid absorption occurs principally, if not

exclusively, by the subperitoneal lymphatics of the diaphragm (Allen, 1956; Casley-Smith, 1964, French et al., 1960; MacCallum, 1903, Odor, 1956). Furthermore, it is known that most of the lymph draining from the diaphragm reaches the mediastinal lymph nodes, a group which includes the thymic haemolymph nodes (Cunningham, 1922; Higgins & Graham, 1929; Olin & Saldeen, 1964; Simer, 1948; Tilney, 1971). The present study has examined:-

i) the pattern and distribution of the intrinsic lymphatics of the diaphragm.

ii) various regional lymphatic routes and corresponding regional lymph nodes of the diaphragm and their relative importance.

iii) the rate of uptake of particulate material from the peritoneal cavity.

iv) the route of uptake of particulate material (including erythrocytes) from the peritoneal cavity, with reference to the concept of peritoneal "stomata", established by scanning and transmission electron microscopy.

v) a comparison of the surface appearance between peritoneal and pleural mesothelium, screened by the scanning electron microscope.

CHAPTER I

GENERAL INTRODUCTION

SURVEY OF THE LITERATURE OF THE LYMPHATIC SYSTEM IN GENERAL

A. HISTORICAL REVIEW

Lymphatic vessels, unlike blood vessels, escaped attention for many centuries, due to their thin wall, their colourless contents and their collapse after death. Although Erasitratus (250 B.C.) along with Herophilus (300 B.C.) are said to have observed lymphatic vessels in a mammal's intestine (cited by Yoffey & Courtice, 1970), it seems that Gasparo Aselli (1622) has the credit for the discovery of the lymphatic system (cited by Drinker, 1942). Aselli described the "lacteals" in the mesentery of a recently fed dog, which contained not blood but a milky fluid which oozed out from these vessels when they were pricked. However, he believed that lacteals drained directly into the liver and that their contents contributed there to the formation of blood. This theory was later disproved by Pacquet (1651) who first described the receptaculum chyli and the thoracic duct, and observed that the contents of the lacteals here transported in this duct to major veins at the root of the neck, and thence to the heart. The term "lymphatics" was first introduced to the literature by Bartholin in 1653, and by the end of the 17th century, it was well established that in man and many of the larger mammals there was this system of vessels, separate from the arteries, which arose from many different parts of the body, and that eventually the fluid which these vessels contained reached the blood through the thoracic and/or the right lymphatic ducts. Regarding the

structure of lymphatics, Von Recklinghausen, in 1862, showed that lymph capillaries, through silver impregnation, displayed distinct outlines of plate-like polygonal endothelial cells, similar to those of blood capillaries described later by Hoyer (1865). Von Recklinghausen thought that the lymphatic capillaries communicated with the tissue spaces by means of canaliculi. However, His (1863) was apparently the first to claim that the lymph-vascular apparatus forms a closed system of tubes. Later, by using injection techniques, Ranvier (1897), MacCallum (1903) and much later Clark and Clark (1937) and Pfuhl (1939-1940) concluded that the lymphatic capillaries formed a closed system and were not in direct communication with the interstitial spaces. Recently, this hypothesis has been challenged by several workers, and Von Recklinghausen's original description of direct communications between tissue spaces and lymphatic capillaries, seems to have an increasing support (Casley-Smith, 1977; 1980; Hauck, 1973; Hauck et al., 1978).

Understanding of the function of the lymphatic system started with the work of William Hunter (1784) and his collaborators, Cruickshank & Hewson, who postulated that the lymphatic vessels are the "absorbing vessels" all over the body. Ludwig (1858) regarded lymph as a filtrate derived from blood, while Heidenhain (1888; 1891; 1896) concluded that lymph was produced by a process of secretion. However, during the last quarter of the 19th century, Ludwig's theory (1858) of filtration was regarded

as obsolete, and Starling (1893; 1896; 1909) was the first to realise the fundamental relationship between the hydrostatic pressure of the blood in the capillaries and the colloid osmotic pressure of the plasma proteins. He found that the blood capillaries leaked protein to a small extent, and showed that, as capillary filtration increased, more interstitial fluid entered the lymphatic capillaries, thereby increasing lymph flow. Later, Drinker and Yoffey (1941) elaborated this concept: "The lymphatic system has evolved in the animal kingdom by physiological necessity, and the probabilities are that the main factor in this necessity has been the need for a specialised mechanism to return to the blood stream blood proteins which have leaked from the blood capillaries".

B. INTERSTITIAL FLUID AND LYMPH FORMATION

In the 19th century, when it was thought that the lymphatic vessels communicated directly with the tissue spaces, the terms "tissue fluid" and "lymph" were used synonymously. Nowadays, however, the term "lymph" is usually restricted to the fluid present in the lymphatic vessels. From most regions of the body the lymph so collected is clear; but that from the walls of the small intestine is milky because of the chylomicrons which it contains, and in this situation it is referred to as "chyle" (Yoffey & Courtice, 1970; Weiss, 1983). Lymph coagulates, but the process occurs much more slowly than in blood and the clot is soft. Lymph normally contains a few red blood cells and a varying number of white blood cells depending on the region from which the lymph is obtained. The predominant white blood cells present are lymphocytes. These are continually recirculating in the lymphoid tissue, many of them entering the lymph stream and passing through the thoracic and right lymphatic ducts before they reach the blood circulation. Therefore, their number in lymph varies depending on the kind of lymph in which they are found, i.e. peripheral or intermediate or central lymph (Yoffey & Courtice, 1970). Peripheral lymph is that which has not yet passed through a node or come into contact with other lymphoid tissue; intermediate lymph is that which has already passed through one or more lymph nodes but has yet to pass through more; and central lymph is that which is on

its way to the blood without further interruption.

It would appear almost certain that most (but not all) of the selectivity that causes the differences in concentrations between the substances in the blood and in the lymph occurs at the endothelial lining of blood capillaries and venules (Yoffey & Courtice, 1970). Further selection may well occur at the blood vessel basement membrane, and in the passage through the tissues. There is, however, the strong possibility that the lymph is concentrated by ultrafiltration in the lymphatics, which is another place where these alterations may occur (Casley-Smith, 1977).

There has been considerable debate about whether lymph is identical to, or more concentrated than tissue fluid. One of the reasons for this long standing controversy is the difficulty of obtaining a sample from the tissue fluid, particularly from sites adjacent to lymphatic capillaries, under normal conditions. The arguments and counter-arguments are too lengthy to discuss in detail (reviewed by Casley-Smith, 1977; 1982). Briefly, it has been shown experimentally that: 1) lymph can alter greatly as it traverses the collecting lymphatics. While Hargens and Zweifach (1976) showed that the concentration of lymph increased as it ascended the collecting lymphatics (due to ultrafiltration), Nicolaysen et al (1975) and Renkin (1979) found that it was constant from the lymphatic capillaries to the adjacent collecting lymphatics in the same tissue. Other workers (reviewed by Casley-Smith,

1982) showed that the collecting lymphatic walls are very permeable to small molecules, although not to those larger than a few thousand Daltons. Therefore, one would expect that, whatever the concentration of the lymph that entered these vessels, it would rapidly come to equilibrium with the tissue fluid in their adventitia. In other words, the concentrated lymph of the lymphatic capillaries maintains its mean concentration in the adjacent collectors but is rediluted in the remote collectors. Thus, to some extent, the lymph comes to resemble tissue fluid, but not necessarily the fluid where it originated but rather the fluid adjacent to the remote collectors. 2) In the lymphatic capillaries, the macromolecules are considerably more concentrated than in tissue fluid. It is estimated that the mean concentration of protein in the lymph in these capillaries is some three times more concentrated than fluid in the adjacent interstitial tissue (Casley-Smith, 1977). 3) It was also found that this lymph concentration varies throughout the "initial lymphatic cycle", i.e. being diluted during the relaxation or filling-phase and concentrated during the compression or emptying-phase. This means the inflowing fluid will dilute the lymph inside the capillaries, which is then reconcentrated during the next emptying-phase by ultrafiltration of fluid via the "closed" junctions (both those normally closed and the openable ones).

C. THE ANATOMY OF LYMPHATIC VESSELS

C.i General

The lymphatic vessels form a system of endothelium lined channels which collect tissue fluid and return it by a circuitous route to the blood stream. The fluid in these vessels, which is called lymph, circulates, unlike the blood, in one direction only, from the peripheral tissues towards the heart. The most peripheral elements of the lymphatic system are innumerable, small thin-walled vessels, which are termed peripheral or initial lymphatics, or more often "lymphatic capillaries". These lymphatic capillaries begin blindly in the tissues from which the lymph is collected, and therefore perform the system's primary function of removing fluid and material from the tissue. They anastomose freely along their course and progressively fuse to form larger lymphatic vessels, the "collecting lymphatics", which perform the system's secondary function of transporting lymph to the blood. Ultimately these collecting lymphatics gather into two main trunks, the large thoracic duct and the smaller right lymphatic duct which empty into the left and right subclavian veins respectively. In the pathways of the lymph vessels there are groups of lymph nodes through which lymph percolates, to be filtered before reaching the thoracic and right lymphatic ducts (Kelly, Wood & Enders, 1984; Weiss, 1983; Yoffey & Courtice, 1970). Lymphatic capillaries and vessels occur in most tissues and organs.

They are most numerous beneath body surfaces such as the skin; the mucous membranes of the gastro-intestinal, respiratory, and genito-urinary tracts; and in subserous tissues. They have not been demonstrated in the central nervous system, the coats of the eye ball (globus oculi), the internal ear, the bone marrow, the intralobular portion of the liver, and the fetal placenta (Weiss, 1983; Yoffey & Courtice, 1970).

C.ii Histology and ultrastructure of lymphatic capillaries

Lymph capillaries, like those of the blood, are delicate tubes with walls which consist of a single layer of endothelial cells. The ultrastructure of lymphatic capillaries generally resembles that of blood capillaries, with some important differences (Leak & Burke, 1966; 1968a; Leak, 1970; Yoffey & Courtice, 1970). These differences are:-

a) Lymphatic capillaries have a wider lumen and more irregular outline than those of blood capillaries, but they may be completely collapsed or narrow, or much distended. In general their calibre is in the range of 15 - 75 um. In sectional profile, the lymphatic wall usually presents a characteristic "scalloped" outline (Fraley & Weiss, 1961).

b) The lymphatic endothelium is very variable in thickness (from several micra in the perinuclear region, to as little as 0.1 um in the attenuated cytoplasmic rims). The shape of the endothelial cells differs from that of blood capillaries. Those of lymphatics are usually

larger with irregular dentate edges and an oval nucleus, which often protrudes into the vessel lumen. In addition to possessing the usual complement of cellular organelles, i.e. mitochondria, Golgi complex, a paucity of endoplasmic reticulum, microtubules, and free ribosomes, Leak and Burke (1968) described the presence of abundant fine filaments throughout the cytoplasm of endothelial cells of dermal lymphatic capillaries. These filaments (40-60Å in diameter) usually appear in bundles, which course along the periphery of the cell , parallel to its long axis. They are similar to the cytoplasmic filaments found in a variety of cell types, including the endothelial cells of blood capillaries (Majno, 1965; Majno et al., 1969; McCuskey, 1971). These filaments in endothelial cells of blood vessels are thought to possess contractile properties (Majno et al., 1969). Similarly, Leak (1970) suggested that these cytoplasmic filaments represent the contractile elements for the lymphatic capillaries.

c) In the lymphatic capillary, a basement membrane is usually absent or is, at most, discontinuous and poorly developed. From the earlier observations on the transfer of particulate materials across the basement membrane, it was suggested that the basement membrane of blood vessels behaved like a filter since the passage of ferritin molecules (Farquhar et al., 1961),

colloidal particles (Majno & Palade, 1961) and also cells (Marchesi & Florey, 1960) was impeded by this dense layer that surrounded the blood vessels. Therefore, the absence of a definitive basement membrane would eliminate a selective filter between the lymphatic capillary wall and the adjacent connective tissue (Leak & Burke, 1966).

d) The endothelial wall of the lymphatic capillary displays numerous cytoplasmic projections, both on the luminal and abluminal surfaces; the latter extending into the surrounding connective tissue and serving to anchor the lymphatic wall to it (Fraley & Weiss, 1961; Leak & Burke, 1966). Lymphatic capillary walls have no fenestrae, and also pericytes are absent from the connective tissue around their walls, unlike the situation around blood capillaries.

e) One of the prominent features of lymphatic capillary endothelium is the presence of numerous pinocytic vesicles, scattered all over the cytoplasm (Casley-Smith, 1964; Leak & Burke, 1966; 1968a; Leak, 1970). Leak and Burke (1966) described two distinct types of vesicles that occur in the lymphatic endothelial cells of the guinea-pig's ear: a) small smooth-surfaced vesicles (around 70nm in diameter) and b) larger coated vesicles (around 200nm in diameter). The small smooth-surfaced vesicles are similar to those described for blood capillaries (Palade, 1953; Wissig, 1958); and to those observed in the lymphatic endothelial cells of

the diaphragm (French et al., 1960). The passage of materials across the cell in small vesicles was first suggested by Palade (1953); and later Bennett (1956) advanced the hypothesis that membrane flow and vesiculation may be important transport mechanisms in carrying particles and molecules within, into, and out of the cells by way of vesicles. Experimental evidence in support of Bennett's hypothesis has since been demonstrated by a number of investigators (Jennings et al., 1962; Palade, 1960; 1961; Simionescu, 1979; Wissig, 1962). Similar observations were reported by Dobbins and Rollins (1970) and Casley-Smith (1964) for the lymphatic endothelial cell. The latter author estimated that approximately 35% of the non-nuclear cytoplasmic volume is occupied by these smooth-surfaced vesicles.

Like the smooth-surfaced pinocytic vesicles, the "coated" or "hairy" vesicles occur along the inside of both luminal and abluminal surfaces and also deep in the lymphatic endothelial cytoplasm (Casley-Smith, 1964; Dobbins & Rollins, 1970; Leak & Burke, 1966; 1968). Leak and Burke (1966) suggested that coated vesicles may represent a specific transport mechanism for transmission of proteins across the endothelial cells of lymphatic capillaries. Other workers regarded these large vesicles as the main passageway for the uptake of fluid by lymphatic capillaries (Dobbins &

Rollins, 1970; Mayerson, 1963).

f) The endothelial intercellular junctions are the single most important feature of the lymphatic capillary, for upon them depends the uptake of material and its retention (Casley-Smith, 1972). These endothelial junctions vary considerably in organisation and structure along the lymphatic wall. Terminal processes of adjacent endothelial cells may overlap each other, interdigitate, or simply end edge-to-edge (Leak & Burke, 1966; Leak, 1970). Leak and Burke (1966) observed, in some lymph capillaries, that the apposed surface membranes, at various points along the intercellular junction, are held in close apposition by adhesion devices: a) Zonula occludens (tight juntions), b) Zonula adhaerens, and c) Macula adhaerens (desmosome) which is the commonest type. While, just as in the blood capillaries, there are many tight and close regions in these unions between the endothelial cells, the lymphatic capillaries are distinguished by also having open junctions, i.e. the endothelial cells may be separated from one another to form intercellular gaps of 0.1 to several um across (Casley-Smith & Florey, 1961; Leak & Burke, 1966; 1968). In the intestinal villi, Dobbins and Rollins (1970) estimated that approximately 2.5% of the total junctions were open. Other workers studying the same tissue (Casley-Smith, 1962; Palay & Karlin, 1959) found greater numbers of open junctions, up to 6% of the total, and

considered this percentage more than sufficient to contribute significantly to the filling of the lymphatic capillary. The number of open junctions increase up to 50% if the tissues are very active metabolically, or during oedema, or even following mild injury (Casley-Smith, 1980). This would suggest that the endothelial cell junctions probably undergo continual changes, depending on the local conditions at the time.

g) Finally, Leak and Burke (1966; 1968) have described fine filaments that are inserted either on the outer leaflet of the trilaminar unit membrane of the endothelial cell wall, or are embedded in an electron dense opaque material associated with the abluminal endothelial cell membrane and which resembles hemidesmosomes. These filaments are 4-11nm in diameter and may appear as individual units, but are usually aggregated into bundles that run parallel to the long axis of the capillary wall and extend for varying distances into the adjoining connective tissue. Leak and Burke (1968) followed the suggestion of Pullinger and Florey (1935), who were the first to describe such filaments in the vicinity of lymphatic capillaries by the use of optical microscopy, that these fine filaments serve to "anchor" the lymphatic capillary wall to the surrounding perilymphatic connective tissue, and that they may play an important role in

maintaining the patency of the lymphatic capillary lumen, particularly under conditions of increased interstitial fluid pressures. This will tend to force the connective tissue elements apart. Therefore, as collagen and elastic fibres are separated by increased interstitial fluid during both normal and inflammatory conditions, areas of the lymphatic wall to which anchoring filaments are attached would also be pulled along with the separating collagen and connective tissue fibres, thus causing a wider lymphatic capillary lumen, by the separation of loosely overlapping endothelial cells, producing patent intercellular junctions. In brief, as the interstitial fluid pressure rises, the anchoring filaments pull on the lymphatic capillary wall like a "guy rope" on a tent and help to keep the vessel open.

C.iii Histology & ultrastructure of collecting lymphatic vessels:-

The lymphatic capillary network drains to the larger collecting lymphatics which have thicker walls and are situated further from the surface being drained. The thickness of the wall and the relative proportions of connective tissue, elastic and muscular elements vary in different regional collecting lymphatics and in different species (Morris, 1970; Yoffey & Courtice, 1970). In general, the lymph vessels resemble the veins in structure, but without definitive layers, and their walls are as a rule thinner than those of veins of a

corresponding calibre. In the smaller lymph vessels, the endothelium is surrounded by collagenous and elastic fibres and a few smooth muscle cells. Carleton and Florey (1927) found that the walls of lymphatic vessels, 100- 200 um in diameter, contained muscle fibres whereas no muscle cells were found in vessels smaller than these. In the larger lymphatic walls muscle cells are found embedded in the connective tissue, while elastic fibres border the endothelial layer and are interspersed amongst the collagen bundles. In the larger lymphatic ducts, three coats may be distinguished as in veins: intima, media and adventitia. The intima is composed of the endothelial lining, underneath which is a delicate network of elastic fibres disposed longitudinally. The media consists mainly of circularly disposed smooth muscle fibres and a few longitudinal ones. Between the muscle fibres are relatively few delicate elastic fibrils. The adventitia, which is the thickest coat, is composed of longitudinally coursing collagenous fibres, among which are bundles of longitudinal muscle and elastic fibres (Weiss, 1983; Yoffey & Courtice, 1970). Blood capillaries and nerves are usually situated in the adventitia of large lymphatic ducts (Weiss, 1983).

Collecting lymphatics contain numerous centrally directed valves, which are of great value in their histological recognition. These valves usually consist of paired cusps, each originating from opposite endothelial

surfaces and extending into the lumen. The base of a single valve cusp takes up approximately 180° of the circumference of the vessel, so that the entire circumference of the vessel wall provides attachment for a valve. In addition to this "traditional" bicuspid valve, occasional tricuspid valves (Weiss, 1983) and truncated cones (Boussauw & Lauweryns, 1969) have been seen in some lymphatic vessels. Cusps are formed as folds of endothelium. Kampmeier (1931) showed that the valves of the smaller lymphatics consisted only of the duplication of the endothelium. In larger collecting lymphatics a few connective tissue fibres and even muscle fibres extend between the folded endothelial surfaces of the cusps from the subendothelial tissue. The cusps project in the direction of lymph flow and prevent back-flow. Valves are responsible for the beaded appearance of lymphatic vessels; the vessel is constricted at the attachment of the base of the valve and dilated beyond (Yoffey & Courtice, 1970). Kampmeier (1928) found that in the human, valves first appeared between the second and the fifth months of foetal life. Also, he noted that the first valves in the developing lymph vessels appeared in the territory of the jugular lymph sacs and in the upper segment of the thoracic duct; this was followed by the development of valves in the plexuses of the extremities, and lastly throughout the entire thoracic duct itself. Furthermore, Kampmeier described three distinct types of valve formation. The first type occurred when one lymph vessel joined another in

the formation of a lymphatic plexus; the second type developed at the mouths of anastomoses already formed; while the third appeared in the course of the principle lymphatic duct long after its continuity has been established.

The ultrastructure of lymph vessels larger than capillaries shows that as they pass centrally, the endothelial cell junctions contain more Zonulae adherentes and occludentes and far fewer open junctions than in capillaries, and the basement membrane becomes well developed. Also, the collecting lymphatics do not have "anchoring" filaments to connect their wall to the perilymphatic connective tissue; hence they can easily be collapsed by excessive external pressures (Casley-Smith, 1967). Larger lymphatic vessels come to resemble small veins. Lymphatic vessels anastomose with one another and tend to travel in company with blood vessels (usually veins), which may be girdled by lymphatics. Lymph is carried to lymph nodes by afferent lymphatic vessels and from them by efferent vessels. It is likely that no lymph reaches venous circulation without passing through at least one node (Weiss, 1983; Yoffey & Courtice, 1970).

D. PERMEABILITY AND FUNCTION OF LYMPHATIC VESSELS

D.i General

In normal tissues the lymphatics drain any fluid in excess of filtration over reabsorption through the blood capillary wall. As the formation of tissue fluid increases, the tissue pressure rises, the lymphatic vessels dilate and the formation of lymph increases (Yoffey & Courtice, 1970). This is better shown in experiments where the filtration rate is increased by increasing the filtration pressure as in intravenous transfusion (Reinhardt, 1952), or by altering the permeability of the cell membrane as in warming a limb in a hot bath (Courtice, 1946). The walls of blood capillaries and venules are semi-permeable membranes that permit the diffusion of small-molecular-weight materials through them and retain larger molecules (such as proteins and fatty complexes) and the cellular elements of the blood. Small but cumulatively significant amounts of plasma proteins do escape from blood vessels, however, with large volumes of fluid (Yoffey & Courtice, 1970). The bathing of perivascular connective tissue by this transudate constitutes an essential physiological process. This is the means by which hormones, antibodies, enzymes and other macromolecules and fluids reach the cells and intercellular matrix of the body. Besides removing the excess tissue fluid, so that normally there is no appreciable amount of free fluid in the tissues, the lymphatic vessels have the special

function of absorbing protein (Yoffey & Courtice, 1970). It is now generally agreed that most of the plasma proteins, if not all, lost from the vascular system, are returned to the blood by lymphatic vessels; as the lymphatic capillaries, unlike their vascular counterparts, are freely permeable to protein, particles, and large colloidal molecules once they become extravascular (Courtice & Simmonds, 1949; Drinker & Field, 1931; Hudack & McMaster, 1932; Leak & Burke, 1968).

There is a small but significant loss of cells from the blood (including red blood cells) into the perivascular tissues. Some are picked up by lymphatics and returned to the blood. In addition there is regular traffic of fluid and cells, especially macrophages, from serosal cavities (such as the peritoneal cavity) via lymph into blood (Yoffey & Courtice, 1970).

Also, the lymphatic vessels are involved in the traffic of lymphocytes through the body, as part of the immune defences. This immunological mechanism is brought about by the passage of lymph through lymph nodes and thence to the blood stream. The lymph nodes are the principal site of immune response to lymph-borne antigens (Weiss, 1983).

D.ii Permeability and function of lymphatic capillaries

The removal of connective tissue fluid by the lymphatic capillaries is of fundamental importance in the normal maintenance of fluid balance for body tissues. In addition to restoring extravascular proteins back to the

blood stream, the lymphatic capillaries are important in the response of the organism to infection and in the spread of disease to various parts of the body (Yoffey & Courtice, 1970). The early studies of Hudack and McMaster (1932) and Drinker and Filed (1933) clearly demonstrated that an intradermal injection of vital dyes or colloidal opaque particles readily passed from connective tissue spaces into lymphatic capillaries. In subsequent studies designed to localise more precisely the pathways by which interstitial fluids and particulate components enter the lymphatic capillaries, suspension of colloidal dyes and electron opaque tracer particles were injected by interstitial and intravenous methods, and found to gain entrance into lumina of lymphatics mainly via two morphologically definable pathways: 1) intercellular clefts of patent junctions, and 2) across the lymphatic endothelium within pinocytotic vesicles (Clementi & Palade, 1969; Leak & Burke, 1966; 1968; Leak, 1972).

The most important path through the lymphatic capillary endothelium is the open junctions. Several workers have observed large amounts of macromolecules, tracer particles, and cells traversing them (reviewed by Allen, 1967; Casley-Smith, 1972; Yoffey & Courtice, 1970). In particular, it has been shown that lymphatics in sites where they are very permeable (e.g. in the diaphragm and the intestinal mucosa) have many more open junctions than those in regions where they are less permeable (e.g. in the

skin of the pinna). The lymphatic endothelial junctions tend to open because: they are poorly supported by adhesive devices; they lack a continuous basement membrane; inflowing fluid pushes the inner flap of cell aside; and anchoring filaments are mainly attached to the outer cell surface, pulling it out (especially under tension as in oedema). The open junctions are only open during tissue relaxation (the filling phase); during tissue compression (the emptying phase) they are closed, and are permeable only to small molecules (Casley-Smith, 1972). In addition to open junctions, the closed ones, i.e. with about 6nm gaps, should also be considered. These are impermeable to large macromolecules but are certainly permeable to fluid, ions and smaller macromolecules (40.000 M.W.) (Casley-Smith, 1972), as are those in blood vessels (Karnovsky, 1968; Majno, 1965).

The other less important path across the lymphatic endothelial barrier is via vesicles. Evidence from studies on vascular and lymphatic endothelium indicates that the movement of these vesicles is relatively slow (about 5 vesicles discharge/um^2/sec.), and consequently they can only transport a very small amount of material compared with that which passes through junctions (Jennings et al., 1962; Perry & Garlick, 1975; Wagner & Casley-Smith, 1981). It has been suggested, however, that interconnected systems of small vesicles may form channels right across the blood capillary endothelium (Simionescu et al., 1975). The only vesicles that may be actively involved in transport of

material are the large vesicles or vacuoles, especially those which ingest Chylomicra as has been described in the lacteals of intestinal villi (Dobbins & Rollins, 1970) and in lymphatic lacunae of the diaphragm (Casley-Smith, 1964; French et al., 1960); but even these vacuoles are relatively infrequent and are much less important than the open junctions.

Therefore, there is a general agreement, as mentioned above, that fluid and macromolecules, particles, cells, etc., pass into the lymphatic capillaries via the open intercellular junctions. This occurs during the phase of tissue relaxation. Similarly the lymph is expelled during the compression of the lymphatic capillaries by the surrounding tissues during muscular contraction, respiration, etc. (Allen, 1967; Casley-Smith, 1977; Yoffey & Courtice, 1970). However, the precise mechanism of entry of fluid and various substances, and the forces involved which may affect this mechanism is unclear, and several theories have been presented:-

a) It has long been held that there is a slight gradient of hydrostatic pressure from the tissues into the lymphatics (MacMaster, 1947; Wiederhielm, 1968; Yoffey & Courtice, 1970). This was often questioned and it seems fairly certain that in normal tissues this gradient cannot exist (except in oedema), and indeed would normally be directed outwards from the lymphatic capillaries (reviewed by Casley-Smith, 1977 and Yoffey

& Courtice, 1970).

b) Also, it has been suggested that fluid could be actively pumped across the lymphatic endothelial cells in the small cytoplasmic vesicles (Dobbins & Rollins, 1970; Mayerson, 1963). This is highly unlikely, since, as mentioned earlier, vesicular transport by small vesicles is very slow, occurs in both directions, and is independent of cellular activity. There are far too few phagocytic or large vesicles to give active directed transport.

c) It is also proposed that the collecting lymphatics, after they have contracted and are filling once more, could exert force at the tissues via the lymphatic capillaries (Guyton & Barber, 1980; Reddy et al., 1975; Taylor et al, 1973). This hypothesis was rejected by Casley-Smith (1982) due to the fact that collecting lymphatics do not have many attached filaments to cause suction from tissue recoil after contraction. Furthermore, the morphology of the lymphatic capillaries in normal tissue indicates that the intralymphatic pressures in them are greater than the tissue hydrostatic pressures, and hence suction is not being applied through them.

d) Recently, Casley-Smith (1977; 1982) concluded that the only possibility is the effective colloidal osmotic pressure (ECOP) of the lymph which seems capable of explaining all the known facts, and accordingly he came up with the hypothetical "initial lymphatic cycle":-

In brief, during the filling-phase, fluid and protein enter because of the high ECOP of the concentrated lymph, which becomes diluted. When the lymph is so dilute that the nett pressure across the open junctions becomes just less than zero, the junctions will be closed to protein by the start of fluid outflow. Thus, the protein of the lymph, and hence the fluid is kept indefinitely in the vessels, until tissue compression occurs. This is the intermediate-phase. During tissue compression, the total-tissue-pressure rises to much greater than tissue hydrostatic pressure, and is transmitted to the lymph. This closes the open junctions to proteins, but ultrafilters the lymph, thus concentrating it again, and also forces some into the collecting vessels. The regurgitated water temporarily dilutes the proteins in the adjacent tissue channels, but rapidly passes through their walls so that the fluid which will next enter the lymphatics is no longer diluted. This indicates that actually only a small part of the lymph is propelled into the collecting lymphatics; most fluid is returned to the tissues.

D.iii Permeability and function of collecting lymphatics

The collecting lymphatics are simply tubes connecting the lymphatic capillaries with the blood circulation and basically transport macromolecules to the blood. Large lymphatic vessels are supplied by vasomotor nerves and, therefore, respond to such powerful constrictor agents as

adrenalin or pituitrin (Weiss, 1983). As collecting lymphatics pass centrally, the junctions become less and less frequently openable, until all are closed. Also, the walls become progressively thicker. Hence, the permeability of the walls to large molecules diminishes, although they are still quite permeable to small ones (Foldi, 1977; Rusznyak et al., 1967). The hydrostatic pressure in these lymphatics, in uncompressed regions, often rises as high as 5-25cm H_2O (Yoffey & Courtice, 1970). If they are obstructed, the pressures can reach about 150cm H_2O. As a result, large amounts of water are likely to be forced out of these vessels while these pressures are being applied, only to re-enter once they are released and the colloidal osmotic pressure of the newly concentrated lymph exerts itself (Mayerson et al., 1962). This process will not occur in the adjacent collecting lymphatics, which are in the same region as the lymphatic capillaries and are similarly compressed during the emptying-phase, but in the remote ones which are outside of this region (Casley-Smith, 1977). When one considers the likely concentration of lymph in the lymphatic capillaries, its further concentration in the compressed collecting lymphatics, its subsequent dilution in the uncompressed ones, and its continual alterations with the varying pressures, it is evident that, eventually, it might bear much resemblance to the tissue fluid from which it originated.

The flow of lymph in lymphatic vessels is promoted by

several possible factors or mechanisms which may act separately or in combination (discussed in detail by Yoffey & Courtice, 1970). In brief, these are:-

1) The remitting compression of the lymphatic vessels by surrounding structures such as muscles and pulsating blood vessels.

2) The massaging effect of respiratory movements. The phasic variations in intrapleural pressure provide a pumping mechanism propelling lymph in valved lymphatics from lower to upper mediastinum (Fry et al., 1952). Therefore, the amount pumped varies, presumably, with the amplitude and frequency of pressure changes which occur in everyday life.

3) The propulsive actions of the lymphatic walls by active contraction of its smooth muscle component. The contractions are segmental, in between the sets of valves. These valvular segments have been termed "lymphangiones" (Mislin, 1967). In mesenteric lymph vessels of the guinea pig, these segments normally measure 0.5-2mm in length and 0.1-0.3mm in width, and exhibit autonomic rhythmic contractions with a frequency of 10-12/minute (Horstman, 1968, cited in Hauck, 1972). These rhythmic propagated contractions were also seen in mesenteric lymphatics of rats, in the diaphragmatic subpleural lymphatics of guinea pigs and in the thoracic duct of recently fed guinea pigs (Florey, 1927; Pullinger & Florey, 1935; Smith, 1949).

The lymphangiones, or segments of the collecting lymphatics, are influenced by a number of factors (Mislin, 1967). When isolated, they have been shown to have varying frequencies that can be influenced by both natural and artificial stimuli. The most important influence is the intravascular pressure. When this is raised, and the vessels dilate, the frequency and the strength of the contractions increase rapidly. Thus, this situation is very similar to Starling's law of the heart, with all its implications of negative feedback and homeostasis (Roddie, 1980). The co-ordination of the activities of adjacent lymphangiones is achieved in different ways. Possibly there is direct passage of the contractile stimulus, but the most important factor is the initiation of contraction in one lymphangion by the dilatation produced by the inflow of lymph from the peripherally adjacent lymphangion, which has contracted slightly earlier (Smith, 1949; Yoffey & Courtice, 1970).

4) The pumping of lymph is also achieved with the aid of the intralymphatic valves which control the direction of lymph flow in one-way traffic; therefore, preventing back-flow (provided the valves are competent). It has been suggested that the endothelial intercellular junctions of peripheral lymph capillaries could act as "inlet valves" and the valves inside the more central collecting lymphatics could act as "outlet valves". Hence, in addition to variations in intralymphatic

pressures, these lymphatic vessels could function as efficient pumps. This has been shown to be the case in subperitoneal lymphatic lacunae of the diaphragm (Allen & Vogt, 1937; Casley-Smith, 1964), and in the intestinal lacteals (Casley-Smith, 1962).

5) The force of gravity, in those lymphatics which lie above the level of entry of the thoracic duct into the venous system is also an important factor in the propulsion of lymph (Weiss, 1983).

The rate of lymph flow varies considerably. Trypan blue injected into the hind foot of a dog reaches thoracic duct lymph in seconds (Yoffey & Courtice, 1970). It has been estimated that the volume of lymph entering the blood stream from the thoracic duct, in resting human subjects, averages 1.38ml/kg/hour. Lymph flow is increased by ingesting food or water or by abdominal massage (Weiss, 1983). Lymph is usually discharged into the veins in the neck from the thoracic and right lymph ducts. Other lymphatico-venous communications exist, at least potentially (depending on relative pressures)(Rusznyak et al., 1967; Yoffey & Courtice, 1970). Although these other communications are usually considered to function only if the more central collecting ducts are relatively overloaded, it appears that rather more lymph is produced than can be collected from the thoracic and right lymph ducts, so those other communications may also function normally to some extent (Yoffey & Courtice, 1970).

E. TISSUE CHANNELS AND PRELYMPHATICS

E.i General

During development, the primitive connective tissue is a transparent, gelatinous cell-free ground substance which is formed as a secretion of the cells of the various germ layers (Baitsell, 1924-1925).

In later life, connective tissue is mainly formed of collagen and elastin fibres, both embedded in a semifluid gelatinous material collectively called the "ground substance" or "interstitial matrix". This is an amorphous poorly stained material, which contains high-molecular-weight polysaccharides called glycosaminoglycans (Goldberg & Rabinovitch, 1983; Hruza, 1977).

The fluid that is present in this tissue, the so-called tissue fluid or interstitial fluid, is not normally "free", since it is associated with the colloid gel which makes up the ground substance of the connective tissue and varies in amount under different circumstances. Only when hydration of the gel is increased above a certain point can accumulations of "free" fluid in pools be observed, as in oedema or inflammation (Yoffey & Courtice, 1970).

Some workers today regard the interstitial tissue as a two-phase system (Casley-Smith, 1982; Granger, 1979; Laurent, 1970; Weiderhelm et al., 1976). They consider that the more "solid gel-phase" has most of the tissue water adsorbed to it and, therefore, contains effectively no free water. The water which is actually free in the tissue

is remarkably little (except in oedema) and found in the "fluid sol-phase", which is usually small in volume and contains a minimal amount of glycosaminoglycans. Furthermore, there are areas of gradation from one state to the other, where the two phases meet. Earlier, Chase (1959) thought that the sol-phase was in the form of many isolated small vacuoles (around 50nm) lying in the gel-phase. However, high voltage electron microscopy of thick sections has shown that, in fact, they form irregular, randomly arranged and interconnected channels (Casley-Smith & Vincent, 1978). Their sizes and numbers can be estimated by filling them with a precipitated tracer, which has a high charge-density and, hence, is retained in the sol-phase rather than entering the gel-phase. It has also been found that their numbers and dimensions increase enormously after injury or in oedema (Casley-Smith, 1978; 1980). These tissue channels appear to be concentrated in the neighbourhood of the blood vessels, as might be expected from the increased fluid flow in these regions. They form a network, which is essentially a very labile and ill-defined "ultracirculatory system", yet which displays an overall orientation and permits much higher fluxes of water and macromolecules between the blood vessels, and between them and the lymphatics (Casley-Smith, 1976; 1978; 1980; Foldi, 1968; 1977; 1982; Hauck, 1972; 1978).

In his extensive study, mainly by use of the electron microscope, of the lymphatic system in many experimental animals, Casley-Smith (1976, 1978, 1980) recognised two

different types of interstitial tissue spaces: (i) "Tissue Channels" and (ii) "Pre-lymphatics".

E.ii Tissue Channels

These are gaps or spaces normally found between the formed elements of the connective tissue all over the body, and which are easily visible when many tracers or proteins are present. They are quite narrow (approx. 100nm) and sparse (approx. 1 per um^2), and relatively short (approx. 0.1mm). While most channels form a "fine circulation" passing from the arterial side of capillaries towards their venous sides, particularly fenestrae (approx. 60 times more channels can be calculated to end at fenestrae), some pass to the lymphatic capillaries. These sometimes end at a lymphatic junction, which is either open or appears openable (Collan & Kalima, 1974). While it is obvious that some small regions, at some times, will contain more fluid than others; Casley-Smith (1980) stated:- "To many workers this hardly seems sufficient to designate those regions that happen to open at a lymphatic junction with the term pre-lymphatic". Therefore, he suggested the term "tissue channels" as most suitable for those short and relatively infrequent channels, remembering that fluid passes in them from and to the blood capillaries, and to the lymphatic capillaries.

E.iii Pre-lymphatics

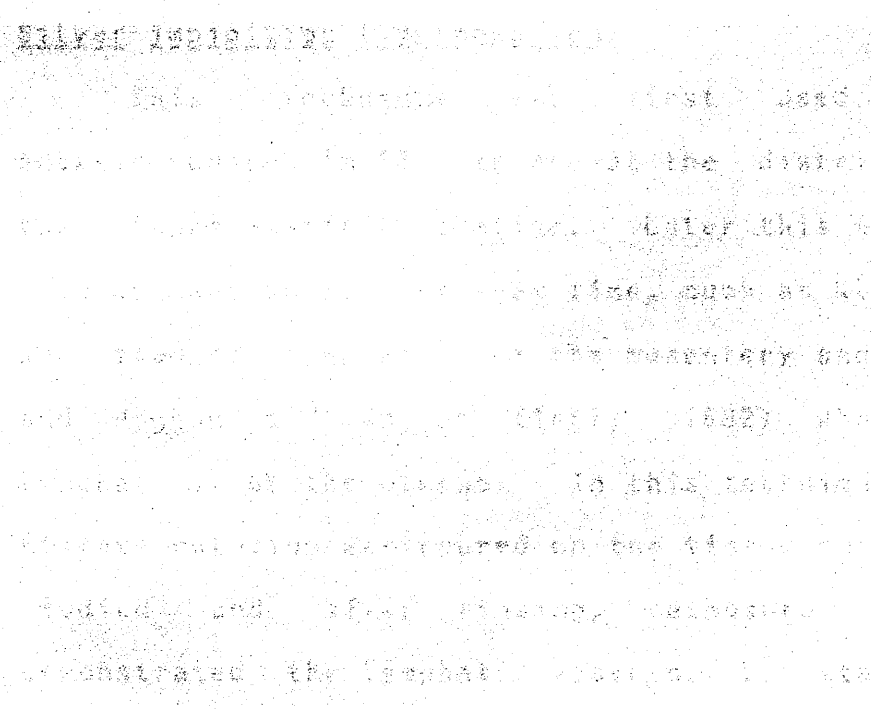
This is a system of long tissue channels, not lined by endothelium, which is particularly important in

regions where there are no true lymphatics, e.g. the brain, retina, bone marrow, etc. In such regions these channels are particularly large, and form a connected series of spaces, or potential spaces, which follow quite well-defined courses in the adventitia of large blood vessels, and also lie adjacent to the basement membranes of blood capillaries. In the brain, these long prelymphatic channels reach from the deeper parts of the cerebral cortex, to the major blood vessels, and eventually discharge into the true cervical lymphatics outside the skull. This course has been traced not only by injecting carbon into the cerebral cortex, but also by ligating the cervical lymphatics and observing the stagnant protein in the very dilated prelymphatics, all the way from the neck to the lymphoedematous cerebral cortex. Thus, in such regions the prelymphatics take over the role of the true lymphatics and pass for many centimeters until they discharge into the true lymphatics. For these long channel systems, which perform such a special role, Casley-Smith suggested the name "prelymphatic system" seems justifiable.

Recently Hauck (1972; 1973; 1978; 1982) investigated in detail the existence of a preformed transinterstitial fluid pathway in the mesenteric tissue of the rabbit, using different methods of vitalmicroscopy including blue light fluorescence combined with dark-field transillumination. His results seem to agree with, and confirm those of Casley-Smith. He recognised: (i) a long transinterstitial pathway and (ii) a short one. The long

pathway included the prelymphatic tissue channels, described by Casley-Smith, in addition to a "preferential fluid pathway" along the surface of the elastic fibres. The nature and mechanism of the latter is not clear, but the transport occurs much faster than is possible by diffusion. Thus, Hauck suggested that the elastic fibres, besides fulfilling their mechanical functions, also have a passive transport or "guide rail" function for fluid and dissolved molecules between microblood vessels and drainage system marking the "low resistance pathway". This has been suggested earlier by Kihara (1956) as an extravascular fluid pathway. Diminution of the elastic fibres, as one finds in atrophic changes of the skin, leads to considerable slowing of the spreading of fluorescent dye in the tissues (Jager et al., 1979). Besides the long transinterstitial pathway, Hauck (1973) also observed a short pathway from the venous microvessels to the paravascular lymphatics which seems to be preferably used by macromolecules leaving the venous microvasculature. Finally, Hauck concluded that the prelymphatic and initial lymphatic systems are continuously connected, forming a common converging drainage system to the larger lymph transport vessels. This led Hauck to suggest that, instead of the traditional concept that lymphatics begin (or end) blindly, the most peripheral part of the lymphatic vessel system is a completely open system of tissue channels, forming a network and represents the primary or elementary

drainage system. This seems to be the beginning of a canalization system for fluid drainage whereby the tissue is divided by the tissue channels in elementary three-dimensional "tissue units". Therefore, his concept of the tissue organisation for supply and drainage is essentially marked by the following preformed elements: microvessels, elastic fibres, tissue units, tissue channels and initial lymphatics.



F. METHODS FOR STUDY OF LYMPHATICS

The discovery by Nuck in 1692 that lymphatics could be directly injected with mercury opened the way for serious study. Cruickshank (1786) who collaborated with William Hunter and published their findings after Hunter's death, improved the method used by Nuck and extended its application to the study of lymphatics in many organs in man. Since these early pioneering studies, many investigators used various material for injection, in human and in different animal species. Various types of injection techniques and other newly adopted methods for the study of lymphatics are discussed briefly:-

1) Silver imbibition (impregnation)

This technique was first used by V. Recklinghausen in 1862 to reveal the distribution of the diaphragmatic lymphatics. Later this method was used by many workers at that time, such as Klein (1873) who studied lymphatics of the mesentery and omentum; and Hoggan and Hoggan (1881; 1882) who studied lymphatics of the uterus. In this technique, silver nitrate solution was poured on the tissue surface to be studied and after rinsing, exposure to light demonstrated the lymphatic vessels, by staining the "intercellular cement" of the endothelial lining of the lymphatics, thus outlining their walls. The main advantage of "silver imbibition" was that the three-dimensional pattern of the lymphatics could be studied.

However, this method could produce false positive results as the blood vessel's walls could also be stained by the procedure (Hoyer, 1865).

2) Local parenchymatous (stab) injection

By about the end of the 19th Century, it was found that it was not necessary to inject directly into a lymphatic vessel to fill it, since injections into the parenchyma was sufficient; thus different substances were used for the injections (e.g. mercury, coloured gelatin, aqueous carbon suspensions, etc.). In this technique the injection was made directly and blindly into the tissue, in the hope of entering lymphatic capillaries and filling the regional lymph vessels by anterograde flow (Higgins, 1925; Job, 1915). This method can give excellent results in tissues such as skin, in which lymphatics are large and abundant (Hudack & McMaster, 1932); where they are small and/or sparse, failure to demonstrate them by injection may give false negative results. Also, false positive results may be given by inadvertent injection of blood vessels or by the creation of artificial spaces in the connective tissue.

This technique has been used to demonstrate lymphatics in a wide variety of sites, e.g.: the dermis and hypodermis; subcapsular injections of solid organs such as kidney, testis, liver; subserosal injections of hollow viscera; and injections into lymph nodes. With care and some luck, this method makes the typical

lymphatic plexus easily visible.

3) Retrograde injection

This method involves injection of collecting lymphatics against the direction of lymph flow, in the hope of filling the smaller tributaries. Once again, false negatives can be produced, because competent valves prevent retrograde flow; and false positives on the other hand, could result from the inadvertent injection of blood vessels.

4) Double injection

As blood vessels may be inadvertently injected by retrograde injection and mistaken for lymphatics, some workers have used a "double injection" technique of contrasting colours (McLean & Scothorne, 1970). A retrograde injection of india ink was used to demonstrate lymphatics in the uterus, after deliberate filling of the blood vessels by intravenous injection of yellow lead chromate.

5) Vascular perfusion

It was Fawcett et al (1969) who first used this method, and it has proved to be by far the most satisfactory method of demonstrating lymphatics. He washed the blood vessels clear by vascular perfusion with saline followed by glutaraldehyde as a fixative. Lymphatic vessels can then be readily distinguished from empty blood vessels, in semi-thin sections of araldite-embedded material, by their content of

precipitated lymph protein. Vascular perfusion produces very rapid and uniform fixation of tissue structures, and has the further advantage that lymphatics are fixed in their natural state, rather than distended as they often are when directly injected. This method also allows the use of both the optical microscope for survey of extensive areas of tissues, and the electron microscope for detailed study of doubtful vessels. The general ultrastructural criteria for differentiating lymphatic capillaries from blood capillaries (previously mentioned in detail) are that lymphatics have: (i) wider lumina and an irregular (scalloped) outline in sectional profile, (ii) attenuated cytoplasm with large protruding nuclei, (iii) abundant cytoplasmic pinocytic vesicles, (iv) a discontinuous or absent basal lamina, (v) open intercellular junctions, (vi) the presence of numerous luminal and abluminal cytoplasmic projections, and (vii) numerous fine filaments at the abluminal surface which serve to anchor the lymphatic wall to the adjoining connective tissue.

6) Injection of tracer material into blood circulation or into serous cavities

This technique involves the injection of small-molecular-weight tracer particles (such as ferritin, thorium dioxide, colloidal carbon, labelled plasma proteins, latex spheres, etc.) into the vascular bed (Clementi & Palade, 1969; Karnovsky, 1967; Leak, 1972),

or into the peritoneal cavity (Allen, 1936; Casley-Smith, 1964; Courtice & Steinbeck, 1950; French et al., 1960; Leak, 1976; 1977; Simer, 1944; 1948), or the pleural cavity (Courtice & Simmonds, 1949; Courtice & Morris, 1953; Wang, 1975), or into the pericardial cavity (Allen, 1958; Drinker & Field, 1931; Kluge & Ongre, 1968; Kluge, 1969; Patek, 1939). The tissue being studied is then excised, fixed and processed for light microscopy and/or electron microscopy. Such a method permits investigation of the transport of tracer particles from the blood, or serous cavities, into the interstitium and its subsequent removal by the lymphatics without having to disturb the normal balance of forces within the connective tissue as is the case in local parenchymatous injection procedures. Thus, the pathway of movement of the tracer substances across the blood-tissue-lymph interface can be followed by electron microscopical observations at successive time intervals after injection. In the present study, it was noticed that if vascular perfusion is also included in the procedure, it gives excellent results; empty blood vessels contrast clearly with lymphatics, which contain lymph protein precipitate, in addition to the artificial injected tracer.

"Autoinjection" of lymphatics, for example by chyle as a natural tracer in the mesenteric lymphatics and the thoracic duct after a fatty meal, is well known

(Yoffey & Courtice, 1970). Chyle contains both chylomicra (0.1 - 1 um in diameter) and lipoproteins (10 - 100 m u in diameter) (Casley-Smith, 1962). It has been estimated that chylomicrons appear in the blood a half to one and a half hours after a fatty meal, and disappear in six to ten hours (Yoffey & Courtice, 1970).

7) Combined injection

This is a technique which we modified during our injection experiments. It involved the intravenous injection of a dye (3% Pontamine Sky blue) 8 - 10 minutes before the animal was killed. During this interval the dye diffused out of the blood capillaries into the interstitium and entered the lymphatic vessels. Then the animal was dissected and the blood was washed out of the circulation by vascular perfusion with saline. Lymphatics retained the blue dye and could be seen under the dissecting microscope by contrast with empty blood vessels. For further detailed study of the lymphatic pattern in a specific organ or tissue, a second injection was then made. This involved a local injection of india ink into selected parts of the tissue being examined, guided by the blue coloured lymphatics, and using a very fine needle. The advantage of this "combined" technique of injections is that the first intravenous injection will act as a guide for the second direct injection, and ensures that it is actually introduced into a lymph

rather than a blood vessel.

The use of intraperitoneal injection of the dye instead of the intravenous one, and the subsequent direct ink injection of the subperitoneal lymphatics of the diaphragm or its draining lymph nodes, proved to be very useful indeed in demonstrating the lymphatics and regional lymph drainage of the diaphragm, as will be shown later.

CHAPTER II

THE THYMIC HAEMOLYMPH NODES

INTRODUCTION

Over one hundred years ago in 1884 a structure which resembled a lymph node, but which had blood rather than lymph in its sinuses, was described by Gibbes. He found these structures, which were probably haemolymph nodes, in human perinephric connective tissue adjacent to the renal blood vessels. Since then, many workers have reported the presence of these nodes in human subjects (Robertson, 1890; Warthin, 1901; Jordan, 1926) and in different animal species such as rats, mice, guinea pigs, goats, cattle and sheep (Robertson, 1890; Clarkson, 1891; Vincent & Harrison, 1897; Drummond, 1900; Lewis, 1902; White, 1904; MacMillan, 1928; Weller, 1938; Selye & Schenker, 1939; Andreasen & Gottlieb, 1946; Erencin, 1948). The term "haemolymph nodes" was first introduced by Robertson in 1890.

Since the beginning of this century widely divergent views have been held about the nature and significance of the haemolymph nodes. In the literature there are contradictory statements regarding the relationship between these haemolymph nodes and the lymphatic system. Some of the apparent contradictions are probably due to species differences, but even within a single species there are still disagreements about their structure and even about the consistency with which they occur. To avoid the confusion caused by species variation, only the literature concerning the haemolymph nodes of the rat will be reviewed in detail here.

The central problems which have arisen during the study of the haemolymph node are:

1) the ultimate origin of the erythrocytes which are found in the sinuses of the node;
2) the route by which these erythrocytes enter the sinuses; and
3) the part played, if any, by afferent lymphatics in the functional anatomy of the node.

The presence of afferent lymphatics to the haemolymph nodes of the rat, was reported by some authors (MacMillan, 1928; Andreasen & Gottlieb, 1946; Nopajaroonsri et al., 1974), but denied by others (Turner, 1969; Olah & Toro, 1970). The route by which erythrocytes enter the lymph sinuses has also been disputed (MacMillan, 1928; Andreasen & Gottlieb, 1946; Turner, 1969; Olah & Toro, 1970; Nopajaroonsri et al., 1974). These differing views can best be summarised by reference to the work of various authors, presented here in chronological order.

MacMillan (1928) reported the presence of red or pinkish nodes in three locations in the rat: dorsal and medial to each kidney; near the pancreas; and at the apex of the pleural cavities lying very close to the thymus gland. He was able to find both afferent and efferent lymphatics in these haemolymph nodes. He suggested that blood could enter the sinuses of these nodes from three possible sources: a) rupture of a blood vessel within a node, b) haemorrhage in the drainage territory of afferent lymphatics, and c) erythropoiesis in the node. According

to his results, he excluded the first and third possibilities, but yet could not establish the source of erythrocytes found in these nodes.

Selye and Foglia (1939) also studied the renal, "pancreatic" and thymic haemolymph nodes in rat, but denied the presence of actual blood or free erythrocytes in their sinuses. They described, instead, the presence of pigment-storing macrophages which gave the nodes a characteristic reddish-brown colour. Therefore, they suggested the term "iron-pigment" lymph nodes. Furthermore, they showed that such "iron-pigment" nodes can be produced experimentally by subjecting the animals to stressful stimuli capable of triggering an "alarm reaction", e.g. excessive muscular exercise, exposure to cold, and injection of large doses of formaldehyde. Under these conditions, ordinary lymph nodes may be transformed into "iron-pigment" nodes within a few hours, and on microscopic examination their sinuses were seen to be full of erythrocytes. These changes were temporary, as erythrophagocytosis soon began and erythrocytes disappeared from the sinuses within a few days. However, Selye and Foglia (1939) were unable to describe the mechanism which leads to this transformation, and where and how erythrocytes leave the blood circulation and enter the lymph nodes. Also, they did not explain the erythrocytic content present in normally occurring haemolymph nodes or "iron-pigment" nodes as they preferred to call them. In a separate paper, Selye and Schenker

(1939) further observed that in young rats (6 weeks old) renal, pancreatic and thymic nodes were not of the "iron-pigment" type, whereas in adult animals these nodes belonged to that category. They suspected that the organs which drain into these nodes might play a role in changing their nature, as age advances. By testing this hypothesis in the case of the renal node, they showed that their characteristic appearance was dependent on the presence of an intact kidney and that nephrectomy led to the disappearance of iron-pigments from the ipsi-lateral renal node. They also noticed that if nephrectomy was performed in young rats, it prevented the renal node from becoming an "iron-pigment" node. However, the exact role played by the kidney in changing the renal node into a haemolymph type and then maintaining this character afterwards was not described.

Andreasen and Gottlieb (1946) carried out an extensive investigation of the haemolymph nodes in 300 albino rats. They examined the renal, splenic, and thymic lymph nodes and specifically denied that the last two groups were of the haemolymph variety, which, therefore, limited their studies to the renal haemolymph nodes. They regarded them as typical lymph nodes with both afferent and efferent lymphatic vessels, differing only in the presence of erythrocytes in the sinuses. They also found that their haemolymph character does not appear until the second half of the first month of life. Before this age, they do not differ, macroscopically and microscopically, from ordinary

nodes. They attributed the origin of the erythrocytes in the haemolymph nodes to reflux of blood from the efferent lymphatic, which Job (1915, 1918) had described as occasionally opening into the renal vein. They further demonstrated that after unilateral nephrectomy the ipsilateral renal haemolymph node lost its red colour. Their interpretation of this result was that the operation probably severed the connection between the renal vein and the efferent lymphatics.

In 1969, Turner described the vascular architecture of the "haemal" node in the rat. He examined renal, "pancreatic" or splenic, and thymic nodes of young rats by light and electron microscopy. In order to determine the route of entry of erythrocytes into these nodes, he injected carbon black suspension into the abdominal aorta, the splenic vein, the tissues around the haemolymph node and the tail vein. His observations and conclusions are summarised as follows:-

1) Microscopic examination of these nodes showed two structurally different regions: a sinusoidal area composed of narrow cords of lymphoid cells with large intervening sinuses, and a non-sinusoidal area made up of a large collection of small lymphocytes.

2) The nodal sinusoids (or sinuses as they are now called) contained only blood. No lymph-filled sinusoids were observed; thus Turner preferred to call them "haemal" nodes.

3) The nodes were very vascular and some capillary blood vessels appeard to lead directly into blood-filled sinusoids. The blood vessels entered and left the node at the hilum. Another thin-walled vessel, resembling an efferent lymphatic and possessing valves positioned to prevent reflux back into the node, was observed here. Vessels resembling afferent lymphatics were not seen in any of the nodes examined.

4) Carbon suspension injected into the aorta and splenic vein outlined the vasculature of the "haemal" node, but did not enter the subcapsular sinus, the sinusoids or the efferent lymphatic. Similarly no carbon was observed in the sinusoidal system when injected into the tissues around the splenic "haemal" nodes.

5) When a dilute suspension of carbon was injected into the tail vein, carbon particles were detected within the sinusoidal macrophages, shortly after the injection (10 minutes). "Thus is would appear that when a less toxic suspension of carbon reaches the vascular tree of the haemal node some of the suspension passes into the sinusoidal system, where it is taken by the macrophages".

6) Turner (1969) described two different types of blood-vascular arrangements in different parts of the "haemal" node: A "fast" circulation through the arterioles, capillaries and venules (a closed blood circulation such as exists in typical lymph nodes); and a "slow" circulation through the tortuous sinusoids.

Thus, Turner (1969) believed that blood capillaries running close to the nodal sinusoids open directly into them, and that a selective mechanism operated to direct a small amount of blood into the sinusoids where it is taken up by the macrophages. However, he could not demonstrate the site of communication between the blood capillaries and the sinusoids.

Olah and Toro (1970) studied the anatomy of haemolymph nodes in male albino rats in semithin and thin sections after vascular perfusion and fixation. A large number of erythrocytes were found lying freely in the marginal sinus; while erythrocytes in the central sinuses were mostly attached to or engulfed by macrophages. All stages of erythrophagocytosis were observed in these macrophages and remnants of red blood corpuscles were present in them. Olah and Toro (1970) denied the existence of a direct communication between the blood circulation and lymph sinuses, as after vascular perfusion, blood vessels were empty in sharp contrast to the sinuses which retained their content of finely precipitated substance. The blood vessels also showed fenestrated endothelium, and lymphocytes were observed between the endothelial cells and between the endothelium and the basement membrane. Olah and Toro (1970) suggested that the direction of migration of lymphocytes, and probably erythrocytes, was from blood vessels into the lymphatic sinuses as well as tissues, as free erythrocytes were also seen in the lymphatic tissues

around blood vessels.

By the use of combined optical, transmission and scanning electron microscopy Nopajaroonsri et al. (1974) studied the ultrastructure of haemolymph nodes in the adult male Wistar rats. They examined haemolymph nodes lying adjacent to: the left adrenal vessels, the superior border of the head of the pancreas, and the inferior aspect of the thymus, in perfused and unperfused animals. Colloidal carbon added to the perfusate or injected retroperitoneally or intravenously was employed to determine the lymphatic and blood-vascular connections of the haemolymph node. Their results and conclusions are summarised in the following points:-

1) Structurally, the haemolymph node resembled a normal lymph node; it was composed of lymphoid parenchyma with intervening lymphatic sinuses. The subcapsular, the cortical and medullary sinuses were interconnected and formed a continuous channel. The lining of the channels lacked a well-defined basement membrane, and gaps were present between the endothelial cells. Lymphocytes and macrophages were often seen in these gaps. All the sinuses contained erythrocytes, macrophages, lymphocytes and, occasionally, plasma and mast cells. The sinusoidal macrophages trapped erythrocytes by pseudopodia, forming "rosettes". Erythrocytes were also present focally in the lymphoid parenchyma, either lying freely or within the macrophages.

2) Afferent and efferent lymphatics were found in the haemolymph node, but Nopajaroonsri saw only a "few" afferent lymph vessels, whose origin he did not determine. He specifically denied the presence of erythrocytes in the afferent lymphatics and observed very few in the efferent lymph vessels.

3) The vascular architecture of the haemolymph node was similar to that of an ordinary node, except that the former was very vascular and had capillaries with attenuated basement membrane and low endothelial cells. Some of the capillaries and post-capillary venules lay adjacent to the lymphatic sinuses and their attenuated basement membrane formed the only partition between the two walls. Erythrocytes were observed in transit across the wall of a capillary and a post-capillary venule.

4) Colloidal carbon perfused through the abdominal aorta did not appear in the lymphatic sinuses, showing the absence of direct communication between the intranodal blood vessels and the sinuses which had been claimed by Turner (1969). However, when injected intravenously, some carbon particles appeared in the lymphatic sinuses as early as one minute; most of these particles were deposited in the deeper sinuses and very few in the peripheral sinus. This observation, together with the finding of erythrocytes in conjunction with carbon particles in the walls of blood capillaries and post-

capillary venules, led Nopajaroonsri to believe that all the erythrocytes entered the sinuses of the haemolymph node by a similar route, i.e. intra-nodal diapedesis.

In recent years, Scothorne and his associates (for references see below) studied the structure and drainage patterns of both renal and splenic haemolymph nodes in the Swiss albino rat. Their investigations, using advanced methods, seem to resolve the long standing controversy surrounding the origin of the sinusoidal erythrocytes. They found that both the renal haemolymph nodes (Kazeem, Reid & Scothorne, 1982; Kazeem & Scothorne, 1982; Hogg, Reid & Scothorne, 1982; Pearce, Reid & Scothorne, 1983); and the splenic haemolymph nodes (Abbas, Reid & Scothorne, 1983) receive their erythrocytic content from afferent lymphatics, draining from the kidney and the spleen respectively; erythrocytes presumably have entered intrinsic lymphatics of both organs by diapedesis through capillaries and veins with which they were frequently in intimate contact.

The histological structure of both renal and splenic haemolymph nodes is, more or less, identical. The only histological difference is that the former is a hypoactive node, with rare or absent germinal centres, while the latter is a moderately active node and regularly contains some germinal centres. The structure of the haemolymph node appears to be essentially similar to that of an ordinary (typical) lymph node. Thus, a haemolymph node has

afferent and efferent lymph vessels, lymphatic sinuses and lymphoid parenchyma divisible into cortex and medulla. Valved afferent lymphatics, entering the subcapsular sinus, were recognized in the majority of the nodes. Variable numbers of free erythrocytes were found in the afferent lymphatics; contrary to the results of Nopajaroonsri et al. (1974). Large numbers of free erythrocytes were present in the subcapsular sinus. Efferent lymphatics, present at the hilum of the node, contained only a few erythrocytes. Medullary sinuses were filled with erythrocytes, mostly attached to macrophages forming characteristic rosettes. The distribution of macrophage-erythrocyte rosettes was uniform in most of the nodes. However, not uncommonly some of the nodes, particularly the splenic, showed polarity, i.e. macrophage-erythrocyte rosettes were abundant at one pole and virtually absent from the opposite pole of the same node. This polarised distribution of rosettes was interpreted as an indicator of erythrocytes entered the haemolymph node principally in the afferent lymph which arrived from the organs which they drain, i.e. the kidney and spleen.

The efferent lymph from the renal and splenic haemolymph nodes drained into the cisterna chyli. Lymphatico-venous communications between the efferent lymphatics and renal or splenic veins were not seen.

All the lymphatic sinuses of both node groups contained stained precipitate of lymph protein, which was

not washed or diluted after vascular perfusion, even in the immediate vicinity of blood vessels, indicating that a direct intra-nodal communication between the blood vessels and lymphatic sinuses, does not exist.

In order to test the hypothesis of extrinsic origin (via afferent lymph) of sinus erythrocytes seen in haemolymph nodes, Pearce, Reid and Scothorne (1983) transplanted the facial lymph node, which is a typical node, of newborn rat as isograft beneath the kidney capsule of an adult recipient. Grafts were recovered at 5 and 10 weeks after operation and studied histologically in serial semithin sections. The grafted node was found to consist mainly of diffuse cortical lymphoid tissue, lacking germinal centres and surrounded by a well-developed subcapsular sinus, which received afferent lymphatics continuous with capsular and subcapsular lymphatics of the kidney. The medulla was poorly developed. Grafts were revascularised through the hilus by blood vessels of the renal cortex. The most striking finding was the presence of numerous erythrocytes in afferent lymphatics and in the subcapsular sinus. Thus, Pearce, Reid and Scothorne (1983) concluded that a "typical" lymph node such as the facial node may be converted into a haemolymph node by grafting it to a site, such as the kidney capsule, in which the lymphatics normally contain erythrocytes.

These findings of Scothorne and his associates were clearly different from those of Turner (1969) and Nopajaroonsri et al. (1974). In the case of the renal and

splenic haemolymph nodes, it has now been clearly established that the erythrocyte content is principally lymph-borne, entering the node via renal and splenic lymph, respectively. Thus, it seemed logical to consider the possibility of an extra-nodal origin of the erythrocytes, found in the sinuses of the thymic haemolymph nodes.

The organs which could be the prospective sites of origin of afferent lymphatics (and hence of erythrocytes) to the thymic haemolymph node group are: diaphragm, thymus gland, heart and lungs. The existing literature was carefully examined for reports on the lymph drainage of these organs and the afferent and efferent lymph drainage of the thymic nodes.

Early authors like Job (1915, 1918); Higgins (1925) and later Green (1935, 1959) were first to describe the anatomy of the lymphatic system in the common rat. They injected various dyes (India ink, soluble blue and Berlin blue) into the skin, different groups of lymph nodes and into viscera. They recognised a group of very small lymph nodes situated ventral to large vascular vessels and anterior to the heart. Each node was spherical in shape and measured approximately 1mm in diameter. For descriptive purposes they referred to this group as the "thoracic nodes". Higgins (1925), who studied the lymphatic system of the newborn rat, found that the posterior group of these nodes receives afferent lymph channels from lungs and pleural cavity; and the anterior

group receives small lymph capillaries from the sternum. Both groups were connected by small lymph capillaries, and a single efferent channel from the more anterior node communicates with the venous system. Harris and Templeton (1968) mentioned that the inferior thymic node, lying deeply in the lower part of the neck, is a focal point in the lymphatic drainage of the thymus gland. Moreover, they observed that a specific part of it appears to be reserved for thymic drainage. From the lower part of this node, the main mediastinal lymph trunk leaves to join the great veins at the root of the neck.

During the last 100 years, many investigators have demonstrated that the parasternal lymphatic trunks are the main lymph drainage pathway from the peritoneal cavity to the "mediastinal" lymph nodes (which include the thymic nodes) (Muscatello, 1895; Durham, 1897; MacCallum, 1903; Buxton & Torrey, 1906; Bolton, 1921; Cunningham, 1922; Higgins & Graham, 1929; Allen, 1936; Simer, 1944; Courtice & Steinbeck, 1951; Saldeen, 1963; Olin & Saldeen, 1964; Whaley et al. 1972). All these authors found that various particles, including erythrocytes, when injected into the peritoneal cavity of different animals, become rapidly absorbed by lymphatics of the diaphragm, and reach the mediastinal lymph nodes, shortly after the injection. (See Chapter IV).

Tilney (1971), using multiple injection techniques, carried out a detailed study of the patterns of lymphatic drainage in the adult rat. It is worthwhile to summarise

his observations in the following points:-

1) The thymus gland lies in the anterior mediastinum, drained directly by the parathymic nodes. Other thymic lymphatics consistently empty into the left posterior mediastinal node only; no drainage to the opposite posterior mediastinal node was ever observed.

2) The parathymic lymph nodes are embedded in fat on the lateral aspects of the thymic capsule, and drain the thymus gland, the peritoneal cavity, and the superior surface of the liver and liver capsule through the large internal thoracic (mammary) lymph channels. These channels collect the lymph from the extensive lymphatic plexus on the pleural surface of the diaphragm and are joined along their course by small radicles from the anterior pericardium and parasternal area.

3) The posterior mediastinal nodes lie adjacent to the oesophagus on the right, and to the left superior vena cava on the left (note that in the rat there are 2 superior venae cavae, one on each side). The right node is usually larger, and drains the right pleural space and lung, the base of the heart, and the thoracic portion of the oesophagus. The left node drains the left pleural space, thoracic viscera, and the thymus gland.

4) Small paravertebral nodes are present inconstantly behind the pulmonary vessels. They receive minor

paravertebral lymphatics from the diaphragmatic plexus, and are joined by intercostal lymphatics and hilar radicles from the thoracic organs and pericardium. Paravertebral lymphatics eventually drain into the posterior mediastinal node group.

5) The mediastinal lymphatic duct, the major efferent channels from the thoracic nodes of both sides, empty into the dorsum of the subclavian veins. The ducts from parathymic and posterior mediastinal nodes may emerge as a single channel or enter the vein separately.

Thus, Tilney (1971) made a clear distinction between two groups of mediastinal lymph nodes: the parathymic lymph nodes (anterior mediastinal) which are embedded in the thymus; and the posterior mediastinal nodes which lie at a deeper plane in the paravertebral gutter. However, he did not describe the precise topography of these node groups, nor, surprisingly, does he mention their mottled reddish colour.

From this review it is clear that there are still disagreements regarding the structure, nature and drainage patterns of the lymph nodes found in the vicinity of the thymus gland. And, since most of the authors who studied the haemolymph nodes in the rat included the thymic nodes, it was then decided to undertake a thorough investigation of the thymic haemolymph nodes in the Swiss albino rat, with particular reference to the following questions:-

(i) Are afferent lymphatics to the thymic nodes absent, as claimed by Turner (1969), or "few" as claimed by Nopajaroonsri et al. (1974)?

(ii) If they are present, what is their regional origin, and do they contain erythrocytes?

(iii) What is the pattern of distribution of erythrocytes within the nodes?

(iv) What is the principal source of erythrocytes to these nodes?

(v) What is the main route of entry of erythrocytes into the nodal sinuses? Is it "intra-nodal" by diapedesis through the endothelial wall of capillaries and post-capillary venules as claimed by Nopajaroonsri (1974); or is it "extra-nodal" via the afferent lymphatics as suggested by Scothorne and his associates?

(vi) At what age do sinusoidal erythrocytes appear in the nodes, and what is the significance of this?

MATERIALS AND METHODS

A total of 75 Swiss albino rats, of both sexes and weighing between 5-250g, were used for these investigations. Their age ranged from newborn to six months. The animals were divided into six experimental groups (see below). The majority of animals were killed with an overdose of anaesthetic ether; the remainder with intravenous injection of Nembutal.

I. TOPOGRAPHICAL STUDY OF THYMIC HAEMOLYMPH NODES IN SITU

In some of the adult animals (a total of 33 rats) to be used for subsequent investigations, a preliminary examination of the thymic haemolymph nodes was made, using the unaided eye and a stereoscopic microscope. The number, position, size, shape, colour and polarity of the nodes were noted.

II. REGIONAL ORIGIN OF AFFERENT LYMPH VESSELS

The following two techniques were employed to demonstrate the afferent lymphatics of thymic haemolymph nodes:-

Technique A - IV injection of pontamine sky blue, followed by vascular perfusion

In 18 adult rats, the lymph vessels of the diaphragm, thymus, heart and lung were demonstrated by this method. Each animal received an injection of 3% pontamine sky blue (0.5ml per 100g of body weight) into the tail vein. Rats were killed 8-10 minutes after the injection; during this interval the dye diffused out of the blood capillaries into

the interstitial fluid and was absorbed by lymph capillaries. This facilitates easy recognition of lymph vessels and nodes (Tilney, 1971). The rats were dissected and the vascular system was perfused via the left ventricle, with Ringer's solution (containing lignocaine as a vasodilator). Diaphragmatic, thymic, cardiac and hilar lymphatics and their regional lymph nodes were then studied under the dissecting microscope.

Technique B - Direct injections of dye

Using a 30G needle, india ink or pontamine sky blue was injected into the following organs (a,b,c,d below), which were suspected to be possible sites of origin of afferent lymphatics to the thymic haemolymph nodes. In groups b, c and d, most of the animals received, prior to direct ink injections, IV injection of 3% pontamine sky blue followed by vascular perfusion as described in technique (A). This enabled mapping of the lymphatics in each organ and made subsequent direct injections much easier.

a) DIAPHRAGM - 6 animals, each of which received a single intraperitoneal (IP) injection of 3% pontamine sky blue. The animals were killed a few minutes later by ether overdosage and the abdominal cavity was opened by a midline incision. Care was taken not to open the thorax, at this stage, and also to leave the diaphragm intact. The residual dye left in the peritoneal cavity, was rinsed away with saline, leaving exposed the peritoneal surface of the diaphragm with its subperitoneal lymphatic plexus labelled

with blue dye. Several direct injections of minute doses of diluted india ink (1:3) was introduced into subperitoneal lymphatics of different parts of the diaphragm. A total of 19 injections was made, 14 of which were successful. Shortly afterwards, the thoracic cavity was opened and the destination of lymphatics leaving each part of the diaphragm and its regional lymph nodes were examined under the dissecting microscope.

b) THYMUS GLAND - In six rats, the thymus gland was directly injected with either 3% pontamine sky blue or freshly prepared india ink (1:3 diluted with saline). A total of 24 injections was made into different parts of the thymus gland, in an attempt to demonstrate the regional lymphatic drainage of the gland. Some injections were made just deep to the capsule.

c) HEART - Diluted india ink (1:3) was injected under the pericardium of the heart in four animals. A total of 16 injections was made into different parts of the heart, 11 of which successfully filled the subpericardial lymphatic plexus.

d) LUNG - In four animals, hilar lymphatics of each lung (labelled with pontamine sky blue) was directly injected with diluted india ink (1:3) giving a total of 8 injections, all of which were successful.

III. EFFERENT DRAINAGE OF THYMIC HAEMOLYMPH NODES

The efferent lymphatics of the thymic haemolymph nodes (including the posterior mediastinal group) were

demonstrated by direct intra-nodal injections in seven animals. This was achieved by injecting minute volumes of either pontamine sky blue or diluted india ink, directly into the parenchyma of the node. Gentle massage of the node caused filling of the efferent lymphatics which were traced under the dissecting microscope. Occasionally some of the afferent lymphatics were filled by reflux of the dye. An attempt was made to inject different thymic nodes in different animals, so that every member of the thymic group was injected in one or the other animal.

IV. HISTOLOGICAL TECHNIQUE

Thirteen thymic nodes, taken from six adult animals, were studied microscopically. The rats were killed by an overdose of anaesthetic ether. A midline incision was made in the anterior abdominal wall and the heart and great vessels were exposed by removing part of the rib-cage. The vascular system was washed free of blood by perfusison with Ringer's solution containing 0.4% lignocaine chloride as a vasodilator. The fluid was perfused through a cannula inserted into the left ventricle, and an outflow was established by incising the right atrium. The preliminary washing took about 2 minutes. Perfusion was then continued for 30-40 minutes with the fixative, which was a 5% solution of glutaraldehyde in Millonig's phosphate buffer at pH7.4. The thymic haemolymph nodes, identified by their reddish or pinkish colour (which was not affected by perfusion) were removed by careful dissection, and immersed overnight intact in the same fixative as that used for

perfusion. The nodes were then rinsed several times in buffer for another 24 hours, post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in Spurr's resin. A continuous series of semithin sections (1-1.5um) were cut with glass knives, through 8 nodes. An interrupted series of sections were cut through the remaining nodes. Alternate slides were stained with Azur II and with Haematoxylin and Eosin, and mounted in DPX. Sections were studied by optical microscopy.

V. COMPARISON OF THE ROUTE OF ENTRY OF CARBON PARTICLES INTO THYMIC HAEMOLYMPH NODES AFTER INTRAVENOUS (IV) AND INTRAPERITONEAL (IP) INJECTION

Two groups of adult rats of different age and sex were used in this investigation:-

1) In the first group, regarded as control, eight animals received an intraperitoneal (IP) injection of india ink (diluted 1:3 with normal saline and repeatedly centrifuged and filtered before injection). 0.12ml per 100g body weight was administered and the rats were killed in groups of 2 at 1, 3, 5 and 15 minutes after injection.

2) A second group of sixteen animals were injected intravenously (IV) with ink, 0.5ml per 100g body weight (i.e. four times the dose used in IP injections), and were killed, also in groups of 2, at 1, 3 and 15 minutes, 6 hours, 2, 5, 7 and 14 days after injection. Thymic nodes from both groups were fixed by whole

animal glutaraldehyde perfusion, preceded by vascular wash-out of blood; the remaining procedure was the same as described before. The majority of nodes were studied in an interrupted series of semi-thin plastic embedded sections. In two nodes, thin sections were obtained (for technique see chapters III & IV) and examined by the electron microscope.

VI. THE AGE AT WHICH ERYTHROCYTES FIRST APPEAR IN SINUSES OF THYMIC HAEMOLYMPH NODES

The thymic nodes of thirteen young rats, of different sex, were examined under the dissecting microscope searching for the presence of areas of pinkish-red colouration within the node. The age of the animals ranged from newborn to six weeks. They were killed in groups of 2 or more of each age. Thymic nodes from three age groups (newborn, one and two weeks) were fixed by immersion in 5% glutaraldehyde, processed in the usual way as described above, and studied in serial semi-thin plastic embedded sections. Intraperitoneal (IP) injections of 3% pontamine sky blue was routinely introduced before examination of the thymic nodes which helped a great deal in their identification.

RESULTS

I. TOPOGRAPHY OF THYMIC HAEMOLYMPH NODES

The topographical features of thymic haemolymph nodes were examined in a total of 33 rats. In all the animals examined, two groups of pinkish or reddish nodes were found lying in the vicinity of the thymus gland in the anterior mediastinum. The first group, the so-called thymic nodes, was invariably found embedded in brown fat and separated from the thymic capsule by a very thin connective tissue layer (Figs. 1, 103 & 105). Their common arrangement was 2-3 nodes situated dorso-laterally to each thymic lobe, but rarely as many as 5 or even 6 nodes were observed on each side. They were irregular in shape and their size ranged between 2-7mm in diameter.

The second group of nodes, known as the posterior mediastinal nodes, consisted usually of a large solitary node on each side, but 2 or 3 nodes were also observed (in 3 out of 33 animals). They were embedded in a mass of mediastinal brown fat and lay posterior to the apices of the lungs, just below and deep to the thymus and between it and the posterior thoracic wall (Figs. 103 & 105). They were located, on the right, between the right superior vena cava and oesophagus, and on the left, adjacent to the left superior vena cava. The right node was usually larger than the left one.

It was observed that both the thymic and posterior mediastinal haemolymph nodes exhibited polarity of their

red colouration in most of the cases. In all 33 animals, the uppermost parathymic node was more red than other members of the same group. In another 22 animals, it was noticed that the posterior mediastinal group, particularly the left node, also had a reddish tinge confined only to one pole, while the rest of the node was completely pale. The relation of these findings to the source of the sinusoidal erythrocytes is discussed below.

II. REGIONAL ORIGIN OF THE AFFERENT LYMPHATICS

Technique A

The method of demonstrating lymph vessels by IV injection of 3% pontamine sky blue followed by blood-vascular perfusion made the identification of dye-filled lymphatics against the empty blood vessels quite easy.

The results obtained by this technique were later confirmed by direct ink injections of the diaphragm, thymus, heart and lung (see technique B). However, it seems worthwhile to emphasise the following findings from the original dye experiments.

1) The uppermost parathymic node was constantly of a haemolymph nature in all 18 animals in which this method was employed. It received its major lymph drainage from the diaphragm via large retrosternal lymphatic trunks (Fig. 106).

2) The lymph nodes draining the diaphragm (which include parathymic and posterior mediastinal node groups) were usually of the haemolymph variety. Most of these nodes, particularly the posterior mediastinal,

exhibited polarity; the red pole of the node received lymphatics from the diaphragm while the pale-greyish part drained the heart and/or lungs (Figs. 20 & 22).

3) The cisternal node (usually a large solitary node, or less frequently anything up to 4 small nodes embedded, to the left of the cisterna chyli, in retroperitoneal fat) was of a haemolymph nature in 16 out of 18 animals studied by this method. Its main lymph drainage came from the posterior diaphragm (Figs. 104, 105 & 106).

Technique B

The method of injecting ink particle suspension directly into the organs succeeded in demonstrating the lymph vessels of the diaphragm, lungs and heart, but failed in the case of the thymus. Detailed results are presented below:

(a) DIAPHRAGM

Out of nineteen diaphragmatic injections in 6 animals, fourteen were successful in demonstrating lymphatics leaving the diaphragm. The results obtained by this method confirmed the findings achieved by IP injection of ink in 50 animals (see Chapter IV). Figures 103-107 show the course of various lymphatic pathways from the diaphragm and the regional nodes in which they terminated. The following conclusions can be drawn from these results:-

1) Subperitoneal lymphatic lacunae of the diaphragm filled beautifully after injection. Four paired groups of diaphragmatic lymphatic pathways were demonstrated; in

order of importance they were -

i) retrosternal, from the anterior diaphragm, into the parathymic nodes (uppermost node);

ii) retroperitoneal, from the posterior diaphragm, into the cisternal node;

iii) intercosto-paravertebral, from the costal part of the diaphragm, into the posterior mediastinal node;

iv) mediastinal, from the peritendinous area of the diaphragm, to the parathymic nodes.

All these lymphatic routes were interrupted by at least one lymph node before entering the great veins or cisterna chyli.

It was generally observed that most of these main lymph trunks end by branching into several small terminal lymphatics before entering regional nodes.

2) These lymphatic routes draining the diaphragm invariably exist as illustrated in Fig. 106. The lymph nodes, which they were destined for, also invariably possess a reddish tinge. In this respect the uppermost parathymic node and, to some extent, the cisternal node were constantly haemolymph in nature, while the remaining parathymic nodes and the posterior mediastinal node of the same animal differ invariably.

3) The incidence of polarity of red colouration was quite high in haemolymph nodes which receive their main lymph drainage from the diaphragm. Polarity was observed in the majority of 33 animals examined for topographical

features. In all animals, it was found that the uppermost parathymic node is the most red in the group. This phenomenon was called polarity in group. Individual polarity (which means the pole of an individual node receiving afferent lymphatics from the diaphragm showed redness, while the rest of the node was pale) was present in about two thirds of the animals.

(b) THYMUS GLAND

Although direct injections of the dyes were tried very carefully into different parts of the thymus, none of the 24 injections in six animals were successful. The soft consistency of the organ and the extremely thin capsule made the injections very difficult. Each time the thymus was injected, the dye spread diffusely into the surrounding tissues making the study of the thymic lymphatics most uncertain. Furthermore, the close proximity of the thymus to the parathymic nodes, made it more difficult to trace efferent lymphatics going from the former to the latter.

(c) HEART

11 out of a total of 16 injections made into the subpericardial lymphatic plexus of the heart, succeeded in filling them. Cardiac lymphatics converged into two large efferent lymph trunks which accompanied the ascending aorta, one on each side, and ended by dividing into two terminal branches: one crossed horizontally deep to the corresponding superior vena cava and drained into the

posterior mediastinal node; the remaining branch continued upwards deep to the thymus and emptied into the lowermost parathymic node (Fig. 22).

The most important, and rather interesting, observation was that these regional lymph nodes were either completely devoid of a red tinge, or in case of polarity, lymphatics from the heart entered the pale part of the node.

(d) LUNG

All direct injections, a total of 8, into lymphatics of the hilum of the lung succeeded in filling a lymph vessel. Hilar lymphatics drained into the corresponding posterior mediastinal node which was pale in colour. When this node showed pinkish or reddish colouration in one pole, hilar lymphatics emptied, as did cardiac lymphatics, into the pale portion of the node (Fig. 22).

III. EFFERENT LYMPH DRAINAGE

Efferent drainage of the parathymic and posterior mediastinal haemolymph nodes was studied by direct intranodal injections. Every node was injected separately and its efferent lymph drainage was traced under the dissecting microscope. The major efferent lymph channels from lymph nodes of both groups entered the corresponding subclavian veins either separately or as a single lymph channel (Fig. 21). These major efferent channels often showed segmental contraction of their wall in a typical beaded manner (suggestive of the presence of valves), which caused filling of the channels away from the nodes towards the

veins.

It was noticed that in addition to major efferent lymphatics, there were also small ones which connect members of the parathymic node group with one another in a descending manner, i.e., when lymph sinuses of an individual node became so full with ink, the excess dye was passed to the node below and so on (Figs. 21, 106 & 107). This pattern of distribution of ink agreed well with that of erythrocytes in some nodes before injection.

Occasional reflux of injected dye into afferent lymphatics of parathymic nodes was observed. These afferents filled partially and were traced backwards to lymphatics coming from the diaphragm as well as from the thymus gland.

IV. HISTOLOGY OF THE THYMIC HAEMOLYMPH NODES

Examination of serial semi-thin sections of thirteen parathymic haemolymph nodes showed that histologically they are similar to ordinary nodes, in general, and to their renal and splenic counterparts in particular. All the basic features of a conventional lymph node, i.e. capsule, subcapsular sinus, cortex, medulla and both afferent and efferent lymph vessels, were recognised (Figs. 2-12).

Capsule and subcapsular sinus - In general, each node was an oval or kidney-shaped structure. In each node, lymphoid tissue was enclosed in a capsule of connective tissue which was pierced at its convex surface by several afferent lymph vessels (Figs. 2, 5, 7 & 8). At the nodal concavity,

however, blood vessels and one or more large efferent lymphatic(s) emerged at the hilum (Figs. 1 & 10).

A subcapsular or marginal sinus was present between the capsule and the nodal parenchyma. It contained a homogeneous pale-stained precipitate of lymph protein and variable numbers of erythrocytes and small lymphocytes. Erythrocytes were usually present in far greater numbers than lymphocytes (Figs. 6, 8 & 9). Some macrophages, occasional medium or large lymphocytes, monocytes and a few plasma and mast cells were observed in the subcapsular sinus. Macrophage-erythrocyte rosettes were rarely found in the marginal sinus. The sinus was slightly enlarged at the site of entrance of afferent lymphatics (Figs. 7 & 9).

Nodal parenchyma - The lymphoid parenchyma of the parathymic haemolymph nodes was clearly divisible into a peripheral cortex composed of densely packed lymphocytes and a central medulla made of branching cords with intervening sinuses (Figs. 1, 2, 3, 5 & 13). The thickness and extent of the cortex varied in different nodes and also in different parts of the same node.

Cortical nodules, composed of heavily packed small lymphocytes, and with well developed germinal centres were found in all the nodes examined (a total of 13 nodes). The number of germinal centres seen in a single section ranged from 1-20. These germinal centres show a pale central and a dark-staining peripheral zone (Figs. 3 & 4). The central pale area was characteristically devoid of any blood vessels, and contained a significant number of dark-stained

tingible-body macrophages (Fig. 4). Since germinal centres are believed to reflect the state of activity of a node, it appears that the parathymic haemolymph node is immunologically a very active node. In any individual animal, the uppermost thymic node was found to have the greatest number of germinal centres (i.e. this node was more active than the rest of the nodes in the same group). This point, and many others, are considered in more detail in the Discussion.

The para-cortex (thymus dependent area), recognised by the presence of post-capillary venules with high cuboidal or columnar endothelial linings was seen in all the nodes examined (Figs. 2 & 5). Small lymphocytes were frequently seen traversing the wall of high endothelial venules, and were present in large numbers in its immediate surroundings (Figs, 15, 16 & 17). However, few erythrocytes were observed within the venules and outside in the surrounding tissue. None were seen in the wall of high endothelial venules.

The medulla extended from the para-cortex to hilum and consisted of anastomosing cords of dense lymphatic tissue with intervening sinuses (Figs. 2 & 3). The cords contained lymphocytes, plasma cells and fixed macrophages. Some erythrocytes were also found in the medullary cords, either attached to or engulfed by macrophages. Blood vessels, both arterioles and venules, traversed the substance of the cords. The medullary sinuses, being

larger than cords, occupied a greater part of the medulla. They contained some free erythrocytes, lymphocytes, macrophages and mast cells; but the most prominent feature here was the macrophage-erythrocyte rosettes which nearly filled the whole field (Figs. 11 & 12). In these rosettes, a single layer of erythrocytes is radially arranged on the surface of the macrophage (Figs. 11, 12 & 23). The macrophages were large and circular or ovoid in shape. They contained fragments of erythrocytes, haemosiderin granules and occasionally intact erythrocytes in their cytoplasm (Figs. 23, 24 & 25). Macrophage-erythrocyte rosettes are the most characteristic feature of a haemolymph node, and serve as a histological criterion for their recognition. Rosettes were rare in the marginal and intermediary sinuses and abundant in the medullary sinuses.

A polarised distribution of erythrocytes and macrophage-erythrocyte rosettes was observed in some nodes. Both were abundant at one pole of the node, and almost absent at the other. This area of polarisation, when present, was found to be located opposite the sites of entry of afferent lymphatics into the marginal sinus.

Afferent and efferent lymphatics - The number of afferent lymphatics present in any one node ranged from 1-4, and they usually entered the nodal capsule at its convex surface (Figs. 2, 5 & 13). The entrance into the subcapsular sinus was guarded by a bicuspid valve, opening towards the node (Figs. 7 & 8). Typical afferent lymph vessels with valves were seen in the majority of the nodes

examined (in 10 out of 13 nodes). The lymphatics were identified by their structure, contents and their continuity with the subcapsular sinus (Figs. 2 & 5-9). The luminal surface of the lymphatic was lined by a single continuous layer of flattened endothelial cells resting on a layer of connective tissue. Endothelial nuclei were ovoid in shape and protruded into the lumen. In large afferent lymphatics, an additional external layer of smooth muscular tissue was frequently observed (Figs. 7 & 8). Valves were made up of a double sheet of endothelium with some connective tissue lying between the sheet at the base of the valve. The afferents contained a pale stained precipitate of lymph protein, and a variable number of cells. Two types of cells present in the majority of the afferent lymphatics were erythrocytes and small lymphocytes. The number of these two cells and their ratio to one another varied in different afferents and in different nodes. Some of the afferents had large numbers of erythrocytes in them (Fig. 6). The erythrocytes were free, intact and appeared fresh. Macrophage-erythrocyte rosettes were never observed in afferent lymphatics.

Large efferent lymph vessels, with bicuspid valves pointing away from the node were recognised in all the thirteen nodes which were studied. These were seen emerging from the hilus, and were continuous with the medullary sinuses (Figs. 2, 3 & 9). They were readily distinguished from neighbouring empty veins by their

content of homogeneously stained coagulum of lymph protein, and by their relatively thin wall. Usually, free erythrocytes, macrophages and plasma cells were present in small numbers in them. However, macrophage-erythrocyte rosettes were never observed in efferent lymphatics.

V. COMPARISON OF THE ROUTE OF ENTRY OF CARBON PARTICLES INTO THYMIC HAEMOLYMPH NODES AFTER INTRAVENOUS (IV) AND INTRAPERITONEAL (IP) INJECTION

Carbon particles (i.e. 1:3 diluted india ink) were injected intravenously (IV) or intraperitoneally (IP) into two separate groups of adult rats, one of 16 and one of 8, animals. The site of entry and the rate of entry of the injected carbon particles into the thymic haemolymph nodes were then compared.

Carbon injected IP reached the thymic haemolymph nodes rapidly and in large quantities via afferent lymphatics which arose principally from the diaphragm. Within minutes of injection carbon particles were abundant in subcapsular, intermediate and medullary sinuses, mostly engulfed by macrophages (Figs. 99-102 & Table 2). Carbon particles, like erythrocytes, were distributed focally opposite the entry point of afferent lymphatics into the subcapsular sinus.

After IV injection, carbon particles entered the nodes much more slowly and in smaller quantities reaching a maximum after 5-7 days. Its distribution, however, was similar to that following IP injection, i.e. carbon particles were confined to the subcapsular, intermediate

and medullary sinuses and to the cells which line them (Figs. 13-18 & Tables 1 & 3). No carbon particles were found crossing the walls of high endothelial venules, nor were seen in the vicinity of these vessels (Figs. 17 & 18).

VI. THE RELATION BETWEEN AGE AND THE FIRST APPEARANCE OF ERYTHROCYTES IN THE SINUSES OF THYMIC HAEMOLYMPH NODES

The parathymic nodes of 13 young animals (ranging from newborn to 6 weeks) were studied both macroscopically and microscopically. Examination under the binocular dissecting microscope, revealed that thymic nodes start to show a slight tinge of reddish or pinkish colouration at the age of 3 weeks and upwards. Before this age no red colour was observed. Serial semithin sections of these nodes, however, showed the presence of small but variable numbers of erythrocytes in the sinuses, particularly in the marginal sinus. In the newborn thymic node, only very few erythrocytes were observed. Some lay inside a valved afferent lymphatic and others in the small sinuses of the node (Fig. 19). The number of erythrocytes in the sinuses increased as the animal grew older. Most of these erythrocytes were free, but some were attached to macrophages forming rosettes.

TABLE 1

NUMBER OF CARBON PARTICLES AT DIFFERENT SITES WITHIN THE THYMIC HAEMOLYMPH NODE AT VARIOUS TIME INTERVALS AFTER INTRAVENOUS (IV) INJECTION.

GROUP	INTERVAL AFTER IV INJECTION	SINUSES		LYMPHOID PARENCHYMA	HEVs
		S/C AND INT.	MED.		
I	1-15 minutes (n = 6)	tr.	tr.	-	-
II	6 hours (n = 2)	+	+	-	-
III	2-5 days (n = 3)	++ to +++	++++	-	-
IV	7 days (n = 3)	+	+++	-	-
V	14 days (n = 2)	tr.	++	-	-

tr. = trace
\+ = small amount
++ = moderate amount
+++ = large amount
++++ = very large amount

S/C = subcapsular
INT. = intermediate
MED. = medullary
} sinuses

HEV = high endothelial venules

TABLE 2

A COMPARISON OF PRESENT RESULTS ON SITE AND RATE OF ENTRY OF CARBON PARTICLES INTO THE THYMIC HAEMOLYMPH NODES AFTER INTRAVENOUS (IV) AND INTRAPERITONEAL (IP) INJECTION.

NO.	ITEM OF COMPARISON	IV (tail vein)	IP
1	Dose of injected ink	0.5ml/100g of body wt. (i.e. four times of dose of IP injection)	0.12ml/100g of body wt.
2	Time of death	1,3,15 mins., 6 hours, 2,5,7 and 14 days after injection	1,3,5 and 15 mins. after injection.
3	Site of first appearance of carbon	Subcapsular sinus	Subcapsular sinus
4	Time of first appearance of carbon	Within hours (6-24 hours)	Within minutes (1-3 minutes)
5	Time of observation of maximum amount of carbon.	5-7 days	5-15 minutes
6	Apparent route of entry	Afferent lymphatics	Afferent lymphatics
7	Site of carbon particles	Mostly in sinus macrophages, some ingested by Littoral cells of lymph sinuses	Some free in subcapsular sinus, many attached to/or engulfed by sinus macrophages

TABLE 3

A COMPARISON BETWEEN THE PRESENT RESULTS OF INTRAVENOUS (IV) INJECTION OF INK AND THOSE OF PREVIOUS AUTHORS WHO STUDIED HAEMOLYMPH NODES IN THE RAT.

NO.	AUTHOR AND SOURCE	TYPE OF INK	DOSE	ROUTE OF ADMIN.	METHOD OF KILLING	TIME OF DEATH	SITE OF FIRST APPEARANCE	TIME OF FIRST APPEARANCE	APPARENT ROUTE OF ENTRY
I	Turner, D.R. J. Anat. (1969), 104 (3).	Commercial india ink diluted 1:10 in saline	Unknown	Tail vein	Ether overdose	10, 20 and 30 minutes after injection	All nodal sinuses	Within 10 minutes	Directly from intra-nodal blood vessels
II	Nopajaroonsri et al. J. Ultrastr. Res. (1974) 48.	"Pelikan" india ink	0.1ml/ 100g body wt.	Right external jugular vein	Ether overdose	1, 5, 15 mins. and 2 hours after injection	Only deep sinuses, and adjacent to H.E.V.s	As early as one minute	Diapedesis through H.E.V.s
III	Present Study	Commercial india ink diluted 1:3 in saline, and repeatedly centrifuged & filtered	0.5ml/ 100g body wt.	Tail vein	1) I.V. injection of Nembutal 2) Ether overdose	1, 3, 15 mins. 6 hours, 2, 5, 7 and 14 days after injection	Marginal sinus opposite entry of afferent lymphatics	Few particles after one day, maximum at 5-7 days	Afferent lymphatics

DISCUSSION

This study has shown that haemolymph nodes, which lie in the vicinity of the thymus gland of the Albino rat, are constant structures. These nodes were regularly found in all the animals in which topographical features and lymphatic drainage patterns were studied. Histological examination of these nodes provided the confirmatory evidence about their haemolymph nature. The marginal sinus contains abundant erythrocytes, and medullary sinuses are packed with characteristic macrophage-erythrocyte rosettes so as to give a red or pink colour to the nodes when examined by the naked eye. The regular presence of the above features in all the rats, selected at random, and the absence of any microscopic abnormality in the nodes examined excludes the possibility that these appearances might be due to any pathology. This group of haemolymph nodes has been identified by Turner (1969, 1970 & 1971); Olah and Toro (1970) and Nopajaroonsri et al. (1974), but the precise number, location, drainage territories and polarity and its relation to afferent lymphatics were either not discussed or even not mentioned at all.

Two node groups were distinguished in the present study. The thymic nodes are a group of 2-3 nodes found embedded on the lateral aspect of each thymic lobe. They are separated from the capsule of the thymus by a very thin layer of connective tissue, which might, therefore, be mistaken for an additional thymic lobule. Blau and Gangas

(1968) estimated their total weight in relation to the thymus in rats and found it to be 1/10 to 1/20 that of the thymus gland in any one animal. The whole group of nodes might be loosely called "thymic" because of their topographical relationship to the thymus gland. The present investigation has shown, however, that the group is not homogeneous: the uppermost node receives lymph mainly from the diaphragm via the retrosternal lymphatic trunks and is constantly of the haemolymph variety. The remaining members of the group are sometimes haemolymph nodes, depending on the area of drainage territory, i.e. whether they receive lymph from the diaphragm via the mediastinal lymph route. The whole group can, therefore, be called with accuracy "parathymic haemolymph nodes". This does not exclude the thymus as a source of afferent lymph, but it shows that in addition to the thymus, these nodes (the uppermost node in particular) receive a considerable amount of lymph from the diaphragm.

The posterior mediastinal nodes, usually one large node on each side, are also invariably haemolymph nodes; their haemolymph nature depending on whether they drained the diaphragm via the intercosto-paravertebral lymph route or not. Because this group is found in the posterior mediastinum, and receives lymph from a wide drainage territory (diaphragm, heart, lungs and possibly the thymus), thus, the name "posterior mediastinal nodes" is most suitable.

Once the normal and regular presence of the

parathymic haemolymph nodes is established, it becomes important to determine whether they are typical lymph nodes having both afferent and efferent lymphatics or whether they are morphologically different from ordinary lymph nodes as regarded by Turner (1969).

The histological structure of parathymic haemolymph nodes was thoroughly examined in serial semi-thin sections of thirteen nodes. All the structural components of a typical lymph node are present with no major architectural differences.

The nodal parenchyma shows the usual distinction into cortex and medulla. The cortex is a peripheral region of densely packed lymphocytes, and contains a variable number of secondary follicles as also described in splenic haemolymph nodes by Olah and Toro (1970). Well developed germinal centres have been invariably seen in all of the nodes examined, i.e. a group of very active nodes. The presence of germinal centres, particularly in the uppermost node, shows that the parathymic haemolymph nodes are capable of an immune response to antigenic stimulation and in this regard are typical lymph nodes. This is, however, not surprising, since they receive afferent lymphatics containing antigens, principally from the diaphragm, through which they drain the peritoneal cavity. It is generally accepted that there are regional differences in the microscopic appearance of lymph nodes, particularly in the degree of development of germinal centres. Nossal and

Ada (1971) pointed out that the formation of germinal centres in rats is less common in distal limb nodes, but most evident in mesenteric and mediastinal nodes. Inguinal, iliac and lumbar nodes occupy an intermediate position. They considered these variations to "reflect a state of background immunological stimulation of the test animal". Similarly, haemolymph nodes should naturally show a variation in the number and degree of development of germinal centres. Some investigators of haemolymph nodes in general (Nopajaroonsri et al., 1974) and of the renal haemolymph nodes in particular (Luk et al., 1973; Kazeem, Reid & Scothorne, 1982) have reported the germinal centres to be either rare or absent, i.e. a hypoactive node. Even in a typical lymph node, germinal centres are not always present (Nopajaroonsri et al., 1971). The scarcity of germinal centres in the renal haemolymph node could be due to the effect of testicular and adrenal hormones, which reach the node via lymph from these organs (Kazeem, 1979). The splenic haemolymph node, on the other hand, contains more germinal centres and thus is regarded as a moderately active node (Abbas, 1984).

The difference in the extent and thickness of the cortex in different sections can be attributed to the plane of section and position of the section in relation to the node. Alternatively, these differences may reflect different levels of immunological activity in various animals, at the time of death.

The medulla of the parathymic haemolymph node, as in

a typical node, is made up of branching cords with large intervening sinuses. Lymphocytes, plasma cells and macrophages, with or without ingested erythrocytes, are the main cellular types found in the medullary cords. Free erythrocytes are rare constituents of these cords. The medullary sinuses were large and contained some free erythrocytes, mast cells and abundant macrophages, each with many attached erythrocytes forming characteristic rosettes. In general, the uptake of particles by macrophages involves two steps: attachment of the particle to the cell membrane, followed by its subsequent ingestion (Rabinovitch, 1967). In the present study, macrophages extend thin cytoplasmic processes between erythrocytes which are ultimately engulfed and phagocytosed by the macrophage (Olah & Toro, 1970; Nopajaroonsri et al., 1974). The presence of fragments of erythrocytes and haemosiderin granules in the cytoplasm of macrophages is a further indicator of their active erythrophagocytic function.

Typical afferent and efferent lymph vessels, containing homogeneous precipitate of lymph protein, have been recognised in the majority of parathymic haemolymph nodes examined in this study. Although the existence of efferents in the haemolymph nodes is generally agreed (MacMillan, 1928; Andreasen & Gottlieb, 1946; Turner, 1969; Nopajaroonsri et al., 1974), the presence of afferents has been denied by Turner (1969) and Olah and Toro (1970). The existence of afferent lymphatics in the parathymic

haemolymph nodes was shown by both injection techniques as well as by microscopic examination. It is usually easier to find slender, thin-walled afferent lymph vessels when they are seen under the dissecting microscope, in continuity and distended with dye, than when they are sought, collapsed and empty, in sections. This could be the explanation of the negative findings of some previous studies. Turner (1969), in his extensive search, relied exclusively on serial sections; it may also be that the cleaning of adventitious tissue from the surface of the node prior to processing tore away the delicate afferent vessels.

In histological sections, the identification of afferents was based on their structure, contents and their continuity with the subcapsular sinus. Their wall is lined by a single continuous layer of attenuated endothelium, which rests on a layer of connective tissue. An additional external layer of smooth muscle was present in larger afferent lymph vessels, which indicates a potential to contract. The ovoid endothelial nuclei bulge into the lumen and their long axis is parallel to the direction of lymph flow. This morphological structure is similar to that of peripheral lymphatics elsewhere and forms the basis by which lymph vessels are differentiated from arteries and veins (Yoffey & Courtice, 1970; Gnepp & Green, 1979; Weiss, 1983). The presence of bicuspid valves, at the entrance of these vessels into the subcapsular sinus, further confirms that these are typical afferents. These

findings agree well with the existing literature on haemolymph nodes of the rat in general (MacMillan, 1928; Nopajaroonsri et al., 1974) and also with the published work on both renal haemolymph nodes (Andreasen & Gottlieb, 1946; Kazeem, Reid & Scothorne, 1982) and splenic haemolymph nodes (Abbas, 1984). Nopajaroonsri et al., (1974), however, found a "few" afferents in the haemolymph node of the rat. In the present study every single parathymic node received at least 2-3 afferent lymphatics and sometimes 5 or even more. The argument against Nopajaroonsri's view is that, broadly speaking, lymph nodes receive a variable number of afferents which depend on the size of their drainage territory, irrespective of whether they are ordinary or haemolymph nodes. In a comparative study, Pearce (1981) examined the number of afferents present in a typical lymph node (of the face) and in a haemolymph node (a renal node). She found that the renal node which drains the testis, kidney and possibly the adrenal gland contains twice the number of afferents (4-8) present in the facial node (2-4), which has a small drainage area (i.e. ear and its surrounding skin). Abbas (1984), on the other hand, found that the splenic haemolymph nodes contain less afferents than its neighbour the renal, in spite of the fact that they drain the spleen which is a large organ. This was explained by the fact that efferent lymph from different parts of the spleen entered separate nodes (usually 3-4 haemolymph nodes).

Thus, one node drains approximately one quarter of the spleen; unlike the renal haemolymph node which is a single node and drains a large area. In this study, different parathymic haemolymph nodes received variable numbers of afferents, e.g. the uppermost parathymic node showed twice the number of afferents (3-6) than that of any other parathymic node (1-3); bearing in mind that this particular node receives its lymph almost exclusively from the diaphragm via the large retrosternal lymph trunks. The posterior mediastinal node occupied an intermediate position (2-4 afferents).

It is evident from the above discussion that the general structure and arrangement of lymphoid tissue in the parathymic haemolymph node does not differ from that of a typical lymph node, except that the haemolymph node is characterised by the presence of abundant erythrocytes in its sinuses.

The origin of the sinusoidal erythrocytes is controversial and various authors have offered different explanations (MacMillan, 1928; Andreasen & Gottlieb, 1946; Turner, 1969; Nopajaroonsri et al., 1974).

Looking at the problem in general terms, erythrocytes can be of intra-nodal or extra-nodal origin, or may arise in both sites. Among the possible intra-nodal sources are (a) erythropoiesis, (b) direct communication between the vascular bed of the haemolymph node and the lymphatic sinuses, and (c) diapedesis across the wall of the blood capillaries and post-capillary venules of the haemolymph

node. The extra-nodal sources include (a) entry by reflux from the venous circulation into efferent lymph vessels; and (b) via afferent lymphatics. In light of the present findings, the above possibilities are discussed separately in the following paragraphs:-

INTRA-NODAL ORIGIN

(a) Erythropoiesis - This has been reported to occur in haemolymph nodes of human (Warthin, 1901) and in some animal species, such as cattle (Winquvist, 1954) and in dogs and rabbits (Jordan, 1926, 1927). However, MacMillan (1928) and Turner (1969) have specifically denied that erythropoiesis occurs in haemolymph nodes of the rat, and other authors who also studied these nodes in the rat, have not reported the presence of erythrocyte precursors in them (Andreasen & Gottlieb, 1946; Nopajaroonsri et al., 1974; Kazeem, Reid & Scothorne, 1982; Abbas, 1984). In the present study, erythropoietic foci or blast cells have not been found in the parathymic haemolymph nodes and therefore it is highly unlikely that erythropoiesis occurs in the haemolymph nodes of this species.

(b) Direct communication between nodal blood vessels and lymphatic sinuses was suggested by some early authors, including Lewis (1902). In recent years this hypothesis has been supported by Turner (1969) who could not recognise the presence of any afferent lymphatics in the rat haemolymph nodes. However, there is no conclusive evidence that the blood vessels of the haemolymph node open

directly into its sinuses. Furthermore, there are good reasons to deny the existence of such communications: 1) the sinuses of the parathymic nodes examined after perfusion-fixation in this study still contain lymph protein precipitate. If a direct link between vascular and lymphatic systems were present within the haemolymph node, one would expect the contents of the sinuses to be washed out or at least diluted after perfusion; 2) when colloidal carbon is injected into the systemic circulation (Turner, 1969; Nopajaroonsri et al., 1974) or into the venous circulation (present results, see tables 1, 2 & 3), it does not penetrate from blood vessels into the sinuses of the haemolymph nodes. This suggests that blood and lymph circulation of the haemolymph node are independent from each other; 3) sinuses of the parathymic haemolymph nodes do not contain the cellular elements of the blood, which one would expect to find if such a communication did exist. Nopajaroonsri et al. (1974) did not find polymorphs in the sinuses of the haemolymph nodes either. All this evidence leads to the conclusion that the lymphatic sinuses of the parathymic haemolymph nodes are separate from the blood-vascular system, and that sinusoidal erythrocytes are not derived directly from intra-nodal blood vessels.

(c) Diapedesis - "Diapedesis" of erythrocytes across the wall of blood vessels is believed to be a passive movement which usually follows the passage of a white blood cell across the endothelium. This should be distinguished from active "emigration" of white cells such as lymphocytes

which are mobile cells and can pass out of blood vessels by active amoeboid movements (Walter & Israel, 1979). Nopajaroonsri et al. (1974) claimed that erythrocytes enter the sinuses of haemolymph nodes by intra-nodal diapedesis through the walls of small nodal blood vessels. They based their hypothesis on two electron micrographs showing the transmural passage of erythrocytes in a capillary and a high endothelial venule. They also presumed the direction of migration to be from the blood vessels to lymphatic sinuses and, without any quantitative studies, concluded that all erythrocytes enter the haemolymph nodes by this route. The present investigation, however, does not give support to the view of Nopajaroonsri et al. (1974). Two main questions must be discussed: Firstly, do most of the erythrocytes found in haemolymph nodes enter the sinuses by the same route as lymphocytes, i.e. diapedesis across the wall of intra-nodal blood capillaries and high endothelial venules? Secondly, do erythrocytes accumulate in large numbers in the lymphoid tissue immediately surrounding nodal blood vessels, as one would expect them to if intra-nodal diapedesis was the main route of entry? Lymphocytes, which form part of the recirculating pool, migrate across the wall of high endothelial venules from blood to lymph stream (Gowans, 1959; Gowans & Knight, 1964). These migrating lymphocytes are seen lying singly or in clusters in the wall (Yoffey & Courtice, 1970). As mentioned in the results, a large number of lymphocytes

were observed in the wall of high endothelial venules of the parathymic haemolymph node. However, no accumulations of erythrocytes were present around nodal blood vessels, nor were "streams" of erythrocytes observed passing from high endothelial venules to lymphatic sinuses. Similarly, in intravenously injected animals, carbon particles did not cross the walls of high endothelial venules at any time during the full experiment (a duration of 14 days), nor were they found in the surrounding tissue.

The virtual absence of erythrocytes and carbon particles from the walls of high endothelial venules and their extreme rarity outwith nodal blood vessels and in the lymphoid parenchyma, make it highly unlikely that intra-nodal diapedesis is the sole, or major, route of their entry into the parathymic haemolymph nodes. Previous investigations on the renal (Kazeem, 1979; Pearce, 1981) and splenic haemolymph nodes of the rat (Abbas, 1984) have yielded similar results.

EXTRA-NODAL SOURCES:

(a) Entry by reflux of blood into efferent lymphatics: In 1915 and 1918, Job described, in the rat, the presence of connections between certain abdominal lymphatics and neighbouring veins, in addition to the familiar lymphatico-venous communications between the thoracic and right lymphatic ducts and the left and right subclavian veins, respectively. It is not uncommon to find the terminal portion (2-3cm) of the thoracic duct coloured with blood in human cadavers (Yoffey & Courtice, 1970). On the basis of

Job's observations, Andreasen and Gottlieb (1946) suggested that the source of erythrocytes present in the sinuses of renal haemolymph nodes was due to reflux of blood from the renal vein into the node via its efferent lymph channels. However, this possibility has already been denied, recently, by Kazeem (1979) and by Abbas (1984) who studied the renal and splenic haemolymph nodes respectively. In the present investigation, although efferent lymphatics from all injected parathymic haemolymph nodes always drained into the corresponding subclavian veins, the presence in them of competent valves directed away from the nodes would tend to prevent reflux of blood. Moreover, the microscopic observation of only a few erythrocytes in efferents of all the nodes examined, speaks against reflux of blood into the nodal sinuses. Additional evidence against the reflux theory is the finding of precipitated lymph protein in the efferent lymphatics following perfusion-fixation and the absence of all the formed elements of blood in these vessels and from the lymph sinuses of parathymic haemolymph nodes. Also, the pattern of distribution of erythrocytes in the nodes is the very opposite of what would be expected if this hypothesis was true, because free erythrocytes are most abundant in the subcapsular sinus, and gradually become fewer in number as they move deeper and become attached to sinus macrophages, until they reach a minimum in the efferent lymphatics. All the above pointers reduce the likelihood that erythrocytes

enter the parathymic haemolymph nodes through efferent lymphatics.

(b) Entry via afferent lymph vessels: The results of the present work suggest that the sinusoidal erythrocytes of the parathymic haemolymph nodes are principally derived from an extrinsic source and enter the nodes via afferent lymph vessels. The main evidence suggesting this is as follows:- The afferent lymphatics to the parathymic haemolymph nodes, identified by having bi-cuspid valves pointing towards the node, usually contain abundant free erythrocytes. Some of these vessels are nearly filled with erythrocytes. The subcapsular sinus also contains many free erythrocytes which commonly lie in contact with the inner wall of the sinus, and appear pressed against it by the inflowing lymph. An exceptionally large number of erythrocytes are noted at the site of entrance of afferents into the subcapsular sinus (Figs. 6 & 9).

The erythrocytes in the intermediary and medullary sinuses are mostly attached to, or engulfed by sinus macrophages; although some are also seen lying freely. The efferent lymph vessels usually contain few free erythrocytes and no macrophage-erythrocyte rosettes. Scattered erythrocytes are occasionally seen in the lymphoid tissue of the medullary cords.

In the majority of the parathymic haemolymph nodes, erythrocytes and macrophage-erythrocyte rosettes are uniformly distributed, but in some nodes their distribution was polarised. In these, one pole of the node contains

many erythrocytes and rosettes, while the opposite pole had few or none at all. This focal distribution is related to the position of entry of afferent lymphatics and hence of erythrocytes into the subcapsular sinus and their subsequent flow through the intermediary and medullary sinuses. This is supported by the finding that some parathymic haemolymph nodes, on stereo-scopic examination, show patchy red colouration, with (injected) afferent lymphatics opening into the red areas (Fig. 20).

Turner (1969), Olah and Toro (1970) and Nopajaroonsri et al. (1974) have denied that erythrocytes enter the haemolymph node through afferent lymph vessels. The first two authors, because they did not find afferent lymph vessels, and Nopajaroonsri et al., because they did not see erythrocytes in the "few" afferents they described. The existence of afferent lymph vessels in thymic nodes of the rat was reported by Higgins (1925), MacMillan (1928), Tilney (1971) and Nopajaroonsri et al. (1974); and they have been demonstrated in the present investigation by both injection techniques, as well as by microscopic examination.

Post-capillary, or high endothelial, venules in lymph nodes are unique among blood vessels, in being composed of high columnar endothelium and permitting rapid migration of lymphocytes through their walls (Gowans & Knight, 1964). It has been claimed by Turner (1969) and Nopajaroonsri et al. (1974), who both studied haemolymph nodes in the rat,

that carbon injected intravenously can reach nodal sinuses by traversing the walls of high endothelial venules shortly after injection (10 minutes and one minute respectively) (see Table 3). This very early appearance of carbon deep in the node, and its scarceness, or virtual absence, within the subcapsular sinus and in the "few" afferents they observed led Nopajaroonsri et al. (1974) to believe that the passage of carbon particles did occur within the node, i.e. by diapedesis through intra-nodal blood vessels; and that this route accounted for all the erythrocytes seen in the sinuses of these haemolymph nodes.

In the present study, we have tested this hypothesis by comparing the site and rate of entry into the parathymic haemolymph nodes of carbon particles injected either intravenously or intraperitoneally, with the animals being killed at various time intervals (see Tables 1 & 2). The results indicate that carbon, injected intraperitoneally, entered the nodes rapidly and in large quantities via the afferent lymphatics and was distributed principally in subcapsular, intermediate and medullary sinuses. Carbon injected intravenously reached the nodes much more slowly and in much smaller amounts, but the pattern of distribution was similar, spreading from the marginal sinus into the intermediate and medullary sinuses. At no time during the experiment, was any carbon found in the cells lining high endothelial venules, nor in the vicinity of these vessels. These findings suggest that intravenously injected carbon particles enter the node <u>indirectly, by way</u>

of its afferent lymphatics, rather than directly through the walls of intra-nodal blood vessels (Table 3). This evidence, in addition to the above mentioned histological appearance, strongly supports the view that the erythrocytes found in abundance in the sinuses of haemolymph nodes are of extrinsic origin and enter the nodes by a peripheral lymphatic route.

On the grounds of these findings, it is concluded that large numbers of erythrocytes enter the parathymic haemolymph nodes through the afferent lymph vessels.

The next logical question relates to the origin of erythrocytes found in these afferent lymph vessels. This could be due to (i) haemorrhage in the area drained by the afferent lymph vessels, or (ii) direct communication between the tributaries of the afferent lymphatics and blood vessels outside the node, or (iii) escape of erythrocytes (by diapedesis) from the blood vascular system, outside the node, and subsequent entry into the tributaries of the afferent lymph vessels.

(i) All the rats examined belonged to a close in-bred colony in the Department, and all were selected at random, from apparently healthy stock. Animals were killed either by an overdose of anaesthetic ether, or by intravenous injection of Nembutal (sodium pentabarbitone), which excludes the possibility of haemorrhage due to physical trauma. Macroscopic examination did not reveal any signs of abnormal bleeding in the vicinity of the parathymic

nodes, nor in the neighbouring organs (thymus, heart and lungs). Thus, it is unlikely that haemorrhage was responsible for the presence of erythrocytes in the afferent lymph vessels. Moreover, the fact that the parathymic nodes of mature rats are regularly of the haemolymph variety (Turner, 1969, 1970, 1971; Olah & Toro, 1970; Nopajaroonsri et al., 1974) is consistent with the normal and regular presence of erythrocytes in the afferent lymphatics to these nodes.

(ii) If a direct link between the tributaries of the afferent lymphatics and extra-nodal blood vessels existed, one would expect to find all elements of the blood in the afferent lymphatics as well as in the nodal sinuses. Nopajaroonsri et al. (1974) have denied the presence of polymorphs in the sinuses. The existence of such a link is also rendered unlikely by the finding that, after vascular perfusion, the afferent lymphatics and all nodal sinuses still contain precipitated lymph protein in contrast to the blood vessels which are empty.

(iii) Since erythrocytes in the afferent lymphatics of the parathymic haemolymph nodes do not arise from haemorrhage or direct entry of blood from extra-nodal blood vessels, they are most likely to enter these vessels in the lymph stream from one or more organs which these vessels drain. Both the renal and splenic haemolymph nodes have been shown to receive erythrocytes from renal and splenic lymph respectively (Kazeem, Reid & Scothorne, 1982; Kazeem & Scothorne, 1982; Hogg, Reid & Scothorne, 1982; Abbas, Reid

& Scothorne, 1983). Four organs were investigated as potential sites of origin of afferent lymph vessels to the parathymic haemolymph nodes: diaphragm, thymus, heart and lungs. The lymphatics of these organs were demonstrated by three different methods; direct subcapsular or subserosal injection of the dye, intravenous injection of pontamine sky blue, and intraperitoneal injection of diluted india ink (see Chapter IV); both the second and third methods were followed by perfusion. Results obtained by these three different techniques were similar.

1. DIAPHRAGM:-

The intrinsic lymphatics of the diaphragm, and their lymph drainage routes, were outlined in eighteen animals after intravenous injection of pontamine sky blue and in fifty animals after intraperitoneal injection of diluted india ink, both followed by vascular perfusion which made the distinction between dye-filled lymphatics and empty blood vessels quite clear. Also, regional lymphatics of the diaphragm were shown by direct subperitoneal injection of india ink in six animals.

Local injection of the dye demonstrates the lymph vessels of the region which is injected, while intravenous and intraperitoneal administration shows all the diaphragmatic lymphatics at the same time. These lymphatics always drained into the red or pink coloured nodes lying in the vicinity of the thymus and also in the retroperitoneum adjacent to the cisterna chyli. When the

node showed polarity of red colouration, lymphatic trunks of the diaphragm opened into the red part of the node (Fig. 20).

The principal regional lymph drainage from the diaphragm reached the parathymic and posterior mediastinal nodes by way of the retrosternal and paravertebral lymph trunks respectively; and also reached the cisternal node by the retroperitoneal lymphatic trunks (Figs. 103-107). The observations of Olin and Saldeen (1964) by lymphangiography, and the injection studies of Tilney (1971) and Saldeen (1963) have shown similar results in the rat. Surprisingly, however, these authors failed to comment on the red colouration of these regional nodes.

2. THYMUS GLAND:-

Both techniques of direct injections (in six animals) and intravenous injection of pontamine sky blue followed by vascular perfusion (in 18 animals), failed to demonstrate the lymphatic drainage of the thymus gland. However, Tilney (1971) has reported that the thymus drains into the parathymic and posterior mediastinal nodes, although he does not mention their reddish colouration.

In the present investigation it was indirectly observed that following intra-nodal injections of the parathymic haemolymph nodes, in order to demonstrate their efferent drainage, occasional reflux of the injected dye partially filled a few afferent lymph vessels. When these afferents were traced under the dissecting microscope, in a retrograde direction, they were found to come from the

thymus gland. When this occurred, these lymph vessels were observed to open into either a complete pale thymic node or, when the node exhibited red polarity, into its pale part.

The presence of lymphatics inside the thymus gland is much debated (see Chapter III). The results indicate that true lymphatic vessels are present in the capsule, interlobular septa, the cortico-medullary region and in the outer medulla; all run, usually, with arterial branches. However, the thymic lymphatics are sparse and small compared with those of lymphoid organs. This might explain the difficulty of injecting and tracing the efferent lymph drainage from the gland. Moreover, the results of serial semi-thin sections through many blocks of different parts of the thymus showed the absence of numerous erythrocytes in the relatively few thymic lymphatics. Only occasional erythrocytes were observed in some of the blocks, while the majority of these thymic lymphatics contained many small lymphocytes. This is unlike the renal and splenic lymph which contained abundant erythrocytes which proved to be the main source of sinus erythrocytes found in their regional haemolymph nodes (Hogg, Reid & Scothoren, 1982; Abbas, Reid & Scothorne, 1983). If, similarly, thymic lymph is the main source of erythrocytes to the parathymic haemolymph nodes, one would expect to find numerous erythrocytes in thymic intrinsic lymphatics. Thus, it is unlikely that the thymus gland is the major source of

erythrocytes in the afferents to the parathymic haemolymph nodes.

3. HEART:-

Lymph vessels of the heart were made visible by direct injections in four animals. The cardiac lymphatics ended by dividing into two terminal branches which entered the posterior mediastinal and the lowermost parathymic nodes respectively. These nodes were pale in appearance, but when one node displayed polarity of red colouration, the lymphatics from the heart were seen entering the pale part of the node (Fig. 22).

4. LUNG:-

Lymph vessels of the lung were studied by direct injection in four animals, and by intravenous injections followed by perfusion in eighteen animals. The lymphatics entered the posterior mediastinal node. In the majority of the animals examined, this node showed red polarity. Lymph vessels from the hilum of the lung opened into the pale part of the node, while the red part was reserved for those lymphatics from the diaphragm (paravertebral lymph trunk).

The above results show that only the diaphragm drains into the parathymic and posterior mediastinal (haemolymph) nodes whereas the heart and lung drain into ordinary grey-coloured members of these groups or into pale parts of the nodes which display polarity of red colouration. Thus, out of the four possibilities examined, the diaphragm remains the most likely source of erythrocytes in the afferent lymphatics of the parathymic haemolymph nodes. This view

is also supported by the following findings:-

(i) Red colouration of the parathymic nodes seems to depend on whether they receive lymph from the diaphragm or not. Thus, the uppermost parathymic node, which constantly drains the anterior diaphragm via the main retrosternal lymph trunk, is consistently the most red in the group. Similarly, the cisternal node, which commonly drains the posterior diaphragm via retroperitoneal lymphatics is also red, but less so. Other members of the parathymic group and also the posterior mediastinal node group have little redness, and even that may be confined to only one pole which drains the diaphragm.

(ii) In some nodes, lymph vessels of the diaphragm drain selectively into the red areas of the node (the rest of the node being completely pale and, presumably, reserved for lymph drainage from either the thymus, lung, heart, or all (Figs. 20 & 22).

(iii) Macroscopic and microscopic results of intraperitoneal injections of dye confirms the above observations. The pattern of distribution of dye in the nodal sinuses, and its focal accumulation opposite sites of entry of afferent lymphatics into the marginal sinus is similar to the distribution and polarity of erythrocytes in the sinuses of the same node.

(iv) In the literature, there is abundant testimony to the complete and fairly rapid removal of erythrocytes by diaphragmatic lymphatics from the peritoneal cavity in

human and animals (Buxton & Torrey, 1906; Bolton, 1921; Cunningham, 1922; Allen, 1936; Hedensteadt, 1947; Simer, 1948; Courtice & Steinbeck, 1950; Bettendorf, 1978; Leak & Rahil, 1978). In rats, the transfer of labelled erythrocytes from the peritoneal cavity to circulation, via lymphatics of the diaphragm, is remarkably rapid and complete (Morris, 1953; Courtice & Morris, 1953). Durham (1897) reported an interesting case of a woman patient, who had died following a ruptured ectopic pregnancy, and had intact erythrocytes in the parasternal lymphatics, and the anterior mediastinal lymph glands were "turgid with blood".

Once they are in the peritoneal cavity, erythrocytes become rapidly absorbed by the so called lymphatic "lacunae" which open into the diaphragmatic lymphatic terminals (MacCallum, 1903). Only recently, several authors demonstrated clearly, by the use of SEM, the presence of preformed openings or "stomata of Von Recklinhausen" in the diaphragmatic peritoneum, through which large cells up to the size of erythrocytes or even larger, could pass into lymphatic lacunae of the diaphragm (Bettendorf, 1978; Leak & Rahil, 1978; Tsilibary & Wissig, 1977, 1983).

In Chapter IV, we re-examined peritoneal absorption and the uptake of intraperitoneal injections of india ink, by lymphatics of the diaphragm. The results showed that peritoneal absorption of ink was remarkably rapid. This rapid uptake of ink, and similarly of erythrocytes, occurred through preformed "stomata", demonstrated by

scanning electron microscopy. These were found only in the diaphragmatic peritoneum overlying lymphatic lacunae, and directly connected the peritoneal cavity with the lymphatic lumen.

The relationship between the age of the animal and the first appearance of erythrocytes in sinuses of parathymic haemolymph nodes:-

Andreasen and Gottlieb (1946) seem to be the only authors who investigated this problem in the haemolymph nodes of the rat. They studied the renal haemolymph node and concluded that erythrocytes did not appear in the node until the second half of the first month of life. Before this age, lymph nodes found in the same location, did not differ microscopically from ordinary nodes. The question which then naturally arises, is "why erythrocytes start to appear at the age of two weeks?" To answer this, we examined serial semithin sections through parathymic haemolymph nodes of young animals of different ages. The results agreed with those of Andreasen and Gottlieb (1946) in that macroscopically the parathymic nodes did not show a visible pinkish or reddish colouration until the age of three weeks. However, microscopic examination of nodes which belonged to earlier ages (newborn, one and two week-old animals) showed that the sinuses contained a small number of erythrocytes, which arrived by afferent lymphatics (Fig. 19). The results suggest that after birth, erythrocytes are continuously released into the

peritoneal cavity and enter the lymphatics of the diaphragm; they finally reach the sinuses of parathymic nodes by several afferent lymph vessels. As the animals grow older, the number of erythrocytes increase proportionally, until the third week, when they become macroscopically visible. This is due to their gradual accumulation in nodal sinuses. The red mottling, which is initially confined to one of the poles of parathymic nodes of younger animals, is presumably related to the afferent lymphatics from the diaphragm and becomes larger and darker as age advances.

It is generally acknowledged that, with normal physiological activities, isolated erythrocytes escape regularly from blood vessels into serous cavities as well as into interstitial spaces of organs, and that these cells are removed, almost exclusively, via lymphatics (Yoffey & Courtice, 1970; Walter & Israel, 1979). The number of erythrocytes entering peripheral lymphatics is increased by experimental conditions such as exercise (Haynes & Fields, 1931), by a warm foot-bath (Engeset et al., 1977) and by exposure to damaging stimuli (Selye & Foglia, 1939). These various experimental procedures were all thought to increase the permeability of the blood vessels. If the number of escaping erythrocytes is large enough, the regional nodes change into the haemolymph type (Selye & Foglia, 1939). However, the mechanism whereby erythrocytes are released into the serous cavities and interstitial fluid remains obscure. Also, the reasons why some organs

of certain species (such as the kidney and spleen of rat) contain more erythrocytes than the same organs of other species is unclear and, until now, remains unsolved.

SUMMARY

1) In Swiss-Albino rats, the parathymic nodes are 2-3 in number, and are embedded on each side of the thymus. They are regularly of the haemolymph type, i.e. their lymph sinuses contain many erythrocytes, both free and attached to, or engulfed by sinus macrophages. This gives the nodes a distinct red colouration which is visible to the naked eye in the adult rat.

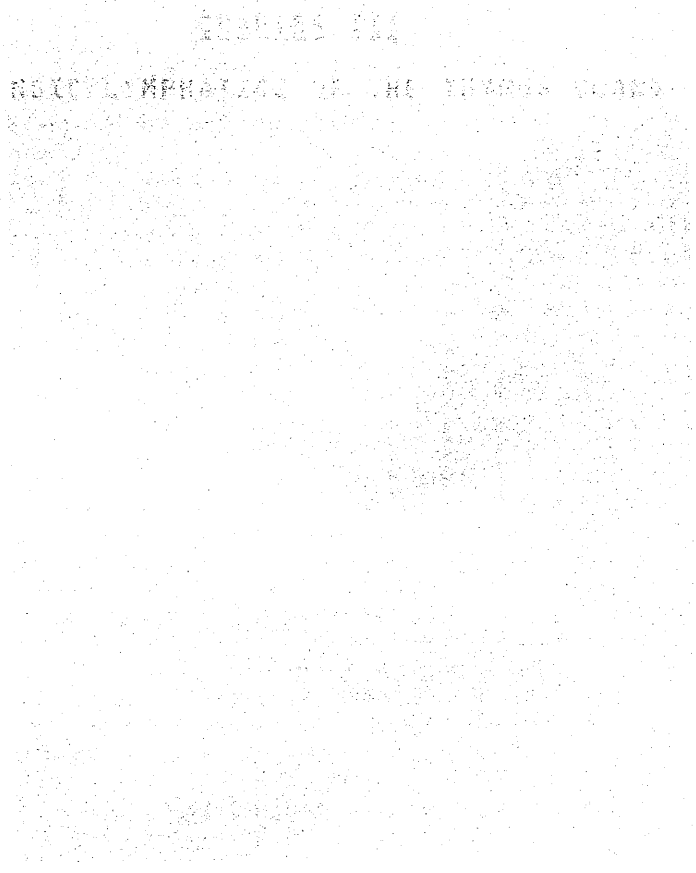
2) The histological structure of these nodes is similar to that of a typical lymph node and they possess valved afferent and efferent lymph vessels.

3) Evidence indicates that the erythrocytes found in the sinuses are chiefly extrinsic in origin, entering the nodes via afferent lymphatics and not by extravasation from intrinsic blood vessels within the node. Erythrocytes were often most common at the site of entry of afferent lymphatics.

4) The results indicate that the diaphragmatic lymphatics, which essentially drain the peritoneal cavity, are the most likely source of erythrocytes in the sinuses of these haemolymph nodes.

5) Microscopic examination of parathymic nodes belonging to newborn, one week and two week-old rats indicates that variable, but small, numbers of erythrocytes are present in the sinuses of the node, before red colouration is visible to the unaided eye. The number of erythrocytes increases in proportion to age until

the nodal red colouration becomes visible to the naked eye at around the second half of the first month of life.



CHAPTER III

INTRINSIC LYMPHATICS OF THE THYMUS GLAND

INTRODUCTION

A. HISTOLOGY AND ULTRASTRUCTURE OF THE THYMUS GLAND

During the last two or three decades, numerous accounts of the fine structure of the thymus in various species have been published, including mouse (Clark, 1964, 1973; Hoshino, 1963; Weiss, 1963), rat (Hwang et al., 1974; Lundin & Schelin, 1965; Van Haelst, 1967), guinea-pig (Izard, 1966; Mandel, 1968), hamster (Ito & Hoshino, 1966), monkey (Chapman & Allen, 1971), dog (Gorgollon & Ottone-Anaya, 1978) and human (Bearman et al., 1978; Bloodworth et al., 1975; Goldstein & Mackay, 1969; Haar, 1974; Hirokawa, 1969; Kameya & Watanabe, 1965; Pinkel, 1968; Von Gaudecker, 1978). The results of these and several other studies are presented in brief in the following paragraphs.

Each lobe of the thymus is invested by a thin capsule of loose connective tissue and is subdivided by primary connective tissue septa into a number of parenchymal lobules. The lobules, 0.5 - 2.0 mm in diameter, appear polyhedral in shape with truncated apices directed centrally (Goldstein & Mackay, 1969). It is a striking feature of the thymus that although the size of the whole organ varies widely in different species, the absolute size of the thymic lobule is remarkably similar in all species (Metcalf, 1966). The uniformity in lobular size is particularly evident with respect to the width of the lymphoid cortex. Metcalf (1966) suggested that there could be a critical size limit for the width of the cortex in

relation to the size of the medulla.

By light microscopy, each lobe of the thymus actually consists of a convoluted parenchymal strand with irregular expansions corresponding to the lobules (Weiss, 1983). Thus, the thymic lobules are not completely independent of one another. By serial sectioning, continuity can be demonstrated from one lobule to another via narrow parenchymal bridges. The thymic parenchyma within each lobule consists of a tridimensional network of stellate epithelial reticular cells, bounding irregular compartments filled with lymphocytes, macrophages and other cells. Each lobule is divided into a peripheral dark zone relatively rich in lymphocytes, called the cortex, and a central pale zone relatively rich in epithelial-reticular cells, called the medulla. In young mice, the cortex comprises about 86% of the thymic volume, and the medulla about 14% (Bryant, 1972). A similar high ratio for cortex versus medulla is found in the young human thymus (Simpson et al., 1975).

By electron microscopy, the stroma of the gland is seen to consist of epithelial-reticular cells with certain ultrastructural features consistently found in these cells: cytoplasmic tonofilaments, well-formed desmosomes which link adjacent cells into a sheet, and a prominent basal lamina associated with the cell membrane whenever these border connective tissue or blood vessels (Bearman et al., 1978; Hwang et al., 1974). The epithelial-reticular cells possess long cytoplasmic processes. Adjacent cell membranes are often held together by desmosomes and

interdigitations; indeed this is how these cells form a three-dimensional meshwork or cytoreticulum of thymic parenchyma.

Several investigators have described ultrastructural differences between cortical and medullary epithelial-reticular cells (Bloodworth et al., 1975; Chapman & Allen, 1971; Hirokawa, 1969; Ito & Hoshino, 1966; Mandel, 1968), as well as various sub-types of medullary epithelial-reticular cells (Bloodworth et al., 1975; Ito & Hoshino, 1966; Mandel, 1968). Very recently, Wijngaret et al (1984) re-examined the ultrastructural heterogeneity of these cells in the human thymus and classified them into six main types. Triangular Type 1 cells lie on a basement membrane that merges with the connective tissue of the capsule and septa of the perivascular spaces. These cells are connected by desmosomes to more pleomorphic Type 2 cells that mainly occupy the outer cortex and seem to form a series of cells with Types 3 and 4 throughout the cortex. Types 5 and 6 are typical of the medulla. However, epithelial-reticular cells have also been classified broadly as pale and dark cells with reference to their electron density (Hirokawa, 1969; Kameya & Watanabe, 1965; Kendall & Frazier, 1979). A further subdivision of each group can be made, depending on the presence in the cytoplasm of variable numbers of vesicles or membrane-bound vacuoles or electron-dense granules (Chapman & Allen, 1971; Clark, 1963; Ito & Hoshino, 1966; Lundin & Schelin, 1965;

Mandel, 1968; Singh, 1980). The significance of the variation in electron-density of the epithelial-reticular cells is not known, but the various intracytoplasmic granules and vesicles suggest that these cells may be secreting thymic hormones (Bennett, 1978; Bloodworth et al., 1975; Clark, 1963, 1966; Ito & Hoshino, 1966). Ultrastructural and experimental evidence suggests that these cells provide not only a three-dimensional supportive framework for the thymic cells but also, by their close contact with lymphoid cells and the production of hormones, induce intrathymic lymphocyte differentiation and possibly influence the events which may be associated with T cell maturation (Bearman et al., 1978; Bloodworth et al., 1975; Clark, 1966; Ito & Hoshino, 1966; Kendall & Frazier, 1979).

Apparently, there are many thymic hormones; at least eight have been isolated in the last two decades (Dardenne & Bach, 1981). However, the intrathymic location of hormone-producing epithelial-reticular cells has not yet been completely established (Goldstein, 1975). It has not been determined whether a single or multiple population of secretory cells are involved in the production of these hormones, or how the secretory cells are involved in the production of these hormones, or how the secretory materials are transported to the surrounding population of developing thymic lymphoid cells and/or to the blood stream (Curtis et al., 1979).

Intercellular epithelial-reticular cysts lined by microvilli or cilia have also been described in some animal

species (Chapman & Allen, 1971; Kendall & Frazier, 1979; Van Haelst, 1967), in the human fetal thymus (Hirokawa, 1969) and in thymomas (Rosai & Levine, 1976). They appear to be functioning in cell disposal, as most authors reported the accumulation of cellular debris, dead cells, macrophages and eosinophils in the lumina of the cysts; although the significance of this is unknown. Cysts are occasionally present in adult human thymus, and may represent remnants of the pharyngeal pouches from which the thymus arises (Weiss, 1983).

Macrophages or mesenchymal reticular cells are generally considered to be responsible for phagocytosis in the thymus. They are basically distinguished from epithelial-reticular cells by the absence of tonofilaments and desmosomes. Macrophages occur in the capsule, septa and perivascular spaces, as well as within the cortex and medulla (Bearman et al., 1978; Hwang et al., 1974; Raviola & Karnovsky, 1972; Weiss, 1983). The origin, residence time in the thymus and fate of thymic macrophages are largely unknown. They appear in greatest concentrations around the cortico-medullary junction (Hwang et al., 1974), and may sometimes be found across gaps in the epithelial-reticular cell sheath which surrounds the perivascular spaces (Bearman et al., 1975). They generally show lymphophagocytosis and inclusions of various types. Some of the macrophages are closely similar to epithelial-reticular cells containing polymorphous membrane-bound

inclusions (Hwang et al., 1974). In addition to their evident phagocytic capacity, macrophages may secrete factors that stimulate T-cell mitosis or differentiation (Weiss, 1983). Their numbers are probably not constant, as they appear particularly numerous in glands involuting under the action of ACTH (Lundin & Schelin, 1966) and also appear to increase with age. Macrophages known as lipid-laden foamy cells (Loewenthal & Smith, 1952) appear to be the same as the PAS-positive reticulum cells of Metcalf & Ishidate (1962) and could be the same as the autofluorescent cells of Sainte-Marie (1968).

THE CAPSULE:-

The thymus is enclosed by a thin but well defined capsule which consists of white connective tissue and parallel bundles of collagen fibres (Weiss, 1983). The connective tissue of the capsule dips down into the gland as fine interlobular septa, to invest incompletely each lobule (Goldstein & Mackay, 1969; Weiss, 1983). It continues into the organ, forming perivascular cuffs around the blood vessels (Kostowiecki; 1967; Pereira & Clermont, 1971). Blood vessels, efferent lymphatic vessels and autonomic nerve fibres are present in the capsule and the connective tissue septa (Goldstein & Mackay, 1969). The inner surface of the capsule and the connective tissue septa are lined by cytoplasmic extensions of epithelial-reticular cells that form a continuous layer resting on a well-defined basement membrane.

THE CORTEX:-

The cortex of the thymus gland appears dark in H & E stained sections due to the crowded nuclei of lymphoid cells, the predominant cell type of the cortex.

Slender cytoplasmic processes of epithelial-reticular cells constitute a thin sheet that bounds the entire surface of the thymic lobules and extends in various directions inside the cortical parenchyma, thus forming a coarse interconnecting meshwork (Bearman et al., 1978; Ito & Hoshino, 1966). Present within the interstices of the cortical meshwork are large numbers of lymphoid cells which show a spectrum of morphology, ranging from large through medium to small lymphocytes. There are few macrophages and plasma cells (Bearman et al., 1978; Hwang et al., 1974).

Although most studies on the ultrastructure of the thymus gland have described the cortex as a single zone, Clark (1973), Gorgollon and Ottone-Anaya (1978), and Hwang et al. (1974) preferred to divide this zone into a subcapsular or "outer cortex", and a deep or "inner cortex". The basis of this division is the predominance of large lymphoid cells, or lymphoblasts, and numerous mitotic figures in the outer cortex and the presence of comparatively smaller lymphoid cells and fewer mitotic figures in the inner cortex. The limit between the two zones is indicated by the presence of blood capillary plexus (Hwang et al., 1974).

The fact that the thymic cortex is a site of extensive lymphoid cell proliferative activity, at least in

early life of experimental animals, has been shown by the studies of Everett and Tyler (1967) and Metcalf (1964). These observations were supported by Clark (1973), Matter (1975) and Metcalf (1966). Clark (1973) suggested that the development of lymphocytes within the thymus was stepwise, rather than continuous, and each step occupied a different region of the thymic lobule. More than 95% of developing lymphocytes lie in the cortex, dominating its appearance (Bryant, 1972). Approximately 10% of thymic lymphocytes are large. These cells are concentrated in the outer cortex beneath the capsule and they actively proliferate. They represent stem cells recently arrived from the bone marrow. The deeper part, and the bulk of the cortex, contains small lymphocytes, about 85% of all thymic lymphocytes (Bryant, 1972). They are maturing cells derived from the large subcapsular lymphocytes. As they mature, they move deeper into the cortex, so that lymphocytes at the cortic-medullary junction and in the medulla are the most mature (Weissman, 1967). Approximately 5% of thymic lymphocytes lie among the epithelial-reticular cells of the medulla. As thymic lymphocytes mature, they acquire distinctive markers on the cell surface and within the cytoplasm that are revealed by special staining (Clark, 1973). By standard light and electron-microscopic stains, developing thymic lymphocytes look like other lymphocytes. However, small thymocytes (i.e. lymphocytes in the thymus) are somewhat smaller than small lymphocytes found elsewhere

in the body (Yoffey & Courtice, 1970). Bach et al. (1979) studied the ultrastructural properties of lymphocytes in general, and classified them into three types:-

1. Small lymphocytes: These constitute the majority of lymphocytes. They are globular in shape measuring 8 um with a spherical nucleus containing very condensed heterochromatin and one to three nucleoli. Its cytoplasmic rim, only 0.2 - 1 um wide, surrounds the nucleus and contains occasional mitochondria, a small Golgi apparatus, centriole, some short rough endoplasmic reticulum, few multivesicular bodies, and a considerable amount of free ribosomes. Their cell surface may be smooth or studded with numerous 1-2 um long microvilli.

2. Medium lymphocytes: They measure up to 12 um in diameter, and have a 2-3 um wide cytoplasmic rim which surrounds the nucleus. Other ultrastructural features are similar to those of small lymphocytes.

3. Large lymphocytes: These are voluminous cells almost identical to immunoblasts. Large lymphocytes do not reach the circulation under normal conditions. They are mobile.

Immunological, morphological and biochemical studies have demonstrated heterogeneity in the thymic lymphocyte population which indicates that medullary small lymphocytes are distinguishable from cortical small lymphocytes (Abe & Ito, 1970; Matter, 1975; Sainte-Marie & Leblond, 1964). In their chick-quail chimaera studies, Le Douarin and Jotereau (1981) found that the cortex and medulla were both populated at the same time from different precursors, thus

challenging the previous view of Clark (1973) and Sainte-Marie and Leblond (1964) that cortical lymphocytes gave rise to medullary lymphocytes which moved to the medulla prior to their release. Whichever view is adopted, there seems to be general agreement that a period of residence in the thymus is necessary for thymocyte maturation, although it is still not known precisely what is involved in intrathymic lymphocyte cell differentiation.

THE MEDULLA:-

This is the lightly-stained central region of the thymic lobule which is continuous, through strands or bridges, with medulla of other lobules of the thymus. It consists also of lymphocytes in an epithelial-reticular lattice or meshwork; but the proportion of epithelial-reticular cells is much greater than in the cortex. Goldstein and Mackay (1969) and Jones et al (1975) reported that the ratio of lymphoid cells to epithelial-reticular cells in the medulla of normal human thymus was 2:5, compared with 3:1 in the cortex.

The basic pattern of the medulla is similar to that of the cortex. However, medullary epithelial-reticular cells are extremely pleomorphic. Some are stellate-shaped, whilst others are large, round or irregular in shape (Pinkel, 1968). They often have voluminous cytoplasm (Mandel, 1968) and shorter cytoplasmic processes, joined to one another by desmosomes, and often show complex interdigitations (Goldstein & Mackay, 1969). They possess

in their cytoplasm either polymorphous membrane-bound inclusions of varying sizes, or small uniform membrane-bound granules (Hwang et al., 1974).

While most workers regarded the medulla as a single zone, Pereira and Clermont (1971) recognized an "inner medulla" that is vascular and made of mesenchymal tissues in distinction to an "outer medulla", which is lymphoepithelial and enclosed within an epithelial boundary layer. It is within the connective tissue of the inner medulla, and in its extensions throughout the gland as it runs along blood vessels, that many of the plasma cells, granulocytes, mast cells, macrophages and other connective tissue-cell types are concentrated. The inner medulla was laced with reticular fibres and contains a mesenchymal reticulum in contrast to the cytoreticulum of the lympho-epithelial portion of the thymus. Pereira and Clermont (1971) also found the so-called blood-thymus barrier to be tight throughout the thymus, except in the inner medulla.

Some epithelial-reticular cells of the medullary parenchyma also share in the formation of Hassall's corpuscles. These structures are unique to the thymic medulla. They consist of nests of concentrically arranged epithelial-reticular cells. Formation of these corpuscles begins with the degeneration of an epithelial-reticular cell indicated by the swelling of its nucleus and cytoplasm. Soon, this cell is surrounded by other epithelial-reticular cells, which become organised circumferentially and connected closely to one another by

numerous desmosomes (Goldstein & Mackay, 1969; Von Gaudecker & Schemale, 1974). New epithelial-reticular cells from the medullary cytoreticulum then wind themselves in a concentric pattern around those already present, leaving very narrow intercellular spaces. The peripheral epithelial cells blend into the adjacent cytoreticulum. In the central cells, keratohyalin granules and numerous tonofilaments appear; the amount and size increasing with the increase in Hassall's corpuscle diameter. As the innermost cells become distant from blood capillaries, they swell, degenerate, and transform into keratinised and/or necrotic material, which may also calcify (Kohnen & Weiss, 1964; Von Gaudecker & Schemale, 1974; Weiss, 1983). Hassall's corpuscles frequently measure over 100 um in diameter, which increase in number and size with age, as well as in some stressful situations (Von Gaudecker & Schemale, 1974). Thymic corpuscles are well developed in humans, dogs and guinea pigs, and poorly developed in mice and rats (Weiss, 1983). Their number in human beings reaches its maximum at puberty; from then on, they decrease in number, especially the smaller ones. With age, however, the remaining corpuscles become larger, and, as the thymus involutes, more prominent (Blau, 1967; Hammar, 1921). The function of thymic corpuscles is poorly understood. Proposed functions include:- 1) as "graveyards" for dead lymphocytes (Blau, 1973; Pinkel, 1968); 2) production or storage of antigens (Blau, 1967; Marshall & White, 1961).

Certainly antigenic stimulation can cause an increase in the number and size of the corpuscles (Kater, 1973); 3) production or storage of antibodies (Blau, 1967; Hammar, 1921; Kater, 1973); therefore they may be involved in immunological reactions; 4) a site of thymic hormone production (Kater, 1973; Kohnen & Weiss, 1964); and 5) without any apparent function, as they are believed to be remnants of the epithelial thymic anlage (Mandel, 1968; Shier, 1963).

Another important cell of the thymic medulla, most probably associated with lymphocyte maturation or differentiation, is the interdigitating reticulum cell. Similar cells are also found in the thymus-dependent areas of the spleen in rats and mice and are believed to be responsible there for a special T-cell microenvironment (Van Ewijk, 1980; Heuserman et al., 1974). Although considered to be derived from monocytes (Klug & Mager, 1979), thymic interdigitating reticulum cells have special tubulovesicular inclusions that distinguish them from macrophages. Phagocytic characteristics of interdigitating reticulum cells have been reported in the guinea pig thymus (Klug & Mager, 1979) and in the human thymus (Singh, 1980). Another characteristic feature of these cells is the long antler-like processes of cytoplasm that can enwrap cells and form complex surface patterns. However, the exact identity and function of this cell type has not yet been established.

Myoid cells in the thymus have been studied for many

years and have received more attention in the last ten years since the realisation of an association between the thymus and myasthenia gravis. Myoid cells are present in the thymus of many species of vertebrate classes. They are more abundant in human fetuses than in adults (Hayward, 1972; Henry, 1966). Some authors state that myoid cells are not present in the postnatal human thymus under normal conditions (Van de Velde & Friedman, 1970); others claim to have observed these cells throughout life; their population being not constant and changing seasonally (Henry, 1966; Ito et al., 1969). Myoid cell ultrastructure was studied by many authors (Bearman et al., 1978; Gilmore & Bridges, 1974; Ito et al., 1969; Raviola & Raviola, 1967). The origin of myoid cells is unknown. The two most frequently proposed theories are: 1) that they are derived from the mesenchyme surrounding the thymus and are secondarily incorporated into the parenchyma (Van de Velde & Friedman, 1970); and 2) that they arise directly from the epithelial-reticular cells; the evidence being the observation of desmosomes between the myoid and the epithelial-reticular cells (Gilmore & Bridges, 1974; Henry, 1972; Toro et al., 1969). However, Bearman et al. (1978) failed to find any desmosomes between the myoid cells and the surrounding epithelial-reticular cells. Moreover, immunofluorescence techniques have shown that myoid cells contain striated filaments with actin and myosin, as found in striated muscle, whereas the epithelial-reticular cells contain

muscle-like myosin only (Gilmore & Bridges, 1974). The function, if any, of myoid cells in the thymus is unknown. It has been suggested that they may play a role in initiating an autoimmune response to striated muscle cells (Ito et al., 1969), and their occurrence has attracted much attention in relation to the pathogenesis of myasthenia gravis (Mackay & Goldstein, 1967; Strauss & Van der Geld, 1966). Mackay and Goldstein (1967) speculated that myoid cells with cytological features typical of striated muscle cells normally would serve to preserve tolerance to muscles and that a disturbed tolerance to muscle self-antigen would result in myasthenia gravis. However, sufficient evidence is not yet available to permit a final conclusion as to whether myoid cells are really involved in the aetiology of myasthenia gravis.

The other cell-types in the medulla consist mainly of lymphocytes, predominantly of the small variety with fewer medium and large lymphocytes and mitotic figures (Abe and Ito, 1970; Hwang et al., 1974; Metcalf, 1966); this is in addition to macrophages which are usually situated close to the cortico-medullary junction, and generally show lymphophagocytosis and inclusions of various types (Hwang et al., 1974). Plasma cells, mast cells, and eosinophils are also found at the cortico-medullary junction, usually adjacent to the medullary venules (Hwang et al., 1974). Occasional myelocytes were also reported in the medulla of human thymus (Bearman et al., 1978).

Controversy exists whether lymphoid follicles with

germinal centres are present in the normal thymus, and if so, how often. Many authors reported the presence of variable numbers of germinal centres in the thymic medulla of human subjects who died suddenly (Sloan, 1943), or of biopsies obtained from patients undergoing surgery for congenital heart conditions (Henry, 1966; Middleton, 1967; Vetters & Barclay, 1973). However, Anderson (1956) and Castleman and Norris (1949) stated that germinal centres did not occur in the thymus glands of normal human subjects. Goldstein and Mackay (1967), in their study of thymus glands obtained either during surgery to correct congenital cardiac defects or at autopsy from patients who died after brief illnesses, detected germinal centres in only 2 out of 94 individuals. Goldstein and Mackay suggested that the variation in the reported incidence of medullary germinal centres in the human thymus glands could be due to the various criteria that have been used to identify these structures by different authors. Focal collection of lymphocytes present in the thymic medulla may not be "true" germinal centres if they lacked "tingible body macrophages" and mitotic figures. Under certain experimental and pathological conditions, however, germinal centres have been observed in the thymic medulla of different animals (Abe & Ito, 1970; Kotani et al., 1967; Marshall & White, 1961). This may suggest that the medulla is immunologically able to respond to antigens, although the mechanism of the formation of these germinal centres

remains undetermined.

The presence of variable numbers of fibroblasts, neutrophils, erythroid cells and multinucleated giant cells has also been reported in the thymus glands of humans and in different animal species (Bloodworth et al., 1975; Weiss, 1983; and also reviewed in "The Thymus Gland" edited by M. Kendall, 1981). This could be explained by the fact that the thymus can host many more cell lines than the T-lymphocyte lineage. Thus, early precursor cells of any of these lines could be present in the thymus and contribute to the range of cells observed with the electron microscope.

B. VASCULATURE OF THE THYMUS GLAND

B.i Distribution

The angioarchitecture of the thymus has been thoroughly studied, both by intravascular injection of dyes and by histological sections, in a variety of animals (Abe & Ito, 1974; Clark, 1964; Ito & Hoshino, 1966; Kardon & Kessel, 1981; Kostowiecki, 1967; Kramarsky et al., 1967; Raviola & Karnovsky, 1972; Weiss, 1963); and in human (Bearman et al., 1975; Blanc et al., 1973; Kameya & Watanabe, 1965; Pinkel, 1968). According to these authors, the disposition of the intraparenchymal blood vessels is linked with the lobulation of the organ. Thymic arteries (branches of the internal thoracic, pericardio-phrenic and thyroid blood vessels) enter the thymus from the surrounding connective tissue, pass down the septa, as the interlobular arteries, and give off lateral branches, the intralobular arteries, situated in secondary septa (Fig. 43). At the cortico-medullary boundary these arteries divide into arterioles that follow the boundary. Arterioles give off capillaries for the blood supply of the cortex, whereas only a few capillaries are destined for the blood supply of the medulla. These capillaries have a radiating course, perpendicular to the cortico-medullary arterioles. After forming subcapsular arcades, cortical capillaries turn towards the medulla, and join to form post-capillary venules. These venules empty into veins which are situated at the cortico-medullary junction and

which in turn drain into the interlobular veins following arterial branching. Some cortical capillaries drain directly into interlobular veins. Finally, interlobular veins empty into the left innominate (brachiocephalic) and thyroid veins.

B.ii Ultrastructure:

Ultrastructural investigations of thymic blood vessels in various laboratory animals (Abe & Ito, 1974; Chapman & Bopp, 1970; Clark, 1963, 1964; Ito & Hoshino, 1966, Raviola & Karnovsky, 1972; Weiss, 1963), and humans (Blanc et al., 1973), suggest that the peripheral part of the thymic cortex is supplied by capillaries of a small diameter, whilst the deep cortex contains vessels of various sizes. The diameter of the capillaries varies from 3.5 - 7.0 um, and their wall consists of endothelial cells, basal lamina, and a thin adventitial layer of collagen fibres and an occasional pericyte (Chapman & Bopp, 1970; Ito & Hoshino, 1966; Raviola & Karnovsky, 1972; Weiss, 1963). The endothelial cell junctions are simple approximations, which occasionally interdigitate. Densities in the cell membrane at cell junctions, suggestive of desmosomes, are present (Abe & Ito, 1974; Raviola & Karnovsky, 1972; Weiss, 1963). The endothelial cells form a continuous lining without any fenestrations or pores (Bearman et al., 1975; Clark, 1963; Hwang et al., 1974; Kameya & Watanabe, 1965; Weiss, 1963). Apart from the presence of luminal cytoplasmic projections, the endothelial cells also extend abluminal cytoplasmic

processes into the adventitia (Raviola & Karnovsky, 1972; Weiss, 1963). The endothelial cells contain, in addition to the usual complement of organelles, a moderate number of micropinocytotic vesicles either communicating with the luminal or basal surface or apparently lying free in the cytoplasm (Raviola & Karnovsky, 1972; Van haelst, 1967).

Arterioles, 10-15 um in diameter, are found at the cortico-medullary region and in the medulla (Ito & Hoshino, 1966; Raviola & Karnovsky, 1972). The endothelial cells display the usual complement of cytoplasmic organelles, a prominent Golgi complex and cytoplasmic filaments about 7-8nm in diameter (Raviola & Karnovsky, 1972; Weiss, 1963). A single layer of smooth muscle cells encircles the tunica intima. The adventitia is thin and consists of bundles of collagen fibres interspersed with elastic fibres (Raviola & Karnovsky, 1972).

Raviola and Karnovsky (1972) defined the post-capillary venules of the mouse thymus as large vessels (10-50 um in diameter), located in the cortico-medullary region and in the medulla. The walls were infiltrated with large numbers of migrating lymphocytes, making the vessels barely distinguishable from the surrounding parenchyma. Opinions regarding the occurrence of post-capillary venules in the thymus seem contradictory. According to Ehrick (1963) and Yoffey and Courtice (1970), post-capillary venules are never seen in the thymus, but Clark (1963) regarded the venules of the thymus as resembling the so-called "high

endothelial post-capillary venules" of lymph nodes, tonsils and Peyer's patches in mice. Sainte-Marie and Leblond (1964), conceiving the post-capillary venules of thymus as sites for the emigration of lymphocytes, observed lymphocyte diapedesis through the (not very high) endothelium of thymic venules. They considered these vessels to resemble the high-endothelial post-capillary venules of lymph nodes.

Ito and Hoshino (1966) reported that the endothelial cells lining the venules or veins of the mouse thymus were usually flattened. They commented on the variability of the electron-density of the cytoplasm of the endothelial cells and reported on the presence of smooth muscle cells and adventitial cells outside the basement membrane. Although Ito and Hoshino (1966) maintained that diaphedesis of lymphocytes was not observed across the vascular wall, in a later publication Abe and Ito (1974) reported that the walls of the medullary venules were sometimes infiltrated with migrating lymphocytes. Raviola and Karnovsky (1972) often found lymphocytes sandwiched between the endothelial cells and their basal lamina or penetrating the endothelium through the intercellular spaces. However, they did not exclude the possibility that lymphocytes could also perforate the endothelial cytoplasm as reported by Toro and Olah (1967) in guinea-pig thymus and Marchesi and Gowans (1964) in the post-capillary venules of lymph nodes. Goldstein et al. (1968), Goldstein and Mackay (1969), and Pinkle (1968) reported that the medullary vessels of the

human thymus had a high endothelial wall surrounded by a basement membrane, and showed the presence of lymphocytes "en passage" through the endothelium of these vessels. They suggested that this could be the morphological evidence of cell traffic between the thymus and the blood vessels. Soderstrom et al. (1970) did not find true high-endothelial post-capillary venules in their study of human thymic material. However, they frequently found venules with ordinary or even rather thin endothelial cells that were infiltrated with lymphocytes. According to these authors, two different types of vessels characteristically showed lymphocyte diapedesis in the normal human thymus: wide venules with a rather thin endothelium, mainly found in the medulla, and pre-capillary arterioles usually seen within or close to the cortex. However, ultrastructural evidence of lymphocyte migration across any vascular endothelium in the human thymus was not observed by Bearman et al (1975), Von Gaudecker (1978) or Kameya and Watanabe (1965).

Fenestrated capillaries: All the thymic blood capillaries so far considered were regarded as continuous, without any pores or fenestrae. Probably the first report on the presence of fenestrated capillaries in the thymus was published by Kramarsky et al. (1967). These authors reported that, in the mouse thymus, fenestrated capillaries were found both in the capsule and in the thymic parenchyma. The fenestrations in the endothelial cells

were usually located at some distance from the cell junction, in attenuated regions of the cytoplasm. The fenestrations measured approximately 30-47nm in diameter and were closed by a membrane about 6.5nm thick. The rest of the structure of the vessel wall was similar to that reported by other authors. The presence of fenestrated capillaries in the thymus was used as an argument against the existence of a blood-thymus barrier (Kramarsky et al., 1967).

Raviola and Karnovsky (1972) found a few blood capillaries with a fenestrated endothelium in the extreme periphery of the thymic cortex. They reported no more than 1-3 fenestrations along the entire circumference of a capillary seen in transverse section. The structure of the fenestrations was similar to that described by Kramarsky et al (1967). Raviola and Karnovsky (1972) stressed that such fenestrated capillaries were more common in the thymic connective tissue capsule especially in association with heterogenous clusters of connective tissue cells such as macrophages, mast cells, adipose cells and "fibroblast-like" elements. As the fenestrated capillaries were commonly located outside the thymic parenchyma, Raviola and Karnovsky (1972) ruled out the possibility of their relationship with the endocrine function of the thymus, although they suggested that they could be related to the development of adipose tissue (as the thymus involutes) and that they may disappear again after the completed histogenesis of the fat lobule. Without going into the

details of their significance, Abe and Ito (1974) reported that a number of capillaries, just under and within the thymic capsule, had an extremely attentuated and fenestrated endothelium. The presence of fenestrated capillaries in the human thymus has also been reported by Singh (1980). He recognised a small number of capillaries, mainly in the medullary parenchyma and less frequently in the cortex, which showed the presence of fenestrations in their attenuated endothelium. Thus, Singh (1980) used that as circumstantial morphological evidence to support the endocrine functions of the thymus gland.

B.iii Perivascular spaces

With improved techniques for reticular fibre impregnation, Strandburg (1917) demonstrated a black-stained double ring around the capillaries in the medulla, as well as in the cortex, of 8 to 9 month old human fetuses. He made no further comment on the origin or significance of these perivascular reticular rings. The presence of a second reticular sheath around the intrathymic arteries, veins and capillaries was also noted by Smith and Ireland (1941), who considered it to be a characteristic feature of the thymic blood vessels of mice. The two rings, the outer and the inner reticular rings, were separated from each other by thymic cells, predominantly lymphocytes. Kostoweicki (1967) confirmed the findings of Smith and Ireland (1941) and suggested that a "space", wide around large blood vessels and narrow or

almost obliterated around the capillaries, was present between the two reticular rings and contained collagenous connective tissue concentrically arranged around the wall of the vessels.

Bearman et al. (1975) and Von Gaudecker (1978), both using human thymic tissue and electron microscopy showed that reticular stains allowed the perivascular space to be easily distinguished from the surrounding parenchyma. Two distinct basal laminae were observed; one was associated with the endothelial cells, and the other with the epithelial-reticular cells of the thymic parenchyma. Between these two basal laminae were varying numbers of reticulum fibres that were continuous with either the vascular or the epithelial basal laminae, although occasionally fibres extended across the entire space.

The presence of a connective tissue space separating the thymic vasculature from the parenchyma resulted in the space being referred to as a separate "compartment" of the thymus (Clark, 1973). Kostoweicki (1967) stated that this perivascular space, enclosed between the two reticular fibre rings, was formed by the envelopment of a vessel and a portion of its surrounding mesenchyme by the growing thymus and was, in fact, extra-parenchymal. Levine et al. (1975) recognised epithelial and mesenchymal components of the thymus, and considered the organ divided into two compartments. They defined an "intraparenchymal" compartment (IPC) composed of lymphoepithelial cortex and medulla, and an "extraparenchymal" compartment (EPC)

composed of blood vessels and surrounding connective tissue. The epithelial boundary layer with its basal lamina excludes the (EPC) and it is, in fact, the border of the (IPC) and is continuous with the subcapsular epithelial border around the perimeter of the gland. Further evidence for the mesenchymal nature of the perivascular space was provided by Pereira and Clermont (1971) and Von Gaudecker (1978) who demonstrated continuity between the space and the extrathymic connective tissue.

Leblond and Sainte-Marie (1960) called the perivascular space a double-walled lymphatic vessel; but Clark (1963), who studied the thymus of mouse with the electron microscope, suggested that in the absence of any lymphatic endothelium lining the space, these could not be regarded as lymphatic vessels. Kobayashi et al. (1964) found the perivascular space communicating with the efferent lymphatic vessels of the thymus. According to Bearman et al. (1975) and Clark (1973) the perivascular space may function as the final pathway for emigration of lymphocytes from the thymus. Also, the space may be the site at which the thymic lymphocytes are first exposed to circulating antigens.

B.iv Blood-thymus barrier

As an explanation of the apparent immunological unresponsiveness of the thymus to circulating antigens, the concept of a hemato-thymic barrier was suggested by Marshall and White (1961). They showed that direct

injection of antigens into the thymus of guinea-pig resulted in the formation of germinal centres and plasma cells and the production of antibodies. According to them the failure of the thymus to react to circulating antigens could be attributed either to a barrier against the entry of antigens into the thymus, or to the lack of a suitable phagocytic mechanism to segregate antigen in the gland. As they found large numbers of reticulo-endothelial cells in the normal thymus and rapid phagocytosis of both vital dye and antigenic material following local injury, they concluded that a barrier existed between blood and parenchyma of the thymus.

Morphologically, the presence of a blood-thymus barrier was suggested by the electron microscopic observations of Clark (1963) on the mouse thymus. He described a continuous layer of epithelial cells connected to each other by desmosomes and lying on the basement membrane between the parenchymal lymphoid cells and the blood vessels. Thus, the combination of endothelial cells with their basal laminae, perivascular connective tissue space (often containing cells), and basal laminae of the associated epithelial-reticular cells, formed the blood-thymus barrier. Similar observations were reported by Clermont and Pereira (1965), Lundin and Schelin (1965), and Van Haelst (1967) in rat thymus; Chapman and Bopp (1970) and Gorgollon and Ottone-Anaya (1978) in canine thymus; and Bearman et al. (1975; 1978), Bloodworth et al. (1975), Kameya and Watanabe (1965), Pinkel (1968) and Rosai and

Levine (1976) in the human thymus. As far as the hemato-thymic barrier of Marshall and White (1961) is concerned, a number of publications clearly contradict the possibility of its existence in the thymus.

Ito and Hoshino (1966) in the mouse thymus and Von Gaudecker (1978) in the human thymus, have shown that the epithelial-reticular cell layer which separated the perivascular space from the thymic parenchyma, in the cortex as well as in the medulla, had frequent intercellular gaps through which the perivascular space communicated with the parenchyma. Thus, lymphocytes and macrophages within the epithelial framework appeared to pass freely between the perivascular spaces and the thymic parenchyma through these inter-epithelial gaps. Hwang et al. (1974) in their scanning electron microscopic study of the rat thymus showed that the side walls of the perivascular channels (as seen in the transmission electron microscope) represented merely the longitudinal sections of the epithelial trabeculae. The "walls" of the perivascular channels were interrupted by a large number of intertrabecular spaces and were, therefore, not truly a continuous partition. A similar epithelial-reticular cell arrangement was seen around the cortical capillaries as well as around the larger blood vessels in the medulla.

A number of substances including some vital dyes, electron-opaque tracers and antigens were found to make their way into the thymus, especially in the medulla,

whether introduced subcutaneously, intravenously, or by direct injection of antigens into the thoracic cavity (Abe & Ito, 1974; Chapman & Bopp, 1970; Clark, 1964; Kostoweicki, 1962, 1967; Kouvalainen & Gitlin, 1967; Sainte-Marie, 1963; Raviola & Karnovsky, 1972). Thus the failure of the thymus to yield antibody forming cells does not result from its non penetration by antigens (Sainte-Marie, 1963). However, the thymic blood vessels generally appeared to have, to some extent, a selective permeability to circulating tracers. The permeability is known to be relatively low compared with that in other lymphatic tissues and in the pancreas (Abe & Ito, 1974; Clark, 1964; Kostoweicki, 1963).

Raviola and Karnovsky (1972) showed that although blood-borne macromolecules did penetrate the thymus, their parenchymal distribution was limited to the medulla by several factors: a) the differential permeability of the various segments (i.e. the arterioles, capillaries and the post-capillary venules and veins) of the vascular tree; b) the spatial segregation of these segments within the lobes; c) the strategic location of parenchymal macrophages along the vessels. The cortical capillaries had impermeable endothelial cell junctions. Although a small amount of tracer was transported by plasmalemmal vesicles through the capillary endothelium (Abe & Ito, 1974), this tracer was promptly engulfed by macrophages stretched out along the cortical capillaries and it did not reach the intercellular clefts between the epithelial-reticular cells. Large

quantities of all injected tracers escaped through the clefts between migrating lymphocytes and endothelial cells in the walls of the post-capillary venules of the medulla. Arterioles also had small numbers of endothelial cell junctions that were permeable to molecules of smaller dimensions, but they did not allow the passage of tracers of higher molecular weights. The tracers released by these vessels penetrated the intercellular clefts of the medulla, but were never found to reach the cortical parenchyma. Thus, Raviola and Karnovsky (1972) concluded that a blood-thymus barrier to circulating macromolecules did exist, but was limited to the cortex. Medullary lymphocytes were freely exposed to blood-borne substances. Kramarsky et al. (1967), who were the first to demonstrate the presence of endothelial fenestrations in the thymic capillaries of mice, suggested that these fenestrated capillaries were permeable, just like other fenestrated capillaries present in a variety of tissues. Thus, Kramarsky et al. (1967) concluded that the blood thymus barrier, if it exists, must lie beyond the capillary endothelium.

C. INTRINSIC LYMPHATICS OF THE THYMUS GLAND

Several studies on experimental animals (Harris & Templeton, 1968; Kotani et al., 1966, 1967; Leblond & Sainte-Marie, 1960; Omori, 1973; Siegler, 1964; Smith, 1955) and humans (Bearman et al., 1975, 1978; Blanc et al., 1973; Goldstein & Mackay, 1969) have reported that the thymus gland has no afferent lymphatic vessels. Efferent lymph vessels leave the connective tissue septa and capsule, and drain to the mediastinal lymph nodes. Kotani et al. (1966, 1967) for the guinea-pig and rat thymus, and Smith (1955) for the mouse thymus, showed that efferent lymphatic vessels formed an extensive network around thymic blood vessels. However, some workers (Bloodworth et al., 1975; Siegler, 1964) claimed that in the human thymus there were no true lymphatics, only tissue spaces which were found in the capsule, and drained to regional lymph nodes.

Early workers established the presence of internal lymphatic vessels in the thymus gland (reviewed by Hammar, 1921). However, Watney (1882), who studied the thymus glands of various non-mammalian and mammalian species including human, specifically showed that lymphatic vessels did not exist in the thymic cortex. He suggested that perivascular spaces, surrounding medullary blood vessels, possibly acted as lymphatic vessels. Similarly, Leblond and Sainte-Marie (1960) regarded the perivascular spaces as lymphatic vessels which enveloped medullary veins. They also concluded that, in the rat thymus, the lymphocytes

that entered the perivascular channels reached the blood circulation either by diapedesis from these channels into the enclosed blood vessels, or by travelling along these channels to the main lymphatic vessels. Harris and Templeton (1968) also regarded the perivascular spaces as one type of the intrinsic lymphatics of the thymus in the guinea-pig. Whilst the other type was the "independent lymphatics" which, although forming part of the neurovascular bundles, were entirely discrete and not intimately related to the walls of blood vessels. On reaching the thymic surface, these independent lymphatics lay at the margins of the thymic lobules and ultimately converged on three main neurovascular bundles; superior, lateral and medial bundles which drain into the thymic lymph nodes which, in turn, drain into the cervical venous system.

Kotani et al. (1967) reported that thymic lymphatics began in the perivascular space and that the septa and capsule both contain efferent lymphatics that could drain from the perivascular space. Blanc et al., (1973) demonstrated the presence of perivascular channels or spaces in the thymus of human neonate, whose structure is similar to that of lymphatic sinuses. They described these channels as surrounding medullary and septal veins, and displayed connections with rare, perilobular and interlobular thymic lymphatics.

While Leblond and Sainte-Marie (1960) regarded the perivascular space as a double-walled lymphatic vessel,

Clark (1963), who studied the mouse thymus with the electron microscope, suggested that in the absence of any lymphatic endothelium lining the space, these could not be regarded as lymphatic vessels.

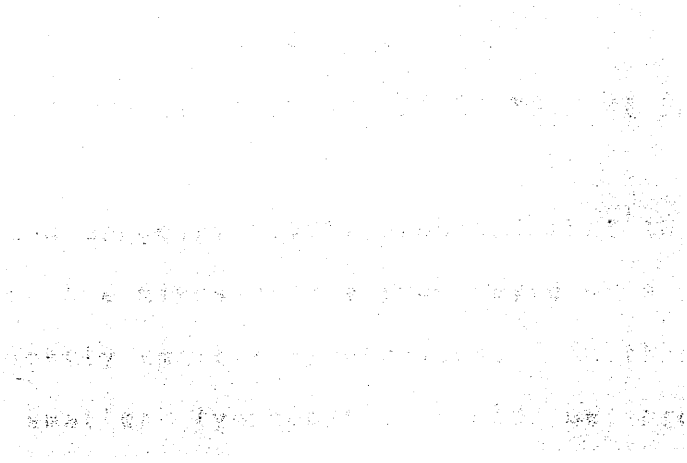
Efferent lymphatic vessels, into which the perivascular spaces may drain, were found in the septa and in the capsule surrounding the thymic lobules (Bearman et al., 1975, 1978; Kameya & Watanabe, 1965; Kobayashi et al., 1964); a number of which were packed with lymphoid cells (Bearman et al., 1978; Omori, 1973).

The ultrastructure of lymphatic vessels in the dog thymus was studied by Omori (1973), who reported that large lymph vessels with valves existed in the capsule and in the interlobular septa and accompanied blood vessels. He suggested that the flow of lymph in the lymphatic vessels was contrary to the flow of blood in the arteries. Two types of lymph vessels were described by Omori (1973) in relation to the parenchyma; "Cystiform" lymphatic vessels were situated close to the surface of the cortex, whilst others, small in size, were found near the cortico-medullary regions accompanying blood vessels. These lymphatic vessels were filled with lymphocytes. Since there were no valves in the lymph vessels that adjoined the parenchyma, Omori (1973) regarded them as lymph capillaries and considered that these represented the origin of lymph vessels in the thymus.

It has been generally accepted that the thymic

parenchyma contains only a few or no lymph vessels (Jolly, 1923; Policard, 1950; Yoffey & Courtice, 1970). However, the presence of small lymphatic vessels characterised by having a wall with attenuated endothelium and the absence of a basement membrane, was reported in the medulla of the rat thymus (Hwang et al., 1974) and recently in the medulla of the human thymus (Singh, 1980). Lymphatic vessels were also found in the cortico-medullary regions, but never in the cortex (Hwang et al., 1974).

Very recently Weiss (1983) summarised the present situation as follows: "Efferent lymphatic vessels lie in the inner medulla of the thymus. They, as do veins, carry T-lymphocytes from the thymus. There appear to be no afferent lymphatic vessels."



D. MATURATION AND EMIGRATION OF THYMIC LYMPHOCYTES

Several studies have provided conclusive evidence that the thymus of normal young animals continuously produces a considerable number of mature thymocytes (Andreasen & Ottesen, 1944; Kindred, 1940; Sainte-Marie & Leblond, 1958a; Yoffey et al., 1961). The production of thymocytes is confined largely to the cortex, from lymphoblastic stem cell derivatives (Clark, 1973). In young animals, the cortical mitotic rate is five to ten times greater in the thymus than in the lymph nodes or spleen (Yoffey & Courtice, 1970). Mitosis occurs most frequently in the subcapsular zone (Clark, 1973) and along the course of the radial blood vessels, especially in their peripheral segments (Metcalf, 1966). The production of thymocytes by mitosis must be balanced by their: a) transformation or differentiation into other cell types, or b) emigration, or c) degeneration.

a) Differentiation and movement of thymocytes inside the thymus

Dustin and Gregoire (1931) proposed that in embryos, as in adults, the mitosis of a lymphocyte of a given size yields two slightly smaller lymphocytes. In this manner, smaller and smaller lymphocytes would be produced by successive mitoses. The small lymphocytes would be at the end of the series. This offered the possibility of arranging thymic cells into a continuum extending from lymphoblastic stem cells to the smallest lymphocyte, thus

suggesting a gradual differentiation ending with the small lymphocyte. Later, decisive support for Dustin's hypothesis has been achieved by radioautography (Cronkite et al., 1959; Everett et al., 1960; Yoffey et al., 1961).

Regaud and Cremieu (1912) and Regaud and Lacassagne (1927) proposed that thymocytes, formed in the thymic cortex, moved towards the medulla and then left the organ, probably in blood capillaries. This conclusion was based on the finding that 3 days after irradiation the medulla still contained abundant small thymocytes at a time when most cortical thymocytes were already pyknotic; the medullary thymocytes subsequently disappeared without signs of pyknosis.

Qualitative and quantitative analyses of cells in the rat thymus led Sainte-Marie and Leblond (1958a,b; 1964) to similar conclusions. They showed that thymocytes do not penetrate the capsule, interlobular septa, or cortical blood vessels, and that pyknotic cells are normally rare in the cortex. Thus, they concluded that the only possibility left for elimination of the large numbers of thymocytes continuously formed in the cortex was for them to move to the medulla. As further studies of the medulla revealed few pyknotic forms but numerous small lymphocytes within venules, Sainte-Marie and Leblond (1958) also proposed that thymocytes left the medulla by entering the circulation. This migration pattern of mature thymocytes was consistent with the decrease of the overall mitotic index from the periphery towards the centre of the thymic lobule; 4.2%,

2.3% and 0.9% in the peripheral cortex, deep cortex and medulla respectively. Proliferation and migration were interdependent here; proliferation creates pressure within a cell population, and the higher the mitotic index the greater the pressure. The greater pressure in the peripheral cortex was reflected by the compressed polygonal outlines of lymphocytes in this zone. Consequently, the greater pressure exerted at the periphery of the lobule forces cells to move away towards the centre. As the mature thymocytes are the smallest it is to be expected that they will be pushed or squeezed away by the larger dividing thymocytes. In turn, the movement of mature thymocytes towards the centre of the lobule progressively diluted the population of dividing cells. Furthermore, Sainte-Marie and Leblond (1958, 1964) found no evidence that thymic cells moved in the opposite direction away from the centre of the lobule towards its periphery. Later, autoradiographic studies supported the view that cortical thymocytes move to the medulla. Cells labelled with ^{3}H-thymidine were first concentrated in the cortex; they disappeared after 2-3 days and became abundant in the medulla (Borum, 1968). The labelled cells subsequently left the medulla, probably in the venous blood.

The movement of thymocytes through the cortex is probably passive, the cells being pushed away as indicated by the rather regular nuclear outlines of cortical small thymocytes; actively moving thymocytes show rather characteristic nuclear distortions (Sainte-Marie & Leblond,

1964). Since most medullary small thymocytes exhibit such distortions, their movement inside the medulla is probably an active process (ameboid-like motion).

Experimentally, the majority of lymphocytes present in the thymus are not immunologically competent. Selected lymphocytes undergo maturation in two stages, both of which are thymus dependent (Stutman, 1977). The first stage takes place within the thymus and leads to the export of thymocytes known as T1 cells, which are immunologically virgin and incompetent (Stutman, 1977). This stage depends upon the presence of the thymic epithelial-reticular framework and mediated by a humoral factor (Mandel, 1968, 1970). The presence of the epithelium is essential to the induction of certain surface antigens in the thymocyte population (McKenzie & Potter, 1979). Before they leave the thymus, and presumably immediately afterwards, T1 thymocytes have poor immune properties; when injected into irradiated animals with skin allografts, they do not produce graft rejection (Walter & Israel, 1979). Most of the thymocytes destined to leave the thymus in the T1 state have not yet undergone antigenic stimulation. When they reach the T-cell areas of the malpighian corpuscles of the spleen, via the blood stream, they tend to remain there, not participating in the recirculation of lymphocytes until they reach full immunocompetence (Jordan & Robinson, in "The Thymus Gland" edt. by M. Kendall, 1981). After export to peripheral T-cell areas, chiefly in the spleen, the

second stage of maturation takes place. It involves the gradual loss of some of the surface antigens present on the lymphocytes (MacKenzie & Potter, 1979). When full immunocompetence is achieved, the cell, now known as a T2 cell, differs from a recirculating T-cell only in that it has not yet met an antigen to which it can react. It now has the potential to make a contribution to the responses to new antigens which the body may meet. T2 lymphocytes join the recirculating T-cell pool although they have not yet responded to antigenic stimulation and are virtually indistinguishable from T-lymphocytes.

In general, it has been estimated that the maturation of thymic lymphocytes, including their proliferation, differentiation and migration from the subcapsular regions into the medulla, may occur within 24 hours (Weissman, 1967).

b) Emigration of thymocytes

Some early investigators thought that many mature thymocytes died in situ (Dustin, 1920; Winiwarter, 1924), in contrast to other authors who believed that cells tended to emigrate from the thymus by probably entering its blood vessels (Hammar, 1921; Regaud & Cremieu, 1912). Those who held the former view provided no evidence of massive cell death in the thymus; proponents of the latter view did not describe the passage of cells into the thymic blood vessels. Although the problem remained unsolved, up to 1958 the thymus was not generally considered as an organ releasing lymphocytes into the circulation. When the

question was re-examined by later investigators, these two views were again the subject of debate (reviewed by Sainte-Marie & Peng, 1971).

In brief, the evidence in favour of emigration of thymocytes is as follows:-

(i) Cell turnover studies: Quantitative histology (Sainte-Marie & Leblond, 1958, 1964) and autoradiography (Borum, 1968; Kallenbach, 1960) have shown that thymocytes have a high turnover rate but a very low pyknotic index. Kindred (1940) found that thymocytes of the cortex in young adult rats had a mitotic index of 7.0% and a pyknotic index of 2.7%. If it is recalled that the duration of mitosis (about 1 hour) is shorter than the time taken for the elimination of a pyknotic cell (several hours to more than 1 day) (Trowell, 1964), it may be estimated that the cell production is about 20 times greater than the cell loss by pyknosis. These findings favour the view that most thymocytes emigrate from the rat thymus (Sainte-Marie & Leblond, 1964). The same argument was taken to conclude to a massive emigration of mouse thymocytes (Mickalke et al., 1969). The probable manner by which thymocytes leave the organ was investigated by Sainte-Marie & Leblond (1958), who observed numerous thymocytes migrating across the medullary venules of the rat thymus. It is unlikely

that circulating lymphocytes move into the organ, as virtually no labelled lymphocytes were recovered from the thymus after their intravenous injection (Gowans & Knight, 1964).

(ii) Perfusate of isolated thymus: In isolated thymuses from young rats, perfused for 12 hours, Folkman et al. (1968) found that they released an amount of small thymocytes into the perfusate corresponding to the production of these cells during the same period. Thus, the released thymocytes most probably left the thymus by migrating across the medullary venules.

(iii) Ablation and irradiation of thymus: Additional evidence for a large-scale emigration of rat-thymocytes was provided by Bierring (1960) and by Schooley and Kelly (1964), who demonstrated that early thymectomy decreases the output of thoracic duct lymphocytes by 40-70%, at least during the 2 months following surgery. Engeset and Schooley (1968) observed a 45% decrease, 5 days after young rats were irradiated over the thymus. Both findings were interpreted as showing that the thymus contributes a large number of cells to the recirculating pool of lymphocytes.

(iv) Thymic blood content: Sainte-Marie and Peng (1971) compared lymphocyte counts in arterial and thymic venous blood in the young rat, a species where the medullary venules appear to be the major pathway for

thymocyte emigration. The cell counts were carried out in sections of quick-frozen vessels to avoid unphysiological changes in the parameter as would be provoked by a stressful experimental procedure. They counted an average of 4.4 ± 0.4 lymphocytes per granulocyte in the arterial blood and 9.4 ± 0.5 in the thymic venous blood. They concluded, therefore, that the increase in the lymphocyte concentration resulted from the entry of thymocytes in the blood circulation through the thymus and this was deemed sufficient to account for the emigration, in the rat, of all mature thymocytes. Ernstrom et al. (1965) compared the lymphocyte content of arterial and thymic venous blood in guinea-pigs and found a difference in the total thymocyte production; but not as impressive as in the rat. Nevertheless, they concluded that the results seem to prove the existence of a venous output of small lymphocytes from the guinea-pig thymus.

(v) Emigration of thymocytes via lymphatics: Kotani et al. (1966) proposed that guinea-pig thymocytes emigrate mostly via lymphatics draining the thymus, a proposal based on the fact that they observed no cells diapedesing through the medullary venules. It was also based on their finding that the daily output of lymphocytes by the right cervical lymph duct was 6×10^6 less after thymectomy than before

the operation. They concluded that this figure represented the amount of thymocytes leaving the right thymus via lymphatics.

In their incidental study of the guinea-pig, Sainte-Marie and Peng (1971) confirmed the observations of Kotani et al. (1966). They noted that the thymic medullary venules were less developed and exhibited fewer diapedesing cells than those in the rat; thus, a substantially smaller percentage of thymocytes emigrate via medullary venules than in the rat. This too could account for the difficulty of Ernstrom et al. (1965) in demonstrating conclusively an emigration of the guinea-pig thymocytes via the blood circulation. In further agreement with Kotani et al. (1966) Sainte-Marie and Peng (1971) observed the presence of lymphatics in the interlobular trabeculae and capsule of the guinea-pig thymus. In this respect, therefore, they concluded: "While the thymus of most species studied possess no, or only few, lymphatics, the guinea-pig would appear to constitute an exception."

c) Cell degeneration in the thymus

The concept of death of thymocytes inside the thymus, the "graveyard hypothesis", was revived by Metcalf and his associates. In 1961, Nakamura and Metcalf reported that the percentage of pyknotic thymocytes nearly equals that of mitotic thymocytes in the normal young mouse. Metcalf

(1964) proposed that a minority of thymocytes became pyknotic and died while the majority emigrated. He considered that as the time taken for mitosis is probably much shorter than that required for pyknosis, the few pyknotic thymocytes present in the thymus could account for the death of only a minority of cells. Metcalf (1966) stated that most thymocytes die in the thymus; he postulated that lymphocyte pyknosis might be a rapid process lasting no longer than mitosis, so that pyknosis would balance mitosis. Metcalf and Brumby (1966) subsequently discarded this possibility and proposed a new mode of destruction for most thymocytes "the thymocyte explosion", a process occurring so rapidly as to afford little visible evidence of its existence. However, Sainte-Marie and Peng (1971) found no evidence to support such a phenomenon. Metcalf's conclusion, that less than 0.5% of thymocytes emigrate, was based on investigations in mice grafted with multiple thymuses (Matsuyama et al., 1966); where a few labelled thymocytes were observed outside these organs. However, Sainte-Marie and Peng (1971) in their extensive review of the problem, gave reasons why they did not consider the reports of Matsuyama et al. (1966) as a satisfactory demonstration that nearly all thymocytes die in situ. While it was initially thought that the observations resulting from an intrathymic injection of tritiated thymidine confirmed the above concept (i.e. less than 0.5% of thymocytes emigrate) (Nossal, 1964), it was

later recognised that this type of experiment can neither confirm nor deny the concept (reviewed by Sainte-Marie & Peng, 1971). Furthermore, the fact that pyknotic nuclei were often encountered in pairs led Sainte-Marie (1962) to propose that the few pyknotic thymocytes could arise mostly from abortive mitoses rather than from the death of mature thymocytes. Dustin and Gregoire (1931) also thought that abortive mitoses do occur frequently.

In summary, the present review reveals a lack of satisfactory evidence in favour of the concept of the death of most thymocytes, but no lack of data in support of the alternative concept of their emigration.

E. FUNCTION OF THE THYMUS GLAND

The thymus does not itself directly participate to a significant degree in immune reactions, but rather releases cells to other tissues that do. Therefore, it is, like bone marrow, classified as a central immune organ (Weiss, 1983). The important functions of the thymus gland may be summarised as follows:-

1) In the newborn the thymus is necessary for the development of the peripheral lymphoid tissues. In its absence, lymphoid aplasia, lymphopenia and a failure of cellular immunity lead to wasting and death from infection (Yoffey & Courtice, 1970). By adolescence the lymphoid system is fully developed and the thymus involutes.

2) In the adult the involuted thymus ensures the supply of virgin, immunologically uncommitted lymphocytes. Removal leads, not to severe illness, but only to a diminished response to new antigens.

3) These two functions are achieved by the essential role of the thymus; that is the differentiation and functional maturation of precursors into circulating immunocompetent T-lymphocytes. This it achieves by the provision of a "microenvironment" for lymphocyte differentiation (Dardenne & Bach, 1981) by direct contact of thymic epithelial-reticular cells with lymphocytes, and by the secretion of thymic hormones. The demonstration of the endocrine function of the

thymus and the existence of a thymic hormone or hormones has been suggested strongly. The evidence for this has been based upon: (i) the action of thymic extracts (reviewed by Dardenne et al., 1974); (ii) the preparation from the thymus of specific substances in varying degrees of purity (Comsa, 1955; Goldstein, 1975; Hooper et al., 1975), and (iii) the results of implanting in thymectomised animals cell-impervious diffusion chambers containing thymic tissue (Levey et al., 1963; Trench et al., 1966). Thymic hormones influence lymphocytes directly within the thymus and, by circulating, they can also affect peripheral lymphocytes (Wara & Ammann, 1978). The role of thymic hormones is illustrated by the restoration of immunocompetence in animals with reduced immune responses due to neonatal thymectomy simply by the addition of thymic hormones (Wara & Ammann, 1978).

4) It is likely that the thymus has considerable autonomy and that its cellular traffic is regulated intrinsically. The most telling evidence for this is the process of involution which no extrinsic agent can induce prematurely, nor arrest once it is underway. Further evidence for thymic autonomy is that thymic grafts behave as they would in their donor and not according to the thymic state of their host. Thus, neonatal thymus grafted into a young adult whose thymus is undergoing involution repopulates normally, while the host thymus continues to involute (Passmore &

Robson, 1986).

It is evident from the foregoing extensive review that most of the published reports on the thymus gland were primarily concerned with the fine structure of its various cellular components and their possible functions; and also concerned with the concept of the presence of a haemato-thymic barrier similar to that described in the brain. However, thymic lymphatics were only mentioned secondarily to these main issues and very briefly. There has been no specific morphological or functional description of the intrinsic lymphatics of the thymus gland. Thus, a detailed histological examination of the thymus in the rat was undertaken to answer specifically the following questions:-

a) Does the rat thymus contain lymphatics? If so,

b) What is their origin and extent in relation to the thymic parenchyma?

c) What do they contain in their lumina?

d) Does a blood thymus barrier exist? If so, is it complete or partial?

e) What is the nature, contents and significance of the perivascular spaces or channels and do they participate in lymph drainage or/and lymphocyte emigration from the thymus?

MATERIALS AND METHODS

A total of 12 Swiss albino rats, of both sexes and from a closed colony in the Department, were used in this study. Their age ranged from 2-7 months and weighed from 100 - 350g. Animals were killed by an overdose of anaesthetic ether, the remaining experimental procedure varied depending on the nature of the study.

I. Semithin sections:

8 adult rats were killed by an overdose of anaesthetic ether. A midline incision was made in the anterior abdominal and thoracic walls, and the heart with its great vessels were exposed by removing part of the rib-cage. The vascular system was washed free of blood, by perfusion for 2 minutes with Ringer's solution containing 0.4% lignocain chloride as a vasodilator, through a cannula inserted into the left ventricle, with an outflow established by incising the right atrium. Perfusion was then continued for 30-40 minutes with the fixative; 5% solution of glutaraldehyde in Millonig's phosphate buffer at pH 7.4. Perfusion solution also contained dextran, 40,000 M.W. (Fison) at a concentration of 3 - 5% W/V, because preliminary experiments showed that this reduced the interstitial oedema which otherwise occurs during vascular perfusion (Bohmann & Maunsbach, 1970) and made thymic lymphatics easier to identify. The intact thymus was carefully removed and immersed overnight in the same fixative as that used for perfusion. The tissues were

rinsed in 2-3 changes of phosphate buffer over a 2-3 hour period. Under the binocular microscope, 2-4mm slices of the thymus were cut with a razor blade. These blocks were post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in Spurr's resin (Spurr, 1969). Interrupted series of sections were cut at 1-1.5 um on a Porter-Blum microtome MT-2 using glass knives, through 5 blocks from each rat, giving a total of about 40 blocks. Continuous series of sections were cut in 6 of these blocks. The sections were stretched, by heating, on numbered glass slides. Alternate slides were stained with Azur II and with Haematoxylin and Eosin, and mounted in DPX and covered with a coverslip. Sections were studied by optical microscopy. In general, lymphatics were followed in a retrograde manner, from the hilum into the parenchyma of the thymus.

II. Transmission electron microscopy:

Semithin sectioning of the blocks continued until appropriate regions were reached. Selected areas of 15 blocks, from 5 thymus glands, were then trimmed down and sections were cut, using a diamond knife, on a Reichert-Jung ultramicrotome. The thin sections, 60-80nm in thickness, were then picked up on uncoated 200 mesh copper grids and double stained with saturated uranyl acetate in 60% ethanol (Watson, 1958) and with lead citrate (Reynolds, 1963). The specimens were then viewed with a JEOL 100S transmission electron microscope.

III. Scanning electron microscopy:

Two thymus glands were studied by this technique. Following vascular perfusion and fixation with glutaraldehyde (as mentioned above), the thymus was removed intact and immersed overnight in the same fixative as that used for perfusion-fixation. The tissues were washed well in fresh buffer and, while still in buffer, one thymus was cut under the binocular microscope into 4 longitudinal slices using a razor blade, while the other thymus was cut into several transverse slices. All specimens were then osmicated in 1% osmium tetroxide in Millonig's buffer, dehydrated through a graded series of ethanol, critical point dried, and mounted on aluminium stubs. The specimens were then coated with gold before being examined in a JEOL T300 scanning electron microscope.

IV. Tracer experiments:

In two animals, primarily used for other experiments as described in Chapter II, diluted india ink (1:3 in fresh saline) was injected intravenously into the tail vein. The animals were then killed, 6 hours and 7 days later, dissected and fixed by vascular perfusion of glutaraldehyde. The remaining procedure was the same as for the previous set of animals. This material is included here because it provided some incidental information on the blood-thymus barrier.

RESULTS

A. ULTRASTRUCTURE OF THE THYMUS GLAND

In the rat, the thymus gland is found in a central position of the anterior mediastinum overlying the pericardium and in front of the great blood vessels as they emerge from the heart. It is composed of two closely applied lobes completely surrounded by a connective tissue capsule consisting of elongated fibroblasts and parallel bundles of collagen which is only broken by the entry and exit of neurovascular bundles (Figs. 26 & 27). The inner surface of the capsule is formed by cytoplasmic prolongations of epithelial cells which form a continuous single layer that rests on a well defined basement membrane (Fig. 27), which separates the epithelial cells from the collagen fibrils.

Each lobe of the thymus is subdivided into small polyhedral lobules by septa that extend from the surrounding connective tissue capsule. Every lobule displays a darker peripheral zone rich in lymphocytes, the cortex, and a central pale zone rich in epithelial-reticular cells, the medulla, with Hassall's corpuscles (Fig. 26). Lobules are continuous with one another via narrow medullary bridges.

The thymic cellular population consists of the principal cell types (epithelial-reticular cells and lymphocytes) and other, minority cells (interdigitating-reticulum cells, macrophages, plasma cells, mast cells,

eosinophils and other granulocytes).

Principal Cell Types:

1) Epithelial-reticular cells

These cells are spider-shaped and possess long branching cytoplasmic processes which form an interconnecting meshwork with lymphocytes occupying the interstices (Figs. 26, 61 & 64). Present in all of the epithelial-reticular cells are tonofilaments and desmosomes (Figs. 34, 35 & 44); both were best developed in the cells forming Hassall's corpuscles (Fig. 41). No desmosomes were found between lymphocytes and epithelial-reticular cells. No basal lamina was associated with the epithelial-reticular cells except where they came in contact with connective tissue. They were usually larger than the cells of the lymphoid series, and the nucleus is usually much more leptochromatic with marginal chromatin and distinct nucleoli. The epithelial-reticular cells showed a great diversity of form in the rat thymus. Dark and pale cell variants were demonstrated (Figs. 34, 44, 47, 48 & 64). The epithelial-reticular cell cytoplasm, apart from the tonofilaments and desmosomes, showed no consistent features. The Golgi apparatus may be well developed. Cytoplasmic inclusions of varying sizes, including vesicles and membrane-bound vacuoles, and small uniformly dark membrane-bound granules were frequently present in these cells (Figs. 34, 35 & 44).

There were variable

numbers of mitochondria, ribosomes and lysosomes, and prominent profiles of both smooth and rough endoplasmic reticulum (Fig. 34). There appear to be some ultrastructural differences between cortical and medullary epithelial-reticular cells. The cytoplasmic vacuoles of the medullary cells were larger in size, aggregated and confluenced to form larger cystic structures with numerous microvilli projecting into their lumina (Fig. 35).

Groups of concentrically arranged and flattened epithelial-reticular cells formed clumps, with a portion of their cytoplasm in an advanced state of degeneration and necrosis, to give Hassall's corpuscles (Fig. 40). The centre of the corpuscles was either solid or cystic. In the cystic type, the lining epithelial cells displayed microvilli, and there was cell debris within the cyst lumen. Degenerative nuclear and cytoplasmic changes were common in the central cells of the corpuscles. Lymphocytes, macrophages and leucocytes were occasionally observed in Hassall's corpuscles. Frequently, smaller intracellular cysts were also noticeable, generally near the periphery of the corpuscle. While Hassall's corpuscles were relatively few in the young rat thymus, they became more frequent as the animal grew older; so that in the adult thymus one or more corpuscles were seen in each medulla.

2) Thymic lymphocytes

Lymphocytes were, quantitatively, the most important component of the thymic tissue; they were packed in the cortex and were less numerous in the medulla (Figs. 26 & 42). They exhibited variable sizes and morphology, depending on their location within the thymus. The majority of the lymphocytes were morphologically quiescent with dark small nuclei containing uniformly dispersed, or sometimes peripherally clumped dark chromatin, an inconspicuous nucleolus and a narrow rim of scanty cytoplasm with electron-dense mitochondria and free ribosomes. These cells had an average diameter of 5 um and were found mainly in the medulla and the deep region of the cortex (Fig. 32). A relatively small number of these small lymphocytes were randomly distributed in the outer cortex. However, the predominant type of lymphoid cells present in the outer cortex (subcapsular cortex) were the large lymphocytes (or lymphoblasts). These cells had an average diameter of 7 um, with an increase in nuclear euchromatin, electron-lucent cytoplasmic mitochondria with well-developed cristae, and numerous polyribosomes (Fig. 27). Isolated cells of this type could be seen scattered throughout the remainder of the cortex, but were less common in the medulla. Mitotic figures were frequently encountered in the cortex, particularly in the subcapsular region and around the septa (Figs. 64 & 66). Large numbers of degenerating

lymphocytes, which consisted of naked pyknotic nuclei, were observed throughout the cortex and the medulla (Figs. 51 & 64), although they were far more common in the cortex. They were also present as ingested material within macrophages (Figs. 28 - 31) and occasionally within Hassall's corpuscles (Fig. 40).

Although, in general, lymphocytes were not in direct contact with the connective tissue of either the capsule or the perivascular space, without being separated from the connective tissue by epithelial-reticular cell cytoplasm (Figs. 27 & 44-48); direct contact was observed in a few instances, particularly in the medulla (Figs. 50 & 52). Occasionally, lymphocytes were observed in transit across the medullary venules. Desmosomes were never observed between adjacent lymphocytes.

Other minority cells

1) Interdigitating reticulum cells

These were large pale cells with a large nucleus and long cytoplasmic processes, which occurred predominantly in the medulla and around its blood vessels (Fig. 49 & 51). The nucleus was large and often indented, with peripheral clumping of the hetrochromatin near the nuclear membrane. The rest of the nucleus was filled mainly with euchromatin which accounted for the electron-translucent appearance of the nucleus (Figs. 36, 37 & 49). One or two prominent

nucleoli could be seen (Fig. 36). The cytoplasm contained a well developed Golgi apparatus, rough endoplasmic reticulum and a few small and large vesicular inclusions (Figs. 36 & 37). Their long cytoplasmic processes surrounded various lymphoid cells forming a characteristic complex surface pattern (Figs. 49 & 51). No signs of active phagocytosis were observed inside these cells, which differentiated them from macrophages.

2) Macrophages

Macrophages were invariably present within the thymus, lying among lymphocytes and epithelial-reticular cells of the cortex and the medulla. Many macrophages were preferentially situated in the cortico-medullary junction as well as in the perivascular spaces. They displayed a morphological spectrum from cells containing few lysosomes and inclusions of various types, to those with large amounts of phagocytosed debris and many phagolysosomes in their cytoplasm (Figs. 28 - 31 & 38). They also often showed lymphophagocytosis with lymphocytes in different stages of degradation (Fig. 30). Some of the macrophages closely resembled epithelial-reticular cells containing polymorphic, membrane-bound inclusions; only to be distinguished by the lack of tonofilaments and desmosomes.

3) Plasma cells

The rat thymus contained a moderate number of

plasma cells, mainly located in the connective tissue of capsule, septa and in the vicinity of blood vessels, particularly those at the cortico-medullary junction (Figs. 27, 56, 57, 62, 63, 66 & 85). They were more prominent in the thymus of older animals, which might suggest that their number increases with age. The great majority of plasma cells were mature cells; this judgement was based on the following ultrastructural criteria: i) their size was 2-3 times larger than small lymphocytes; ii) they were usually ovoid in shape with round or oval nucleus which tend to lie eccentrically; iii) coarse clumps or blocks of nuclear chromatin spreaded irregularly around the periphery, which resembled spokes of a wheel; iv) progressively dilated endoplasmic cisternae which, in some cells, united to form intracytoplasmic vacuoles filled with amorphous material (Figs. 66 & 85). Immature plasma cells were rarely observed. Transition forms between lymphoid cells and plasma cells have not been observed in this study.

4) Mast cells

Mast cells were regularly present in the capsule, interlobular septa, and perivascular spaces surrounding blood vessels, especially large septal vessels. Occasionally, a few mast cells were also noticed scattered in the thymic parenchyma (Figs. 32, 57, 66 & 87). In the Azur II stained semithin-plastic sections,

mast cells were easily detectable by the metachromasia of their granules. Their ultrastructural appearance was similar to mast cells described elsewhere in other tissues (Weiss, 1983). In general, mast cells observed in the present study were of the mature type, and their number seemed also to increase with age. Transformation of lymphoid cells and epithelial-reticular cells into mast cells was not observed. However, on one occasion, an unusual mast cell with long cytoplasmic processes was observed in close contact with a fibroblast in the space surrounding a venule (Fig. 66).

5) Other cells, including eosinophils, monocytes, neutrophils, erythrocytes and fibroblasts were regularly present within the perivascular spaces (Figs. 33, 57, 61, 66 & 89). Erythrocytes were also frequently observed in the medulla, particularly around its blood vessels; in a few instances there were so many that it resembled a haemorrhagic area (Fig. 68).

On two occasions only, multinucleated giant cells were identified in the medulla. A close inspection of electron micrographs containing these cells indicated two possibilities: i) either these cells actually contained more than one nucleus, or ii) the nucleus in a cell could be highly lobated so as to reveal several nuclear profiles in one plane of section. These cells could be either macrophages (Von Gaudecker, 1978) or epithelial-reticular cells (Singh, 1981).

A special search was made in an attempt to find myoid cells (cells containing numerous intracytoplasmic thick and thin myofilaments arranged in a haphazard fashion) similar to those described in the human thymus (Bearman et al., 1978). With the exception of rare cells in Hassall's corpuscles, we failed to identify myoid cells in the rat thymus.

B. THYMIC BLOOD VESSELS

Electron microscopy showed that the thymic cortex is supplied mainly with capillaries whilst the medulla, in addition to the capillaries, contains many arterioles and venules (Figs. 43 & 67).

The capillaries of both the cortex (Figs. 44, 45 & 46) and medulla (Figs. 47, 48 & 54) had a very narrow lumina bounded by a wall consisting of continuous endothlium, a basal lamina, and pericytes in a thin adventitial layer of collagen fibrils. In a cross-section, portions of one to three endothelial cells bounded the lumen. Their cell junctions were simple approximations, or overlapping, and occasionally were interdigitated. Endothelial cells displayed an irregular luminal and abluminal contour. The cell membrane on the luminal side has numerous slender and finger-like cytoplasmic projections that enclosed vacuoles on contact with opposing membrane (Figs. 45, 47 & 54). Some vacuoles contained dense granular material, but most were empty. In animals which were injected intravenously with india ink, particles of carbon were seen captured and invaginated within these vacuoles. Numerous micropinocytotic vesicles were present scattered in the endothelial cell cytoplasm. Other cytoplasmic organelles included: mitochondria, occasional profiles of rough endoplasmic reticulum, a few fine cytoplasmic filaments and a few coated vesicles. One frequent observation was the presence of electron-lucent and

electron-dense endothelial cells constituting the walls of the capillaries (Figs. 44, 47 & 54). The capillary endothelium has a continuous basal lamina. Endothelial fenestrations were never observed, either in cortical, or in medullary, capillaries. Pericytes were always present outside the endothelial cells, which sometimes enclosed as much as half of the perimeter of a capillary or, as tiny processes of pericyte cytoplasm together with a few longer ones (Figs. 44-48 & 54). The pericytes were never found to make contact with epithelial-reticular cells, which is the most peripheral element of the capillary wall, or their basal lamina from which they were separated by an adventitial layer of collagen fibres.

Venules in the medulla had wider lumina, and their walls consisted of tall endothelium, basal lamina, layers of pericytes and a connective tissue adventitia (Figs. 49, 51, 62, 63 & 87). The fine structure of the endothelial cells was similar to that of the capillaries. Micropinocytotic vesicles were variable from cell to cell, so were the numbers of ribosomes, giving different electron-density to the cytoplasm of adjacent cells (Figs. 49 & 66). The basal lamina of the endothelium was surrounded by cytoplasmic processes of pericytes, bundles of collagen fibres, and occasional elastic fibres. The walls of the medullary venules were sometimes infiltrated with migrating lymphocytes, but the direction of migration could not be determined.

Arterioles at the cortico-medullary boundary had a lining of flattened endothelial cells, which were surrounded by a layer of smooth muscle cells (Figs. 52, 53 & 66). Peripheral to the smooth muscle cells was a sleeve of loose connective tissue, consisting of a few fibroblasts, collagen and elastic fibres and a variable number of lymphoid cells.

Perivascular spaces: Examination, by light microscope, of Haematoxylin and Eosin-stained preparations, revealed the existence of a space bounded by an eosinophilic basal lamina separating the thymic blood vessels, particularly the large septal vessels and those at the cortico-medullary junction, from the thymic parenchyma (Figs. 55, 58, 59, 60 & 79). This stain allowed the perivascular space to be easily distinguished from the surrounding parenchyma.

The presence of a distinctive epithelial basal lamina, bordering perivascular spaces, was confirmed by both scanning (Fig. 69) and transmission electron microscopy (Figs. 49, 51, 61-66, 85 & 87). The use of transmission electron microscopy showed that the thymic vessels were surrounded by a perivascular interstitial space of varying width, and appeared to be completely surrounded by elongated epithelial-reticular cell processes linked together with desmosomes and the associated basal lamina so that several well delineated layers were produced. These were formed by:-

1) endothelial cell cytoplasm, with a muscular coat in

arterioles and veins, bounding the vascular lumen;

2) endothelial cell basal lamina;

3) a perivascular space, of varying width, containing collagen fibres and cells;

4) epithelial-reticular basal lamina; and

5) epithelial-reticular cell cytoplasm.

Thus, the thymic parenchyma was separated morphologically from the perivascular space, and therefore the vascular lumina (Figs. 44, 46, 47, 51, 66, 69 & 87). However, around smaller blood vessels, particularly in the medulla, epithelial-reticular cells did not enclose the entire circumference of the perivascular space, leaving gaps often occupied by different cells (Figs. 45 & 50). Where this occurred, the basal lamina associated with epithelial-reticular cells also disappeared abruptly (Figs. 50 & 52).

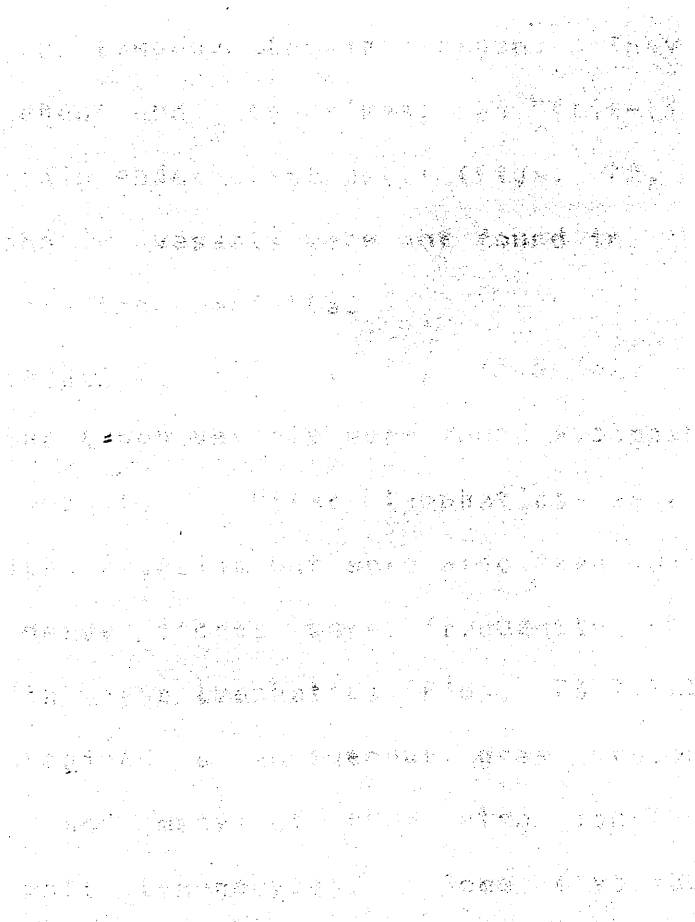
There may be two or more concentric perivascular spaces around a blood vessel (Fig. 69). Where this occurs, processes of epithelial cells extend across these spaces to enclose perivascular lymphocytes and into the thymic parenchyma to surround cortical lymphocytes (Figs. 61 & 64). The width of the perivascular space around thymic blood vessels is variable. This was clearly appreciated when the outer limit of the space was bounded by a continuous layer of epithelial-reticular cells. The perivascular space was particularly wide around the arteries in the connective tissue septa and the venules at the cortico-medullary junction (Figs. 49, 55, 59, 61 & 64).

The deeper, and smaller, the thymic vasculature became, the closer the two basal laminae (of both endothelial cells and epithelial-reticular cells) lay to one another, and as a result, the narrower the perivascular space became (c.f. Figs. 55, 59 & 49 with Figs. 58, 60 & 50 respectively). While the space was considerably narrower in the cortical and medullary blood capillaries, two separate basal laminae could still be demonstrated (Figs. 44, 48 & 54). The space around the capillary walls usually contained some collagen fibres embedded in amorphous, sometimes mottled, ground substance of low electron-density (Figs. 47, 48 & 54). However, profiles of a few lymphoid cells could also be seen occasionally.

While perivascular spaces around arterioles and venules contained large amounts of collagen and some cells (Figs. 51 & 52), those around septal arteries and veins were packed with a wide range of cells including lymphocytes, macrophages, plasma cells, mast cells, neutrophils, eosinophils, monocytes, erythrocytes and fibroblasts (Figs. 49, 57, 61, 66, 87 & 89). On some occasions, the spaces were so full with lymphoid cells, that it could easily be mistaken as an extra thymic lobule (Figs. 49 & 59).

It was constantly observed that perivascular lymphocytes were stained more deeply than those in the parenchyma of the thymus (Figs. 55, 59 & 79). This observation was further confirmed by electron microscopy

(Figs. 64, 65 & 91). Perivascular lymphocytes were characteristically smaller than the general parenchymatous lymphocytes. They were arranged in concentric rows, ranging from one to several, around the blood vessels (Figs. 55, 58 & 64). They were often surrounded by collagen fibres and fibroblastic or mesenchymal-like processes; all bathed in interstitial fluid.



C. THYMIC LYMPHATIC VESSELS

Each lobe of the thymus had at least one neurovascular bundle or hilus. The hilum was usually found at the postero-lateral aspect of the thymic lobes. Large efferent lymphatic vessels were recognised to form part of the hilum as they reach the thymic surface (Figs. 72, 73 & 74). Under the light microscope, lymphatic vessels, when traced retrogradely, followed the branching of the vascular tree and, therefore, were identified in the hilum, capsule, interlobular and intralobular septa, cortico-medullary junctions, and in the outer medulla. In general, thymic lymphatics were relatively few and less than would be expected for a lymphoepithelial organ. They appeared irregular in shape and, sometimes, as "slit-like" spaces surrounded by thin endothelial walls (Figs. 70, 79, 81, 82 & 83). Lymphatic vessels were not found in the thymic cortex nor in the inner medulla.

Hilar lymphatics: (Figs. 72, 73 & 74). Multiple, large irregular lymph vessels were found accompanying the hilar blood vessels. Hilar lymphatics were usually associated with arteries but were also seen with veins. Profiles of nerve fibres were frequently observed in association with these lymphatics (Figs. 73 & 74). Hilar lymphatics contained a homogenous gray precipitate of lymphoprotein, and many of them also contained large numbers of small lymphocytes. Some erythrocytes and macrophages were also seen in them (Fig. 74). Other cell

types, other than these, were not observed. These lymph vessels were lined by a single layer of endothelium, resting on a connective tissue layer. Endothelial nuclei bulged into the lumen. Typical bi-cuspid valves were found in them (Fig. 73).

Septal lymphatics: (Figs. 75-80). Examination of serial sections showed that hilar lymphatics were continuous with similar vessels in the interlobular and intralobular connective tissue septa. The septal lymphatics were usually smaller and less numerous than the hilar lymph vessels. They, too, mostly lay with arteries, however a few small vessels were associated with veins (Fig. 76). Small lymphocytes and a few erythrocytes were also seen in their lumina (Figs. 77, 78 & 80). A medium sized lymph vessel suddenly disappeared, sometimes 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. Some of the lymphatics were seen to branch, or re-unite. They followed either a straight or a spiral or a tortuous course.

Cortico-medullary lymphatics: (Figs. 81 & 82). As the septal lymphatics were traced serially towards the medulla, it was observed that they progressively became smaller at the cortico-medullary junction but they were still associated with blood vessels, mainly arterioles.

Medullary lymphatics: (Fig. 83). Subsequently, it was also observed that lymphatics at the cortico-medullary junction slightly extended into the medulla, but only as

far as the outer region of the medulla (Fig. 83). No lymph vessels were seen in the deep medulla. Some of the medullary lymphatics also were found to contain variable numbers of small lymphocytes.

Under the electron microscope, two types of lymphatic vessels were identified; lymphatic capillaries (usually present in the cortico-medullary junction and medullary parenchyma, Fig. 87) and collecting lymphatic vessels (that were found in the capsule and interlobular septa in addition to some lymphatic capillaries, Figs. 84 & 85). The fine structure of the thymic lymphatic vessels was, in general, similar to that of the lymphatic vessels present elsewhere in the body (Leak & Burke, 1966, 1968; Yoffey & Courtice, 1970; Weiss, 1983).

Ultrastructurally, the lymphatic capillaries were distinguished from blood capillaries by their attenuated endothelial walls, their relatively larger lumina, the presence of a discontinuous basal lamina, and the absence of pericytes around the capillary wall (Fig. 87). The endothelial cells had a greatly attenuated cytoplasm. The nucleus was often ellipsoid in profile with a thick rim of heterochromatin inside the nuclear membrane. On some occasions, the nucleus bulged into the capillary lumen (Fig. 87). In addition to the usual complement of cytoplasmic organelles, numerous micropinocytotic vesicles were also observed along both the luminal and abluminal surface of the endothelium. Larger vacuoles were always

found near the lumen, probably representing infoldings of cell membrane. The endothelial cells contained numerous fine filaments in the cytoplasm. The luminal surface of the endothelium frequently displayed small cytoplasmic processes. Similar, and occasionally longer, abluminal cytoplasmic projections from the endothelial cells extended into the surrounding connective tissue. Adjacent endothelial cells made contact with each other by simple overlaps or by interdigitations (Fig. 71). However, endothelial discontinuities, perhaps produced by the opening of an endothelial flap, were also observed in these lymph capillaries. Numerous extracellular anchoring filaments forming an irregular meshwork, were often found between the abluminal endothelial surface and collagen of the surrounding connective tissue (Fig. 71). The basal lamina beneath the endothelial cells was often discontinuous and poorly developed (Figs. 71 & 86).

The walls of the collecting lymphatic vessels consisted of tunica intima, composed of endothelial cells; a tunica media, formed by randomly arranged smooth muscle cells, and a tunica adventitia, containing collagen fibres that merged with the surrounding connective tissue (Fig. 84). Adventitial collagen fibres were abundant, apparently arranged in a haphazard manner. Numerous cells (small lymphocytes, macrophages, plasma cells, mast cells, eosinophils and fibroblastic-like elements) were observed lying in the connective tissue around the lymphatic vessels. Nerve bundles were also found in association with

the lymphatic vessels. The lumina of lymphatic vessels were filled with a gray precipitate of lymph protein, in contrast to the accompanying empty blood vessels due to vascular perfusion; and also contained a variable number of cell profiles (Figs. 77, 84 & 85). The cells were identified as lymphocytes (predominantly of small size), macrophages and eosinophils. Occasional erythrocytes were also seen. The lymphocytes were the most numerous cells in the lymphatic vessels (Figs. 77 & 84). These circulating small lymphocytes were round in profile with a thin rim of cytoplasm around the nucleus and a few cytoplasmic protrusions, or microvilli, emerging from the cell surface. The nuclei of these lymphocytes were sometimes deeply indented and, as a rule, stained darker than parenchymatous lymphocytes. They had a great deal of resemblance to perivascular small lymphocytes, from which they might originate.

Pre-lymphatic channels: (Figs. 89-92). These were non-endothelialized spaces or channels, frequently seen by the electron microscope, and were found in the perivascular connective tissue surrounding blood vessels, particularly large septal vessels. Their walls were mainly formed by thin cytoplasmic extensions of mesenchymal cells or fibroblasts and surrounded by bundles of collagen fibres. They contained gray homogenous interstitial fluid and numerous cells mainly small lymphocytes. These small lymphocytes were similar in shape and in the degree of

staining to those circulating lymphocytes present in the septal lymphatic vessels, except that the former had a smoother outer surface (c.f. Fig. 64 with Fig. 84). Other cells found in these fibroblastic spaces included macrophages, mast cells, and eosinophils. Quite often these spaces seemed to join together as they travel distally towards the hilum forming continuous channels which eventually entered the nearest septal lymphatic vessel. Thus, they acted as a "pre-lymphatic" pathway which channeled small, and presumably mature, lymphocytes and interstitial fluid from deep perivascular spaces, and became continuous with true lymphatic vessels, which they are so often associated with, in the perivascular connective tissue.

DISCUSSION

This study has dealt with the light and electron microscopic appearance of the histology, vasculature, and intrinsic lymphatics of the rat thymus gland. Previous studies, in different animals and in humans, have concentrated mainly on particular aspects of the structure of the thymus (Bearman et al., 1978; Clark, 1964, 1973; Haar, 1974; Hammer, 1921; Hirokawa, 1969; Hwang et al., 1974; Ito et al., 1969; Kameya & Watanabe, 1965; Lundin & Schelin, 1965; Mandel, 1968, 1970; Sainte-Marie & Leblond, 1964; Van Haelst, 1967), and also on the permeability of the thymic vascular tree and on the validity of the concept of a blood-thymus barrier (Abe & Ito, 1974; Bearman et al., 1975; Kramarasky et al., 1967; Pereira & Clermont, 1971; Raviola & Karnovsky, 1972; Weiss, 1963). Furthermore, although several authors have secondarily included or mentioned lymphatics in their original studies of the fine structure of the thymus (Bearman et al., 1978; Harris & Templeton, 1968; Hwang et al., 1974; Kotani et al., 1967; Omori, 1973; Smith, 1955), most of these studies have been based on light microscopic observations.

This part of the thesis confirms and extends these observations at both light and electron microscope levels.

A. STRUCTURE OF THE THYMUS GLAND

Light microscopy:

The rat thymus resembled that of other mammals, in being a lymphoepithelial organ, the supporting framework of which is composed of epithelial-reticular cells with interconnecting cytoplasmic extensions. Present within this framework are large numbers of lymphoid cells, a variable number of macrophages and interdigitating reticulum cells, and a small number of other cells that include plasma cells, mast cells, and eosinophils. The architecture of the cortex and medulla is basically similar. However, the cytoplasmic processes of epithelial-reticular cells are much finer in the cortex and are difficult to appreciate in hematoxylin and eosin stained sections. On the other hand, the medullary epithelial-reticular cells have a voluminous cytoplasm, and these form a much denser network in the medulla. A characteristic feature of the thymic medulla is the presence of Hassall's corpuscles with a structure suggestive of derivation from medullary epithelial-reticular cells. The light microscopic features of the rat thymus gland, as found in the present study, added little to the previous investigations by a multiplicity of authors.

Ultrastructure:

Lymphoid cells -

The ultrastructure of lymphoid cells in the rat thymus was, in general, similar to that reported by others

in avian (Kendall, 1981); mammalian (Abe & Ito, 1970; Clark, 1973; Hwang et al., 1974; Lundin & Schelin, 1965; Murray et al., 1965; Van Haelst, 1967); and human thymus (Bearman et al., 1978; Bloodworth et al., 1975; Von Gaudecker, 1978) and also applied in a major way to the lymphocytes of other lymphoid organs (Weiss, 1983; Yoffey & Courtice, 1970). Their sizes varied from 5 to 15 um in diameter, with all intermediate sizes represented. The sizes and ultrastructural differences of different forms of lymphoid cells corresponded to that reported for rat thymus by various authors (Hwang et al., 1974; Lundin & Schelin, 1965; Murray et al., 1965; Van Haelst, 1967). Large lymphocytes predominated in the peripheral cortical region; the deeper cortex and the medulla contained mainly small lymphocytes. Mitotic figures were most numerous in the subcapsular cortex (Bearman et al., 1978).

It seemed logical to assume, as others have done, (Murray et al., 1965; Sainte-Marie & Leblond, 1964) that the differences in size and other features noted among the thymic lymphoid cells were representative of a range of maturity. Thus, the large lymphocytes with leptochromatic nuclei, broader cytoplasm and more obvious nucleoli might be the least mature among these cells (Murray et al., 1965). However, it may not be justifiable to say that all small lymphocytes with pachychromatic nuclei and a thin rim of cytoplasm were highly differentiated and represented the end stage in a maturation process, as many small lymphocytes in the cortex as well as in the medulla

contained ring-shaped nuclei. Cells with ring-shaped nuclei have been regarded as resting cells capable of re-stimulation with respect to RNA synthesis (Smetana & Ivanyi, 1972).

The origin of lymphocytes in the thymus has long been a source of controversy. Although in some ultrastructural studies transitional forms and desmosomal junctions between lymphocytes and epithelial-reticular cells have been demonstrated and an origin of lymphocytes from eptithelial-reticular cells has been proposed (Ackerman & Knouff, 1965; Auerback, 1961), this has not been supported by other electron microscopical reports (Bearman et al., 1978; Lundin & Schelin, 1965; Mandel, 1968; Murray et al., 1965). No evidence of such a transformation of epithelial-reticular cells into lymphocytes was found in the present study, neither were desmosomal junctions observed between these two types of cells.

Experimental studies using interspecies nuclear differences in chimaeras (Le Douarin & Jotereau, 1981) have shown that the entire lymphoid cell population of the thymus appears to arise from blood-borne stem cells that immigrate into the thymic epithelial anlage. It is not clear where stem cells enter the thymus. Working with parabiotic rats, Brumby and Metcalf (1967) found that blood-borne stem cells accumulated near cortical blood capillaries. However, Sainte-Marie (1973) reviewed cell migration into and out of the thymus and suggested that the

stem cells could enter via the capsule and move along the connective tissue septa into the thymus.

Epithelial-reticular cells -

The present study confirmed that certain ultrastructural characteristics were consistently found in the epithelial cells of the thymus, namely, many well-formed desmosomes, bundles of branching tonofilaments and their association with a basement membrane whenever they came in contact with connective tissue.

The long branching epithelial cells formed the framework of the thymic parenchyma. Although the framework appeared equivalent to that found in the lymph nodes and spleen (Clark, 1962), there is abundant evidence from animal and human studies that unlike the framework of the lymph nodes and spleen which is mesenchymal-reticular (Weiss, 1983), the thymic framework is composed of epithelial cells (Bearman et al., 1978). Evidence in favour of this interpretation includes: the endodermal derivation of the thymus (Le Douarin & Jotereau, 1981), the keratinising nature of Hassall's corpuscles (Von Gaudecker & Schmale, 1974), the growth of thymic cells in sheets in tissue culture (Shelton, 1966) and most convincingly, the demonstration of desmosomes and tonofilaments in the framework cells in the normal animal (Chapman & Allen, 1971; Clark, 1963; Hoshino, 1963; Hwang et al., 1974; Mandel, 1968a,b; Van Haelst, 1967) and in the human thymus (Bearman et al., 1978; Bloodworth et al., 1975; Golstein & Mackay, 1969; Haar, 1974; Hirokawa, 1969;

Rosai & Levine, 1976). Thus, the term "epithelial-reticular" adequately describes these cells since it denotes both their epithelial nature as well as the tendency of these cells to have long cytplasmic processes. In general, the epithelial-reticular cells of the rat thymus varied greatly in morphology. Several investigators have described ultrastructural differences between cortical and medullary epithelial-reticular cells (Bloodworth et al., 1975; Chapman & Allen, 1971; Hirokawa, 1969; Hoshino, 1963; Hwang et al., 1974; Lundin & Schelin, 1965; Mandel, 1968; Van Haelst, 1967), as well as various sub-types of medullary epithelial-reticular cells (Ito & Hoshino, 1966; Kendall, 1986; Mandel, 1968). In human material studied by Bearman et al. (1978), the authors considered that the epithelial-reticular cells were identical throughout the thymus except when they were located within and immediately adjacent to Hassall's corpuscles. However, epithelial-reticular cells have been classified generally as pale and dark cells with reference to their electron density (Jarplid, 1974). In accordance with this worker who studied the thymus of mice, dark and pale epithelial-reticular cells of the rat thymus were also observed in the present study. Apart from other conventional cytoplasmic organelles, epithelial-reticular cells also contained many vacuoles and vesicles and a variable number of membrane-bound dense granules. Similar structures were noted by Clark (1963) and Lundin and Schelin (1965) in thymic

epithelial-reticular cells of mouse and rat respectively.

The function of the characteristic vacuoles and vesicles found in the cortical and medullary epithelial-reticular cells is still not clearly understood. Clark (1966) provided radioautographic evidence that after injection of ^{35}S-sulphate and ^{14}C-glucosamine, the label was incorporated into the vacuoles of medullary vesicular epithelial-reticular cells of mice; according to him these results indicated that the cells were manufacturing and perhaps secreting a sulphated mucopolysaccaride or glycoprotein. Subsequently, Clark (1973) showed a correlation between the amplitude of lymphopoiesis and the degree of ^{35}S-sulphate incorporation.

Considerable evidence has accumulated in recent years that the thymus secretes one or more hormones with lymphopoiesis-stimulating activity and/or immunocompetence inducing properties (reviewed by Dardenne & Bach, 1981). In experiments in which thymus-deprived animals were immunologically restored by thymic implants enclosed in cell-impermeable millipore chambers, the examination of the remnants in these chambers showed that the main component of the remnants was epithelial cells (Shelton, 1966). The results suggested that the epithelial-reticular cells were the principal source of a thymic hormone. In recent studies on serum thymic factor (TF) (a factor which is constantly present in the blood of normal animals, but disappears from blood a few hours after adult thymectomy) it was found that TF could be made to appear in the blood

by grafting a millipore chamber enclosed non-lymphoid thymoma or purely epithelial thymic tissue (Dardenne et al., 1974). Conversely, Garaci et al. (1976) found that when an antiserum prepared specifically against thymic epithelial cells was injected into a normal mouse, the level of circulating TF dropped quickly. Similarly, immunofluorescence studies by Teodorczyk et al. (1975) have shown that an antibody against a soluble thymic factor (STF) inhibited T-cell maturation in the thymus; and furthermore this STF was localised to thymic reticular-epithelial cells.

These results strongly suggest that the epithelial-reticular cells are responsible for the production of one or more thymic hormones.

The intrathymic location of the hormone-producing epithelial-reticular cells has not yet been completely clarified. Clark (1973) suggested that since both the proliferation and differentiation of thymocytes takes place mainly in the outer cortex, any hormone that induces these processes may be secreted by the cortical epithelial cells. In the present study, the most promising candidate for such a secretion appeared to be the granule-containing epithelial reticular cells which, besides other cell organelles, contained a number of homogenously electron-dense, membrane-bound granules.

However, in another reported experiment, a specific antibody to the thymic hormone, thymosin, was prepared and

applied to sections of bovine thymus. The antibody appeared to be localised to the epithelial cells of the thymic medulla (Mandi & Glant, 1973). Thus it was the medullary epithelial cells that were considered to be the cells involved in the synthesis of this hormone. However, Goldstein (1975) injected thymus-derived extracts containing other thymic hormones, thymopoietin I and II, into two rabbits separately to produce antisera. When these antisera were tagged with fluorescein and applied to sections of guinea pig thymus, the antiserum of one rabbit was localised to the cytoplasm of cortical epithelial cells only, while the antiserum of the other rabbit was localised only to medullary epithelial cells. Goldstein (1975) speculated that the antisera may have been produced against thymopoietin I and II respectively, and that these two hormones may thus arise from different cell types, one from the cortical and the other from the medullary epithelial-reticular cells.

Thus, it is likely that the epithelial-reticular cells may be producing several different hormones and these could be synthesised by different epithelial-reticular cell types. Some of these hormones, produced by cortical epithelial-reticular cells, could regulate lymphopoiesis and differentiation of lymphocytes whilst in the thymic cortex. Other hormones, produced by medullary epithelial-reticular cells could act to induce further differentiation of thymic lymphocytes in the medulla and/or be secreted into the blood to act in other lymphoid organs. None of

the thymic hormones so far documented have been associated with any particular type of epithelial-reticular cell.

Hassall's corpuscles -

The light and electron microscopic evidence presented here was in agreement with the observations of other authors which led to the suggestion that Hassall's corpuscles developed as a result of the specific differentiation of some thymic medullary epithelial-reticular cells into keratinising cells (Bearman et al., 1978; Chapman & Allen, 1971; Hwang et al., 1974; Kameya & Watanabe, 1965; Mandel, 1968b,c, 1970; Toro et al., 1965; Von Gaudecker & Schmale, 1974).

A spectrum of morphology ranging from Hassall's corpuscles (showing irregular shape and pyknosis of nuclei and a marked increase of tonofilaments and vacuoles in the cytoplasm) to multilayered large corpuscles were seen. Although the central elements of corpuscles were often difficult to identify, the great majority of cells involved were clearly epithelial in nature, as evidenced by an abundance of well developed desmosomes and tonofilament bundles. Secondary developmental changes occur, however, which modify the basic structure of the corpuscles (Mandel, 1968c). These changes included the development of a microvillous epithelial lining, cleft-like spaces around the periphery of some Hassall's corpuscles, and the occasional incorporation of macrophages and lymphocytes within their central cysts. It is these features derived

from light microscopical evidence that probably led to the suggestion that the Hassall's corpuscles were developed from blood vessels (Jaroslow, 1967). The cytoplasmic processes from the outer cells of the Hassall's corpuscles that joined to cytoplasmic processes of medullary epithelial-reticular cells and the findings that Hassall's corpuscles were not surrounded by a basement membrane were evidence against the concept that Hassall's corpuscles originated from thick walled venules.

The significance of cystic Hassall's corpuscles is open to speculation. The fact that experimental lymphocyte depletion by cortisone results in a great increase in the numbers of cystic Hassall's corpuscles (Cowan & Sorenson, 1964) possibly suggested that some cysts may represent currently unoccupied interstices of the cytoreticulum, accounting for both intracellular and intercellular cysts (Kohnen & Weiss, 1964).

The lining epithelial-reticular cells of cystic Hassall's corpuscles displayed microvilli; cilia were never observed, hence, no evidence was seen in the present study to support the hypothesis that these were remnants of thymopharyngeal ducts (Shier, 1963). However, the presence of ciliated cysts have been reported in the thymus of birds (Kendall & Frazier, 1979), guinea pigs (Mandel, 1968) and of the human fetus (Hirokawa, 1969). Hassall's corpuscles at various stages of development were seen and it is thus likely that new corpuscles are formed constantly. The development of clefts lined by microvilli may represent the

terminal events in a process of degeneration of the corpuscles.

Some investigators have described secretory granules in the epithelial-reticular cells forming Hassall's corpuscles, and proposed these as sites of thymic hormone production (Kater, 1973; Kohnen & Weiss, 1964). In this study, dark dense, membrane-bound granules scattered singly or in small groups were occasionally observed in the epithelial-reticular cells forming Hassall's corpuscles that might resemble secretory granules but, on the other hand, they also closely resemble the keratohyalin granules as pointed out by Bloodworth et al. (1975). Evidence of granules emptying into the lumen of cystic Hassall's corpuscles, as reported by Van Haelst (1967), were not seen in this study, nor was there found an intimate contact between Hassall's corpuscles and blood vessels, as described by Kater (1973). However, an extensive search for confirmation of these findings was not made. If there is always an intimate contact between Hassall's corpuscles and blood vessels, then perhaps the corpuscles represent a significant secretory mechanism for humoral substances (Kater, 1973). However, the significance of this remains to be determined.

A few investigators have shown that particulate material and antigens promptly reach the Hassall's corpuscles (Blau, 1967; Chapman & Bopp, 1970; Kater, 1973). Hassall's corpuscles with large numbers of granular

lymphocytes were shown by some authors (Kohnen & Weiss, 1964; Mandel, 1968c; Von Gaudecker & Schmale, 1974) who suggested the possibility that these substances could find their way into the corpuscles within the infiltrating leucocytes. However, this observation was not confirmed here.

While no positive evidence for the function, if any, of Hassall's corpuscles was obtained in this study, there was little morphological evidence to support the suggestion that the corpuscles were the site of degeneration of numerous lymphoid cells and thus act as a "graveyard for dead thymocytes" (Bearman et al., 1978; Blau, 1967). Indeed, the presence of numerous macrophages and pyknotic cells in the cortex and medulla suggested that removal of lymphoid cells most probably occurred outside the Hassall's corpuscles. These observations were in agreement with those obtained by Bloodworth et al. (1975) in the human, and Mandel (1968c) for the guinea pig thymus.

Macrophages -

Macrophages were readily distinguished from epithelial-reticular cells, since they lacked tonofilaments and desmosomes but contained many lysosomes, phagolysosomes, and vesicular inclusions. The variations in the morphological appearance of macrophages were interpreted as reflecting the state of their phagocytic activity (Bearman et al., 1978).

Many vacuoles near the cell membrane were possibly pinocytotic. Using electron-dense markers Cohn et al.

(1966) showed a regular flow of pinocytotic vesicles into the cells. Within the cells there were variable numbers of coated vesicles, such vesicles could be associated with absorption of protein into the cell (Carr, 1970). Other uncoated vesicles within the cells contained moderately electron-dense material. It has been suggested that these may represent stages in lysosome synthesis (Carr, 1967). Dense granules present in the thymic macrophages were similar to those in macrophages elsewhere - in splenic, or lymph node macrophages (Carr, 1970). Carr (1970) suggested that at least some of these granules were primary lysosomes. The rough endoplasmic reticulum was often rather prominent. The prominent Golgi apparatus and abundant rough endoplasmic reticulum suggested the possibility that these cells could be engaged in the secretion of some cell product (Beller & Unanue, 1977). The role of macrophages in thymic function is speculative. Phagocytosis may be one of the important functions of thymic macrophages. This is more evident under conditions of stress in which there is selective steroid-mediated destruction of cortical lymphocytes (Cowan & Sorenson, 1964; Ito & Hoshino, 1966). In the absence of stress, there is still a regular phagocytosis of some lymphocytes by macrophages (Bloodworth et al., 1975). It is speculated that these macrophages may play a monitoring role, removing those lymphocytes that are programmed against "self" (Bloodworth et al., 1975). An association between mitotic

lymphocytes and "PAS-cells" suggested to some authors that the role of the macrophage was more than that of a non-specific phagocyte (Metcalf & Ishidate, 1962). These cells, called "nurse cells" (Metcalf, 1964), were thought to trigger off mitosis in neighbouring lymphocytes. It is known, however, that in any rapidly proliferating tissue a number of the cells produced are defective; macrophages must then move in to engulf the defective cells, the fragments of which form PAS-positive inclusions. Macrophages may thus become automatically surrounded by one or more cells in mitosis (Miller & Osoba, 1967). The thymic macrophages may thus be simply engaged in phagocytosis of defective lymphoid cells.

Macrophages are known to play a role in the uptake, localisation and "processing" of antigens (Salman & Cordingley, 1980; Weiss, 1983). However, macrophages also are known to degrade antigens and soluble macromolecules into non-immunogenic forms (Friedman, 1978). The presence of macrophages in the parenchyma, often along blood vessels as shown here, and also reported by Raviola and Karnovsky (1972), may suggest that these cells perhaps serve a similar function in the thymus.

Interdigitating reticulum cells -

There is little disagreement about the essential ultrastructural features of an interdigitating-reticulum cell. The cells have been well described by Bennett (1978) and Olah et al. (1968). Where lack of accord exists, it is concerned with such details as the amount of endoplasmic

reticulum and the presence or absence of phagocytosis by these cells.

The present study confirmed that the interdigitating-reticulum cells occurred predominently in the medulla (Bennett, 1978; Olah et al., 1968; Von Gaudecker, 1978). Cells with identical morphology in the cortex have not been identified here. The well developed Golgi apparatus and rough endoplasmic reticulum found in the "special cells" of Olah et al. (1968) led them to speculate that these cells were involved in secretion, perhaps of thymic humoral substances.

All previous workers have commented on the lack of phagocytosis by these cells. On the basis of the absence of any phagocytosed material in the cytoplasm of these cells, Bennett (1978) distinguished them from macrophages. However, Klug and Mager (1979) demonstrated pyknotic lymphocytes inside interdigitating cells of guinea-pig thymus, and concluded that they may be classified as a special type of macrophage. The present study, however, confirmed the notion that interdigitating-reticulum cells were generally not phagocytic cells.

The known information on interdigitating-reticulum cells provides some indication concerning their function. The observation that these cells occurred exlusively in T-cell regions of peripheral lymphatic tissue and in thymus (Heusermann et al., 1974; Veerman, 1974) offers a strong argument in favour of their importance as a specific

microenvironment for T-cell maturation and differentiation. On the basis of the precise location of these cells in the thymic medulla and their absence in the cortex, Kaiserling et al. (1974) suggested that the functionally immature thymocytes developed in the thymic cortex, further differentiate into mature thymocytes in the medulla, perhaps under the influence of interdigitating-reticulum cells.

Mast cells -

Various investigators into thymic morphology have shown that prior to involution the distribution of mast cells in the thymus is mainly extra-lobular with only occasional cells inside the parenchyma, situated close to blood vessels (Bearman et al., 1978; Hwang et al., 1974; Weiss, 1983). During involution their numbers increase (Singh, 1980).

The present study confirmed the distribution of mast cells in the connective tissue of the capsule, septa, and perivascular spaces, with occasional cells scattered in the parenchyma of the rat thymus. The origin of thymic mast cells is not precisely known. Suggested sources of these cells have been mesenchymal cells, fibroblasts, lymphocytes, epithelial-reticular cells and plasma cells (Burnet, 1965; Weiss, 1983). The majority of mast cells observed in the present study were mature cells. Transformation of lymphoid cells and epithelial cells into mast cells was not judged likely as no cell of intermediate morphology was seen. However, the observation of the

unusual mast cell with long cytoplasmic processes, overlapped by processes of a fibroblast in the vicinity of a blood vessel (Fig. 66), might indicate some kind of close connection between both cells.

Plasma cells -

Although the thymus is sometimes said to lack plasma cells (Azar et al., 1963), the present study confirmed the findings of several workers on human and rodent thymus glands (Bearman et al., 1978; Bloodworth et al., 1975; Goldstein, 1966; Goldstein & Mackay, 1969; Hwang et al., 1974; Sainte-Marie, 1965; Van Haelst, 1967; Von Gaudecker, 1978), who suggested that the organ contained a few plasma cells located mainly in the capsule, interlobular septa, and around blood vessels in the thymic parenchyma. Most plasma cells were mature cells; immature plasma cells were rarely observed.

It was not possible to ascertain whether these plasma cells had originated in the thymus, or whether they were cells that had migrated into the organ after their formation elsewhere. However, several observations that suggested the latter possibility were:

(i) their presence mainly in the perivascular spaces surrounding blood vessels, and in the capsule and interlobular septa;

(ii) a large majority of these cells being morphologically mature (large ovoid cells, eccentrically placed nucleus, wheel-shaped appearance of nuclear chromatin, progressively

dilated endoplasmic cisternae); and

(iii) the absence of groups of plasma cells at various levels of maturation (presence of such islands were shown by Burnet and Holmes (1964) in NZB mice thymus, and by Kendall and Frazier (1979) in the avian thymus).

A migratory origin of plasma cells in normal rat thymus was suggested by Sainte-Marie (1965) and by Hwang et al. (1974), who proposed the presence of two-way traffic of lymphocytes across the wall of venules at the cortico-medullary junction; an outward migration of T-lymphocytes from the thymus, and an influx of B-lymphocytes from the blood. A similar two-way traffic of lymphocytes has also been suggested across the wall of post-capillary venules of rat lymph nodes (Sainte-Marie et al., 1982).

However, Goldstein (1966) observed an increase in the numbers of plasma cells in the thymus gland of patients suffering from various diseases. A high proportion of immature plasma cells in electron micrographs taken from patients suffering from certain autoimmune diseases led to the suggestion that they could have developed *in situ*, although the possibility of their migration from the blood stream was not ruled out (Goldstein, 1966). Possible transition forms between lymphoid cells and plasma cells were found by Van Haelst (1967) in the normal rat thymus. Electron microscopical evidence for such transformation was not secured in this study.

Eosinophils -

The observations of the present investigation indicated that some perivascular spaces showed eosinopoietic activity. Scattered eosinophils were occasionally seen in the thymic parenchyma. Sin and Sainte-Marie (1965) reported that, in the rat thymus, granulopoietic islands (independent islands showing eosinophil and neutrophil development) occurred in the peripheral cortex, cortico-medullary regions and in the medulla. However, they specifically mentioned the association of granulopoiesis with the perivascular spaces. This has also been observed in the human thymus (Bearman et al., 1978; Von Gaudecker, 1978). It seems likely that the perivascular spaces may be sites at which the thymic lymphoid cells are first exposed to circulating antigens (Clark, 1973), and in response to antigenic challenge, may modulate eosinopoiesis (McGarry et al., 1971).

The main function of eosinophils in the thymus, as elsewhere in other tissues, is not clear. There is sufficient experimental evidence that the eosinophils phagocytose antigen-antibody complex (Litt, 1964). Archer and Jackas (1965) have provided evidence that the eosinophil granules contain a potent mast cell degranulating and histamine-releasing agent namely, eosinophil-peroxidase.

B. THYMIC BLOOD VESSELS

In both humans and experimental animals, the blood vessels of the thymus, their permeability, and the blood-thymus barrier have been extensively studied. It has been suggested that the capillaries in the thymic cortex are largely impermeable to the circulating macromolecules whilst the veins present in the medulla do not impede the passage of macromolecules across the endothelial walls (Abe & Ito, 1974; Bearman et al., 1975; Blanc et al., 1973; Clark, 1964; Raviola & Karnovsky, 1972; Weiss, 1963). Around the thymic vessels, perivascular spaces have been described and it has been suggested that these spaces could channel lymphocytes into post-capillary venules (Bearman et al., 1975; Kostowiecki, 1967; Sainte-Marie & Leblond, 1964). The outer limit of the perivascular space formed by the epithelial-reticular cells and their basal lamina has been claimed to form a barrier against the passage of circulating macromolecules into the thymic parenchyma (Clark, 1963). The observations of the present study regarding the blood vessels will therefore be discussed with respect to their ultrastructure and their relationship with the thymic parenchyma.

In agreement with several observations on various species (Abe & Ito, 1974; Clark, 1964; Ito & Hoshino, 1966; Raviola & Karnovsky, 1972; Smith et al., 1939; Weiss, 1963) including human (Blanc et al., 1973), the present study showed that the majority of vessels in the thymic cortex

were capillaries, unlike the medulla, which contained arterioles and venules as well as capillaries. The ultrastructure of the blood capillaries showed the same features as are known for mammalian blood capillaries (Karnovsky, 1967; Palade, 1953; Simionecu & Simionecu, 1983) and agreed with the findings of Ito and Hoshino (1966). Endothelial cells contained pinocytotic vesicles, that were numerous along the abluminal cytoplasmic border (Van Haelst, 1967). Cytoplasmic densities that occurred at the endothelial cell apposition have been referred to as attachment belts (Bennett, 1956), and were similar to those present in the capillaries of the mouse thymus (Raviola & Karnovsky, 1972). Morphological evidence for the presence of carbon particles in the pinocytic vesicles, after intravenous injection of ink, as shown in the present study, and the evidence of accumulation of some ultrastructural tracers after intravenous injection (Abe & Ito, 1974; Raviola & Karnovsky, 1972) suggested that the pinocytic vesicles were to some extent, engaged in taking up circulating macromolecular elements.

The ultrastructure of venules present in the medulla correspond in general to the findings of Ito and Hoshino (1966) who studied the blood vessels of the mouse thymus. The large numbers of pinocytotic vesicles, large cytoplasmic vacuoles and at times dilated intercellular clefts between the endothelial cells of thymic venules, as seen in this study, might suggest that the endothelium of thymic venules do not form a barrier against the passage of

circulating macromolecules into the medullary parenchyma - an observation greatly emphasised by Raviola and Karnovsky (1972). In the present study, a limited vesicular transport of carbon particles occurred through the venular endothelium, but the vascular adventitia and thymic parenchyma were free of carbon, even at long time intervals after the intravenous injection. This is probably due to the large size of carbon particles. The venules of rat thymus were slightly different in their ultrastructure from the post-capillary venules described in the thymus glands of some experimental animals (Clark, 1963; Raviola & Karnovsky, 1972) and in human (Goldstein & Mackay, 1969; Pinkel, 1968). They had an ordinary, rather than a high, endothelial wall; and their endothelium was regularly infiltrated by lymphocytes. The lack of typical high endothelial walls in the normal thymic venules was also noted by Soderstrom et al. (1970) and Abe and Ito (1974). These authors also found that the "ordinary" or "thin" walled thymic venules and small arterioles were frequently infiltrated with lymphocytes. Thus, although the thymic post-capillary venules differed in morphology from the typical high endothelial post-capillary venules found elsewhere in lymphatic tissues (Weiss, 1983), these still retained at least the characteristics of diapedesis of lymphocytes across the endothelial wall (Soderstrom et al., 1970) and could be considered analogous to the high endothelial post-capillary venules of lymph nodes (Sainte-

Marie & Leblond, 1964). The present findings, and those of Soderstrom et al. (1970) and Sainte-Marie and Leblond (1964), were in contrast to those obtained by Bearman et al. (1975), Kameya and Watanabe (1965) and Von Gaudecker (1978), who did not observe ultrastructural evidence of lymphocyte migration across a vessel wall in the normal human material. In this study, diapedesis of lymphocytes across the vascular wall was observed, as also suggested by Hwang et al. (1974), Raviola and Karnovsky (1972) and Sainte-Marie and Leblond (1964).

In most accounts of the ultrastructure of the thymus gland, it is generally stated that the endothelial cells of the thymic vessels do not show pores or fenestrations (Bearman et al., 1975; Clark, 1963; Hwang et al., 1974; Kameya & Watanabe, 1965; Weiss, 1963). However, the presence of fenestrated capillaries in the thymic parenchyma, was first described by Kramarsky et al. (1967) in the thymus gland of the mouse and its presence was used as an argument against the existence of a blood-thymus barrier. The subsequent observations of Raviola and Karnovsky (1972) that the fenestrated capillaries occurred in the perilobular connective tissue and only exceptionally in the outermost regions of the thymic cortex led to the suggestion that these did not represent a significant leak in the vascular tree, at least for macromolecules since they were scarcely permeable to peroxidase. However, Raviola and Karnovsky (1972) suggested that this particular finding could be related to the development of adipose

tissue during thymic involution. A relationship to the endocrine function of the thymus was ruled out as these authors found that the fenestrated capillaries were commonly located outside the parenchyma. However, Singh (1981) demonstrated the presence of fenestrated capillaries in the parenchyma of human thymus. Since the thymus is regarded as an endocrine gland (Dardenne & Bach, 1981), in which a thymic hormone of low molecular weight (Goldstein, 1975) is released into the circulation in appreciable amounts; the presence of these fenestrated capillaries in the thymic parenchyma were used by Singh (1981) as a circumstantial morphological evidence to support the endocrine function of the thymus gland. The present study, however, did not confirm the presence of any fenestrated capillaries in the vascular tree of the rat thymus.

This study demonstrated the presence of a connective tissue perivascular space around thymic vessels that separated the vessels from the thymic parenchyma. The presence of a connective tissue space separating the thymic vasculature from the parenchyma has resulted in the space being referred to as a separate "compartment" of the thymus (Clark, 1973). However, the embryonic origin of this "compartment" is uncertain. Kostowecki (1967) stated that the perivascular space was formed by the envelopment of a vessel and a portion of its surrounding mesenchyme by the growing thymus and was in fact, extra-parenchymal. Further

evidence for the mesenchymal nature of the perivascular space was provided by Periera and Clermont (1971) in the mouse and Von Gaudecker (1977) in the human thymus glands. These authors demonstrated continuity between the perivascular spaces and the extrathymic connective tissue.

Several studies (Bearman et al., 1975; Bloodworth et al., 1975; Clark, 1963; Lundin & Schelin, 1965; Van Haelst, 1967) have demonstrated that two separate basal laminae bound this perivascular space and are each uninterrupted. This was considered to be the morphological evidence of the presence of a blood-thymus barrier, proposed by Marshall and White (1961).

The present study showed that whilst most cortical capillaries and some medullary vessels had a perivascular space bordered by a complete layer of epithelial-reticular cells and their basal laminae, others showed frequent interruptions of the epithelial-reticular cell layer, particularly in the medulla. Such discontinuities of the epithelial-reticular cell layer around thymic vessels were observed in a variety of experimental animals (Hwang et al., 1974; Ito & Hoshino, 1966; Van Haelst, 1967) and in humans (Von Gaudecker, 1978). Lymphocytes and macrophages lay in direct contact with the perivascular connective tissue space (Raviola & Karnovsky, 1972; Van Haelst, 1967). Moreover, erythrocytes were often seen in the vicinity of some blood vessels or at a short distance between thymic lymphocytes (Van Haelst, 1967). Consequently, it is clear that the blood-thymus barrier is not a complete

anatomically defined structure. Furthermore, since it has been shown that the epithelial-reticular cells and their basal laminae impede, but do not form an absolute barrier to the penetration of antigens (Clark, 1964; Kouvalainen & Gitlin, 1967) or ultrastructural tracers (Abe & Ito, 1974; Chapman & Bopp, 1970; Raviola & Karnovsky, 1972) it seems doubtful that much functional significance can be ascribed to the histological appearance of the blood-thymus barrier.

The function, if any, of the perivascular space is unknown. It has been proposed that the structures separating the vascular lumen from the parenchyma function as a blood-thymus barrier similar to the blood-brain barrier (Kameya & Watanabe, 1965). However, the present study, and other similar studies in rats (Hwang et al., 1974; Van Haelst, 1967) and in mice (Ito & Hoshino, 1966), demonstrated that the haemothymic barrier is structurally incomplete, due to apparent interruptions in the epithelial cell basal lamina. This was further confirmed by the variable permeability of the thymic vasculature to various ultrastructural tracers depending on the vessel size and location, and the molecular weight of the tracer particles (Raviola & Karnovsky, 1972). The endothelium of the cortical capillaries was essentially impermeable to particulate material, while that of the venules in the medulla and cortico-medullary junction was permeable (Raviola & Karnovsky, 1972).

It is well established that the thymus produces a large number of small lymphocytes (Kindred, 1940; Sainte-

Marie & Leblond, 1958, 1964; Weissman, 1967; Yoffey et al., 1961); and that they leave the organ by entering its blood vessels (Clark, 1963; Toro & Olah, 1967) and lymphatics (Kotani et al., 1966, 1967). It has been suggested by Bearman et al. (1975) and by Sainte-Marie and Leblond (1958) that perivascular channels could function as the final pathway for the migration of mature lymphocytes from the thymus. The light microscopic and ultrastructural observations of the present study provide further evidence in support of the idea that the perivascular spaces act as part of the pathway for emigration of small and mature lymphocytes leading to both vascular and lymphatic routes (discussed later).

Also, the perivascular space may be the site at which lymphocytes are first exposed to circulating antigen (Clark, 1973). Normally the thymus gland does not contain any lymphoid follicles with germinal centres, similar to those observed in lymph nodes (Yoffey & Courtice, 1970). However, under certain experimental and pathological conditions, germinal centres have been observed in the thymic medulla of different animals (Abe & Ito, 1970; Kotani et al., 1967; Marshall & White, 1961). This might suggest that the medulla is immunologically able to respond to antigens, although the mechanism of the formation of those germinal centres remains unknown. No typical germinal centres (with tingible body macrophages and mitotic figures) were observed in the present study of normal rat thymus. However, collections of parenchymatous

lymphocytes and foamy-like macrophages, containing degenerating lymphoid cells, and enclosed by a prominent epithelial basal lamina, were observed on two occasions (Fig. 88). The lack of immune responsiveness of the thymus, particularly of the medulla in which the blood thymus barrier is discontinuous may be because thymic lymphocytes are less responsive to antigens than are lymphocytes in peripheral lymphoid tissues (Weiss, 1963).

Finally, the perivascular space could be of diagnostic value in thymic diseases, as in thymomas, in which it frequently becomes dilated and contains large amounts of plasma and red blood cells, in addition to the cells normally present (Rosai & Levine, 1976).

C. THYMIC LYMPHATIC VESSELS

The general consensus is that lymphatic vessels of the mammalian thymus gland, including man, are confined to the interlobular connective tissue septa and the capsule, and that the parenchyma contains few or no lymphatics (Goldstein & Mackay, 1969; Harris & Templeton, 1968; Kameya & Watanabe, 1965; Kobayashi et al., 1964; Kotani et al., 1967; Rosai & Levine, 1976; Smith, 1955). However, it has recently been reported that the thymic medulla of man (Singh, 1980; Weiss, 1983) and of rat (Hwang et al., 1974) contained a few lymphatic capillaries, although none were found in the cortex. In the present study of the rat thymus, intrinsic lymphatics were found in the connective tissue of capsule, interlobular and intralobular septa, cortico-medullary junction and in the outer medulla. Lymph capillaries, characterised by an attenuated endothelium and discontinuous basement membrane, were identified in the cortico-medullary junction and in the outer medulla. Lymphatic capillaries, as well as collecting lymphatic vessels, constantly accompanied the blood vessels, particularly arteries, in the connective tissue septa. However, no lymphatics were found in the inner medulla, or in the cortex. Identification, by optical microscopy, of intrinsic thymic lymphatics in perfusion-fixed material (Fawcett et al., 1969) was based on the following criteria: (i) the structure of the lymphatic wall: irregular contour of sectional profiles, bounded by an endothelial lining.

(ii) the luminal contents of a palely stained precipitate of lymphoprotein, whilst blood vessels were empty. (iii) Cell types present in the vessels did not match with their ratios in the whole blood. (iv) Continuity between hilar, septal, and cortico-medullary lymphatics when traced in serial semi-thin sections.

There is much evidence that the thymus produces large numbers of lymphocytes that migrate to the thymus-dependent areas of the spleen and lymph nodes (Sainte-Marie & Leblond, 1958, 1964; Sainte-Marie, 1973; Weiss, 1983; Weissman, 1967; Yoffey & Courtice, 1970). This view is supported by cell labelling studies (Weissman, 1967); differences in thymic arteriovenous lymphocyte concentration (Sainte-Marie & Peng, 1971); the rarity of thymocytic pyknoses (Metcalf, 1964); substantial diapedesis of thymocytes across the thymic venules (Clark, 1963; Toro & Olah, 1967); the abundance of thymocytes in the perfusate of isolated thymuses (Folkman et al., 1968), and the depletion of thoracic duct lymphocytes following ablation or irradiation of the thymus (Engeset & Schooley, 1968; Schooley & Kelly, 1964). Emigrating lymphocytes appear in thymic veins (Clark, 1963; Toro & Olah, 1967) and lymphatics (Kotani et al., 1966, 1967) on their way to colonise peripheral lymphoid organs. The most obvious sites for thymocyte emigration are the medullary venules (Toro & Olah, 1967).

In the present study, numerous mitotic figures were observed, especially in the cortex, which reflects and

confirms the high mitotic activity of lymphocytes in the rat thymus (Hwang et al., 1974; Van Haelst, 1967). Furthermore, the ultrastructural evidence of direct entry of small lymphocytes into the venules of the cortico-medullary region (Hwang et al., 1974) was confirmed here. Although one cannot determine, in routinely sectioned material, the direction of movement of migrating lymphocytes seen in the walls of these venules, it is unlikely that the quantitatively small immigration of bone marrow stem cells into the thymus would be so conspicuous (Clark, 1973).

The necessity of considering the lymphatic drainage of the organ when accounting for the total thymic lymphocyte output has been pointed out by a few authors (Kotani et al., 1966, 1967; Joel et al., 1974). The perivascular spaces have been regarded by some authors as true lymphatic vessels through which both lymphocytes and, in the case of haemorrhage, erythrocytes leave the thymus (Harris & Templeton, 1968; Smith, 1955). 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". They described them as bounded by thin endothelium-lined walls, barely visible under the light microscope, and contained rows of lymphocytes. Furthermore, the walls of these channels

often showed small lymphocytes undergoing diapedesis, as did the walls of medullary blood vessels. However, electron microscopic observations of these spaces (Bearman et al., 1975; Clark, 1963) have shown that they do not have the morphology of lymphatic vessels. Lymphatic capillaries in the rat thymus have been shown to arise in the perivascular spaces (Kotani et al., 1967). Perivascular spaces surrounding the medullary vessels communicate with the interlobular septa (Kotani et al., 1967; Von Gaudecker, 1977) so it is possible that cells in the perivascular spaces could move in the septal connective tissue and find their way into the lymphatic vessels present there (Kobayashi et al., 1964). Thus, the perivascular spaces may function as part of the pathway for thymic lymphocyte migration (Bearman et al., 1975; Sainte-Marie & Leblond, 1958, 1964).

The ultrastructure of the rat thymic lymphatic vessels was similar to that described elsewhere for other mammalian tissues (Fraley & Weiss, 1961; Leak, 1970; Leak & Burke, 1966; Weiss, 1983). The interdigitation of the endothelial cell membranes with few desmosomes and the discontinuities in the endothelial cell wall, suggest that these vessels act as the main pathways for the removal of interstitial fluid and proteins, as elsewhere in the body (Leak, 1970) and possibly facilitate migration of lymphocytes from the medulla and the perivascular connective tissue. It has been suggested that only small

lymphocytes emigrate from the thymus (Ernstrom & Larsson, 1967). This study confirmed that the lumina of the lymphatic vessels contained predominantly small, and morphologically mature, lymphocytes. The thymic lymphocytes may mature during passage through the medulla (Sainte-Marie & Leblond, 1958, 1964). It was also generally observed in this study that circulating lymphocytes found in the lymphatic lumina, were smaller and stained more deeply than those found in the parenchyma of the thymus. Also, the thymic lymphocytes in the perivascular spaces were frequently small and deeply stained, as were circulating lymphocytes. The only difference between circulating and perivascular lymphocytes is that the former display a microvillous, whereas the latter have a smooth, cell surface (Van-Ewijk, 1980).

It is generally accepted that in some tissues (e.g. brain, retina, bone marrow, etc.) true lymphatic vessels are not found (Weiss, 1983; Yoffey & Courtice, 1970). However, Foldi et al. (1968) expressed the view that, in spite of the fact that there are no lymph vessels in the brain substance, the cervical lymphatics play a role of great importance in the drainage of cerebral interstitial fluid. The "perivascular spaces", first described by His in 1865, have been shown to be long "prelymphatic tissue channels" connecting the deep cerebral tissues with the cervical lymphatic system. Later, these "prelymphatic tissue spaces" were confirmed and repeatedly demonstrated in these organs when the lymph flow in the collecting

lymphatics in the neck has been obstructed (reviewed by Casley-Smith et al., 1976; Casley-Smith, 1978, 1980, 1982; Foldi, 1977; Hauck, 1972, 1973, 1982; Hauck et al., 1978). These "prelymphatic pathways" form a system of non-endothelialised and potential spaces, lying adjacent to the basement membrane of blood capillaries and in the adventitia of the larger blood vessels. They perform the function of lymphatics which empty into the true lymphatics, and which cause the same changes to be seen in the tissues they drain as are seen in tissues drained by true lymphatics when lymphostasis occurs. Thus, in such regions, the pre-lymphatics take over the role of the true lymphatics and travel for many centimeters until they discharge into the true lymphatics.

This concept of "prelymphatics" has aroused some controversy. While similar systems have been claimed to exist in various situations, e.g. the intestine (Kalima & Collan, 1976); mesentery (Hauck et al., 1978); cortical bone (Deysine, 1976) and the tongue (Casley-Smith, 1976), it has been objected that these are simply spaces in the interstitial tissue, which are present everywhere, and therefore do not justify any special title. A more serious objection has been that of Fiedler (1975), who considered that many of these spaces were artifacts caused by immersion fixation. However, Casley-Smith et al. (1976) re-examined this problem and still could demonstrate similar spaces in perfused-fixed material.

The findings of the present study showed the presence

of a specific system of tissue spaces situated in the perivascular connective tissue channels around thymic blood vessels, particularly the large ones. These formed irregular, randomly arranged, and interconnected channels which were frequently associated with collagenous bundles and other connective tissue elements. Some of these spaces were observed to end at a lymphatic endothelial junction, which was open or appeared openable (Collan & Kalima, 1974). The walls of these special spaces were formed by long fibroblastic or mesenchymal cell processes which joined or overlapped each other, as they travelled peripherally, forming a continuous system of spaces which eventually entered the nearest septal lymph vessel. They contained gray homogenous interstitial fluid and numerous cells, mainly small lymphocytes. These small lymphocytes were similar in shape and in the intensity of the staining to circulating lymphocytes found in the adjacent septal lymphatics. This suggests that small lymphocytes found in the lymphatic vessels had arrived from the "prelymphatic" channels. This is further evidenced by the presence of small lymphocytes in the lumina of lymphatics whose numbers gradually increased as the lymphatics were traced towards the hilum, so that many lymphocytes were present in septal and hilar lymphatics. Other cells found in these fibroblastic spaces included macrophages, mast cells, and eosinophils.

Very recently, similar fibroblastic channels have

been demonstrated in the portal tracts of the liver (Al-Jomard, Reid & Scothorne, 1985). They found that these channels drained lymph, and intravenously injected tracers, from the space of Disse to the portal lymphatics. Thus, they concluded that these fibroblastic channels acted as, and should be considered as, "pre-lymphatics".

In the thymus, it seems that as the septal blood vessels reach the cortico-medullary junction, and then branch into arterioles and capillaries in the medulla, their connective tissue sheath is reduced in thickness until it becomes very thin in the medulla (Bearman et al., 1975). As true lymphatic vessels ran in these connective tissue spaces in the vicinity of blood vessels, we were able to trace these lymphatics up to the cortico-medullary junction and less frequently in the outer medulla, but never in the inner (central) medulla where they disappeared. By the use of the electron microscope, concentric layers of small and darkly stained lymphocytes were frequently observed in these perivascular connective tissue spaces. They were enclosed by long fibroblastic processes which appeared to form a channel or "prelymphatic" pathway for lymphoid cells and interstitial fluid to travel through, from the deep parts of the thymic medulla to the nearest true lymphatic in the perivascular connective tissue.

It thus appears as if the connective tissue of the perivascular space, including the fibroblastic pre-lymphatic channels frequently observed within it, could

afford a significant route for the migration of small mature lymphocytes from the rat thymus gland. This perhaps helps to explain the relative lack of success of some authors in establishing diapedesis of lymphocytes through blood vessel walls as an important means of disseminating lymphocytes from the gland (Bearman et al., 1975; Ito & Hoshino, 1966).

SUMMARY

1) The intrinsic thymic lymphatics were investigated by both optical and electron microscopy. This was carried out by tracing them systematically in a retrograde manner from the hilum into the thymic parenchyma in serial semi-thin sectioned material. True lymphatics were identified in the connective tissue of the capsule, interlobular and intralobular septa, cortico-medullary junction and in the outer medulla. The cortex as well as the inner medulla contained no lymphatics. Lymph capillaries characterised by an attenuated endothelium and discontinuous basement membrane were demonstrated in the cortico-medullary junction and in the outer medulla. Collecting lymphatics, as well as lymph capillaries, constantly accompanied the branching of the vascular tree in the connective tissue septa, particularly large sized arteries.

2) Using electron microscopy, the present study also showed the presence of a system of irregular tissue spaces, situated in the perivascular connective tissue. These tissue spaces were frequently associated with collagenous bundles and other connective tissue elements. Their walls were formed by long fibroblastic or mesenchymal cell processes which joined or overlapped each other, to form a system of spaces that could be traced to enter a nearby true lymph vessel.

Thus, the name "pre-lymphatics" may be justified because of their function in transporting interstitial fluid and cells to the lymphatic circulation. They contained gray homogenous interstitial fluid and numerous cells, mainly small lymphocytes.

3) It was observed that circulating lymphocytes, found in the lumina of lymphatic vessels, were consistently smaller and stained more deeply, than those in the thymic parenchyma. Furthermore, the thymic lymphocytes in the perivascular channels were frequently deeply stained as were lymphocytes within lymphatics. Thus, the perivascular spaces, including the fibroblastic "pre-lymphatic" channels present in them, appear to play a role in carrying thymic lymphocytes from the medulla to the lymphatic circulation. This provides a significant route for the migration of lymphocytes from the rat thymus, supplementing that provided by blood vessels.

CHAPTER IV

LYMPHATICS OF THE DIAPHRAGM AND THEIR ROLE IN THE UPTAKE OF PARTICLES FROM THE PERITONEAL CAVITY

INTRODUCTION

Fluid, particles and cells such as erythrocytes when introduced into the peritoneal cavity are rapidly absorbed (Yoffey & Courtice, 1970). This rapid absorption occurs principally, if not exclusively, by the subperitoneal lymphatic plexus of the diaphragm (Allen, 1936, 1956; Casley-Smith, 1964; Cunningham, 1922; French et al., 1960; MacCallum, 1903; Odor, 1956). Lymph drains from the diaphragm predominantly into the mediastinal lymph nodes; the parasternal lymphatic trunk appears to be the main lymph pathway, with the thoracic duct usually being of minor significance (Higgins & Graham, 1929; Olin & Saldeen, 1964; Simer, 1948; Tilney, 1971).

The principal aim of the present study was to analyse the regional lymphatic drainage routes of the rat diaphragm and their relative importance, and also determine the speed of uptake of india ink following its injection into the peritoneal cavity. We also investigated the route of uptake of carbon particles by subperitoneal lymphatics of the diaphragm, with special emphasis on the role and function of peritoneal stomata; whether stomata exist in other serous membranes.

Starling and Tubby (1894) demonstrated that water and salt solutions containing vital dyes were absorbed from the serous cavities more rapidly than particulate substances. It was not until the work of Heidenhain (1895) on the "secretion" of lymph that the blood vessels were considered

as the major routes for absorption of fluid from the peritoneal cavity, with only small amounts being removed by the lymphatics (Orlow, 1895; Starling & Tubby, 1894). The cytological studies at that time revealed that the serous cavities were lined by a special type of flattened cells (Henle, 1866; Von Recklinghausen, 1863). Early investigators were also aware of the morphological similarities between cells lining the serous cavities and those lining blood and lymphatic vessels, and, in general, both groups were classified as endothelial cells (His, 1903; Paladino, 1883; Von Recklinhausen, 1863). Other workers, however, considered the serous lining cells to be more like epithelium than endothelium and classified them as epithelial cells (Bichat, 1827; Neumann, 1875; Waldeyer, 1883). Mall (1891) and Minot (1890), on the basis of comparative studies, used the term mesothelium in describing those cells lining the serous cavities, while the term endothelium was reserved for those cells lining the blood and lymphatic vessels.

The way, however, by which large particles and cells enter subserous lymphatic plexus of the diaphragm has been strongly debated for over a century.

Von Recklinghausen (1862) was one of the first to study, in some detail, the phenomenon of absorption from the peritoneal cavity. He claimed to demonstrate, by silver nitrate staining of the mesothelial lining, the existence of openings between the peritoneal mesothelial cells leading into a subserous lymphatic plexus, whose

terminal lymphatics were large, flattened, blind-ending cisterns, which Von Recklinghausen termed "lacunae". He demonstrated clearly, and for the first time, that the peritoneal cavity, like the blood and lymphatic vessels, is lined by single flattened cells with characteristic boundaries that could be sharply delineated. These flattened mesothelial cells were continuous except for small openings at the junctions of cell borders, which were called "stomata". These were considered as pores, which opened on the peritoneal surface on one side and communicated directly on the other with the underlying lymphatics within the diaphragm. He also observed that various tracers, such as milk, cinnabar, ink and cobalt blue entered the subserous lymphatics of the diaphragm via the stomata.

Von Recklinghausen's concept of open channels between the peritoneal cavity and the underlying lymphatics received much support from early studies of the structure and function of mesothelial cells, such as Beck (1893), Ludwig and Schweigger (1866), and Klein (1873). But there were those who disputed the concept of peritoneal stomata (Hertzler, 1901; Kolossow, 1893; MacCallum, 1903; Muscatello, 1895) and regarded the peritoneal cavity as being completely enclosed by a continuous layer of mesothelial cells, and interpreted Recklinhausen's stomata as artifacts resulting from silver salts reacting with serous fluids and forming deposits which adhered to the surfaces and boundaries of cells to give the impression of

pores or stomata. MacCallum (1903) emphasized that no one had succeeded in repeating Recklingausen's experiments. MacCallum (1903) thought that, when carbon particles were injected into the peritoneal cavity they were mainly taken up by phagocytes, which wandered through the serosa to reach the subserous lymph plexus in the diaphragm. He considered further that the connections between the cells of the peritoneal mesothelium and lymphatic endothelium were so frail that the powerful pumping action of the diaphragm is sufficient to force the material between the cells. However, MacCallum (1903) took the view that there was no difference in principle between his own and Recklinghausen's picture of the process, at any rate from a practical point of view. According to the one theory, direct and open connections were present between the peritoneal cavity and the lymph vessels, having the form of channels filled with the tracer substance; according to the other, the same functional relationship exists, on account of loose connections between the cells. The frequent appearance of particles within the cytoplasm of mesothelial cells led MacCallum (1903) further to suggest that there could be an alternative pathway of absorption, namely through the cytoplasm of the cellular layers. Thus, he concluded that more than one mechanism may be involved in the process of peritoneal absorption: intercellular, intracellular, and phagocytosis, and he regarded all these mechanisms as influenced by diaphragmatic movements during respiration.

Later, intracellular transport of particles into and through the cell mesothelial cytoplasm was supported by Cunningham (1920, 1922). In a later review, Cunningham (1926) summarised the position as follows: "The earlier work all tended to establish the concept of the presence of actual preformed physical openings between the peritoneal cavity and the diaphragmatic lymphatics. This idea was gradually eliminated, and in its place the concept of potential physical openings between cells was offered. In turn this hypothesis is being replaced by one which assumes that most, if not all, of the particulate material that is absorbed from the peritoneal cavity passes directly through the living cytoplasm of the mesothelial cells".

Since 1926, further evidence in favour of passage of cells and particles between temporary separation of mesothelial and endothelial cells at their borders, has been brought forward by Florey (1927), Florey and Witts (1928), Allen (1936) and Simer (1948). Florey (1927) and, later, Simer (1948) confirmed MacCallum's (1903) observations that the cell boundaries of the diaphragmatic peritoneum were outlined by carbon particles during absorption, with little evidence of passage through cytoplasm. Allen (1936) went a step further back towards Recklinhausen's original concept of preformed stomata. He used a variety of techniques and found that modified stomata do occur in the mouse diaphragm at the junction of mesothelial cells leading down through fenestrations in the basement membrane (Muscatello, 1895) to the intercellular

junctions of lymphatic endothelium. It is, according to Allen (1936), very significant that these stomata only occur at the sites of the subserous lymphatic lacunae, and not on other parts of the peritoneum. He also showed a beautiful photograph of many nucleated red blood corpuscles passing through the roof of the lacunae, following their injection into the peritoneal cavity. In a preparation of this kind Allen (1936) could count up to 600 such corpuscles in the act of being absorbed. The lymphatic lacunae, as well as collecting lymphatic vessels emerging from them, also contained many nucleated red blood corpuscles. Simer (1944), however, could not find such stomata in the diaphragms of cats or rats, but Hedenstedt (1947) observed what appeared to be a stoma of this type in a histological section of human diaphragm.

Allen and Vogt (1937) described the mechanism of lymphatic absorption from the peritoneal cavity as a diphasic mechanism, and their hypothesis is summarised as follows:- Lymphatic absorption takes place almost entirely through certain restricted areas of the serous lining of the diaphragm. In these areas there are fenestrations in the basement membrane and connective tissue layers, so that subserous lymphatics are virtually in contact with mesothelial cells. Tension on the serous lining and connective tissues during inspiration or expiration pulls the mesothelial and lymphatic endothelial cells slightly apart at their points of contact and dilates the subserous

lymphatics. Fluid, colloids and particles enter the lymphatic lumina, due to the gradient of pressure set up. With relaxation of tension the functional "stomata" close and the subserous lymphatics empty into the collecting and efferent lymph vessels. Thus, Allen and Vogt (1937) considered that the essential mechanism of lymphatic absorption was rhythmic stretching of the diaphragm during ordinary respiratory movements (MacCallum, 1903). This view was recently supported by Bettendorf (1978, 1979) and Wang (1975). Accordingly, paralysis of the diaphragm, by uni- or bilateral phrenectomy, resulted in a delay of absorption, by lymphatics of the diaphragm, of carbon particles injected into the peritoneal cavity of dogs (Higgins et al., 1930). Quantitative experiments have also shown that these findings apply, in rats, to the absorption of labelled red blood cells and protein molecules (Morris, 1953). Variations in respiratory pattern, as in different types of anaesthesia, could also affect the rate and speed of absorption in different experimental animals such as dogs (Mengle, 1937) and rabbits and rats (Courtice & Simmonds, 1949; Courtice & Morris, 1953; Morris, 1953). These authors also showed that increased ventilation, produced by breathing 5% CO_2 in air or O_2, increased the rate of absorption of protein and erythrocytes in anaesthetised or unanaesthtised animals. Apart from the respiratory movements, the intra-peritoneal pressure conditions play a role; decreased intraperitoneal pressure reduces resorption (Yoffey & Courtice, 1970), while

increased pressure promotes resorption (Florey, 1927; Higgins & Graham, 1929; Mengle, 1937; Tsilibary & Wissig, 1983). The effect of posture on lymphatic absorption from the peritoneal cavity has also been considered an important factor. It was shown that Fowler's position (pelvis-down, trunk at 45° to the horizontal) definitely impaired lymphatic absorption in rabbits (Courtice & Steinbeck, 1951; Dandy & Rowntree, 1914). Bangham et al. (1953) showed that radioactive glass particles were more slowly absorbed in rats in the head-up than in the head-down position, while absorption rates were intermediate in animals moving about normally.

In order to enter the lumen of a lymphatic lacuna materials absorbed from the peritoneal cavity must pass through a composite structure, which for convenience MacCallum (1903) referred to as the "roof" of the lacuna. It has been generally accepted that each roof consists of three main layers; a sheet of mesothelial cells facing the peritoneal cavity and in continuity with the mesothelium of the rest of the peritoneum; a lattice of connective tissue fibres; and an inner layer of endothelium in continuity with the endothelium in the walls and floor of the lacuna and ultimately with the endothelium of the efferent lymphatic vessels (Allen, 1936; Efskind, 1940; French et al., 1960; MacCallum, 1903; Muscatello, 1895).

As regards the rate of absorption from the peritoneal cavity, Cunningham (1926) claimed that the lymphatic removal of fluid from the peritoneal cavity is slow and

that significant amounts of fluid are not removed by this route. Courtice and Steinbeck (1950, 1951), however, found that the capacity of the diaphragmatic lymphatics to remove plasma proteins or 0.9% Nacl solution from the peritoneal cavity is considerable. As much as 20ml/Kg, about half the normal plasma volume, of whole plasma was absorbed in 3-5 hours in the rat, in 5-8 hours in the rabbit, and in 16-24 hours in the guinea pig. In addition to plasma protein and fluid, there is abundant testimony to the complete and fairly rapid removal of whole blood by diaphragmatic lymphatics from the peritoneal cavity in man and animals. A full review of the literature was given by Hedenstedt (1947). In experimental animals, the lymphatic transfer of labelled erythrocytes from the peritoneal cavity to circulation may be remarkably rapid and complete (Courtice & Morris, 1953; Hahn et al., 1944; Morris, 1953; Siperstein & Snasby, 1923). Species differences were also found in relation to the rate of removal of erythrocytes; thus absorption was most rapid in rats, intermediate in rabbits and slowest in guinea pigs when these three species were compared (Courtice et al., 1953). The fairly rapid lymphatic absorption of erythrocytes from the peritoneal cavity has suggested this route for blood transfusion in human, especially in infants (Hedenstedt, 1947; Clausen, 1940; Cole & Montgomery, 1929; Siperstein & Snasby, 1923).

In general, absorption of erythrocytes and plasma seems to be slower from the pleural than from the peritoneal cavity (Yoffey & Courtice, 1970). It also seems

that the passage of lymph and of particles and cells from the peritoneal cavity exhibits one-way traffic. MacCallum (1903) retroinjected the lymphatics of the diaphragm and failed to force suspended particles back into the peritoneal cavity except when using pressures sufficient to rupture the lymphatics.

In the last three decades many investigators, using electron microscopy and electron opaque tracers, have studied in detail the permeability of mesothelial lining cells (Baradi & Hope, 1964; Casley-Smith, 1964, 1967; Cotran & Karnovsky, 1968; Cotran & Majno, 1967; French et al., 1960; Fukuta, 1963; Leak, 1976, 1977; Marchesi, 1965; Odor, 1956). Odor (1956) studied the uptake and transfer of colloidal mercuric sulphide and thorium dioxide across the peritoneal cavity of the rat, and found that these particles were localised within mesothelial cells, in vesicles and within bodies having a relatively dense structure. Using colloidal metallic tracers, Fukuta (1963) and Marchesi (1965) found that these tracers crossed the mesothelium by way of pinocytotic vesicles. Baradi and Hope (1964) reported further on the fine structure of the rabbit peritoneal mesothelium; they emphasized that the intercellular spaces were often tortuous and labyrinthine, and that side branches took origin from the main intercellular channels and opened into the lumen or into lateral or basal ends of the cell. Cotran and Karnovsky (1968) confirmed Baradi and Hope's (1964) findings, by studying the permeability of mesothelial cells lining the

rat crotal sac using horseradish peroxidase as a tracer. They showed further that particles were localised in the intercellular cleft of the mesothelium often along their entire lengths, in vesicles adjoining or continuous with the cleft, and along the peritoneal and basal surfaces of the cell, and also in intracytoplasmic vacuoles. They also observed open intercellular junctions between some mesothelial cells. They concluded that their findings were consistent with physiological data which postulate that mesothelial transport can be accounted for, at least in part, by passive diffusion through a system of pores located in the intercellular clefts.

It is clear that these mechanisms of trans-mesothelial transport, whether intercellular or intracellular, still do not explain satisfactorily the rapid absorption of large particles and cells by the lymphatics of the diaphragm when injected into the peritoneal cavity, and were considered as secondary routes concerned mainly with the absorption of fluid and fine particles.

Recently, however, several workers studied the precise topography, using SEM, of cells lining the diaphragm and their 3-dimensional relationship with the submesothelial connective tissue layers and lymphatic lacunae; and proved beyond any doubt that openings or pores, which correspond to the "stomata" of Von Recklinghausen (1862) do occur between adjacent mesothelial

cells lining the peritoneal (Leak & Rahil, 1978; Tsilibary & Wissig, 1977, 1983) as well as the pleural surfaces (Wang, 1975) of the diaphragm.

Wang (1975) studied the ultrastructure of the pleural mesothelium of the rabbit diaphragm, and demonstrated the presence of stomata between mesothelial cells overlying lymphatics. He also observed that the mesothelium and lymphatic endothelium were continuous with one another at the rim of the stomata. Tsilibary and Wissig (1977), who studied the mouse diaphragmatic peritoneum by SEM, found that the lymphatic lacunae occur exclusively on the muscular portion of the diaphragm. They also observed that the mesothelial cells overlying non-lacunar areas were extremely flat, and their boundaries indistinct; whilst mesothelial cells overlying lacunae were cuboidal in shape and protruded towards the peritoneal cavity and had distinct outlines. Openings or stomata, 4-12 um in diameter, were found between these cells. Leak and Rahil (1978) studied the morphology and ultrastructure of mesothelial cells lining the peritoneal surface of the mouse diaphragm. Their observations agreed, in general, with those of Tsilibary and Wissig (1977). The stomata were special circular pores found between the margins of several neighbouring mesothelial cells. They suggested that these circular pores were formed when cell margins of lymphatic endothelial cells extend upwards to reach the mesothelial surface and form intercellular junctions with cell margins of several neighbouring mesothelial cells lining the

peritoneal surface of the diaphragm. This will provide an open channel between the peritoneal cavity and the lymphatic lumen. Intraperitoneally injected colloidal carbon and erythrocytes were rapidly removed from the peritoneal cavity via the circular pores or stomata. In addition to preformed stomata, Leak and Rahil (1978) recognised intercellular gaps between mesothelial cells overlying lymphatic lacunae. These gaps existed between interlacing filamentous processes at the lateral borders of the lacunar cuboidal cell.

Very recently, it was found that the ovarian bursa of the golden hamster also has stomata connecting the bursal cavity and lymphatic lumina (Shinohara et al., 1985, 1986). The existence of such stomata was regarded as functionally important for draining bursal fluid as well as cell components.

In the last hundred years, many investigators have studied the absorption of fluid, particulate matter, and cells from the peritoneal cavity. They used a variety of tracer substances in many different animal species. The most important of these observations are summarised in table 4.

A detailed study of the pathways of lymph drainage from the diaphragm of dogs after intraperitoneal injection of colloidal graphite was reported by Higgins and Graham (1929). They recognised five routes through which such particulate matter was removed: the parasternal, the

pulmonary, the thoracic duct, the retroperitoneal, and the direct peritoneal lymphatic routes. About 80% of the material was absorbed along the "parasternal" lymphatics, the most conspicuous and effective route. It arose from the diaphragm and ran along the internal mammary blood vessels parallel to the sternum, and drained into a series of lymph nodes between the first and third ribs in the upper thorax. The second route, the "pulmonary", consisted of small lymph vessels which drained the central part of the diaphragm to lymph nodes in the hilus of the lung, and occasionally to the anterior mediastinal nodes by a small lymph channel accompanying the phrenic nerve and blood vessels. The third route appeared to have no great significance for the transport of particles, and consisted of small lymph vessels which emptied directly into the thoracic duct. The remaining routes were also of no great significance; of the two, the "retroperitoneal" lymphatic route was more extensive and more constant in its distribution. It drained the diaphragm downwards to a large lymph node in the retroperitoneal space just above the kidneys, from which separate lymph channels passed obliquely to the cisterna chyli. The fifth and final lymphatic route, the "direct peritoneal", comprised a small lymph channel which passed directly from the diaphragm to the peritoneal cavity, and was traced to lymph nodes near the spleen or pancreas.

By means of lymphangiography, Menville and Ane (1932) and Bennet and Shivas (1953, 1954) examined the lymphatic

pathways from the peritoneal cavity of the rat. Menville and Ane (1932) injected thorium dioxide (Thorotrast) intraperitoneally and radiographed the animals at 24 hours and later at 2 weeks. Right and left lymphatic ducts, running from the diaphragm, were demonstrated in the chest, but their exact location was not described. They also observed connections between the intercostal lymph vessels and nodes, and the above mentioned lymphatic ducts. Bennet and Shivas (1953), however, injected 1ml of Angiopac (ethyl iodostearate) into the peritoneal cavity of ten rats. Each day one rat was sacrificed and radiographed. On lateral chest films a large lymph trunk was seen behind the upper part of the sternum. Moreover, some mediastinal lymph nodes were filled.

Simer (1948), after injecting india ink in the peritoneal cavity of the rat, reported that the pathways of lymph from the diaphragm corresponded to those described by Higgins and Graham (1929) in the dog. Most of the lymph passed by way of the parasternal lymph trunks, and drained into the mediastinal nodes, but some followed a dorsal course through the lymph vessels in the crura of the diaphragm to lumbar lymph nodes. He also noticed the presence of dense ink spots in the greater and lesser omenta, in the peritoneal fat, and in the mesentery. Further, he examined the anterior mediastinal and lumbar nodes microscopically and found abundant free ink particles in the peripheral nodal sinuses and a few ink-laden

phagocytes. He concluded that the major portion of lymph from the diaphragm passed along the parasternal route, since the anterior mediastinal nodes contained much more ink than did the lumbar nodes.

Courtice and Steinbeck (1950, 1951) studied the absorption of labelled plasma protein from the peritoneal cavity of rabbit, cat, guinea pig and rat. They found that in the rat the lymphatic drainage of the diaphragm included both ventral (parasternal) and dorsal (paravertebral) pathways, which ultimately reached the anterior mediastinal nodes. The dorsal route was a pair of lymphatic trunks of variable calibre, which at times appeared larger than the parasternal lymphatics, lying in the paravertebral position. At the intercostal levels there were irregular dilatations continuous with the corresponding intercostal lymphatics. In addition, they observed the occurrence of extra peritoneal collections of dye in the greater omentum and some areas of the mesentery.

Saldeen (1963) studied the anatomy of the lymphatic pathways from the peritoneal cavity in the rat by injecting colloidal silver, and recognised four lymph routes: parasternal, paravertebral, mediastinal and retroperitoneal routes. The first three ran cranially to drain into the mediastinal lymph nodes, which were situated adjacent to the thymus gland and were usually 6-10 in number. These nodes were stained after 5 minutes. The fourth and last lymph route "the retroperitoneal" ran from the diaphragm caudally to drain into a group of nodes situated

immediately to the left of the cisterna chyli, i.e. the cisternal lymph nodes which were 1-5 in number. Sometimes 1-5 lymph nodes, situated immediately to the right of the cisterna chyli (intestinal lymph nodes), were also stained. These nodes, whether the cisternal or the intestinal nodes, were stained after 10 minutes. However, after an interval of 15 minutes, small amounts of silver could also be demonstrated in the cisterna chyli. He added that the bulk of silver had reached the mediastinal lymph nodes, more often, via the parasternal lymph vessels. Histological examination revealed large accumulations of the injected silver in the mediastinal lymph nodes. The deposits were usually smaller in the cisternal and smallest in the intestinal, lymph nodes.

In the following year, Olin and Saldeen (1964) confirmed these findings by lymphography after intraperitoneal injection of thorium dioxide (Thorotrast) in the rat. However, in addition, the thoracic duct was never visualised on the radiograms following the injection, and they therefore concluded that most of the lymph in the thoracic duct originated from the intestine, and not from the diaphragm. They also found that both the liver and spleen were frequently opacified in the radiograms, which on microscopic examination, revealed the presence of thorium dioxide deposits in the parenchyma of both organs.

As part of a general study of lymphatic drainage in the rat, Tilney (1971) investigated drainage from the

peritoneal cavity by injecting pontamine sky blue or colloidal carbon into the peritoneal cavity. He found: 1) that the parathymic lymph nodes which are embedded in fat on the lateral aspect of the thymic capsule, drained the thymus gland, the peritoneal cavity and the superior surface of the liver and its capsule through the large parasternal lymph channels. These channels collected the effluent lymph from an extensive lymphatic plexus on the pleural surface of the diaphragm; 2) the posterior mediastinal lymph nodes lay adjacent to the oesophagus on the right, and to the left superior vena cava on the left side. The right node was usually the larger; both drained the thoracic viscera, pleural space, pericardium and the thymus gland; 3) small paravertebral nodes were found inconstantly behind the pulmonary vessels. They received lymph from the diaphragmatic plexus and were joined by intercostal lymphatics, and sent efferent lymph channels to the posterior mediastinal nodes; 4) the cisternal group, an inconstant cluster of minute lymph nodes, lay rostral to the left renal vein, and drained the suprarenal gland, retroperitoneum or diaphragm, and emptied into either renal node.

The role played by the thoracic duct in peritoneal absorption seems to differ slightly between various species and for different substances (Abdou et al., 1952; Courtice & Steinbeck, 1950; Courtice et al., 1953; Saldeen, 1963). In the rat 72% of intraperitoneally injected plasma protein reached the blood by routes other than the thoracic duct;

at the same time as 28% was found in the thoracic duct (Abdou et al., 1952). The corrresponding values for erythrocytes, however, were 75% and 25% (Saldeen, 1963). In the cat 20% of intraperitoneally injected labelled plasma protein appeared in the thoracic duct (Courtice & Steinbeck, 1950) and only a minor portion of a dose of erythrocytes, injected by the same route (Courtice et al., 1953). Olin and Saldeen (1964), however, could not visualise the thoracic duct on radiograms following intraperitoneal injection of Thorotrast in the rat.

MATERIALS AND METHODS

A total of 61 Swiss albino rats, of both sexes and from a closed colony in the Department, were used in this study. Their ages ranged from newborn to six months and their weights from 5-350g. They were divided into 3 groups, A, B and C.

Group A:

In a study of the lymphatic pathways from the peritoneal cavity, each of 50 rats received a single intraperitoneal (IP) injection of either diluted india ink or 3% pontamine sky blue, at a dose in each case of 0.5ml per 100g body weight. The site of injection was in the midline of the infra-umbilical region. A commercial ink was used, diluted 1:3 with freshly prepared saline, centrifuged at 3000 R.P.M. for 30 minutes and the supernatant was filtered through a Whatman No.1 filter paper. This procedure was repeated three times and the residual ink was then left uncovered overnight to eliminate, as far as possible, toxic impurities such as ammonia usually present in commercial ink. Before injection, the final suspension was once more filtered so that when it was spread on a slide and examined under the optical microscope it was shown to consist chiefly of uniformly sized carbon particles, about 0.5-1 um in diameter.

Most animals were killed, at intervals varying from 5-60 minutes after intraperitoneal injection, by an

overdose of anaesthetic ether; when the experiment required quick sacrifice, i.e. 1, 3, or 5 minutes after injection, animals were injected via a tail vein with Nembutal (Sodium pentabarbitone) at a dose of 0.25ml.

In all cases, the abdominal and thoracic cavities were opened and any residual ink in the peritoneal cavity, particularly over the peritoneal surface of the diaphragm, was rinsed away by saline. The vascular system was washed free of blood by perfusion for about 2 minutes with Ringer's solution containing 0.4% lignocain chloride as a vasodilator, through a cannula inserted in the left ventricle, and an opening made in the right atrium to allow wash-out of the blood. The lymphatic plexus of the diaphragm, containing the tracer, and the draining nodes in both abdomen and thorax, were then examined under a Wild M400 binocular dissecting photomicroscope with a magnifying power of, as a rule, about 10. Photographs of the desired area, at the desired magnification, were taken on 5" x 4" sheet film.

All abdominal organs and tissues covered with visceral peritoneum were also examined in situ for evidence of staining; special attention was paid to the diaphragm, liver, spleen, mesentery and omentum.

Group B:

For microscopic studies, 5 adult rats were killed 1, 3, 5, 15 and 20 minutes after intraperitoneal injection of india ink at the same dose and in the same manner as described above. After washing out the blood by vascular

perfusion with Ringer's solution for about 2 minutes, vascular perfusion was continued for 30-45 minutes with the fixative, a 5% solution of glutaraldehyde in Millonig's phosphate buffer at pH7.4. The perfusion solution contained dextran, 40.000MW (Fison) at a concentration of 3-5% W/V, to limit the development of interstitial oedema. Organs and tissues of special interest, i.e. intact diaphragm, the draining lymph nodes, liver, spleen, omentum, mesentery and peritoneal fat were quickly excised and immersed overnight in the same fixative as that used for perfusion. The tissues were rinsed several times in phosphate buffer and, while still in buffer, 2-4mm pieces of the diaphragm were cut with a razor blade under the binocular dissecting microscope. The blocks were orientated in such a way that the peritoneal surface of the diaphragm faced upward and sections were cut through the full thickness of the diaphragm in a plane transversely to its muscular fibres. These blocks were then post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in Spurr's resin. Semithin sections were cut at 1-1.5um in an interrupted series through each of about 5 blocks from each rat, giving a total of about 25 blocks. Alternate slides were stained with Azur II and with Haematoxylin and Eosin, and then studied under the optical microscope.

For transmission electron microscopy, selected areas from 7 blocks were trimmed down and ultrathin sections (60-80nm in thickness) were cut, picked up on uncoated 200 mesh

copper grids and double stained with uranyl acetate and lead citrate. The specimens were then viewed with the transmission electron microscope. Some perfused-fixed diaphragms were also processed for scanning electron microscopy (see later).

Group C:

In eleven animals a comparison was made, using SEM, between the visceral and parietal peritoneum and between these and the visceral and parietal pleura. The animals were killed, dissected and vascularly washed out as already described. Tissues of special interest were excised and fixed by immersion in 5% glutaraldehyde for 48 hours. In some tissues the serous fluids and proteins were removed from the mesothelial lining by a quick rinse in normal saline prior to fixation. The tissues included: peritoneal and pleural surfaces of the diaphragm, liver, spleen, abdominal wall, lungs, and costal wall. The tissues were washed well in fresh buffer, osmicated in 1% osmium tetroxide in Millonig's buffer, dehydrated through a graded series of ethanols, critical point dried in a Polaron carbon dioxide bomb, and mounted on aluminium stubs using silver or carbon electron conductive paint. The specimens were then coated with gold in a polaron sputter coater and observed in a JEOL T300 scanning electron microscope.

Two of the animals used in this group were 18 and 20 day-old rat embryos, in which the diaphragm was removed and fixed intact by immersion in glutaraldehyde and processed for SEM as described before. This was in order to study

the difference, if any, between surface mesothelium lining the diaphragm of adult and embryo rats, with particular reference to the existence of peritoneal stomata.

RESULTS

A. GENERAL ARRANGEMENT OF LYMPHATICS IN THE DIAPHRAGM

Both peritoneal and pleural surfaces of the diaphragm were covered by a single layer of flattened mesothelial cells. India ink injected intraperitoneally rapidly entered lymphatics of the subperitoneal plexus of the diaphragm; indeed, as early as one minute after injection, they were easily seen with the naked eye, because of their content of ink particles. After one hour they became intensely blackened and more clearly defined. When examined with the dissecting microscope, the subperitoneal lymphatic lacunae, filled with ink particles, had a characteristic appearance (Fig. 93). They were large elongated spaces running parallel to the muscle fibres of the diaphragm. They were separate and not continuous with one another. Their borders were sharply demarcated, so that lucanar areas could be easily distinguished from inter-lacunar areas (Fig. 93). They occurred exclusively in the muscular part of the diaphragm and were never observed in the tendinous part. In general, ink particles were distributed evenly in lymphatic lacunae of both sides of the diaphragm. The lymphatic lacunae intercommunicated at regular intervals by transverse anastomoses at a slightly deeper level and they emptied at a still deeper level, into a rich plexus of valved collecting vessels, which may be designated, according to their position, as intermuscular and perivascular collecting lymphatics.

A similar arrangement was found on the pleural surface of the diaphragm, where a subpleural lymphatic plexus also drained into the deeper collecting vessels located between muscle fibres of the diaphragm. Drainage from the deeper collecting lymphatics of the diaphragm was via several efferent lymph trunks to lymph nodes located in thoracic, as well as abdominal, cavities (Figs. 103-107). A small plexus of thin-walled lymph vessels was frequently observed behind the xiphi-sternum.

B. LYMPHATIC DRAINAGE ROUTES FROM THE DIAPHRAGM

Ink particles were absorbed rapidly by the subperitoneal lymphatics of the diaphragm and distributed via four lymph drainage routes; in order of importance they were:

1) Retrosternal: These paired lymphatic trunks were constantly seen in all the animals studied, usually as one trunk on each side of the sternum. Occasionally, either trunk may divide into two lymph vessels. These retrosternal lymph trunks ran, classically, parallel to the internal mammary blood vessels. The intensity of the dye and the calibre of these vessels were usually greater in comparison with other routes from the diaphragm. In almost all the animals, the retrosternal lymph trunks drained into the uppermost parathymic lymph node by branching into 3-8 terminal small afferent lymphatics. Descending distribution of dye from the uppermost parathymic node to nodes below in the same side, was frequently observed (Figs. 106 & 107).

2) Retroperitoneal: The left retroperitoneal lymphatics, usually 1-3 small trunks, were observed in more than three quarters of the animals injected intraperitoneally (Figs. 103-107). They pass caudally from the posterior part of the diaphragm and descend over its left crus to drain usually into one large, cisternal node. Efferent lymph from this node drained

into the left renal node (Figs. 103-107). No direct lymph drainage was observed from the diaphragm to the left renal node. The corresponding retroperitoneal lymphatics on the right side were seen less frequently (in about 20% of injected animals), but they often drained directly into the right renal node.

3) Intercosto-paravertebral: The paravertebral lymphatic trunks were observed in two thirds of the animals, following IP injection of ink, predominantly on the left side (Figs. 103, 105 & 106). These were paired lymphatics of variable calibre, occasionally larger than the retrosternal lymph vessels, and lying in the paravertebral position behind the pulmonary vessels. When examined carefully under the dissecting microscope, the paravertebral lymphatics showed small dilatations located at the level of posterior intercostal spaces and joined to one another by a network of small anastomosing lymph vessels. The paravertebral lymphatics finally drained, as a rule, into the posterior mediastinal node by 2-3 vessels. No transverse connections were observed between the paravertebral lymphatics of the two sides. The intercostal lymphatics were seen in about one third of the intraperitoneally injected animals (Figs. 103, 105 & 106). They were small vessels, 1-3 in number, which drained the dorso-lateral part of the diaphragm and ran posteriorly in the intercostal spaces to empty into the paravertebral lymph vessels (Fig. 105). In effect,

therefore, the paravertebral and intercostal lymphatics formed one system which drained into the posterior mediastinal nodes.

4) Mediastinal: This lymphatic route was observed in half of the injected animals, usually as narrow paired vessels. They accompanied the phrenic nerve and blood vessels in the mediastinal fat, and drained into one of the parathymic nodes (Figs. 106 & 107).

In about 20% of the injected animals, the posterior gastric node showed a tinge of darkening with ink particles, and their afferent lymph vessels seemed to come from the omentum. No connections whatsoever were observed between the diaphragmatic lymphatics and the thoracic duct. No other lymph nodes in the abdomen showed any degree of blackening with ink.

In all injected animals, the thoracic cavity was free from colouration except for the parathymic and posterior mediastinal nodes and their corresponding afferent lymphatic trunks arising from the diaphragm (Figs. 105, 106 & 107). However, in the abdominal cavity, in addition to the diaphragmatic lymphatics and their draining nodes, other organs and tissues showed a variable degree of black colouration, i.e. omentum in 100%; peritoneal fat in 80%; mesentery and mesovarium in 70%; and liver and spleen in 50% of intraperitoneally injected rats. The visceral peritoneum of the liver and spleen retained the ink in a characteristic mosaic pattern, which was even more evident after injecting pontamine sky blue, rather than india ink.

In around one quarter of the injected animals, the parietal peritoneum lining the abdominal wall also showed some retention of the ink, but in an irregular fashion.

The general pattern of lymph drainage from the peritoneal cavity of newborn and young rats was identical to that in the adults.

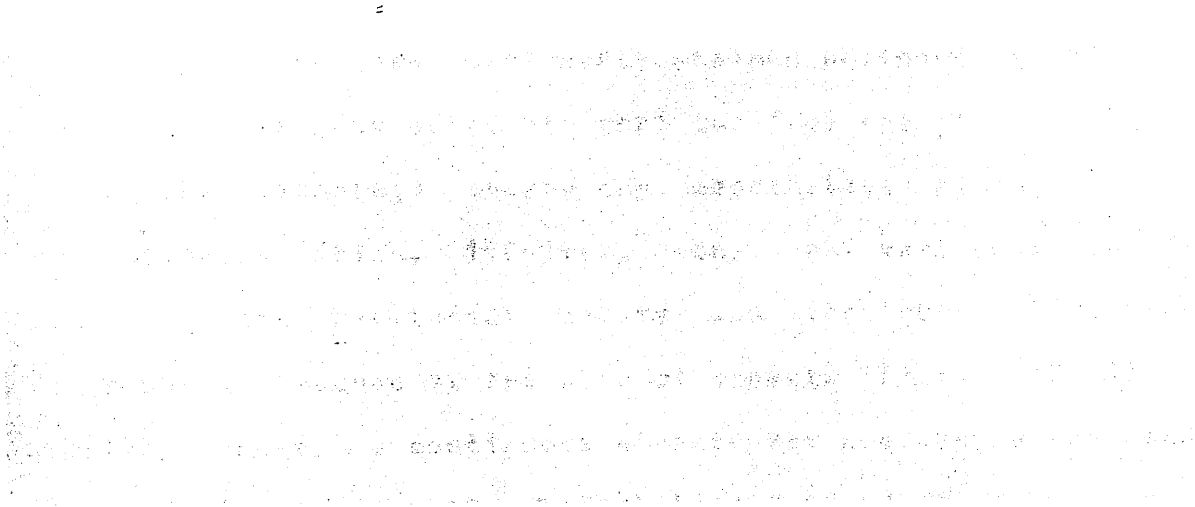
C. LIGHT MICROSCOPIC OBSERVATIONS

In semithin transverse sections of the diaphragm, a single layer of mesothelial cells was observed lining its peritoneal and pleural surfaces (Figs. 94-98). The cells rested on a connective tissue matrix within which lay a rich plexus of lymphatic lacunae. In sections from various areas overlying the muscular portion of the diaphragm, two morphologically different profiles of mesothelial cells were seen: In one (inter-lacunar area), the cytoplasm appeared extremely attenuated over major areas of the cell, while in the other (lacunar area), the cells had a cuboidal profile, and formed dome-shaped contours projecting above the surface of the diaphragm (Figs. 97 & 98). For the most part, the cuboidal cells were observed directly over the submesothelial lymphatic lacunae, while the more flattened cells covered the remaining areas of the diaphragm which lack submesothelial lymphatic lacunae.

1-3 minutes after intraperitoneal injection of india ink, traces of carbon particles were visible within the submesothelial lymphatic lacunae of the diaphragm; in the retrosternal lymphatic trunks and within the sub-capsular sinuses of the parathymic and cisternal lymph nodes (Figs. 96, 99 & 100). By 5 minutes these deposits were heavy and extended into the medullay sinuses of both nodal groups, being smaller in the cisternal node. Most carbon particles were free; however, some were engulfed by sinusoidal macrophages (Figs. 101 & 102). The number of carbon-laden

macrophages increased in proportion to the time intervals after injection.

The visceral peritoneum covering various abdominal organs and tissues was diffusely coated with aggregates of carbon, as was evident macroscopically 5-15 minutes after intraperitoneal injection. The omental milk spots, in particular, were quite heavily laden with carbon at this time interval; and microscopically there were small aggregates of carbon within the omentum. The boundaries of mesothelial cells were outlined by carbon particles. Some of the particles were also observed within interstitial spaces, surrounding fat cells of omentum, mesentery, and peritoneal fat. Intrinsic lymphatics of the omentum and mesentery contained only a few fine particles. At 20 minutes after injection, Kupffer cells of the liver were also heavily laden with carbon.



D. TRANSMISSION ELECTRON MICROSCOPIC OBSERVATIONS

The light microscopic observations were confirmed and extended by transmission electron microscopy. The lumina of the submesothelial lymphatic lacunae of the diaphragm were separated from the peritoneal cavity by a tissue barrier consisting, principally, of cuboidal mesothelial cells with an underlying thin basement lamina; a fenestrated layer of elastic tissue; bundles of collagenous fibres of variable thickness, and a rather thin endothelial cell layer (Figs. 110-114). These four principal layers formed the so-called roof of the lymphatic lacuna.

The nuclei of cuboidal mesothelial cells were large and palely stained, with peripherally arranged chromatin. Their cytoplasm was abundant in the area of the nucleus with many intracytoplasmic vacuoles, and very thin at the cell margins (Figs. 113-117). These cells displayed many microvilli on their free surface. On the other hand, mesothelial cells from inter-lacunar regions were more flattened and had more darkly stained nuclei (Fig. 109).

While many ultrathin sections from the lacunar region of the diaphragm showed the mesothelial lining to be continuous (Figs. 110-114), many areas were observed in which the peritoneal cavity was continuous with the lymphatic lacunae at the site of stomata (Figs. 117, 118 & 119). Here, a continuous channel was produced where the underlying lymphatic endothelium extended up to the peritoneal surface of the diaphragm to become directly

continuous with the surface mesothelium (Figs. 117 & 118). Here the mesothelial basal lamina and the collagenous fibres were absent. The junctional contacts between these two different cell types were maculae adherentes (desmosomes) similar to those observed between adjacent mesothelial cells and between adjacent lymphatic endothelial cells. This intimate association between mesothelial and lymphatic endothelial cells provided a direct passageway between the peritoneal cavity and diaphragmatic lymphatic lacunae. In such areas, intraperitoneally injected carbon particles had direct access to the lymphatic lumina (Figs. 117, 118 & 119).

The ultrastructure of lymph vessels and capillaries of the diaphragm was, in general, similar to lymphatics elsewhere as described by several workers (Casley-Smith, 1964, 1967; Leak & Burke, 1966, 1968; Weiss, 1983; Yoffey & Courtice, 1970). Their endothelial cell cytoplasm contained numerous pinocytotic vesicles, and the cell margins were joined by overlapping and interdigitating junctions (Figs. 123 & 124). Patent endothelial intercellular junctions were also observed (Fig. 125). They showed anchoring filaments between their endothelial cells and the surrounding collagenous fibres (Figs. 123 & 124).

Incidentally, blood capillaries with few fenestrations were observed in the diaphragm (Figs. 121 & 122). Their ultrastructure was identical to that reported elsewhere by other workers (Simionescu & Simionescu, 1983).

E. SCANNING ELECTRON MICROSCOPIC OBSERVATIONS

The 3-dimensional images of the peritoneal surface of the diaphragm also confirmed a non-uniform distribution of the mesothelial cells over its surface (Figs. 126, 127, 132 & 134). Areas in which the cell apices appeared rounded were clearly distinguishable from those areas of the diaphragm that were populated with very flattened cells. These areas are classified as lacunar and inter-lacunar respectively:-

1) Lacunar areas

Individual mesothelial cells overlying lymphatic lacunae were easily distinguished (Figs. 126, 127 & 134). Their boundaries were prominent and the central region of each cell, containing the nucleus, protruded (Figs. 128, 130 & 133-137). On the apical surfaces of these cuboidal cells were many microvilli. They also displayed elaborate cytoplasmic processes which extended from their lateral borders, so that processes from adjacent cuboidal cells interlaced to form a very fine anastomosing network with numerous intercellular channels between them (Figs. 128 & 136).

Many areas of the mesothelial lacunar roof were populated with pores or openings that occurred at the junction of several mesothelial cells (Figs. 130-137). These were oval or round-shaped preformed openings which measured 4-10 um in diameter. Their size and shape varied in different specimens depending on the state of the

diaphragm at the time of fixation. Some of the pores appeared as shallow pits which exposed parts of cells of the submesothelial connective tissue, while others were quite deep, too deep to be adequately examined with the SEM, forming channels which appeared to open into the underlying lymphatic lacunae (Figs. 130-137). These special openings between adjacent cuboidal mesothelial cells correspond to the "stomata" described originally by Von Recklinghausen (1862). In animals injected intraperitoneally with india ink, circular pores or stomata were very prominent in the peritoneal surface of the diaphragm. Carbon particles and erythrocytes were observed at the portals of the circular stomata apparently in the process of entering the pores (Figs. 128 & 139), and within the deeper recesses of the underlying lymphatic lacunae (Fig. 129).

2) Inter-lacunar areas

Mesothelial cells overlying inter-lacunar regions formed a continuous flat surface (Figs. 126 & 127). They were covered with variable numbers of microvilli, and the outlines of individual cells were ill-defined.

Incidentally, peritoneal mast cells were not uncommonly seen in the diaphragmatic peritoneum (Figs. 142 & 143) and in the visceral peritoneum of the liver (Fig. 155).

Other serous membranes

The scanning electron microscopic images of mesothelial cells covering the diaphragm differed from

those lining the rest of the serous cavities and visceral organs in that they contained stomata opening directly into a rich plexus of subperitoneal lymphatic lacunae. Stomata were only found in between mesothelial cells covering the peritoneal surface of the adult rat diaphragm and to a lesser extent in between those lining its pleural surface (Figs. 144 & 145). Also, they were identified in diaphragmatic peritoneum of 18 and 20 day-old rat embryos (Figs. 140 & 141). The number and patency of peritoneal stomata appeared to increase slightly following IP injections. No stomata were found in the parietal peritoneum or pleura; nor in the visceral peritoneum or pleura as shown in Figs. 146-155, and summarised in Table 5.

The visceral mesothelium covering the lungs and spleen was formed of a sheet of prominent cells with well-defined boundaries and their cell surfaces were studded with numerous short microvilli (Figs. 148, 149, and 152, 153 respectively).

Mesothelial cells of the liver surface were more flattened with less defined borders (Figs. 154 & 155). However, those of the parietal peritoneum and pleura formed a sheet of much flattened mesothelial cells with ill-defined boundaries and with many long microvilli on their surface (Figs. 146, 147 and 150, 151 respectively).

TABLE 4

Summary of previous studies on lymphatic absorption of various particulate matter and cells from the peritoneal cavity of different animals.

NO.	SUBSTANCE	APPROX. DIAMETER IN MICRA	EXP. ANIMAL	INVESTIGATOR	YEAR	APPROX. TIME TO REACH MEDIASTINAL LYMPH NODES
1	Milk, cinnabar & egg-yolk	1 um	Rabbit	Von Recklinghausen	1862	-
2	Carmine granules	1 um	Rabbit	Muscatello	1895	-
			Cat	Cunningham	1922	3 minutes
3	Chick erythrocytes	7 um	Rabbit	Buxton & Torrey	1906	15 minutes
			Cat	Cunningham	1922	3 minutes
			Mouse	Leak & Rahil	1978	-
			Rat	Bettendorf	1978	-
4	Frog erythrocytes	23 um	Mouse	Allen	1936	-
			Rat	Simer	1948	-
5	Homologous blood	8 um	Cat, rat, rabbit & guinea-pig	Courtice & Steinbeck	1950	-
			Human	Hedenstedt	1947	-
6	Human erythrocytes	7.22 um	Cat	Bolton	1921	-
7	Bacteria	?	Rabbit	Durham	1897	-
			Guinea-pig	Buxton & Torrey	1906	15 minutes
8	Parasites	?	Mouse	Tsilibary & Wissig	1983	-
9	Tumour cells (live & killed)	?	Rat	Saldeen	1963	-
10	Mould spores	10 um	Rabbit	Allen	1956	-
11	Yeast cells	6 um	Rat	Simer	1948	-
12	Tritiated thymidine labelled cells	?	Rat	Steer	1983	-
13	Carbon particles (india ink)	1 um	Dog	MacCallum	1903	-
				Higgins et al.	1930	5 minutes
			Rabbit	French et al.	1960	-
			Cat	Cunningham	1922	3 minutes
				Simer	1944	-
			Guinea-pig	Durham	1897	-
			Rat	Simer	1948	-
				Bettendorf	1978	-
				Takahashi & Patrick	1987	-
			Mouse	Casley-Smith	1964	-
				Whaley et al.	1972	5 minutes
				Tsilibary & Wissig	1977	-
				Leak & Rahil	1978	-
14	Colloidal silver	1 um	Rat	Saldeen	1963	5 minutes
			Dog	Brown	1928	10 minutes
15	Lampblack particles	1-2 um	Cat	Bolton	1921	-
				Cunningham	1922	3 minutes
16	Latex particles	1.1 um	Rat	Bettendorf	1978	-
17	Glass beads	12.5 um	Rabbit	Allen	1956	-
18	Paraffin-asphalt-spheres	22.5 um	Rabbit	Allen	1956	-
19	Chylomicra	1 um	Mouse	Casley-Smith	1964	-
20	Ferritin	0.1 um	Mouse	Casley-Smith	1964	-
21	Horseradish peroxidase	0.5 um	Rat & Mouse	Cotran & Karnovsky	1968	-
22	Thorium dioxide (Thorotrast)	0.3 um	Rabbit	French et al.	1960	-
			Mouse	Casley-Smith	1964	-
			Rat	Odor	1956	-
				Olin & Saldeen	1964	1 hour

TABLE 5

The occurrence of stomata, in peritoneum and pleura as viewed by SEM.

NO.	TYPE OF MESOTHELIUM	NO. OF STOMATA	FIGURES
1	Diaphragmatic peritoneum	Many	128-137
2	Diaphragmatic peritoneum after IP injections	Many	138 & 139
3	Diaphragmatic peritoneum of 18 & 20 day-old rat embryos	V. few	140 & 141
4	Diaphragmatic pleura	Moderate number	144 & 145
5	Parietal peritoneum (of abdominal wall)	Absent	150 & 151
6	Parietal pleura (of thoracic wall)	Absent	146 & 147
7	Visceral peritoneum of spleen	Absent	152 & 153
8	Visceral peritoneum of liver	Absent	154 & 155
9	Visceral pleura of lungs	Absent	148 & 149

DISCUSSION

It is well established that particulate matter and various cells when introduced into the peritoneal cavity are rapidly absorbed by the subperitoneal lymphatic plexus of the diaphragm (Courtice & Morris, 1953; Leak & Rahil, 1978; MacCallum, 1903; Weiss, 1983; Yoffey & Courtice, 1970). The terminal lymphatics or lacunae, which form this plexus, are separated from the peritoneal cavity by a thin wall, the roof of the lacuna (Allen, 1936; French et al., 1960; MacCallum, 1903). The location and general arrangement of the diaphragmatic subperitoneal lymphatic lacunae varies in different species. Lacunae are particularly abundant in the central tendon of dogs and rabbits (Florey, 1927; French et al., 1960; MacCallum, 1903), whereas in the mouse they are present exclusively in the muscular portion (Allen, 1936; Tsilibary & Wissig, 1977).

Our findings in the rat diaphragm were similar to those of the mouse. Due to rapid absorption of intraperitoneally injected ink, subperitoneal lymphatic lacunae appeared as separate blackened elongated channels with sharply demarcated borders running parallel to muscle fibres of the diaphragm, in a characteristic radial fashion.

Job (1915) and later Green (1935) first described the general anatomy of the lymphatic system in the rat, but did not discuss in detail the lymphatic drainage from the

peritoneal cavity. The only lymphatic route from the peritoneal cavity demonstrated by early workers was the retrosternal (parasternal) lymph trunks (Bennet & Shivas, 1953-1954; Brown, 1928; Courtice et al., 1953; Durham, 1897; Menville & Ane, 1932).

Our examination has revealed several other lymph pathways running from the diaphragm in cranial, as well as caudal, directions. All the lymphatic pathways and corresponding drainage lymph nodes are summarised in Figs. 105, 106 & 107.

The retrosternal lymphatics were found to be the most important route of drainage of lymph from the peritoneal cavity, since they were intensely stained with ink in all animals studied. As a rule, these lymphatics drained the anterior diaphragm into the uppermost parathymic node, which was blackened within one minute after injection. Higgins and Graham (1929) estimated that 80% of the lymph from the diaphragm does in fact pass through the retrosternal lymph trunks.

The drainage system through the intercostal and paravertebral lymphatics was mainly left sided. They drained the costal part of the diaphragm into the posterior mediastinal nodes. These lymphatic routes were not seen in all animals, either because they were absent or because they did not convey enough ink to be seen. The former is the more likely explanation, since lymphatics could be identified, under the dissecting microscope, even when they contained no ink particles. Thus, the intercostal-

paravertebral lymphatics were of less importance than the retrosternal lymphatics. The same was true of the mediastinal lymphatics, which drained the peritendinous area of the diaphragm to the parathymic nodes.

Drainage caudally by way of the retroperitoneal lymphatics was also greater on the left than on the right. These lymphatics drained the left posterior diaphragm into the cisternal node. Efferent lymphatics from this node then drained downwards into the left renal node. The corresponding retroperitoneal lymph drainage on the right side was less frequently seen; when present, it went directly into the right renal node. Thus, intraperitoneally injected materials spread more frequently to the cisternal and left renal than to the right renal nodes.

Olin and Saldeen (1964) studied the lymphatic pathways from the peritoneal cavity in the rat following IP injection of thorium dioxide (Thorotrast) and subsequent radiography of the animals 1-10 hours later. The pattern of the lymphatic system obtained, agreed well with that found in the present investigation.

The role, if any, played by the thoracic duct in absorption from the peritoneal cavity, appears to vary between different species and for different substances (Abdou et al., 1952; Courtice et al., 1950, 1953; Saldeen, 1963; Olin & Saldeen, 1964). In this study, we were unable to visualise any direct lymphatic connections or pathways

between the diaphragm and the thoracic duct. This difference may be due to the barrier function of lymph nodes through which the ink has to pass before reaching the thoracic duct or the cisterna chyli.

It is clear that in most of the species which have been studied, the drainage of lymph from the peritoneal cavity is probably similar in general to that in rats. Thus, the cranial route, mainly by way of the retrosternal lymphatics, dominates over the caudal route and the thoracic duct (Brown, 1928; Courtice et al., 1950; Higgins & Graham, 1929; Pomeranz, 1934; Saldeen, 1963). The intercostal-paravertebral, mediastinal and retroperitoneal lymphatics are usually only secondary pathways.

Tilney (1971) has defined the general drainage pattern of the lymph nodes in rats by using colloidal carbon and the dye pontamine sky blue. The present results partially support his findings. They differed only in the number of parathymic nodes which varied between individuals, but was normally 2-3 on each side; and to the location of the posterior mediastinal lymph node on the left side, which in this study was found to be located more ventrally, adjacent to the thymus gland (see Chapter II). We identified the structures described by Tilney (1971) as "paravertebral nodes" but found that they were in fact simply local dilatations of the paravertebral lymphatic channel opposite each intercostal space. We proved this by the simple expedient of probing them with the tip of the forceps, when they emptied. As to the distribution of

colloidal carbon to the lymph nodes after IP injection, in this study a relatively large amount of carbon was deposited not only in the parathymic lymph nodes but also in the posterior mediastinal nodes, whereas Tilney (1971) has reported that all the colloidal carbon injected intraperitoneally and draining to the thorax was collected in the parathymic lymph nodes.

Absorption from the peritoneal cavity by peritoneal surfaces of abdominal organs and tissues, other than the diaphragm, does not appear to be of much significance quantitatively. Courtice and Steinbeck (1951) working on rabbits, observed extra-peritoneal collections of dyed fluid in the greater omentum and some areas of the mesentery. Simer (1948), following the injection of india ink into the peritoneal cavity of rats, noted the presence of ink accumulations in the greater and lesser omenta. He also observed smaller spots of ink on the peritoneal fat and mesentery. The results of the present study showed similar macroscopic observations; various peritoneal surfaces retained the tracer, with a variable degree, in the form of dense spots or accumulations. This retention of the tracer was particularly prominent in the omentum, mesentery and peritoneal fat.

The extent of lymphatic absorption through the omentum has been disputed for a long time. Several workers demonstrated clearly the presence of a rich plexus of lymphatics, generally associated with blood vessels, in the

omentum of different animals and of man (Buxton & Torrey, 1906; Casparis, 1918; Rubin, 1911; Simer, 1934, 1935; Suzuki, 1910). Some, however, have doubted the existence of omental lymphatics (Shipley & Cunningham, 1916), or have been unable to observe them (Higgins & Bain, 1930; Tilney, 1971). Simer (1944) considered that omental lymphatics, although present, were of little importance in the removal of particles from the peritoneal cavity compared with those of the diaphragm. In this study, microscopic examination of semithin sections revealed the presence of many lymphatics in both omentum and mesentery, but with very few particles of carbon inside their lumina. The bulk of carbon deposits were found adherent to mesothelial cell surfaces, and also phagocytosed by interstitial connective tissue macrophages and macrophages of the omental milky spots. Thus, the action of macrophages which are present throughout these tissues, particularly the omentum, forms an efficient defence mechanism against particulate matter and, presumably, bacterial invasion. The power of the omentum to fix irritant particles by elaborating a fluid with marked coagulative properties and then of the macrophages, of milky spots, to ingest these particles is no doubt more important than removal by the omental lymphatics.

A few authors have reported, also, on the involvement of the liver and spleen in the absorption mechanism of materials injected into the peritoneal cavity (Olin & Saldeen, 1964; Roser, 1970; Whaley et al., 1972). Olin and

Saldeen (1964) found large amounts of Thorotrast in the liver and spleen of the rat 10 hours after its intraperitoneal injection, which presumably arrived via blood. Whaley et al. (1972) noted that Kupffer cells of the liver and perifollicular zones of the mouse spleen were laden with carbon deposits 30 minutes after its IP injection. Roser (1970) followed the fate of peritoneal macrophages labelled with radioactive colloidal gold in normal mice and found that, although 12 hours after cell transfer, labelled cells were confined to the subcapsular sinus area of parathymic lymph nodes, by 24 hours labelled cells were present within the parenchyma of the liver and spleen. Roser (1970), thus concluded that these labelled cells must have migrated through the parathymic nodes in order to enter the blood circulation. In this study, light microscopy showed some sinusoidal Kupffer cells of the liver were packed with fine particles of carbon 20 minutes following its intraperitoneal injection. No trace of carbon particles, however, were found before this time interval (i.e. after 5, 10 or 15 minutes). This suggests that 15-20 minutes are required for carbon particles to travel from the peritoneal cavity via subperitoneal lymphatic lacunae of the diaphragm and for subsequent distribution to draining lymph nodes in the thorax and abdomen. Particles would then leave the nodes via their efferent lymphatics, entering the venous circulation and finally reaching the liver sinusoids via the blood circulation.

In a number of early morphologic studies at light microscopic level, extensive use was made of tracer particles and various cells to study the absorption and permeability of the diaphragmatic peritoneum (Table 4). However, many of the findings remained controversial because the resolution provided by light microscopy was not adequate to give satisfactory information about the precise relationship between adjacent cells.

In subsequent studies at the electron microscopic level, tracer particles were also employed to study the permeability of mesothelial cells. When mercuric sulphide or Thorotrast was injected into the peritoneal cavity (Odor, 1956), the particles were observed within cytoplasmic vesicles in mesothelial cells of the diaphragm and the mesentery, but not in pores between adjacent cells. Similar findings were reported for colloidal metallic tracers by Fukuta (1963) and Marchesi (1963). The most extensive study designed to shed light on the problem of diaphragmatic mesothelial cell permeability was that carried out by French et al. (1960). They used the combined techniques of the classical "Hautchen" preparation (i.e. staining the intercellular cement with silver nitrate) at the light microscopic level and tracer experiments at the electron microscopic level. Their studies showed that the diaphragmatic mesothelial cells could easily separate from one another at the base of the intercellular junctions to produce gaps. Such gaps were found to be large enough to accommodate particles of

colloidal carbon, thorium dioxide, saccharated iron dioxide, and erythrocytes.

Other morphological studies on the permeability of the mesothelium at the electron microscopic level have provided information on the structure of intercellular junctions between mesothelial cells lining the scrotal sac (Cotran & Karnovsky, 1968). More recently, using combined thin-sectioning, scanning electron microscopy and freeze-fracture methods, Leak and Rahil (1978) demonstrated successfully the presence of pores or stomata between adjacent mesothelial cells within the roofs of lymphatic lacunae of the mouse diaphragm.

Our results corroborate the observations of Leak and Rahil (1978); mesothelial cells of the peritoneal surface of the rat diaphragm were organised into two discrete populations. A population of rounded or cuboidal cells was found in the roof covering submesothelial lymphatic lacunae, while the remaining non-lacunar (or inter-lacunar) areas of the diaphragm were lined by flattened (squamous) cells. The apical surfaces of both types of mesothelial cells were covered with many microvilli. The lacunar cuboidal cells displayed many cytoplasmic processes which extended from their lateral borders to form an anastomosing network with numerous intercellular gaps and channels. In addition to intercellular gaps, special preformed openings or stomata were found between the margins of several cuboidal mesothelial cells. Von Recklinhausen's (1862) original concept of stomata within the mesothelial lining

was based upon the appearance of circular deposits of silver nitrate within the intercellular junctions of several cells. Although other staining methods failed to confirm that these localised sites of silver deposits within circumscribed regions represented permanent openings (Hertzler, 1901; Muscatello, 1895), Allen's (1936) studies of diaphragms of mice impregnated with silver nitrate demonstrated that peritoneal stomata appeared as oval openings at the junction of three or more cells and were always located within the roofs covering the lymphatic lacunae.

The earlier studies of MacCallum (1903) had suggested that the superficial lymphatics in the diaphragm constituted the initial component of a separate and closed drainage system. He further defined the lumina of these lymphatic vessels as lacunae, suggesting that each was covered by a roof consisting of three distinct layers: 1) the lymphatic endothelium, 2) a thin layer of connective tissue, and 3) the surface layer of mesothelial cells. The observations in the present study confirmed the exclusive presence of peritoneal stomata in the roof of the lymphatic lacunae. Also, in addition to the 3 basic layers constituting the lymphatic lacunar roof, a fourth additional layer of fenestrated elastic tissue was frequently observed in the rat diaphragm.

The rapidity with which particles and cells of various dimensions are removed from the peritoneal and pleural cavities by the lymphatics of the diaphragm is well

documented (Allen, 1936; Cunningham, 1920, 1022; French et al., 1960; MacCallum, 1903; Simer, 1944, 1948; Von Recklinghausen, 1862; Wang, 1975; Yoffey & Courtice, 1970). However, the precise pathways taken by fluids, particles and cells had been more difficult to delineate, until Leak and Rahil (1978) settled this problem, in the mouse diaphragm, once and for all. In the present study, it is clear from scanning electron microscopic preparations and sectioned material taken from areas of the lymphatic lacunar roofs of the rat diaphragm that many of the stomata represent a true passageway between the peritoneal cavity and the lymphatic lumen. These stomata were formed when cell margins of several lymphatic endothelial cells span the submesothelial connective tissue to form intercellular junctions with cell margins of several neighbouring cuboidal mesothelial cells lining the peritoneal surface of the diaphragm. This uninterrupted pathway would facilitate the free and rapid uptake of fluids, particles, and cells of varying sizes from the peritoneal cavity directly into the underlying lymphatic lacunae.

In providing an explanation for the filling and emptying of lymphatic lacunae, previous investigators suggested that during rhythmic contraction and relaxation of the diaphragm, mesothelial and lymphatic endothelial cells within the roofs of lacunae are alternately separated to form intercellular gaps and brought together again (Allen & Vogt, 1937; Allen, 1956; Yoffey & Courtice, 1970). This hypothesis of functional stomata was recently

supported by Bettendorf (1978, 1979) who suggested that peritoneal stomata open passively to allow the passage of abdominal fluid and of latex beads injected into the peritoneal cavity during expiration, and that they close passively during inspiration; thus acting as valves. In contrast, Leak and Rahil (1978) reverted to the original description of Von Recklinhausen (1862) and suggested that stomata in the diaphragmatic peritoneum are permanent structures that are open irrespective of the movements of the diaphragm. More recently, Tsilibary and Wissig (1983) returned to the suggestion that peritoneal stomata of the diaphragm are not stable openings, but that their patency may vary in response to changing conditions in the peritoneal cavity; actin components in the cytoplasm of mesothelial and lymphatic endothelial cells may control their patency. Although the dimensions of the stomatal orifices may change with the rhythmic contraction and relaxation of the diaphragm, the observations in the present study suggest that they are stable structures and, therefore, support the view of Leak and Rahil (1978). Patent stomata connecting the peritoneal cavity with the lumina of lymphatic lacunae were consistently observed throughout all the diaphragms examined (a total of 11) regardless of the method of fixation (whether by immersion or by vascular-perfusion) or the state of contraction or relaxation of the diaphragm at the time of fixation. Peritoneal stomata were even observed in two rat embryos

aged 18 and 20 days at which time the diaphragm was, presumably, motionless (Figs. 140 & 141). Tsilibary and Wissig (1983) claimed that peritoneal stomata open when the diaphragm is relaxed by administration of succinyl choline and close, or disappear, when it is contracted by administration of carbachol. Thus, they speculated that the patency of stomata could be similarly altered during the normal respiratory cycle. Whether the patency of stomata does, in fact, change during the ordinary respiratory cycle is a difficult point to establish, because fixation of the diaphragm with conventional methods is, first, a relatively slow process and, second, necessitates major alterations of the environment within the peritoneal cavity. Such alterations in themselves, and any additional experimental manipulations similar to those produced by Tsilibary and Wissig (1983), might initiate reflex changes in patency of the stomata, and thus give false information.

It should be emphasised that stomata, with their associated channels, which connect the peritoneal cavity with the lumina of the lymphatic lacunae of the diaphragm, are structurally differentiated units: 1) Along the margin of a stoma, mesothelial and lacunar endothelial cells are joined to one another so that they line a channel that leads from the peritoneal cavity into the lumen of the lacuna (Leak & Rahil, 1978; Wang, 1975). 2) The submesothelial connective tissue at the site of a stoma is interrupted and in addition, is structurally modified to

accommodate the channel. 3) Both the mesothelium and lymphatic endothelium lack a basement membrane at the site of the channel.

This high degree of ultrastructural complexity of the stomata and associated channels, in addition to the reasons discussed above, lead us to conclude that they are stable structural units whose number and location are fixed.

The presence of lymphatic stomata in the diaphragmatic peritoneum was initially reported by Von Recklinghausen (1862), but their presence was confirmed ultrastructurally only recently by Leak and Rahil (1978) after more than a century of controversy (Baradi & Hope, 1964; Cunningham, 1926; Hertzler, 1901; MacCallum, 1903). Today, it is known that stomata are also present in the parietal pleura (Wang, 1975). By the use of scanning electron microscopy we have demonstrated the presence of peritoneal, as well as pleural, stomata between mesothelial cells lining both surfaces of the rat diaphragm. However, no stomata or openings were found in the remaining mesothelial lining of the peritoneal and pleural cavities (Table 5). This is probably due to the fact that mesothelial cells covering the diaphragm overlie a rich plexus of lymphatic lacunae which is strategically located within the submesothelial connective tissue, thus justifying the presence of stomata or openings that provide a rapid removal of fluid, particles and cells from the peritoneal and pleural cavities into the diaphragmatic

lymphatic vessels. Once fluid and particulate materials are within the submesothelial lymphatic lacunae, a contraction of the diaphragmatic muscles would also cause a contraction of the lymphatic lacunae, compressing the mesothelial cells of its roof and expelling its contents towards the larger collecting lymphatic vessels.

SUMMARY

1) The lymphatic drainage from the peritoneal cavity of the rat was studied following intraperitoneal (IP) injection of india ink. Peritoneal absorption of ink was remarkably rapid, indeed as rapid as one minute after its IP injection.

2) Absorption of ink from the peritoneal cavity was carried out by terminal lymphatics called lacunae, located immediately subperitoneally in the diaphragm. Within 1 minute, lymphatic lacunae filled with ink were seen running radially between muscular fibres of the diaphragm.

3) This rapid uptake of carbon occurred through preformed stomata, found only in the diaphragmatic mesothelium overlying the roof of lymphatic lacunae.

4) Lymphatic lacunae communicated directly with the peritoneal cavity via these stomatal openings between cuboidal mesothelial cells that lead into channels formed by juxtaposed processes of mesothelial and lacunar endothelial cells.

5) Stomata or openings, identified by scanning electron microscopy, were consistently and exclusively demonstrated in the diaphragmatic mesothelium, predominantly on the peritoneal surface; none were observed in the mesothelium lining other parts of the peritoneal and pleural cavities.

6) The regional lymph drainage from the diaphragm was

predominantly to the mediastinal lymph nodes (parathymic and posterior mediastinal nodes) by way of the retrosternal and intercosto-paravertebral lymph trunks, and also to retroperitoneal lymph nodes (cisternal and renal nodes) by the retroperitoneal lymphatic trunks.

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STUDIES ON THE LYMPHATIC SYSTEM

(i) The intrinsic lymphatics of the thymus gland;

(ii) The thymic haemolymph nodes;

(iii) Lymphatics of the diaphragm and the uptake of particles from the peritoneal cavity.

By

DR. MARWAN F.M. ABU-HIJLEH, MB.B.Ch.

A thesis presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow.

VOLUME II: FIGURES

Department of Anatomy,
University of Glasgow.

May, 1987.

Fig. 1

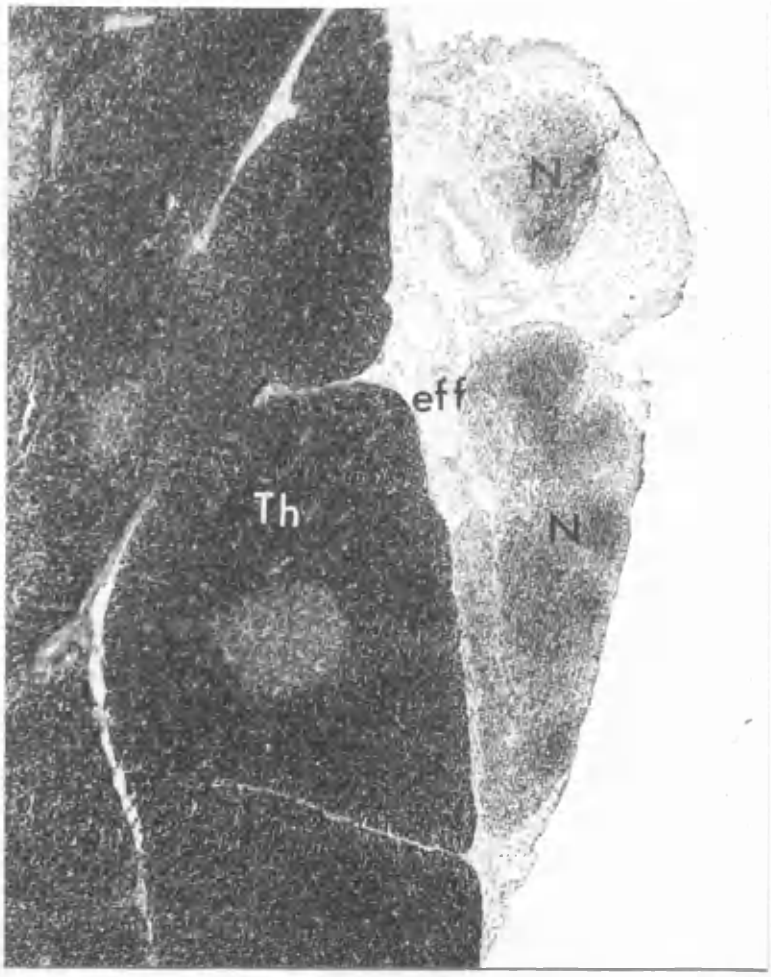
Low power view showing the normal relationship between thymus gland (Th) and thymic haemolymph nodes (N) embedded in its capsule. Note efferent lymphatic (eff) and blood vessels leaving the nodal hilum.

x 40 Young adult rat H & E

Fig. 2

The general topography of a thymic haemolymph node. Note: afferent (aff) and efferent (eff) lymphatics; intra-nodal blood vessels (bv); cortex with cortical nodules and germinal centres (g); medullary cords (c) and sinuses (s).

x 20 Adult rat H & E


N
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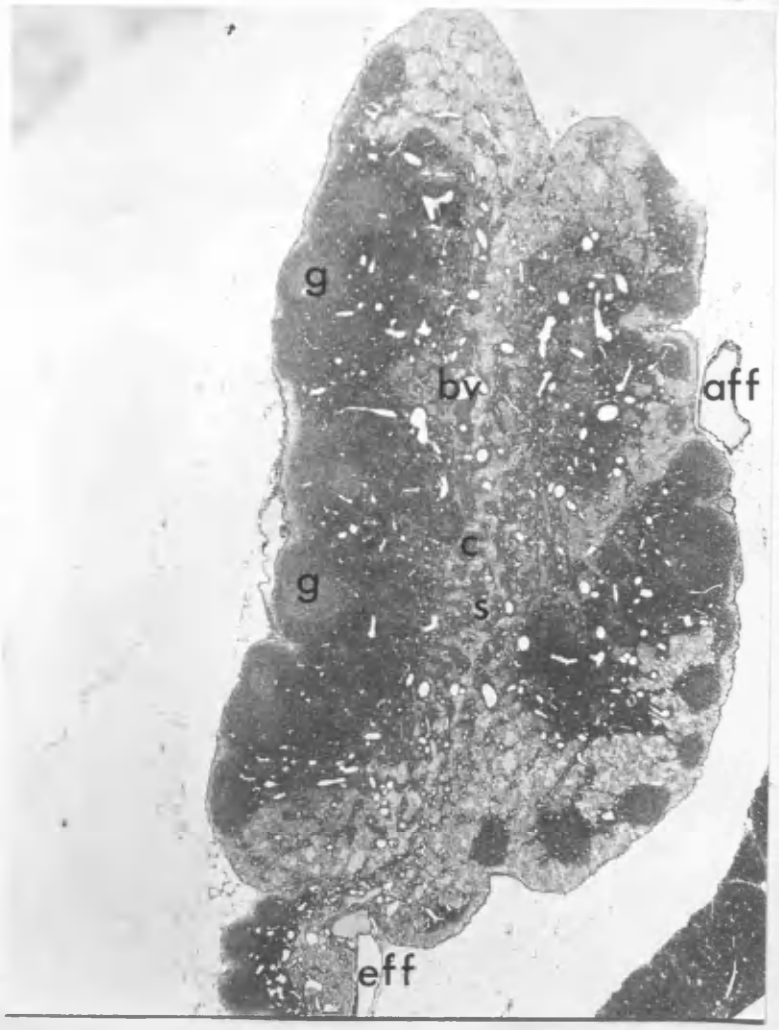
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Fig. 3

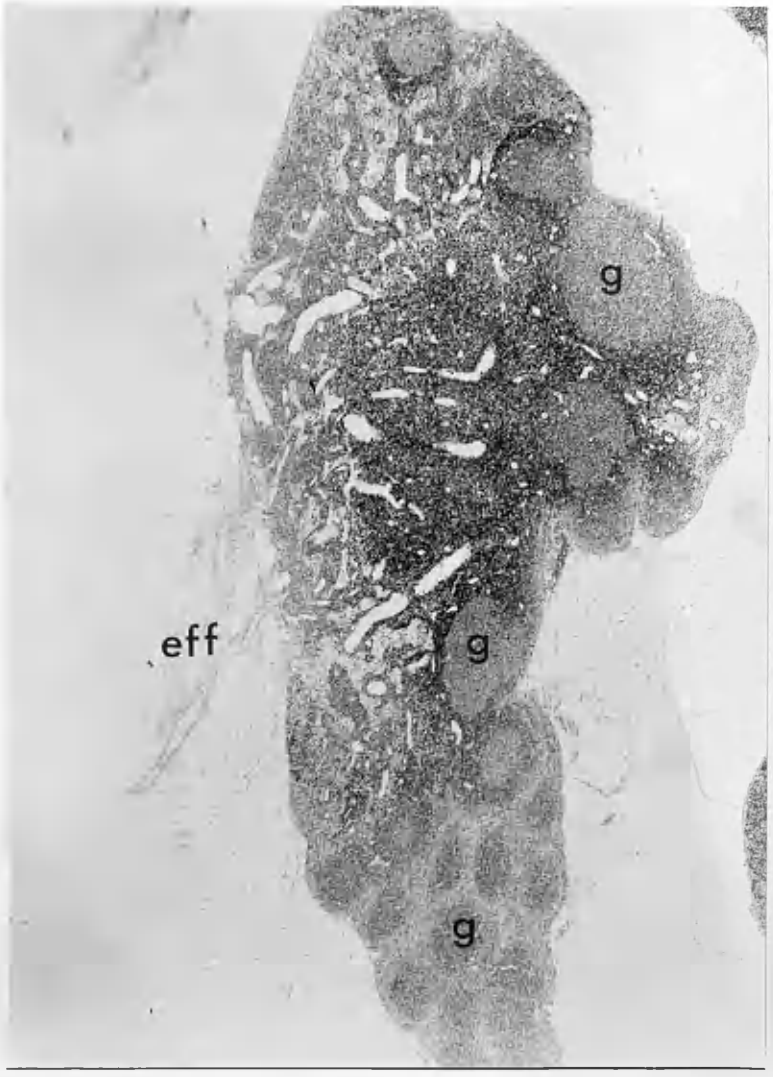
The general topography of another thymic haemolymph node. Note:
1) the peculiar shape of this profile
2) the well-developed cortex with many germinal centres (g)
3) efferent lymphatics (eff) and veins leave the hilum
4) Medullary sinuses converge on the hilum

x 32 Adult rat H & E

Fig. 4

A high power view of a germinal centre from the node shown in Fig. 3. It contains a pale-staining central zone, which is composed of medium and large lymphocytes and "tingible body macrophages" (arrows). The central zone is surrounded by dark-staining small lymphocytes, which constitute the mantle zone.

x 400 Adult rat H & E


g
eff
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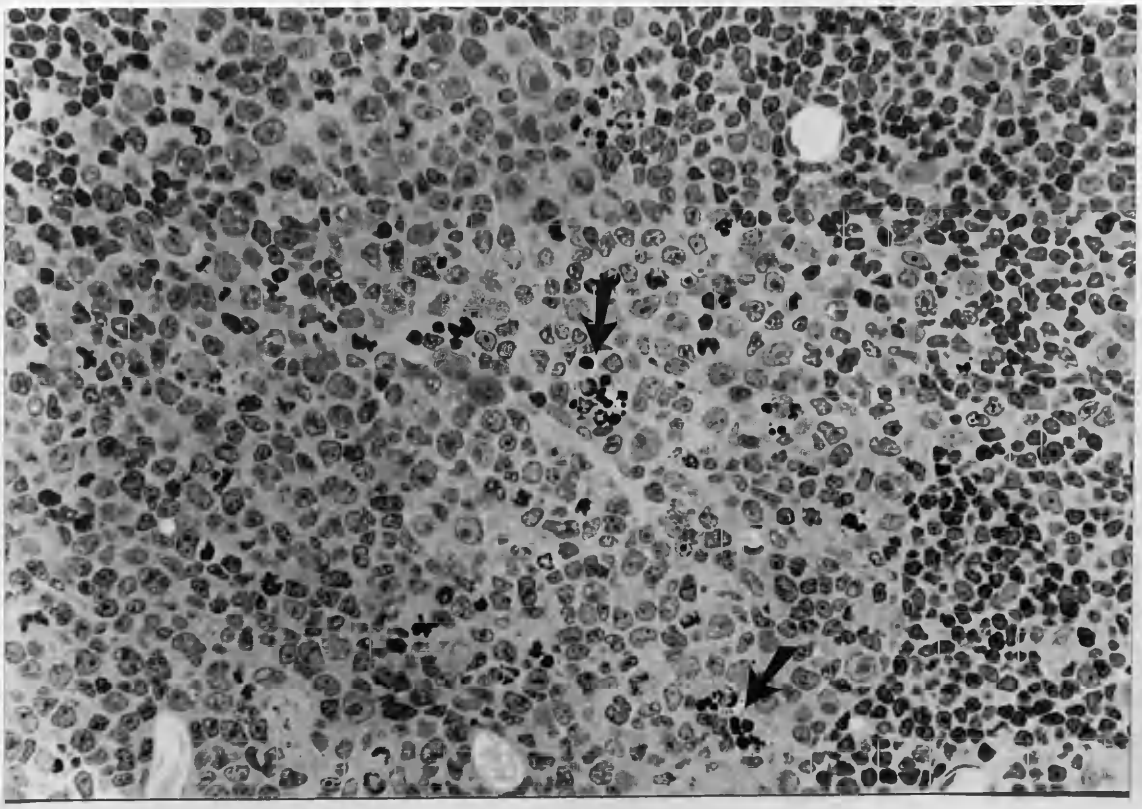


Fig. 5

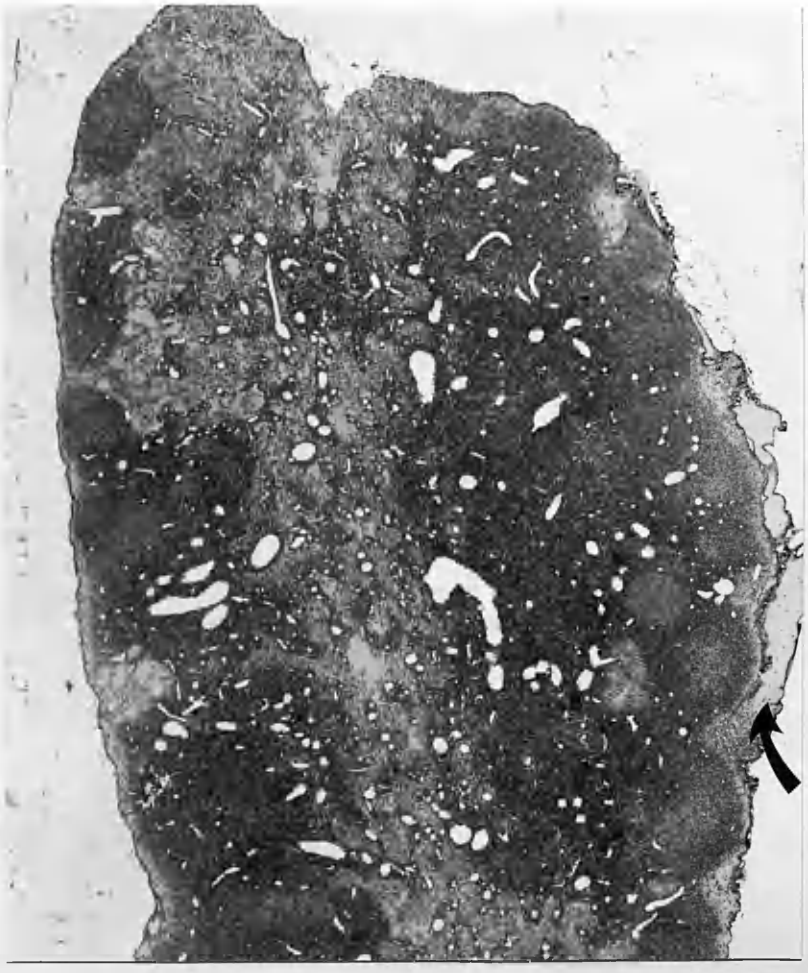
Similar to Figs. 2 & 3, but showing afferent lymphatic (arrow) containing many cells.

x 30 Adult rat H & E

Fig. 6

Higher power of afferent lymphatic, shown in Fig. 5. It contains lymphocytes, macrophages and free erythrocytes (arrows); all to be released into the subcapsular sinus (SS).

x 320 Adult rat H & E



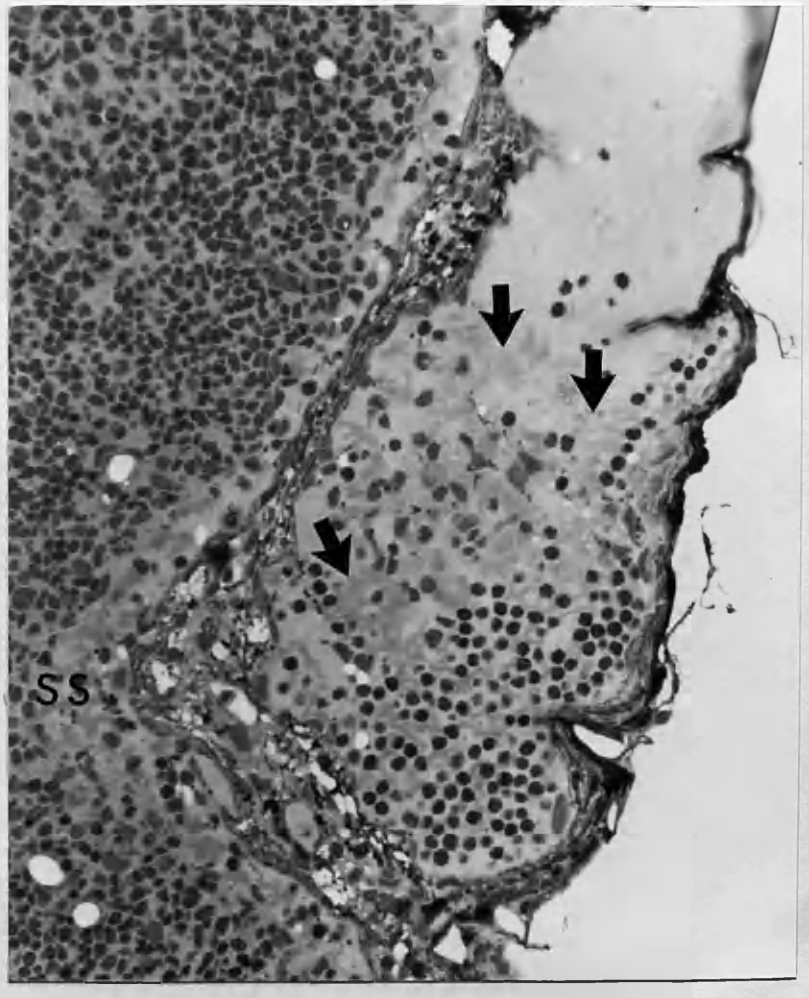

SS

Fig. 7

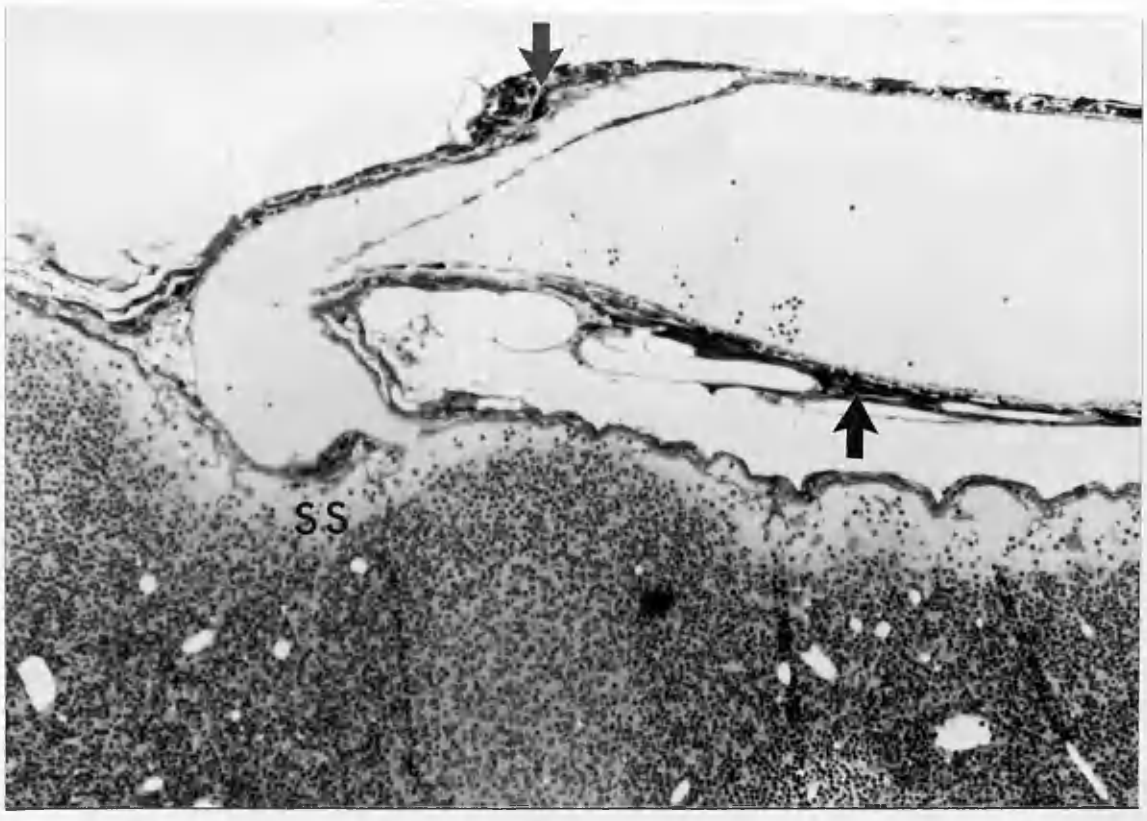
A medium power view of another afferent lymphatic. The bi-cuspid valve is opening towards the subcapsular sinus (SS). It contains a grey precipitate of lymph protein and a few cells. Note its large calibre and thick wall which contains a smooth muscle component (arrows).

x 125 Adult rat Azur II

Fig. 8

An afferent lymph vessel, with two valve cusps is shown here. Small lymphocytes and free erythrocytes are present in the subcapsular sinus (SS).

x 250 Adult rat H & E


SS

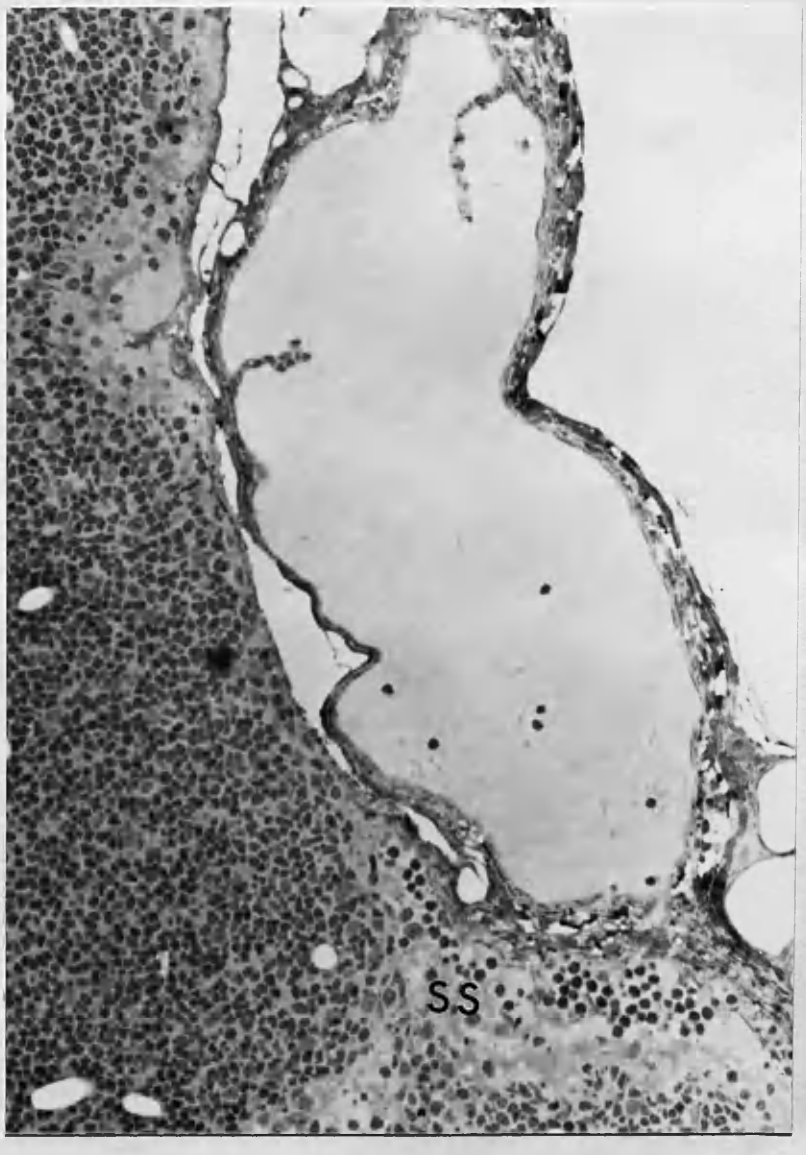

SS

Fig 9

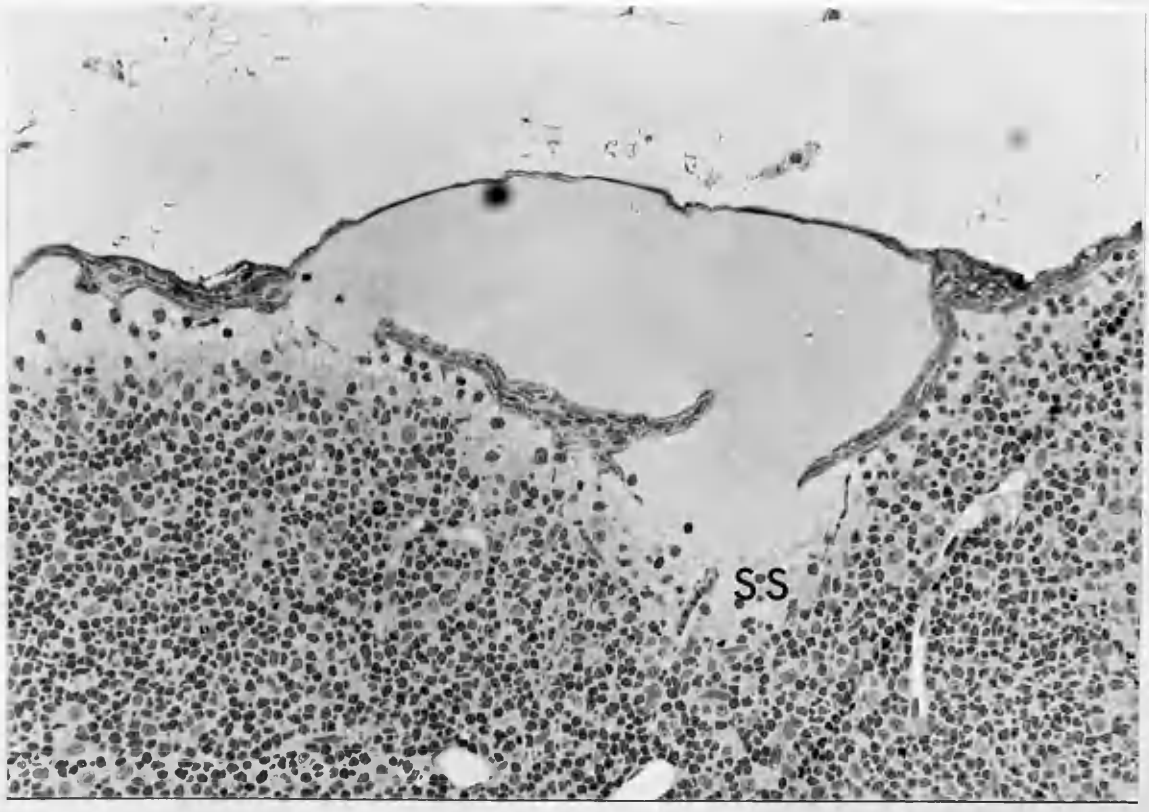
The subcapsular sinus (SS) is enlarged at the site of entry of the afferent lymphatic. It contains large numbers of free erythrocytes, which are pressed against the inner wall of the sinus.

x 250 Adult rat H & E

Fig. 10

The hilum of a thymic haemolymph node with a valved efferent lymphatic (eff) emerging from it. The bi-cuspid valve (arrows) is pointing away from the node. Very few cells are seen in the lumen of the efferent lymphatic.

x 80 Young adult rat Azur II


S.S

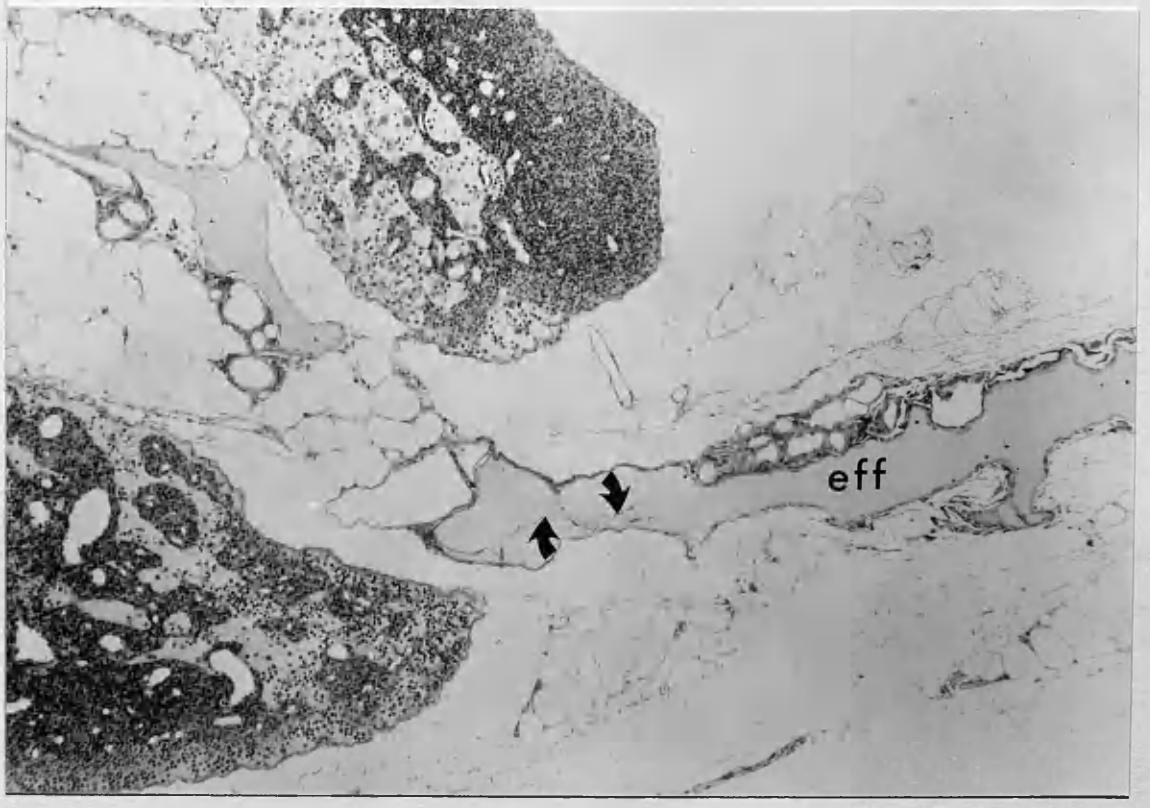

eff

Fig. 11

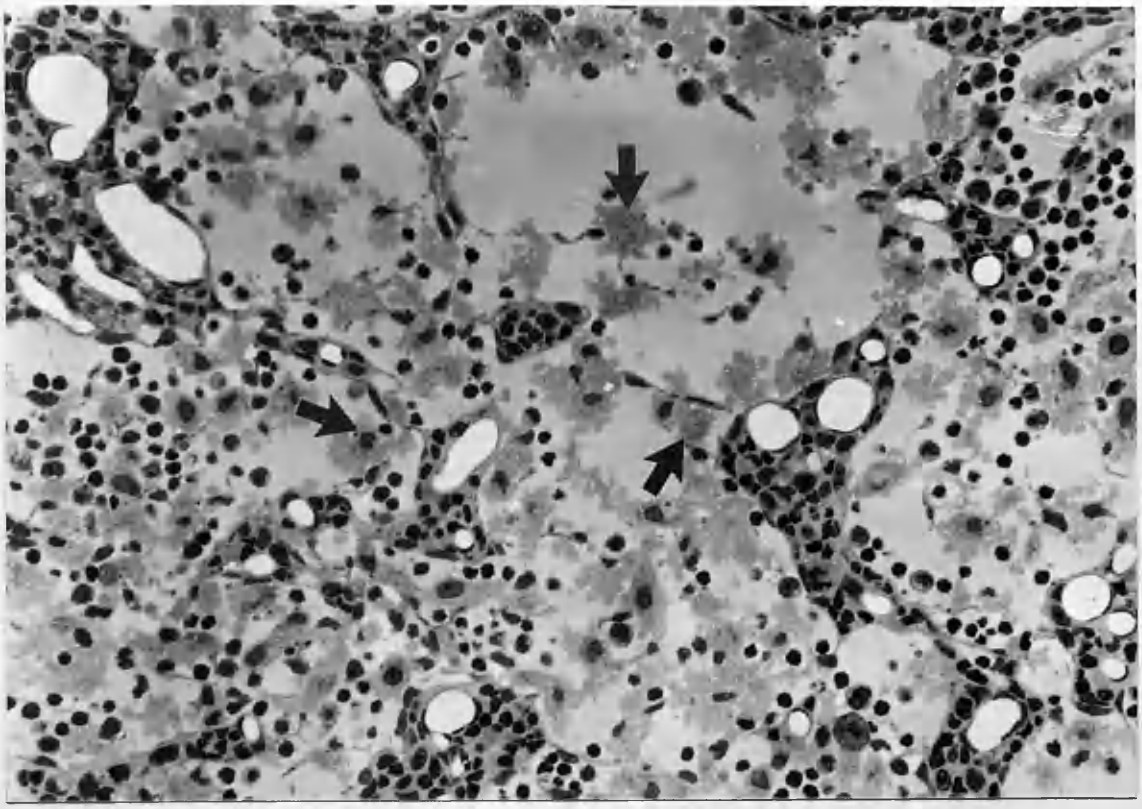
A high power view of the medulla, from Fig. 2, showing numerous erythrocytes, both free and phagocytosed by sinus macrophages (arrows). Note the contrast between blood vessels, emptied by vascular perfusion and lymph sinuses, filled with stained lymph protein.

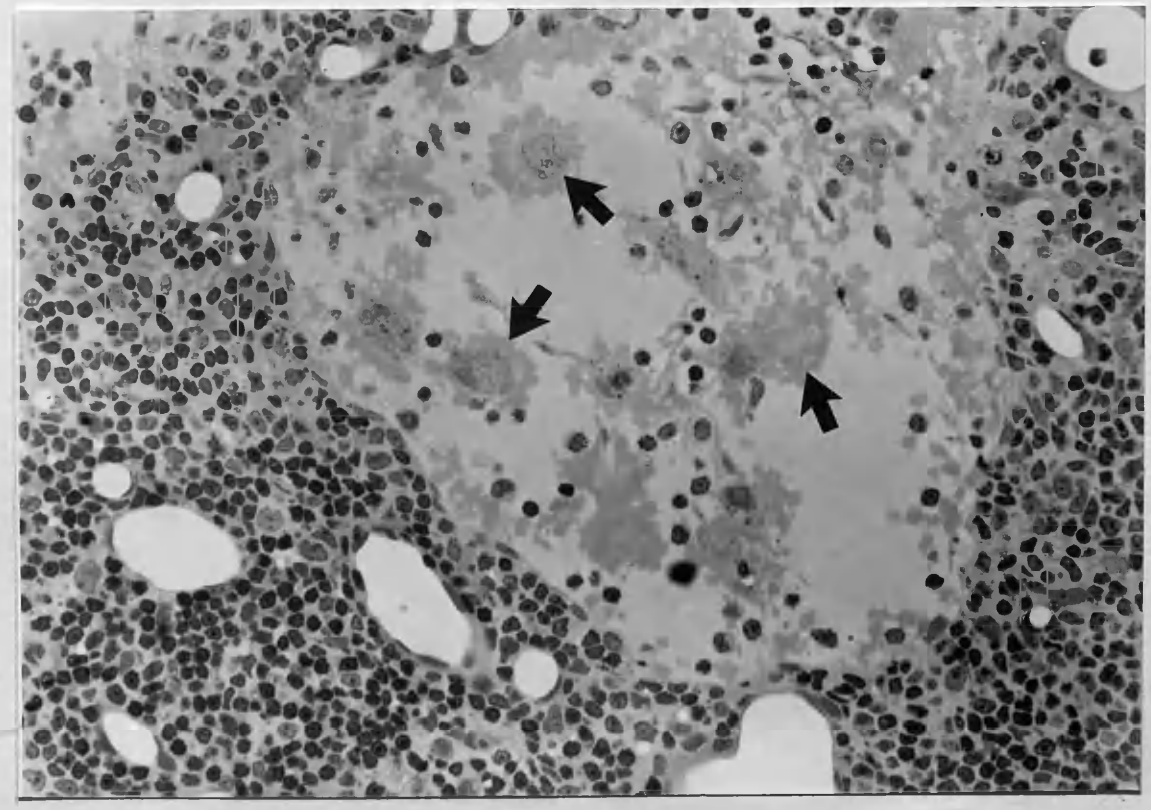
x 200 Adult rat H & E

Fig. 12

A medullary sinus with erythrocytes mostly attached to macrophages forming characteristic rosettes (arrows).

x 400 Adult rat Azur II





Figs. 13, 14 & 15

Parathymic haemolymph nodes of adult rats, 6 hours after intravenous (IV) injection of ink.

Fig. 13

General low power view. Ink is so sparse that it cannot be seen at this magnification (c.f. with Fig. 101, where ink is abundant 5 minutes after IP injection).
INSET: See Fig. 14

x 50 Azur II

Fig. 14

High power view of area outlined in Fig. 13. Particles of ink still cannot be seen in the sinuses at this magnification.

x 240 Azur II

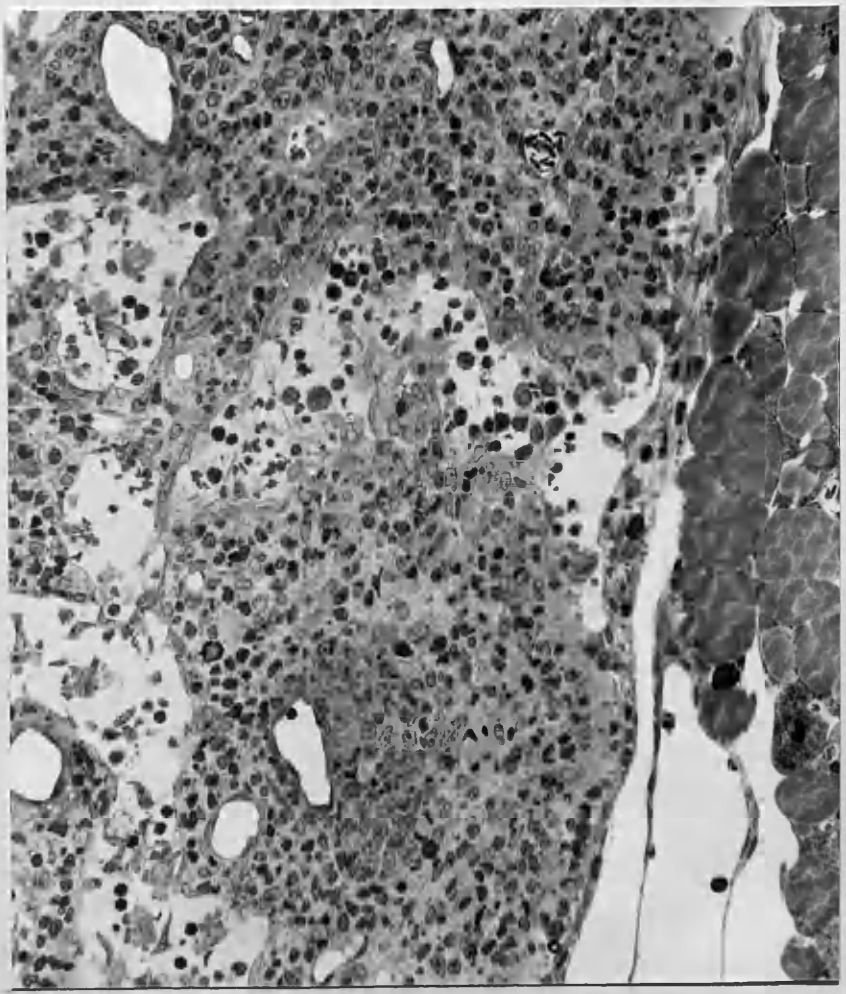


Fig. 15

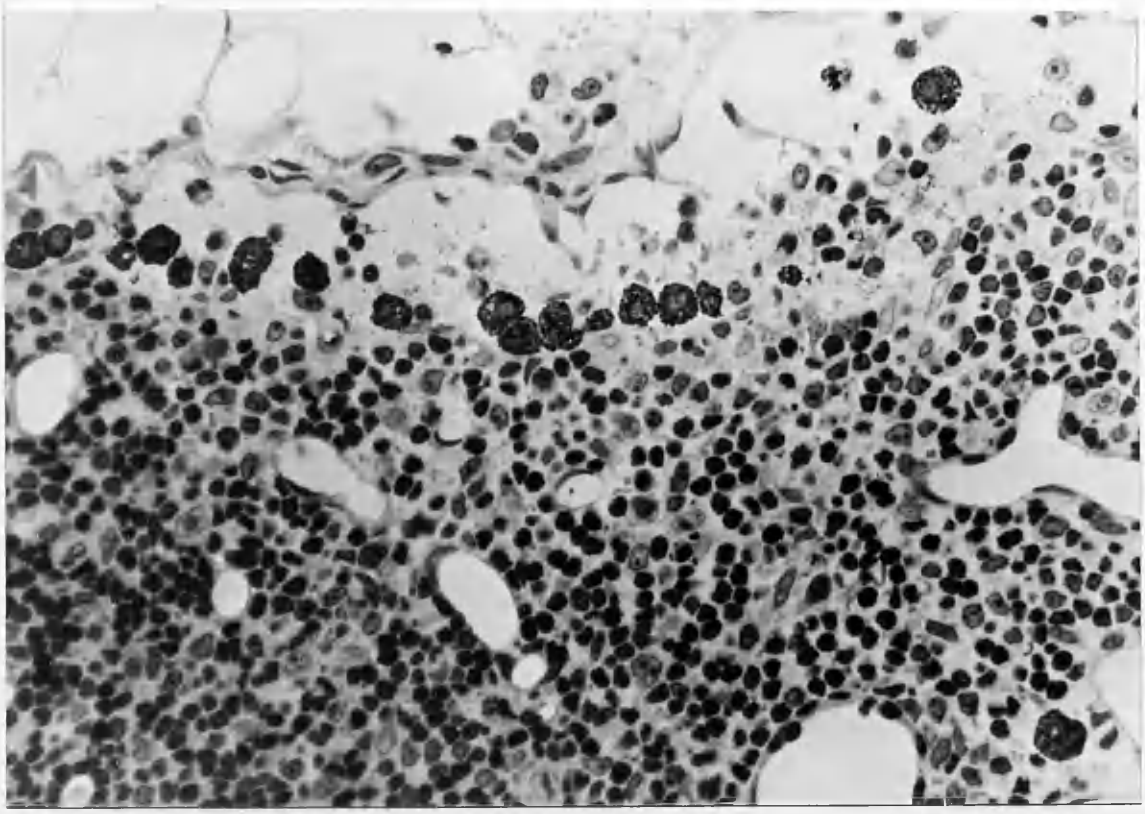
High power view of subcapsular sinus of another node. Ink particles are more numerous than in Fig. 14, but much less abundant than following IP injection (Fig. 102). All the large darkly stained cells are mast cells, not to be confused with ink-laden macrophages.

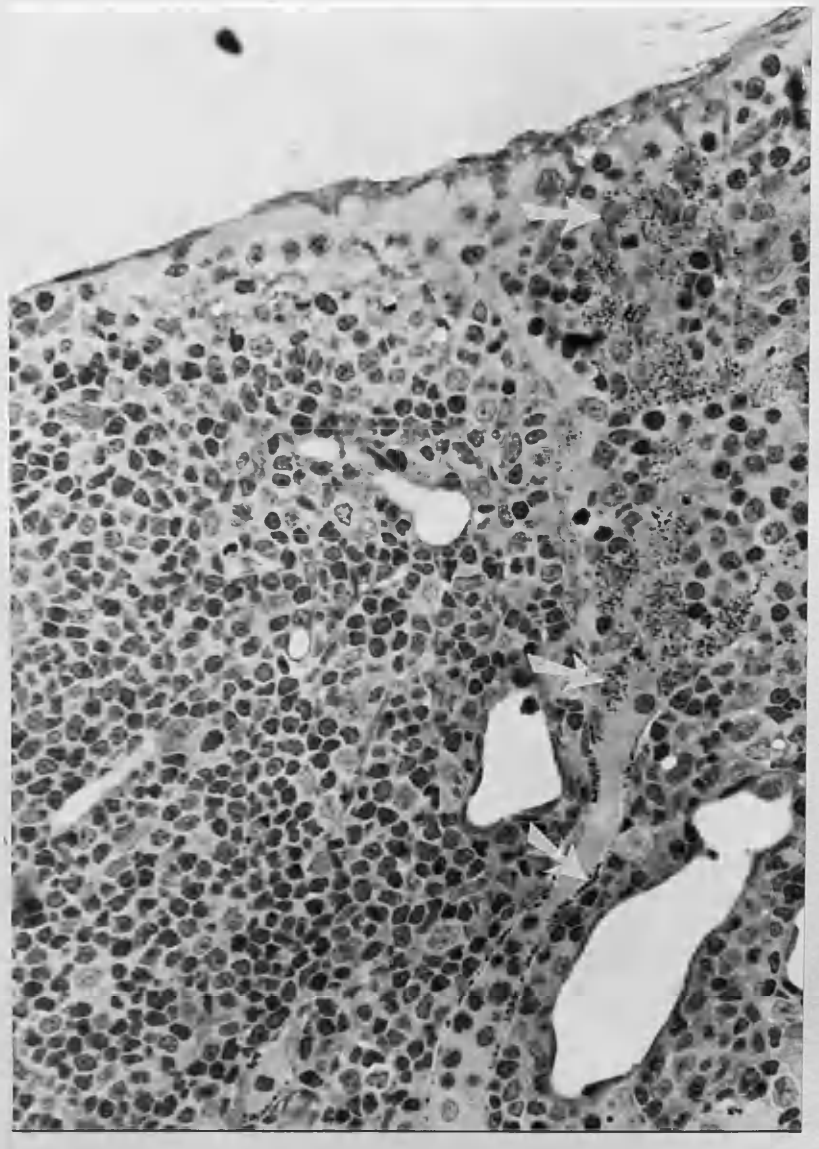
x 300 Azur II

Fig. 16

Subcapsular and intermediate sinuses, 2 days after IV injection of ink. Carbon particles are moderately abundant in sinus macrophages and littoral cells (arrows).

x 400 Adult rat H & E





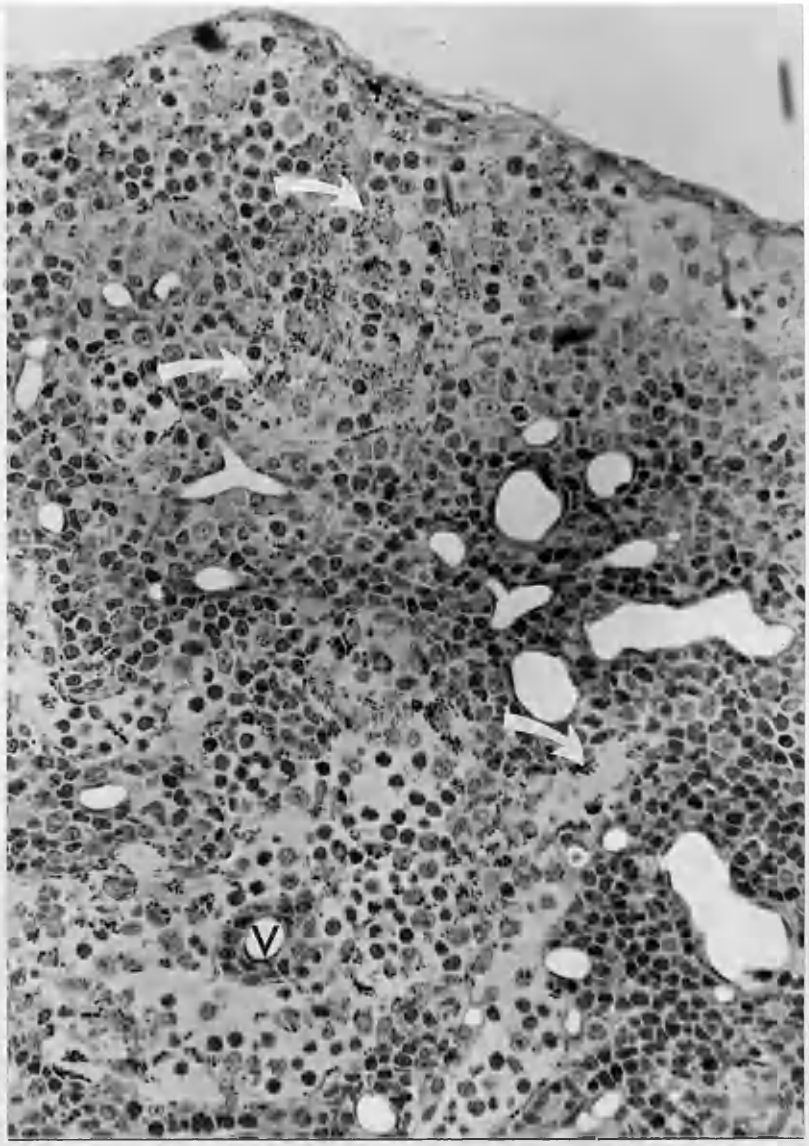
Figs. 17 & 18

5 days after IV injection of ink. Note:

1) More abundant ink particles in sinus macrophages and littoral cells (arrows).

2) Absence of ink particles in and around endothelial cells of high endothelial venules (V).

x 300, x 400 Adult rat H & E


V

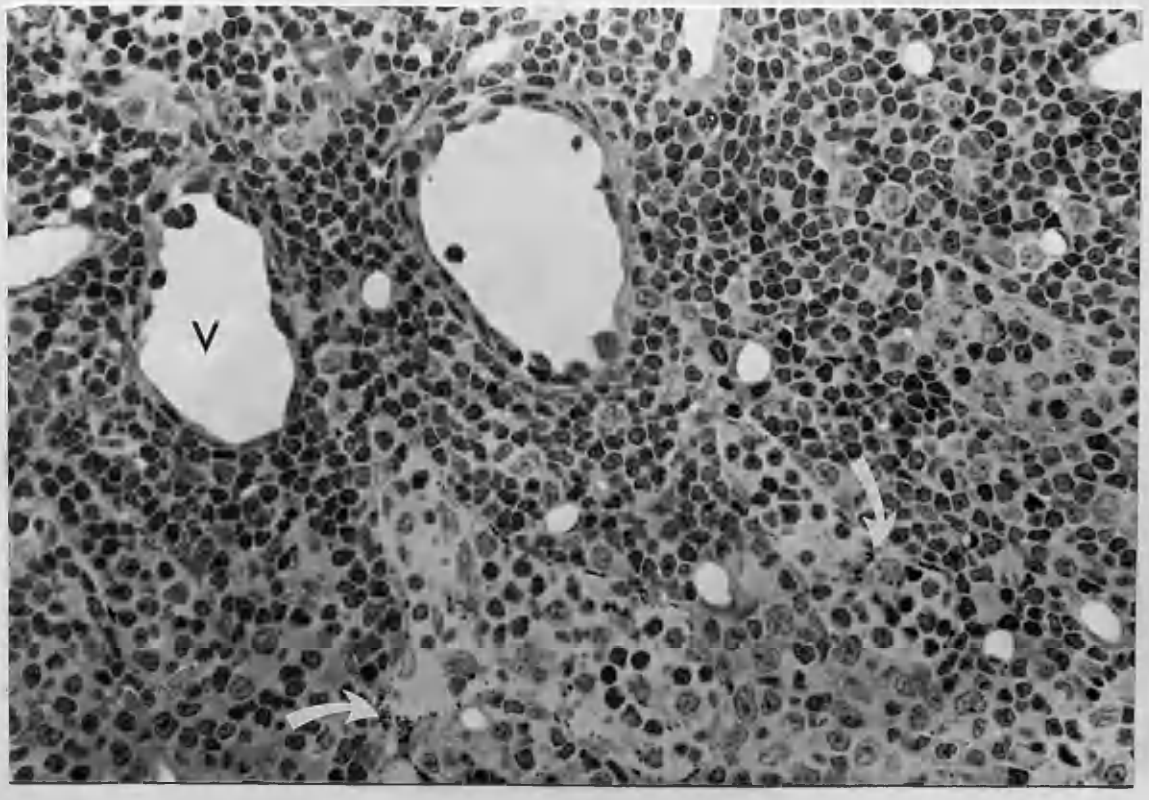

V

Fig. 19

Thymic haemolymph node of newborn rat, showing an afferent lymphatic with a valve (aff) approaching the capsule. The nuclei of the endothelial cells of the lymphatic wall and valves, are visible. Several erythrocytes are seen in the lymphatic lumen.

x 400 H & E Newborn rat

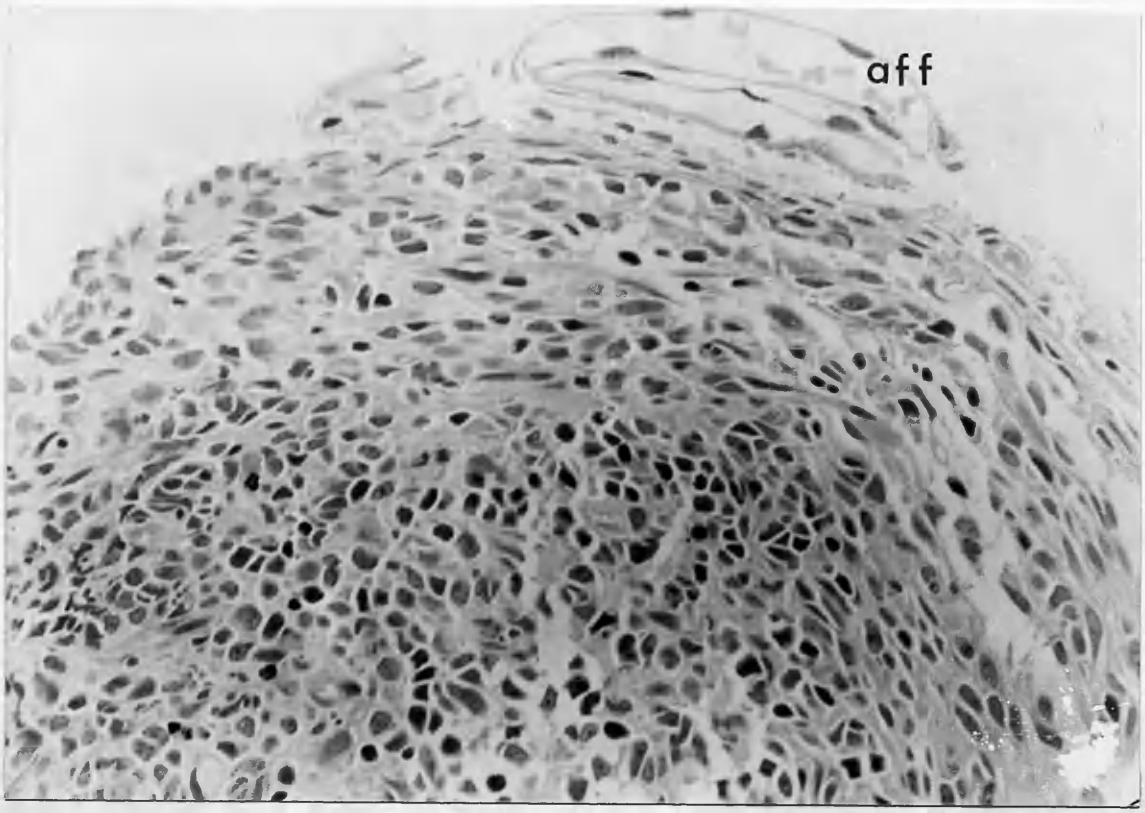
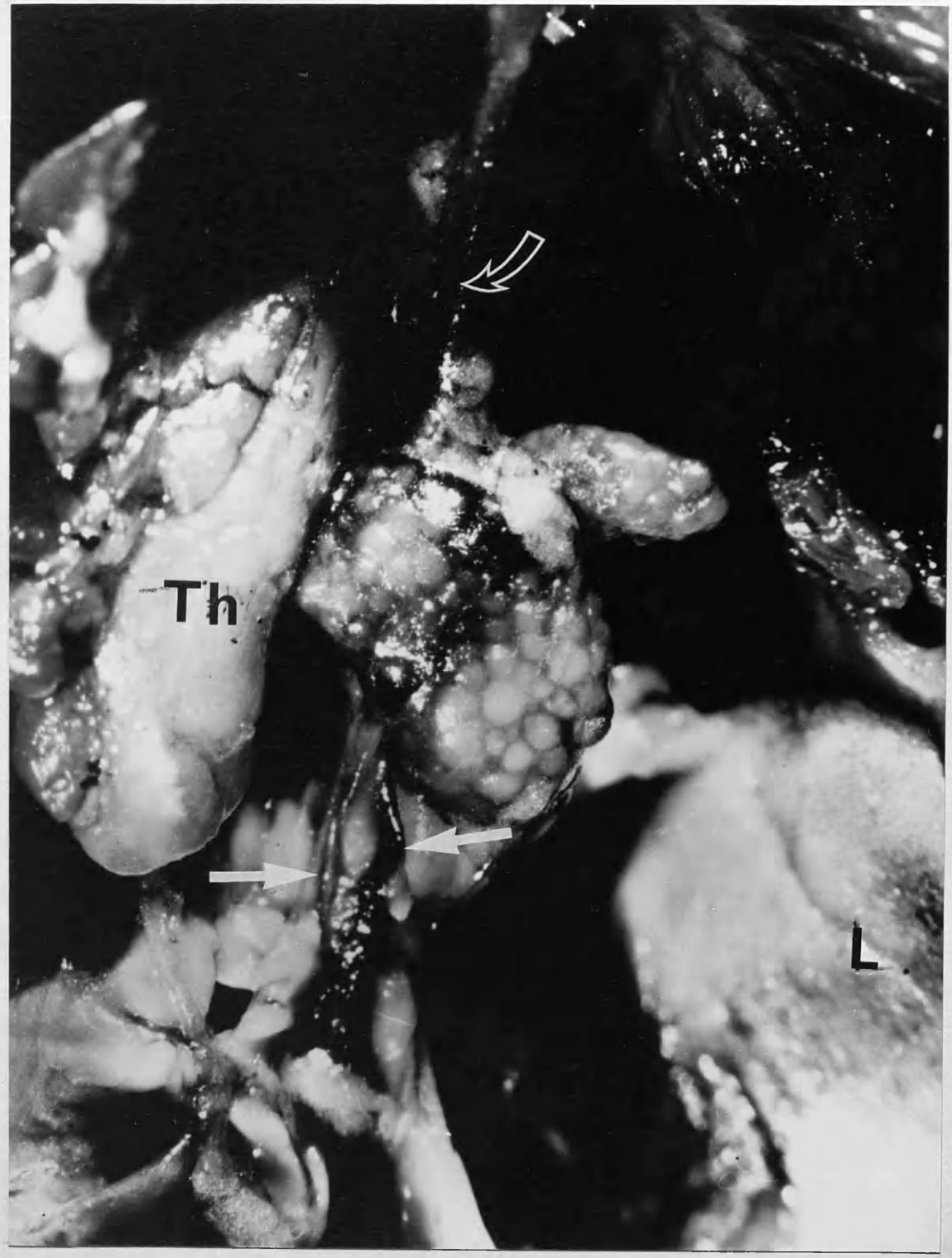

aff

Fig. 20

Dissection of a thymic haemolymph node, showing 3 afferent lymph vessels from the diaphragm (retrosternal - open arrow, and mediastinal - closed arrows) demonstrated by intravenous injection of pontamine sky blue, followed by vascular perfusion. Note afferent lymphatics enter the dark (reddish) part of the node.
Th - thymus gland, L - lung.

x 10 Adult rat


Th
L

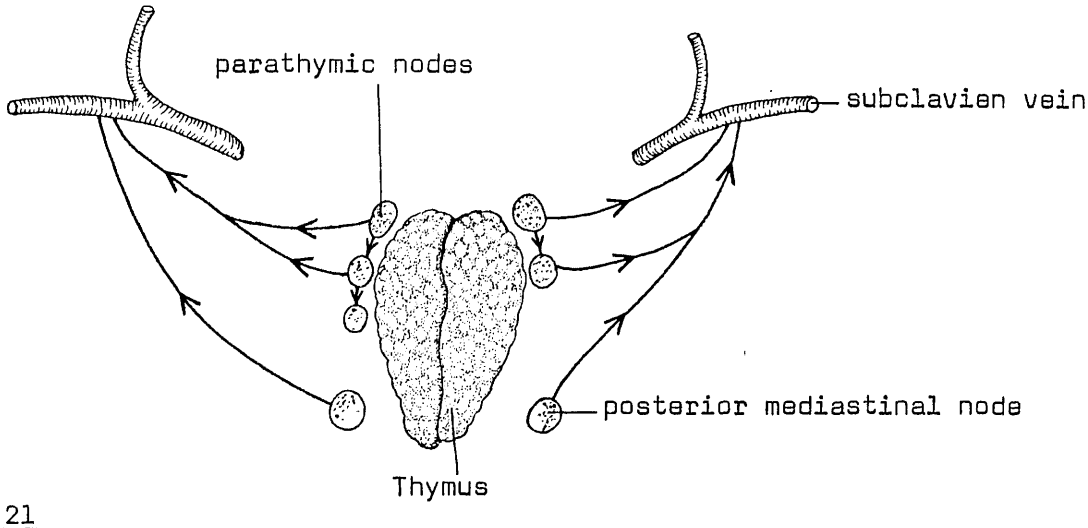


Fig. 21

Efferent lymph drainage of parathymic and posterior mediastinal haemolymph nodes.

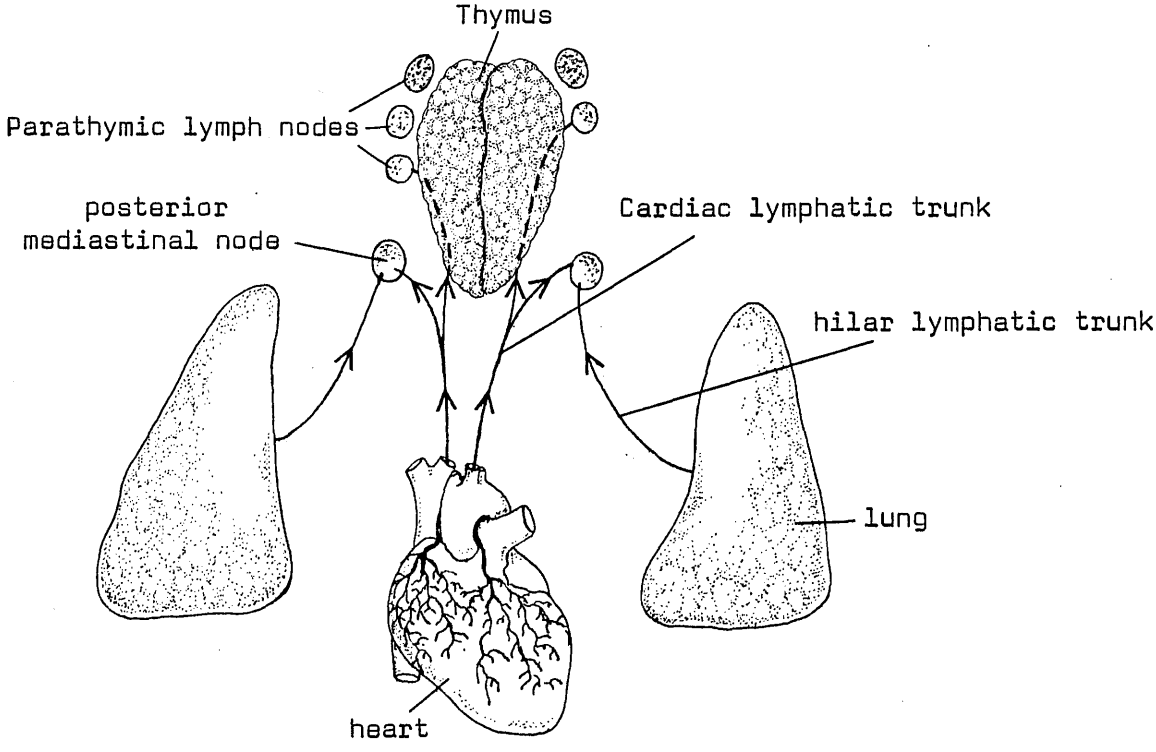


Fig. 22

Regional lymph drainage of heart and lung.

Fig. 23

TEM micrograph of medullary sinus (Ms) of thymic haemolymph node. Pseudopodia (arrows) of a large macrophage (Ma) partly surround four red blood cells (Rbc). The cytoplasm of the macrophage contains numerous mitochondria (m) and many phagosomes of varying size (Ph). N - nucleus.

Ly - lymphocyte, M - mast cell, Mc - medullary cord, End - endothelial lining of medullary sinus.

x 2,100 Adult rat

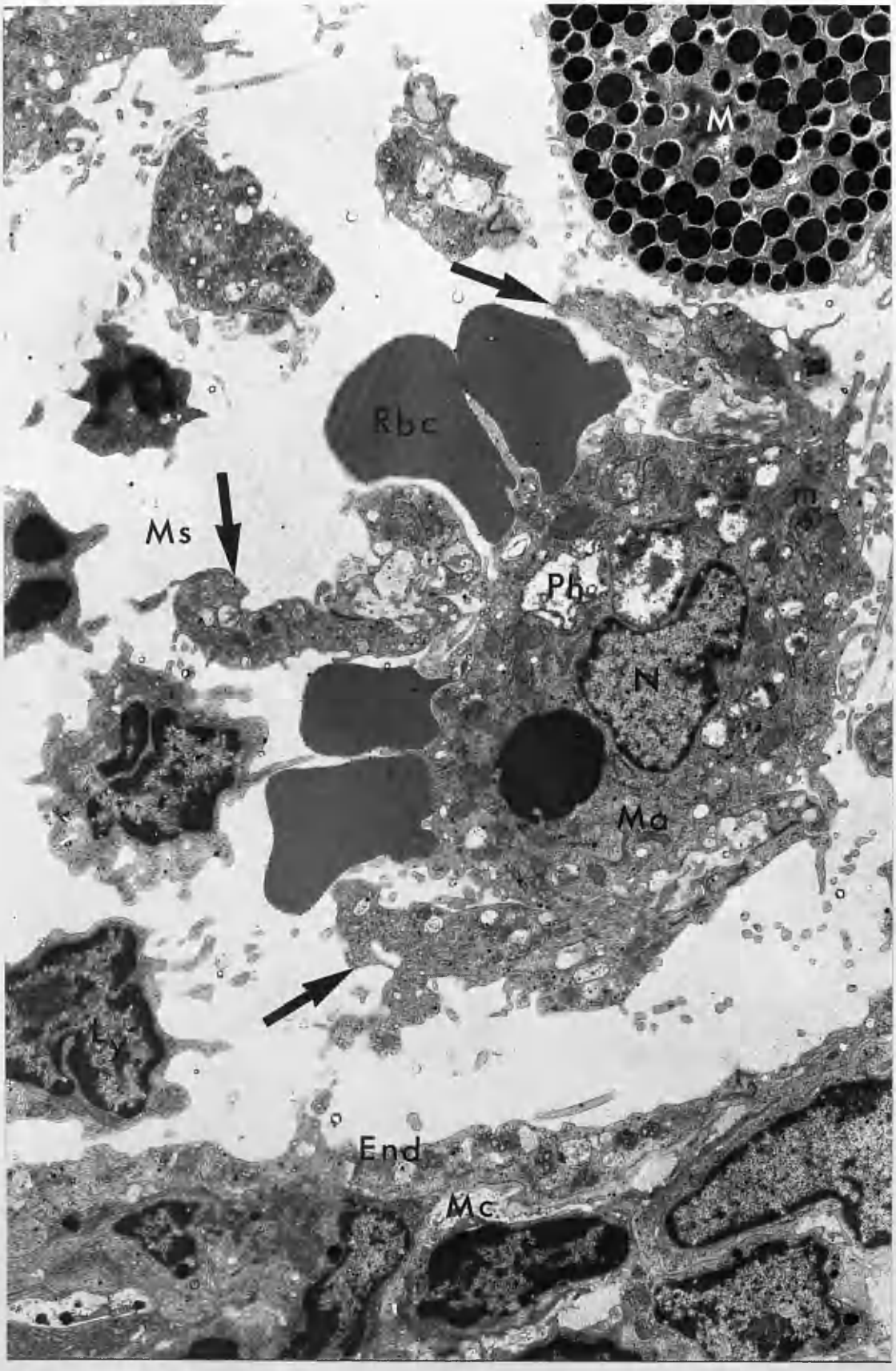

M
Rbc
Ms
Pb
N
Ma
Ly
End
Mc

Fig. 24

TEM micrograph of another macrophage (Ma) in the medullary sinus (Ms). In the cytoplasm of the macrophage many large phagosomes representing degradation products of red blood cells are seen (asterisks). The nucleus is not seen in the plane of this section.

m - mitochondria, E - eosinophil.

x 2,800 Adult rat.

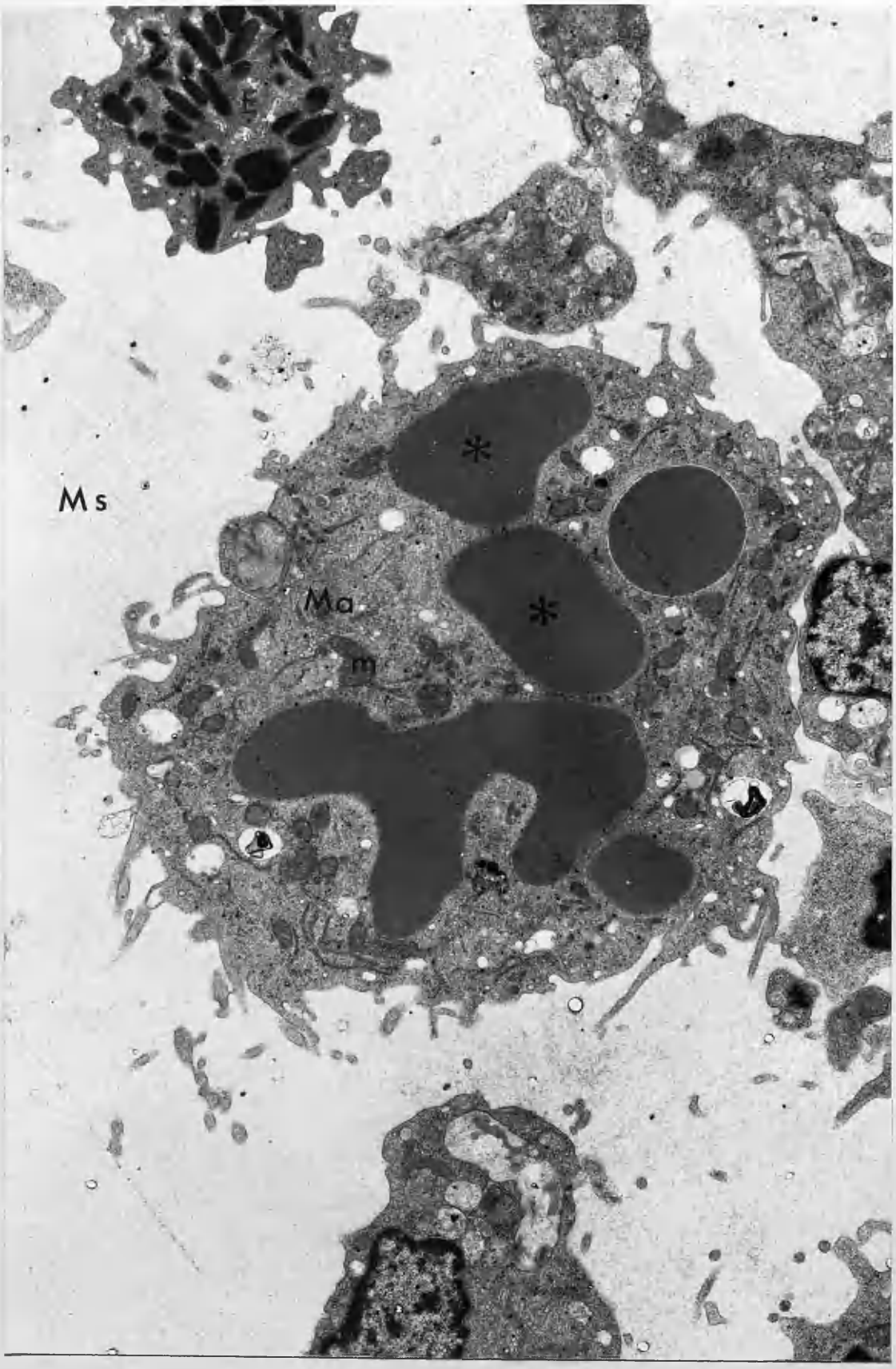
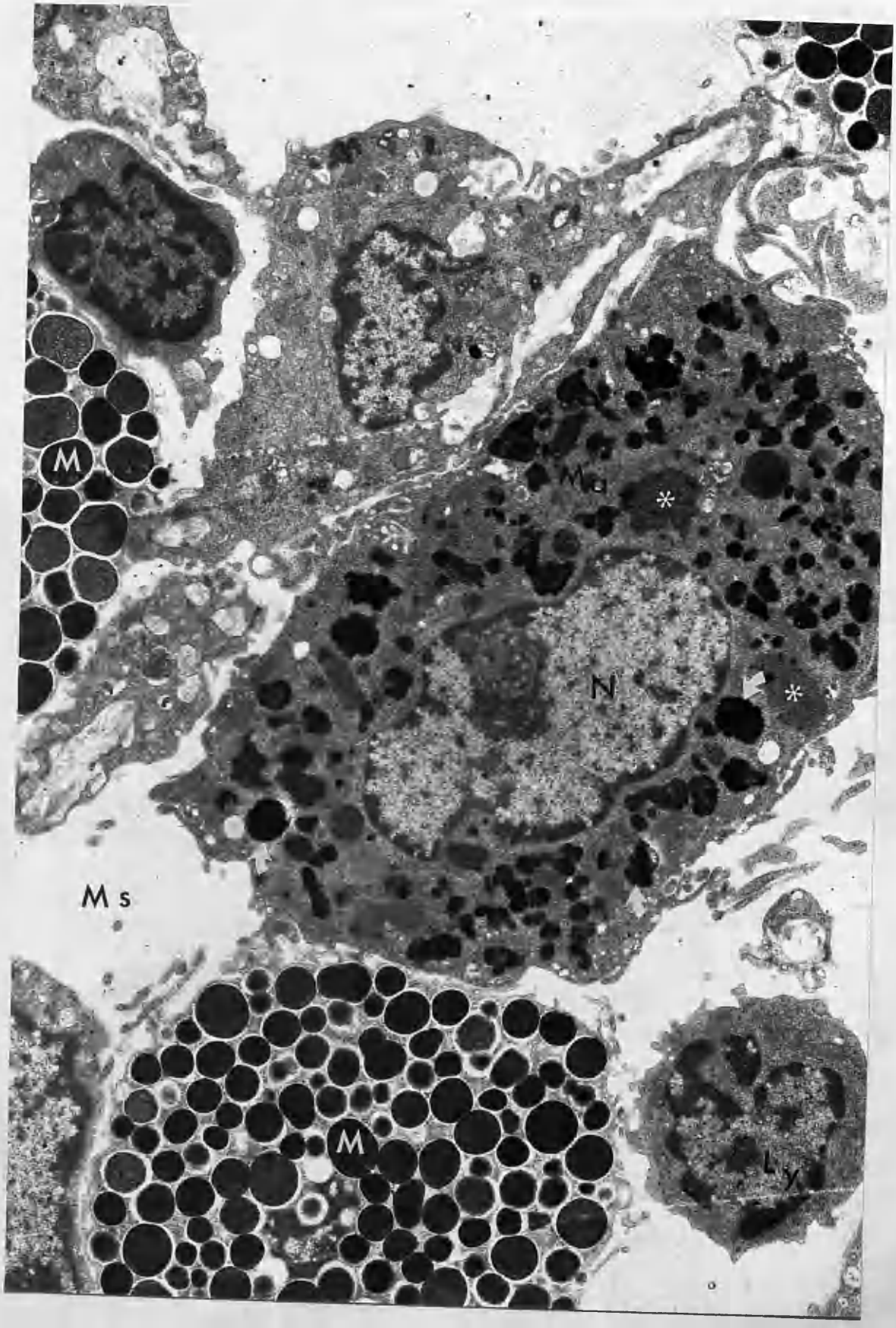

Ms
Ma
m

Fig. 25

Medullary sinus (Ms) of a thymic haemolymph node 7 days after IV injection of ink. A sinusoidal macrophage (Ma) contains many dark ink-ladened phagosomes (arrowheads). The phagosomes containing ink should not be confused with dark staining haemosiderin granules (asterisks).

M - mast cell, N - nucleus, Ly - lymphocyte.

x 2,800 Adult rat


M
Ma
N
Ms
M
Ly

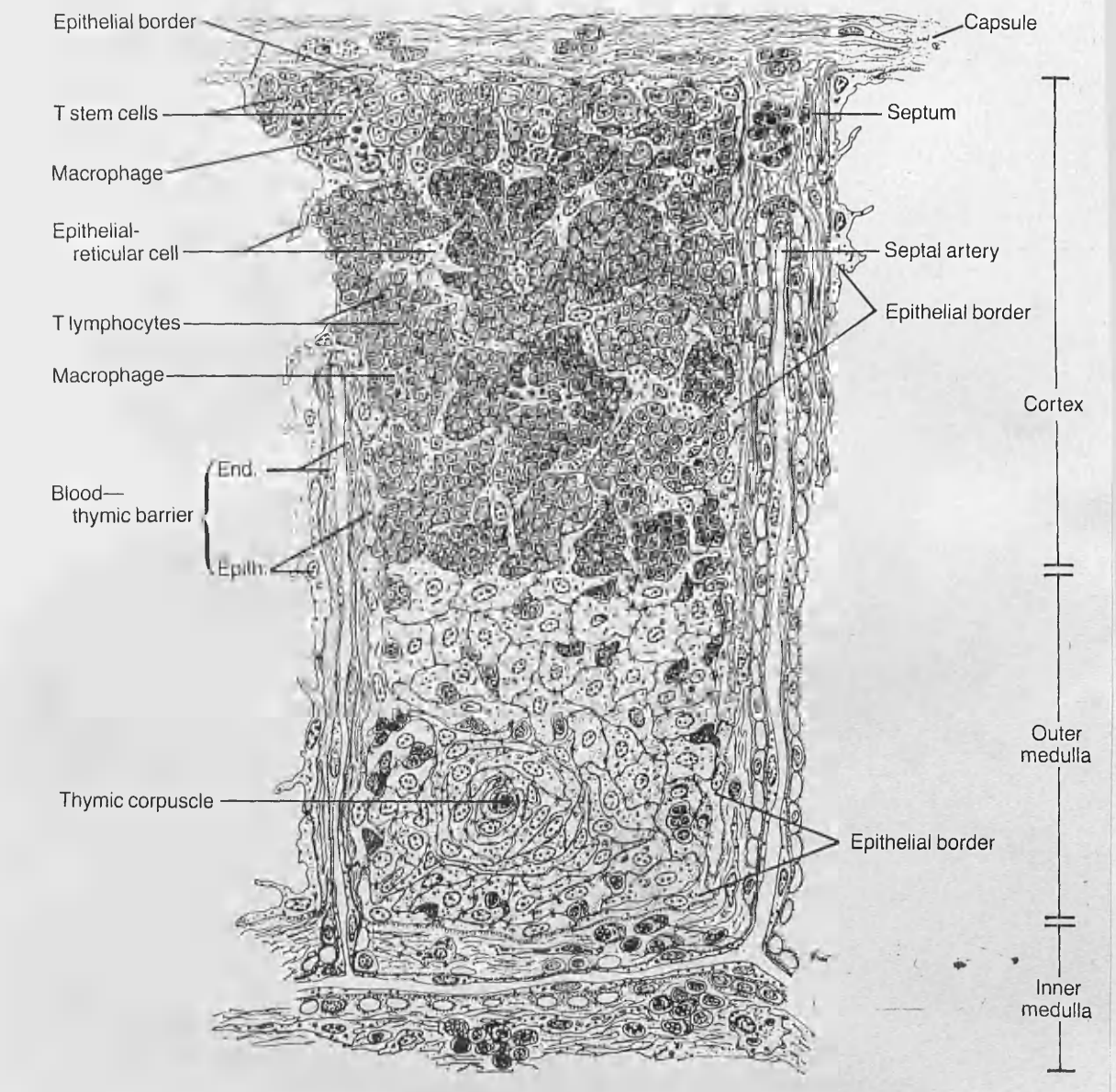


Fig. 26

Diagramatic representation of a lobule of the thymus gland.

(From Weiss, L., 1983)

Fig. 27

The lymphoblasts (Ly) in the peripheral cortex are separated from the collagen fibres (Col) of the capsule by a thin process of an epithelial-reticular cell (Ep) resting on a basement membrane (arrows). The abundant cytoplasm of the lymphoblasts contain numerous free ribosomes, and the mitochondria (asterisks) show well developed cristae.

P - plasma cell

x 5,600 Rat 6 Bl. 12

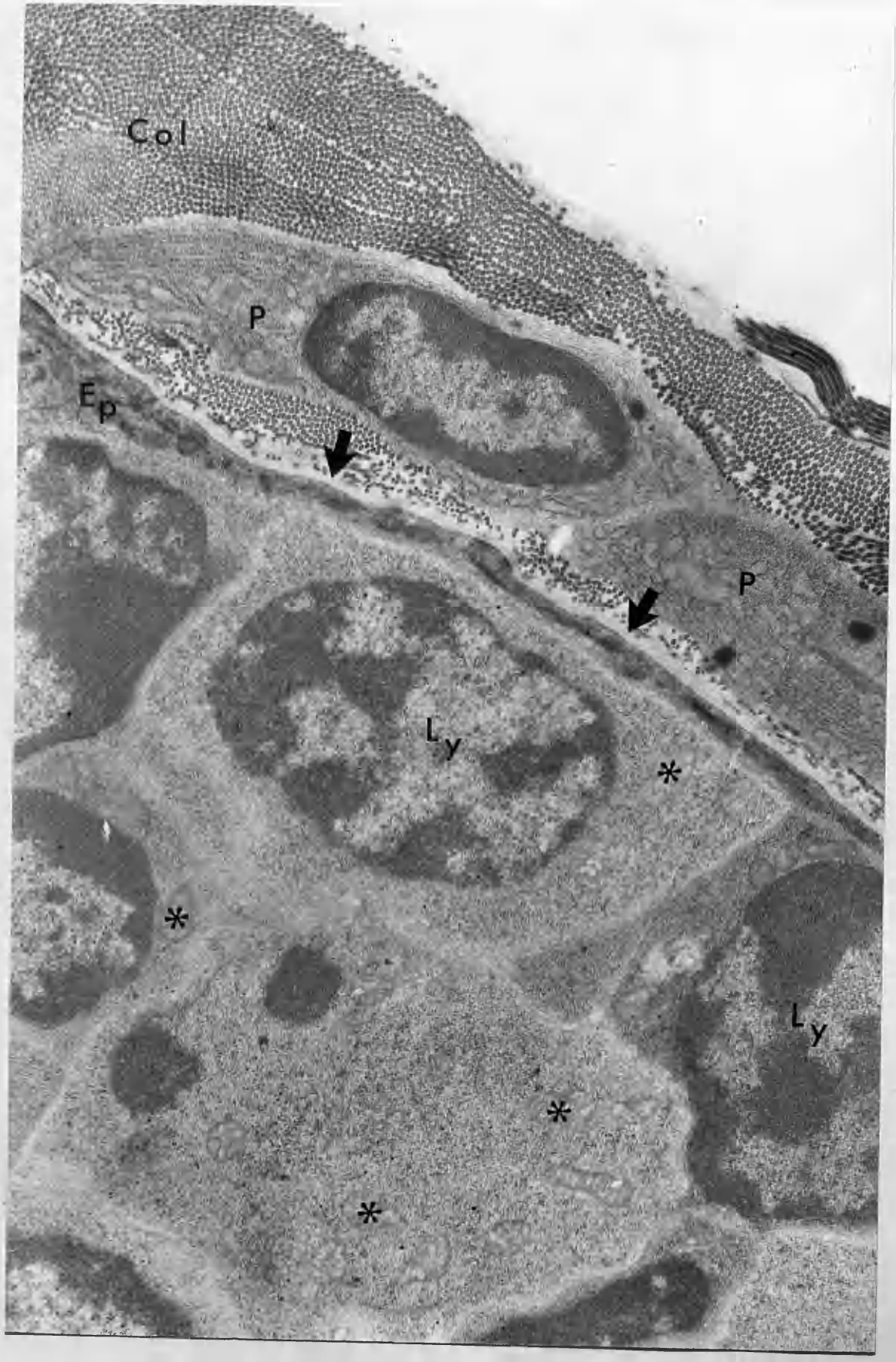

Col
P
Ep
P
Ly
Ly

Fig. 28

A cortical macrophage (Ma) containing an apparent degenerate lymphocyte (long arrow) and numerous vacuoles containing amorphous material (short arrows). The macrophage is surrounded by lymphoid cells (Ly) and processes of one epithelial-reticular cell (Ep) with prominent tonofilaments (arrow head).

x 2,100 Rat 7 Bl. 6

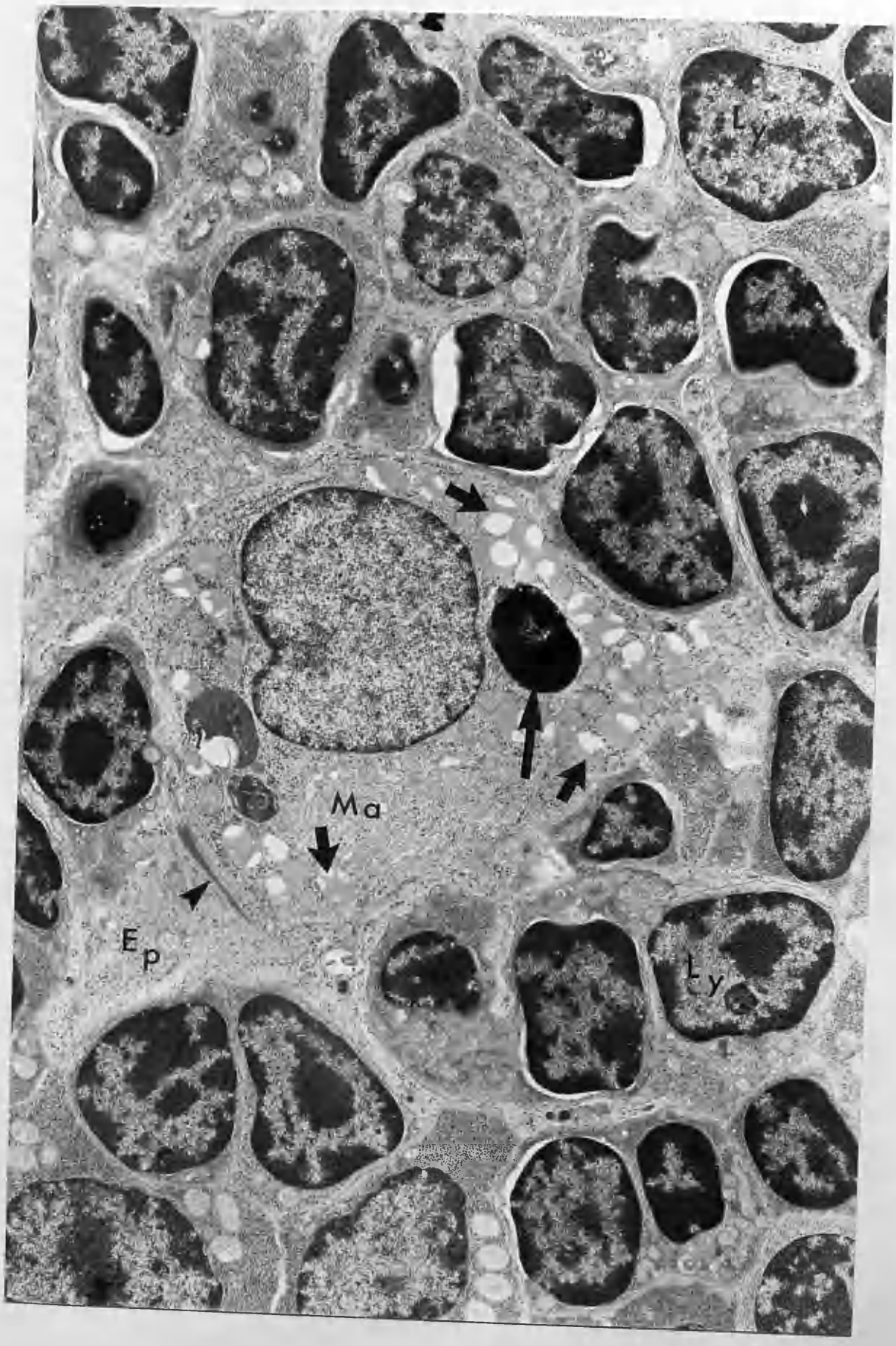

Ly
Ma
Ep
Ly

Fig. 29

Electron micrograph of another cortical macrophage (Ma) with engulfed lymphocytes (arrows) and a great number of electron-dense cytoplasmic inclusions. Bordering the macrophage is part of an epithelial-reticular cell (Ep) containing large vacuoles (Va), some of which are partially filled with a fine granular material.

Ly - lymphocyte.

x 4,200 Rat 4 Bl. 14

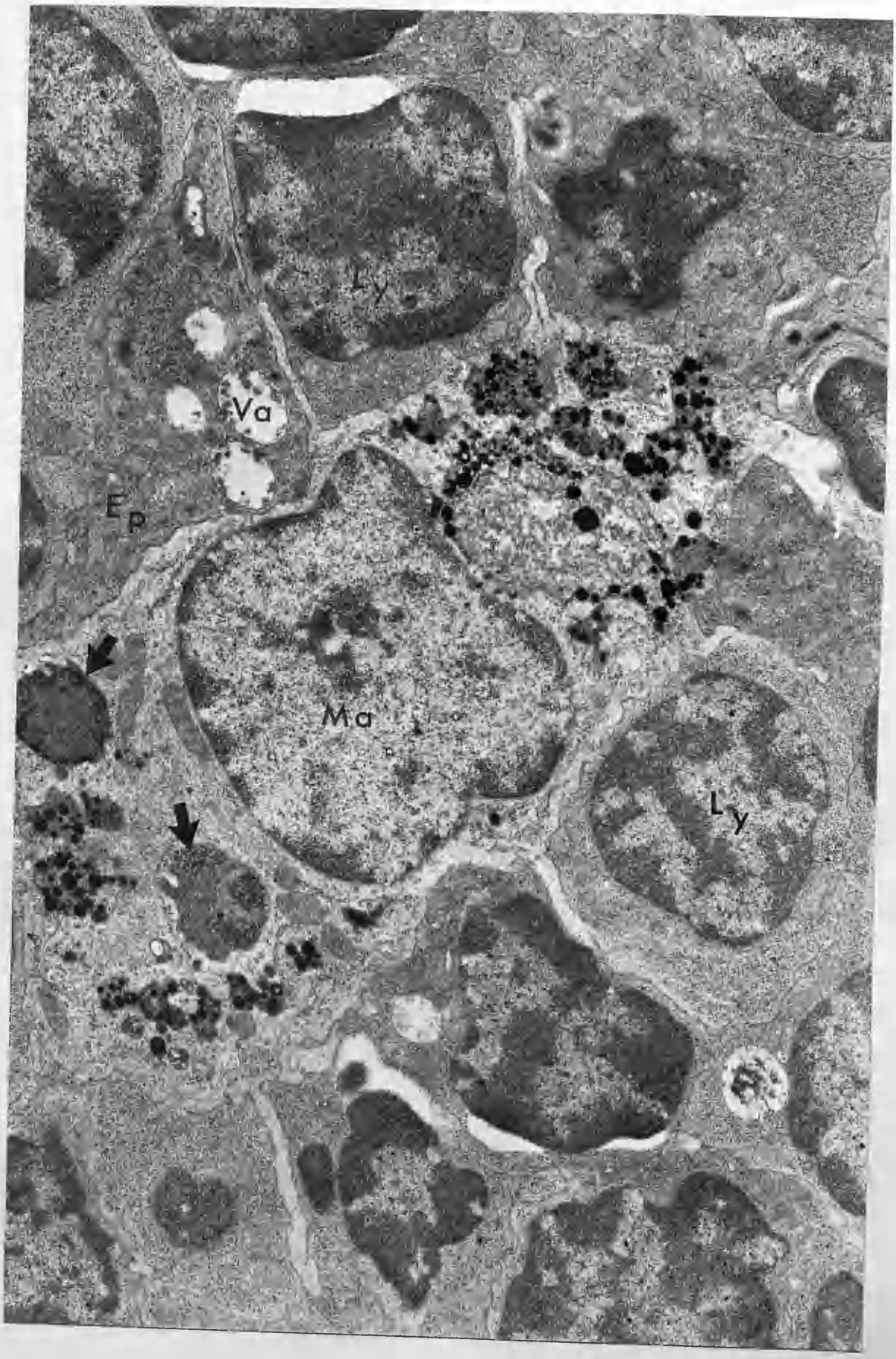

Ly
Va
Ep
Ma
Ly

Fig. 30

A medullary macrophage (Ma) containing portions of engulfed lymphocytes showing varying degrees of degradation (arrows), and inclusions of varying densities. A portion of a dendritic epithelial-reticular cell (Ep), with its pale nucleus, is seen close to the macrophage.

x 2,100 Rat 1 Bl. 3

Ep
Ma

Fig. 31

A medullary macrophage (Ma), containing one degenerated lymphocyte (de) and many intracytoplasmic inclusions.

Ly - lymphocyte.

x 5,600 Rat 1 Bl. 2

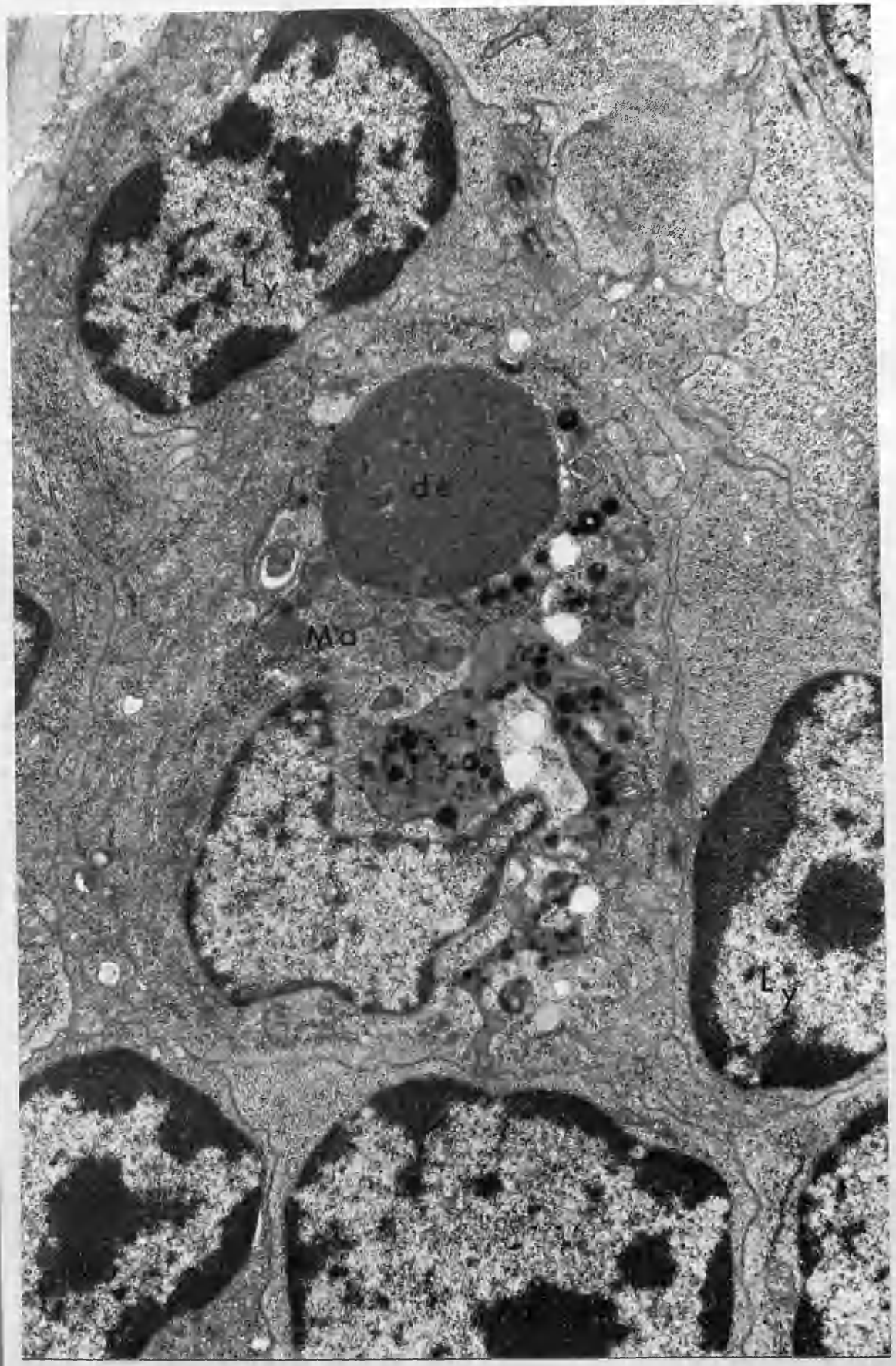

Ly
dé
Mo
Ly

Fig. 32

A mast cell (M), with its characteristic electron-dense granules, is located in the deep cortex of the thymus. Surrounding it is a range of intermediate (T) and small (S) lymphocytes.

x 2,100 Rat 5 Bl. 9

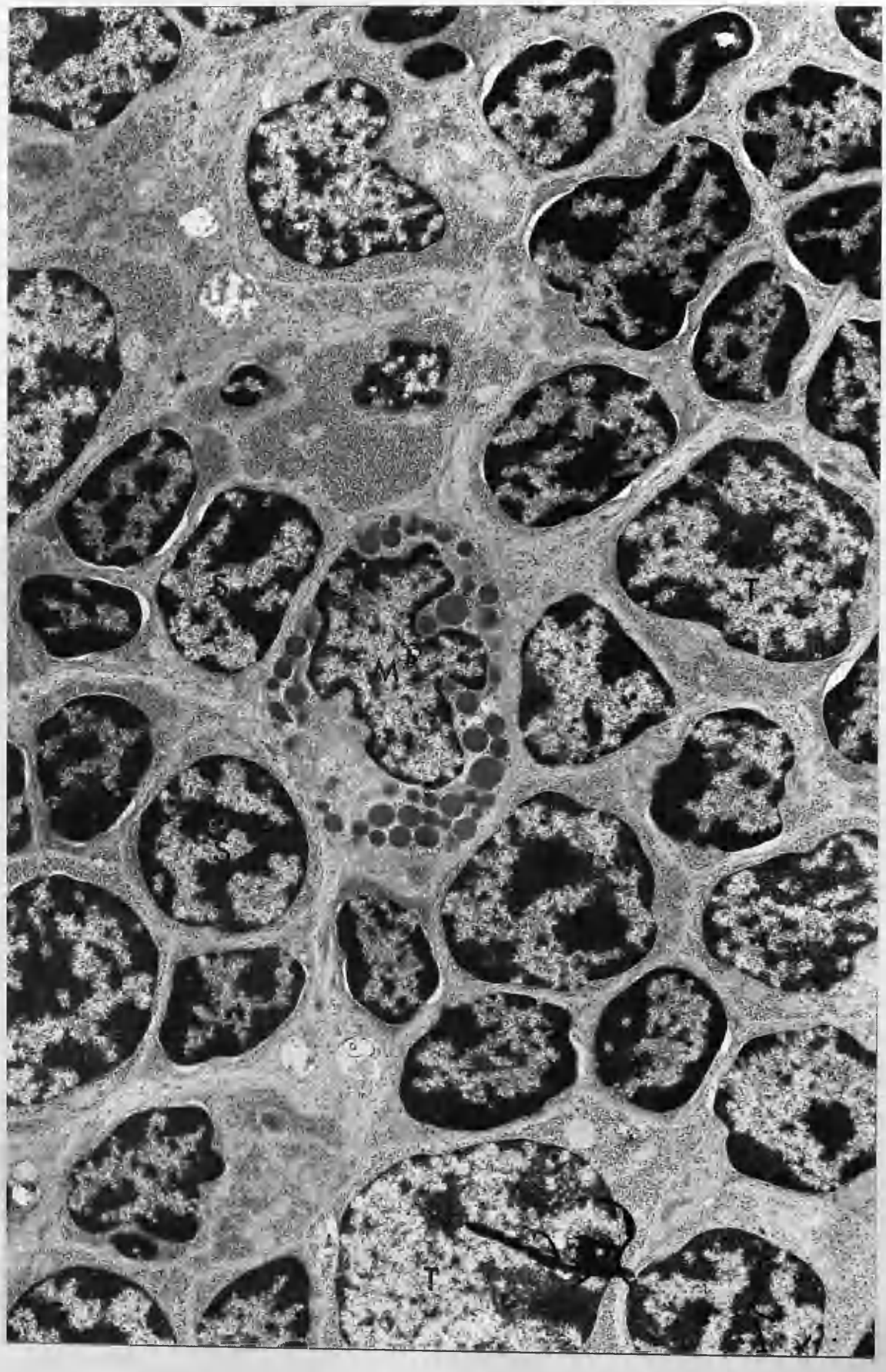

UP
M
T
T

Fig. 33

Electron micrograph of an eosinophil (E) with its typical spindle-shaped banded granules in the thymic medulla.

Ly - lymphocyte.

x 7,000 Rat 1 Bl. 2

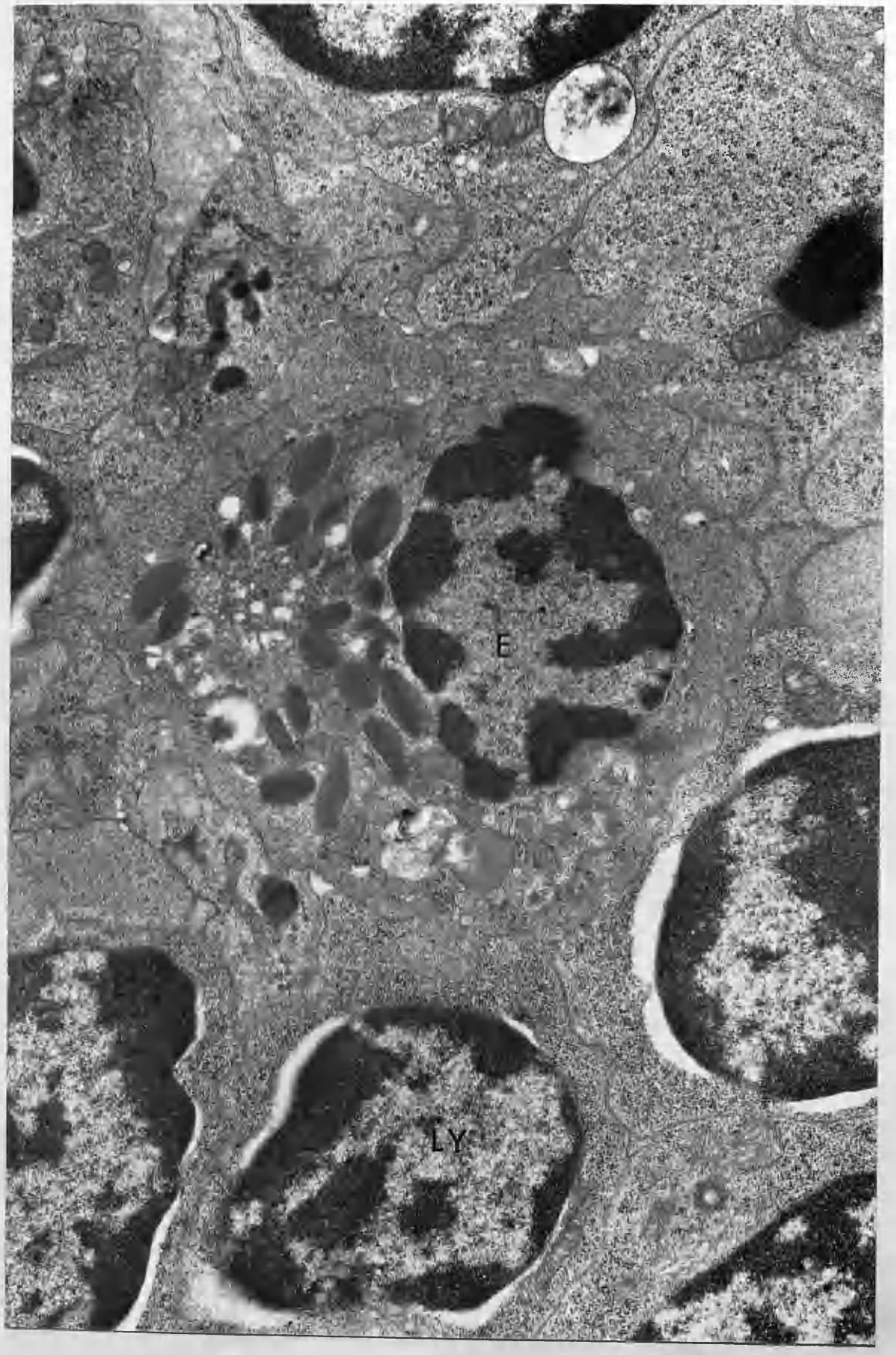

E
LY

Fig. 34

Electron micrograph of a medullary epithelial-reticular cell (Ep). It shows a large pale nucleus with dispersed chromatin, numerous bundles of tonofilaments (short arrows), rough endoplasmic reticulum (arrowheads) and scattered electron-dense cytoplasmic inclusions (long arrows).

x 2,800 Rat 1 Bl. 2

Fp

Fig. 35

Electron micrograph of a medullary epithelial-reticular cell containing large intracellular dilated vacuoles (Va). Tonofilaments (arrows) and a prominent Golgi apparatus can be seen. The nucleus is not in the plane of section.

Ly - lymphocyte.

x 5,600 Rat 4 Bl. 14

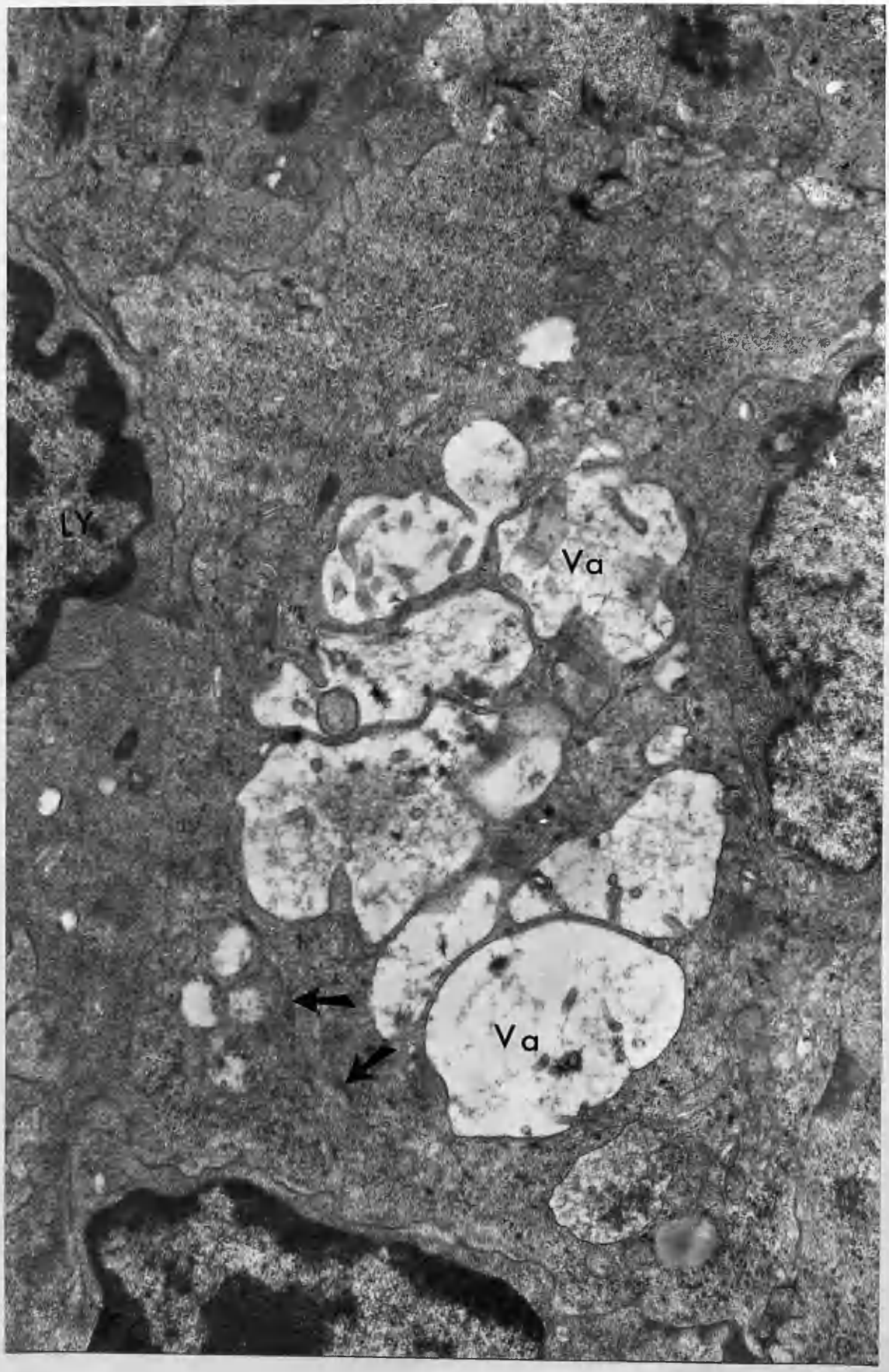

LY
Va
Va

Fig. 36

An interdigitating-reticulum cell in the thymic medulla. It contains an irregularly shaped nucleus (N) with a prominent nucleolus, a well-developed system of smooth-surfaced vesicles (V), and a few tubulo-vesicular structures (Tv) in the centre.

Ly - lymphocyte, m - mitochondria,
Ep - epithelial-reticular cell.

x 2,800 Rat 6 Bl. 7

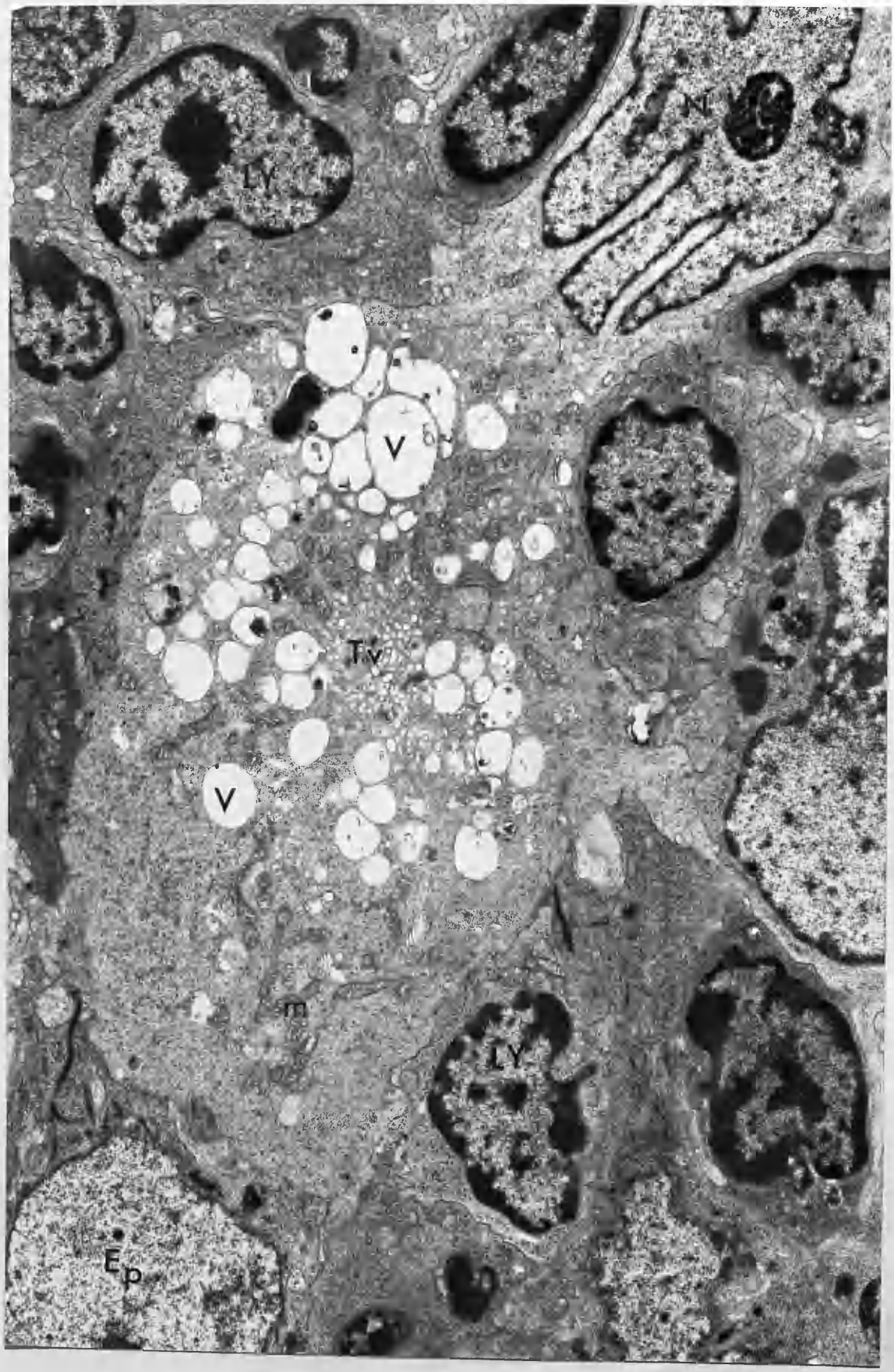

LY
N
V
Tv
V
m
LY
Ep

Fig. 37

An interdigitating-reticulum cell extends finger-like cytoplasmic projections between neighbouring medullary lymphocytes (Ly). N - nucleus, m - mitochondria, V - vesicle, Tv - central tubulo-vesicular structure.

x 5,600 Rat 1 Bl. 2

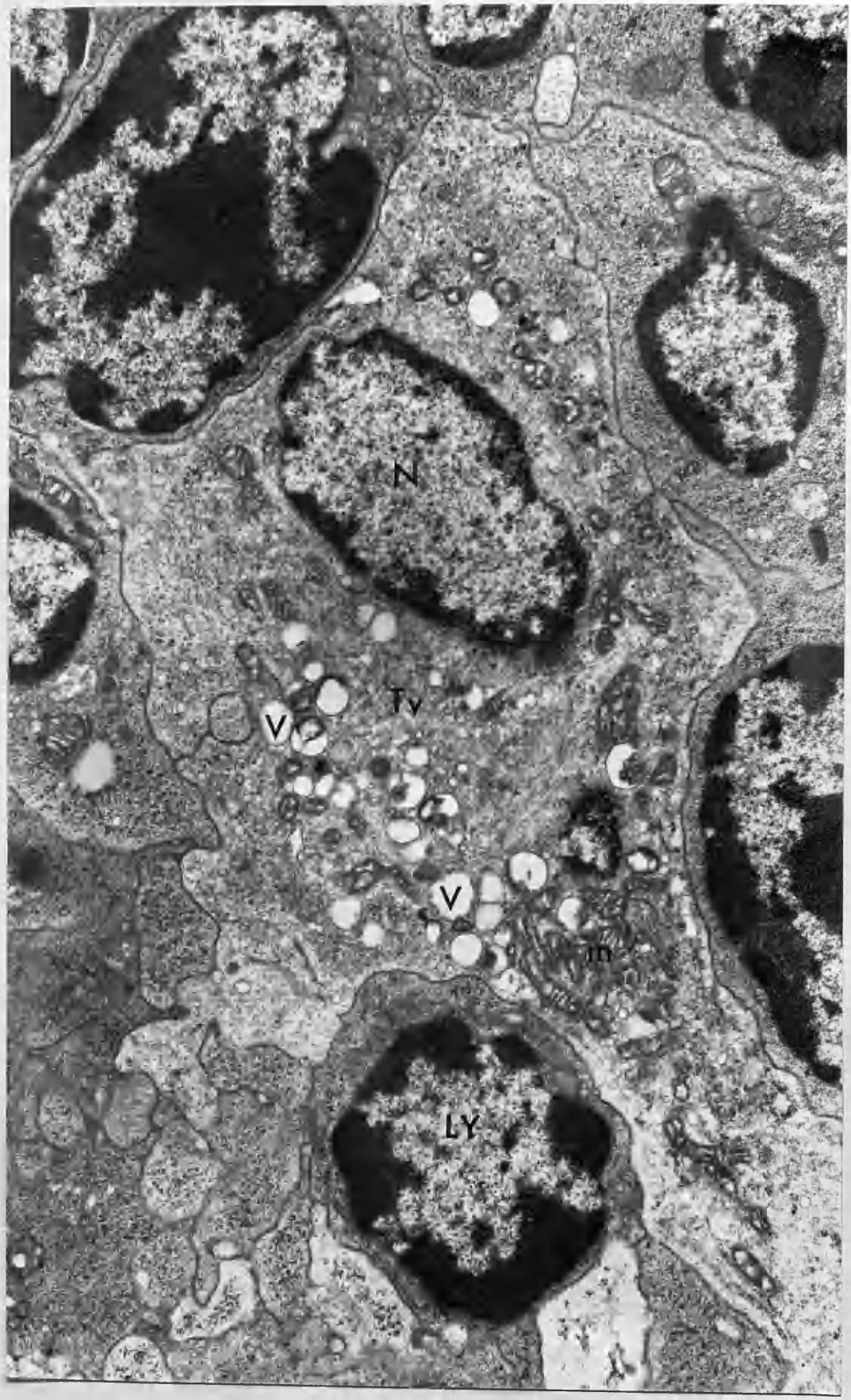

N
Tv
V
V
in
LY

Fig. 38

Electron micrograph of a large, unusual macrophage located in the thymic medulla. The dark elongated nucleus (N) is pushed to the periphery by many intracellular cysts (Cy), some of which contain microvilli (Mv), and by smaller vacuoles (Va) giving the cell a foamy appearance.

Ly - lymphocyte.

x 2,800 Rat 1 Bl. 2

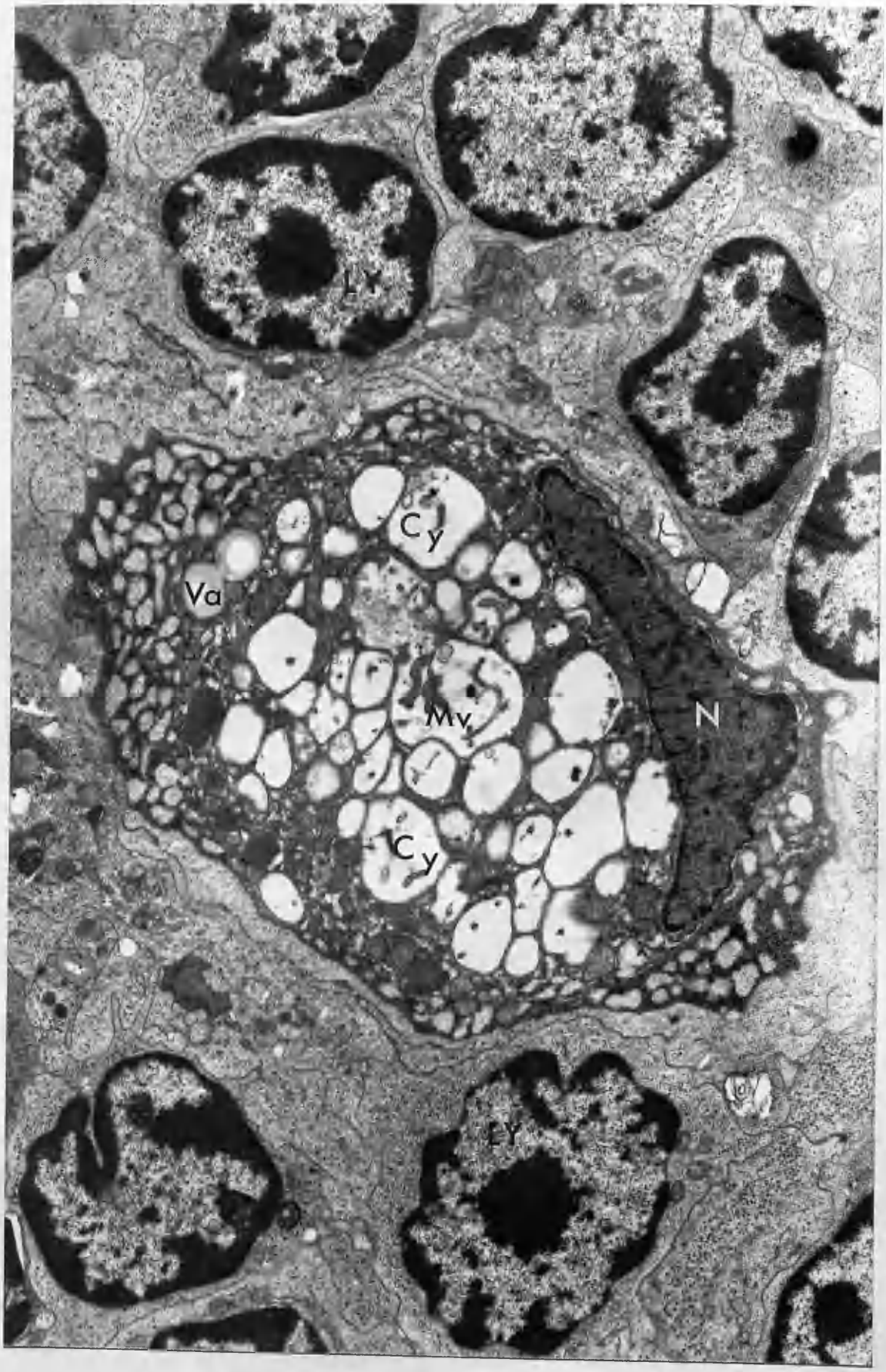

Ly
Cy
Va
Mv
N
Cy
Ly

Fig. 39

Electron micrograph of another dark, unusual cell present in the medulla surrounded by lymphocytes (Ly) and processes of interdigitating-reticulum cell (IDC). Note the dark nucleus (N), the dense cytoplasm and the presence of many spaces and vacuoles (Va) containing lipid-droplets. These features might indicate some kind of degenerating process of an epithelial-reticular cell.

x 4,200 Rat 1 Bl. 2

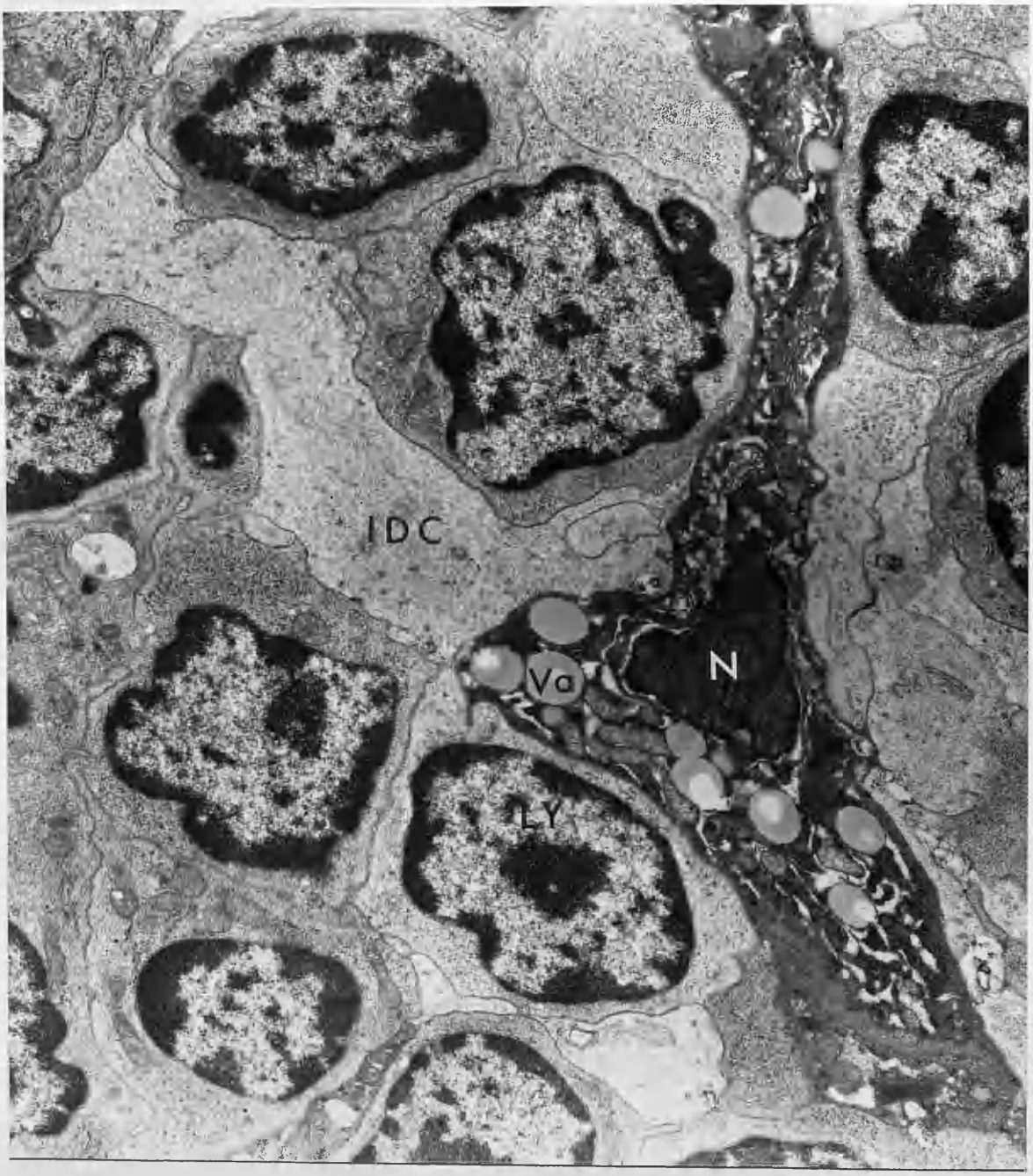

IDC
Vo
N
LY

Fig. 40

A low power electron micrograph of a cystic Hassall's corpuscle. Elongated epithelial cells (Ep) with prominent tonofilaments, are concentrically arranged around the central cystic epithelial cell. There are 3 peripheral intracellular cysts (Cy) containing amorphous material. The centre of the corpuscle is cystic and the lining epithelial cells have microvilli (long arrows). The central cyst is often filled with degenerative cellular debris, including lymphocytes (short arrows).

x 1,400 Rat 7 Bl. 11

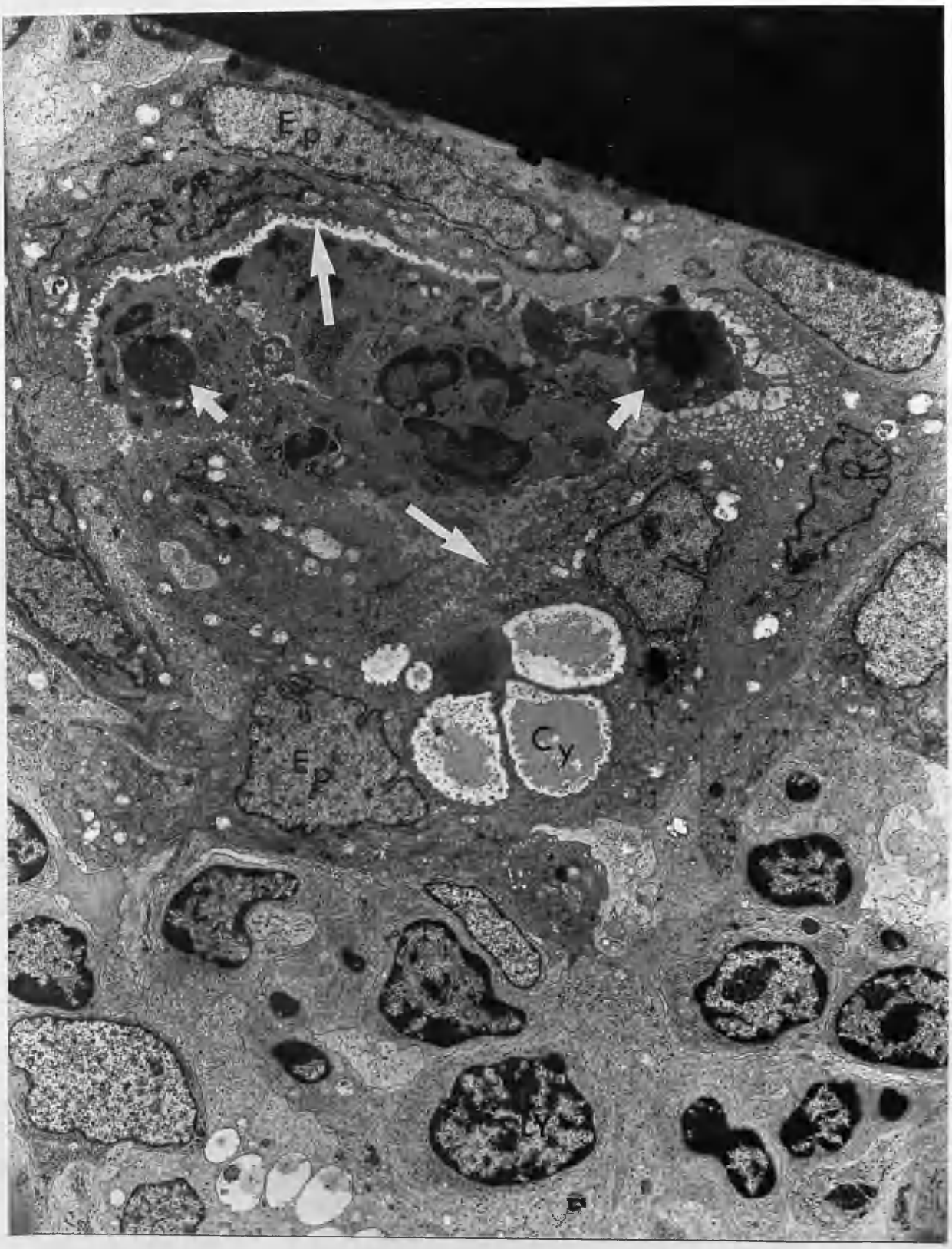

Ep
Ep
Cy
Y

Fig. 41

Numerous prominent bundles of tonofilaments in an epithelial-reticular cell forming part of a Hassall's corpuscle.

x 10,000 Rat 1 Bl. 3

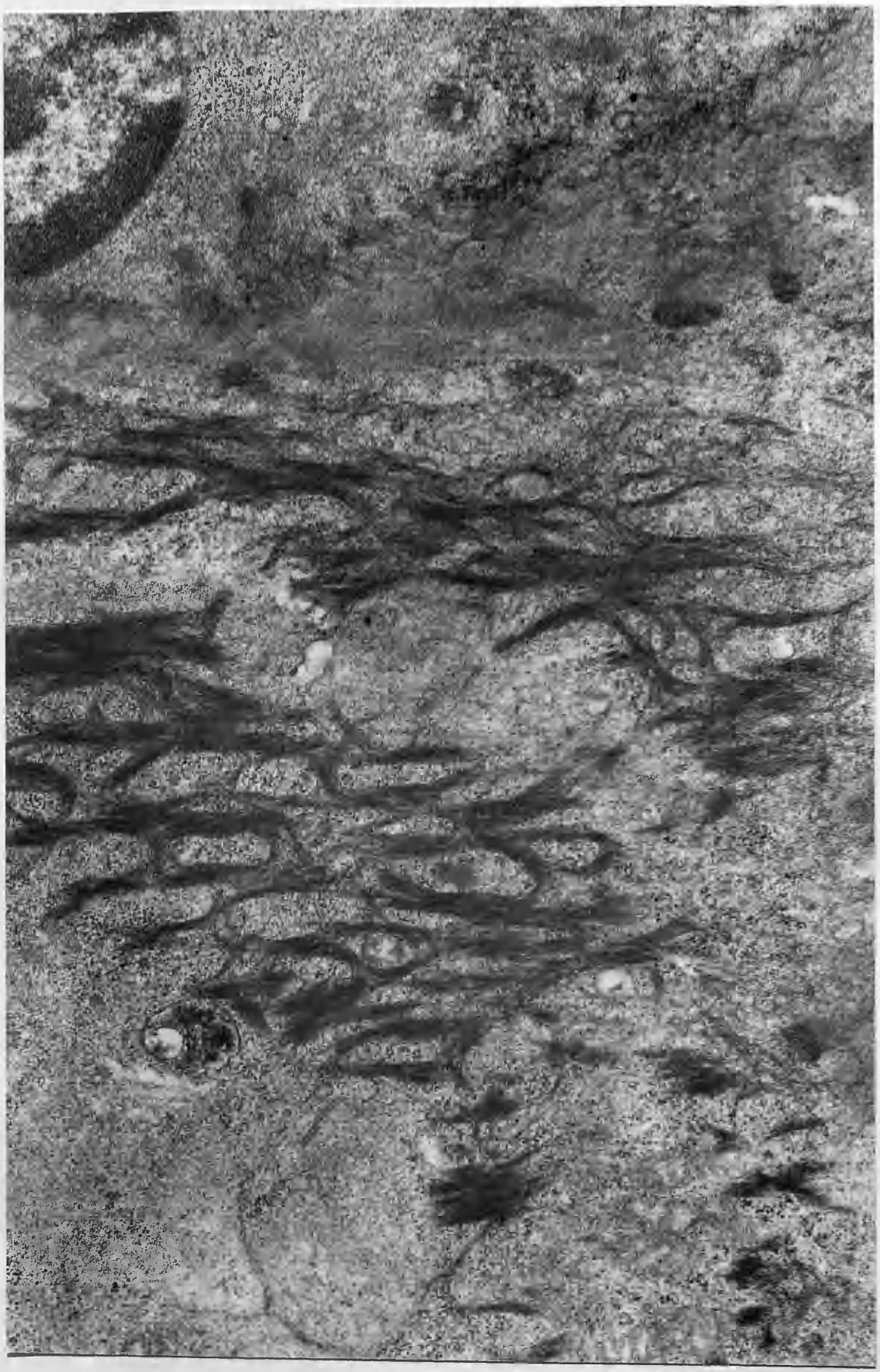
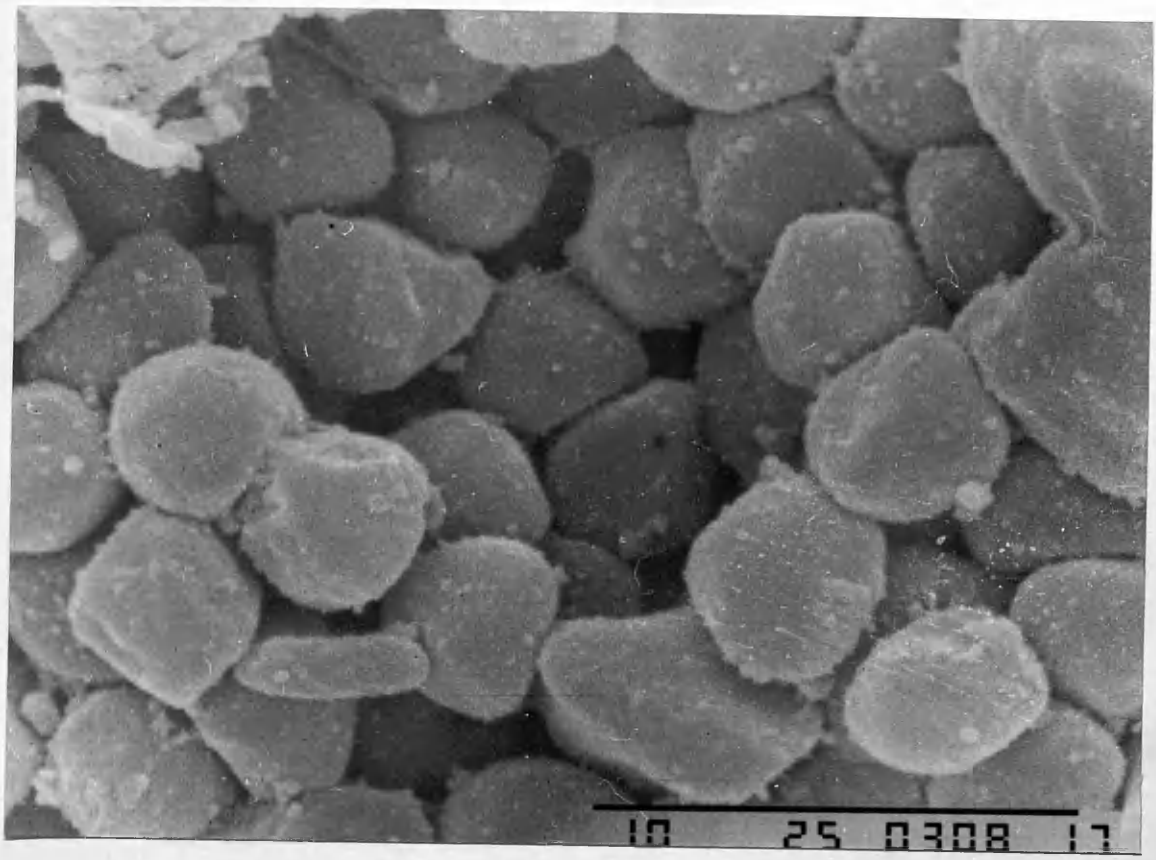


Fig. 42

A scanning electron micrograph of lymphocytes present in the cortex of the thymus. These lymphocytes closely resemble the seeds inside a pomegranate.

Rat 10


10 25 0308 17

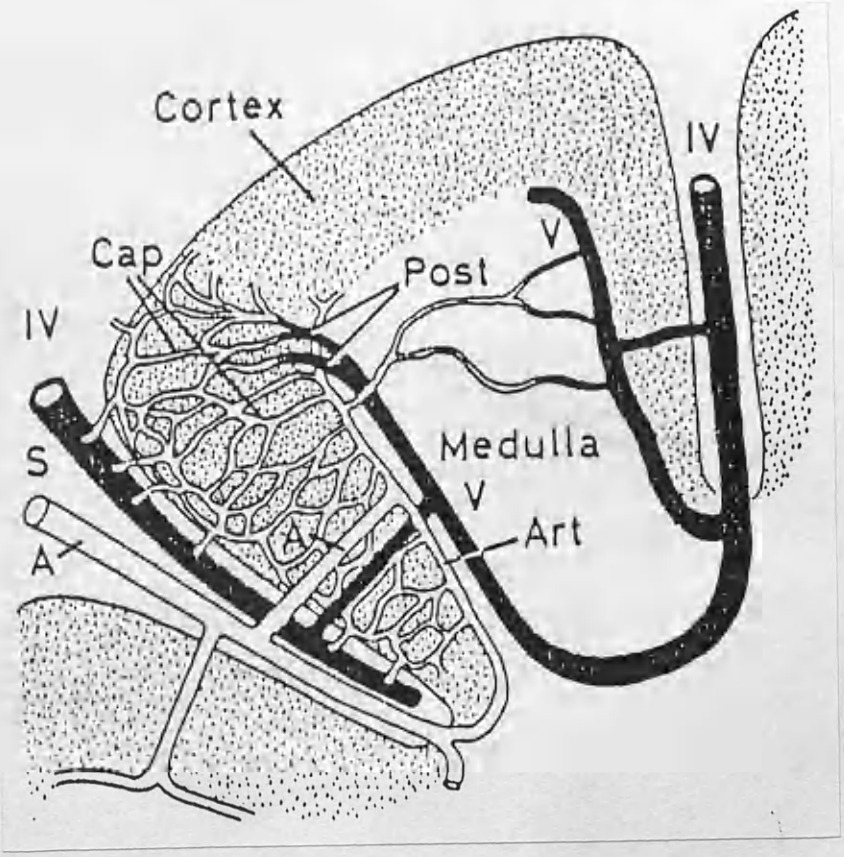


Fig. 43

Scheme of the distribution of blood vesels in a thymic lobule.

(From Krstic, R.V., 1984)

S = Connective tissue septum

IA = Interlobular artery (or primary septal artery)

IV = Interlobular vein (or primary septal vein)

Art = Arteriole

A = Intralobular artery (or secondary septal artery)

V = Intralobular vein (or secondary septal vein)

Cap = Capillary

Post = Post-capillary venule

Fig. 44

A transverse section of a blood capillary in the cortex of the thymic lobule. Note the presence of electron-lucent and dense forms of the capillary endothelial lining. The capillary lumen is completely surrounded by a thick layer of cytoplasmic processes (arrows) of epithelial - reticular cells (Ep). The perivascular space (Pvs) at this level of vessel size contains only collagen fibres and processes of a pericyte (Per).

Ly - lymphocyte.

x 2,100 Rat 7, Bl. 6

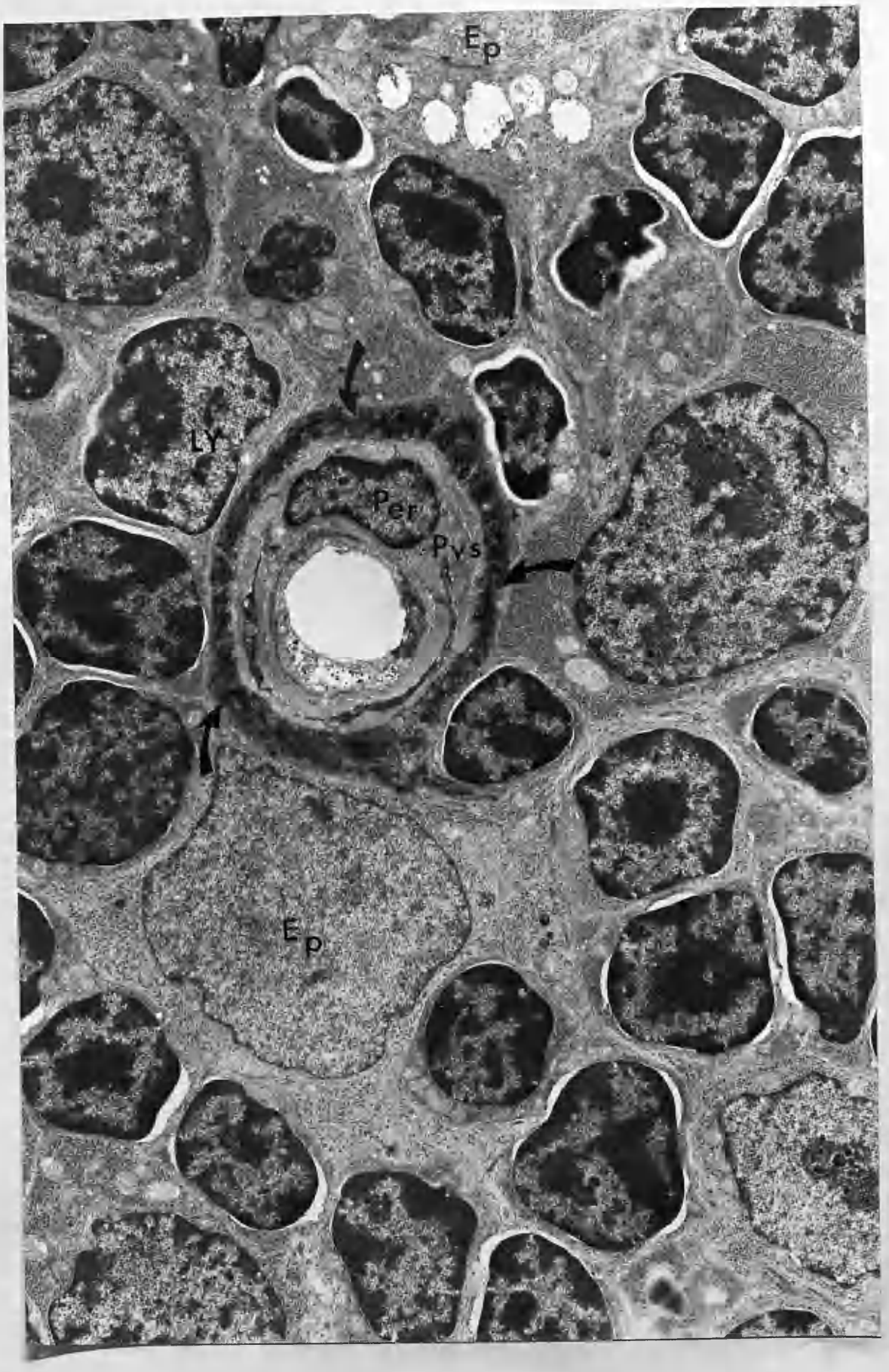

Ep
LY
Per
Pvs
Ep

Fig. 45

A cortical blood capillary partially surrounded by cytoplasmic processes of epithelial-reticular cells (long arrows). Lymphocytes (Ly) and processes of pericyte (Per) surround the capillary endothelium. The endothelial cells contain pinocytotic vesicles (short arrows) and show luminal cytoplasmic projections (asterisk).

x 3,500 Rat 7 Bl. 6

LY
Per
*
LY

Fig. 46

A transverse section through 3 cortical blood capillaries (probably branches of one vessel). Endothelium (End) is continuous with no fenestration, and contains vesicles of various sizes. The perivascular spaces are outlined by processes of epithelial-reticular cells (arrows).

Per - pericyte, Ly - lymphocyte.

x 2,100 Rat 7 Bl. 6

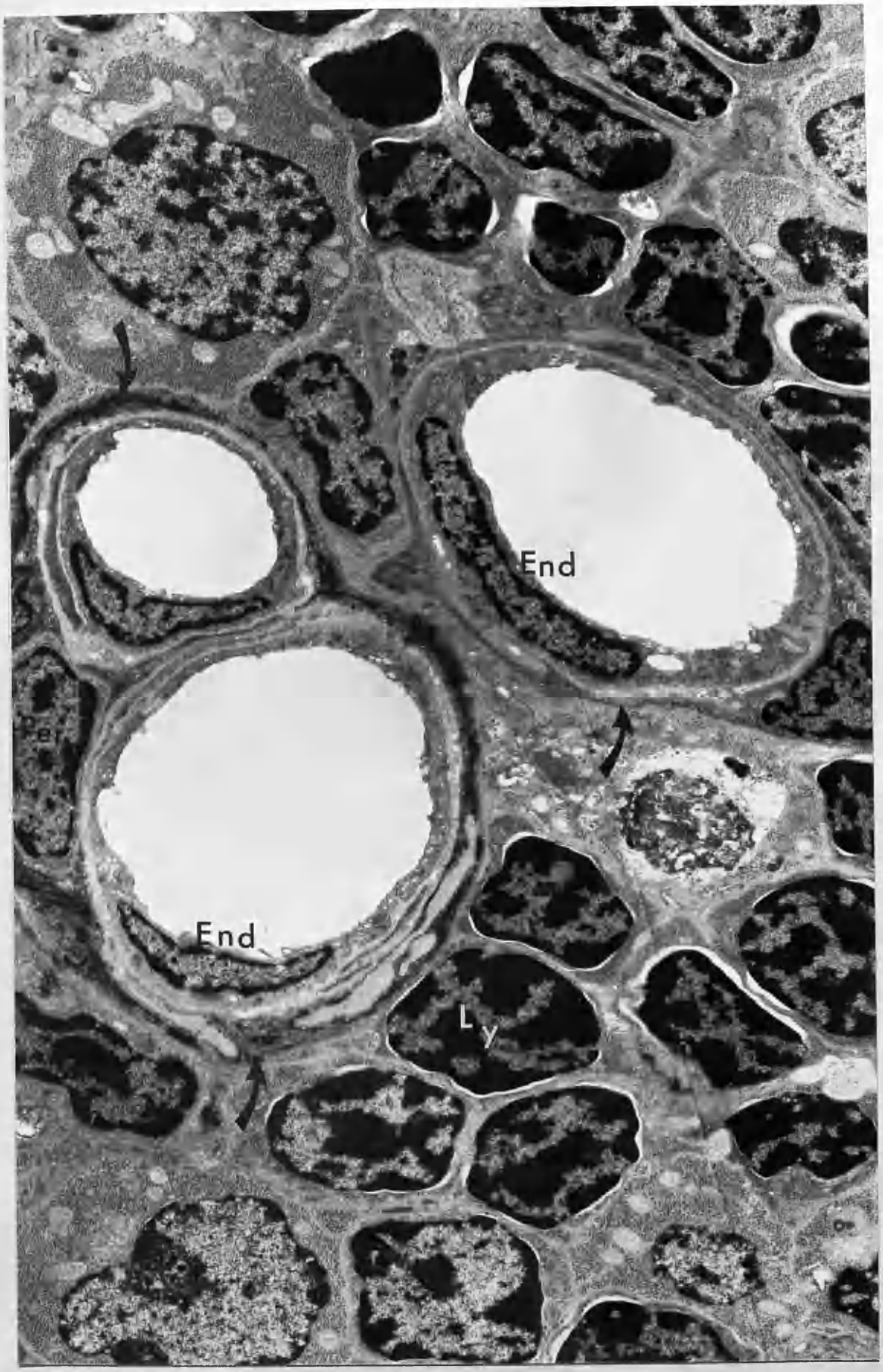

End
End
Ly

Fig. 47

A medullary blood capillary surrounded completely by basal lamina (large arrows) of two epithelial-reticular cells (Ep). The small arrow points to endothelial cell basal lamina. Several processes of pericytes (Per) and collagen fibres are present in the perivascular space (Pvs). The endothelial cell (End) contains vesicles (asterisks). No endothelial fenestrations are present. Note the variable electron-density of the capillary endothelium.

Ly - Lymphocytes.

x 2,100 Rat 5 Bl. 9

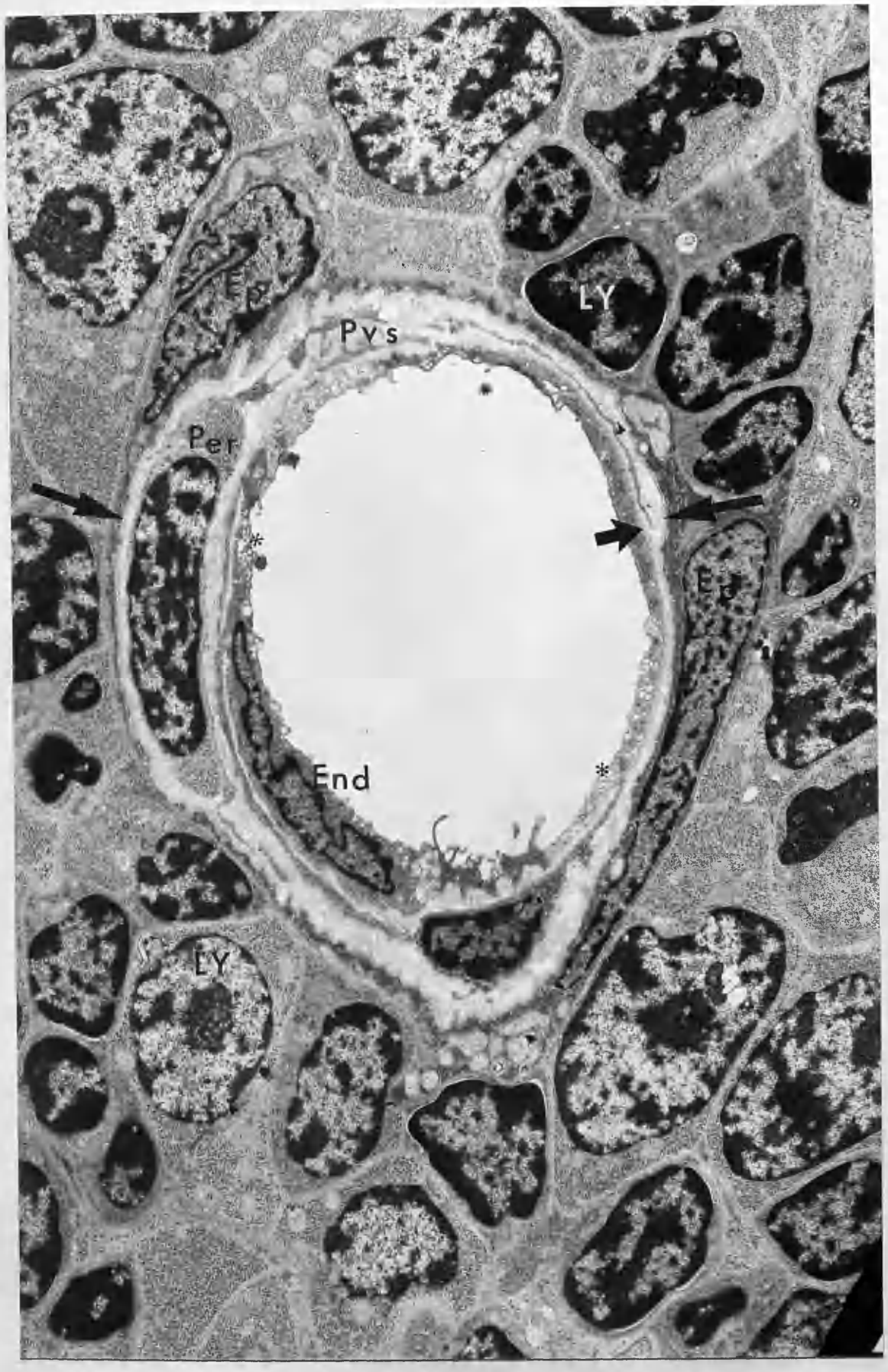

Ep
LY
Pvs
Per
*
Ep
End
*
LY

Fig. 48

A medullary blood capillary showing similar features to Fig. 47. The perivascular space (Pvs) is between the arrows.

Ep - epithelial-reticular cell, Per - pericyte, Ly- lymphocyte.

x 2,800 Rat 5 Bl. 9

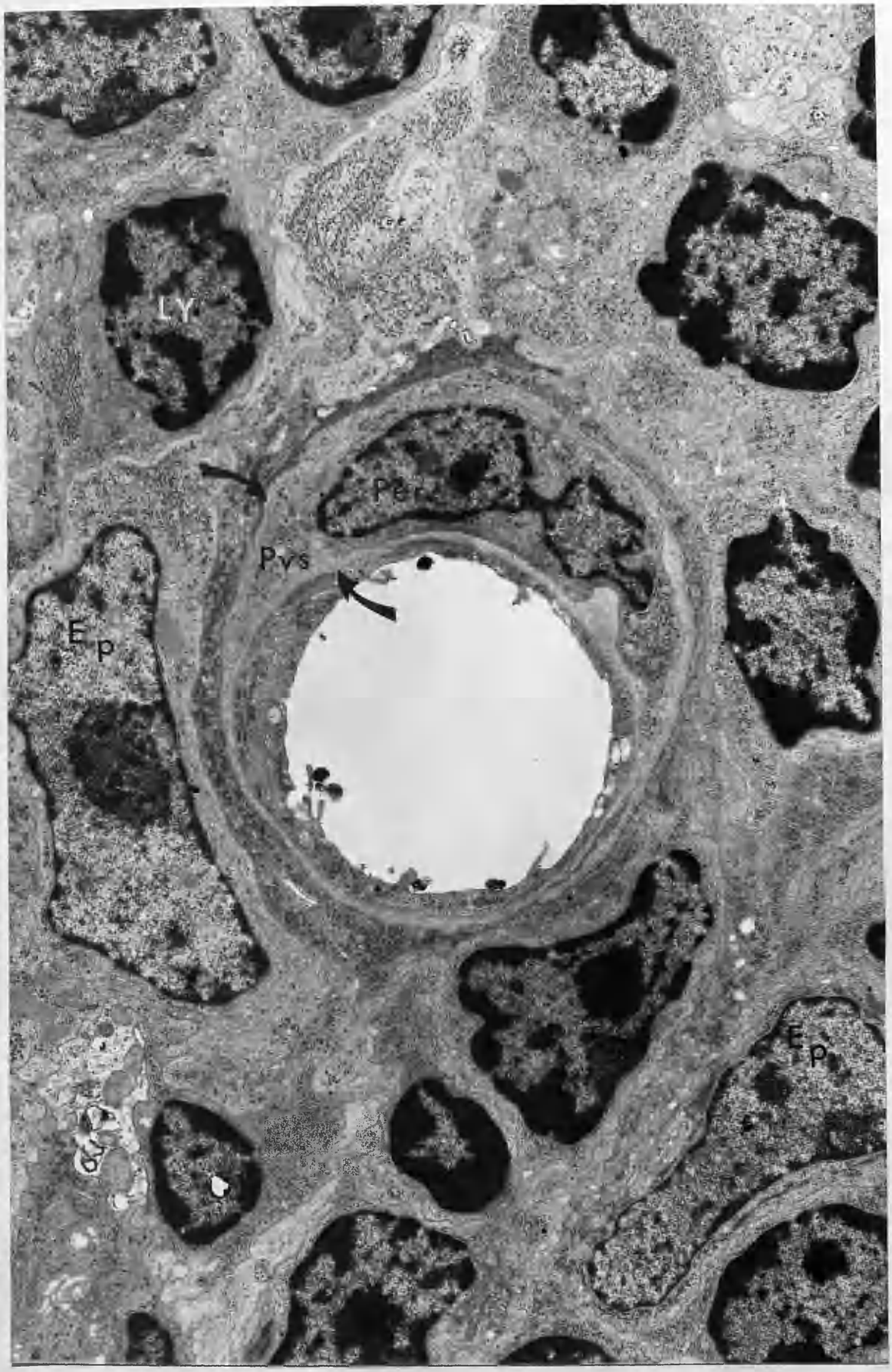

LY
Per
Pvs
Ep
Ep

Fig. 49

A low power electron micrograph of a medullary venule and part of thymic medulla. The processes of epithelial-reticular cells and their basal laminae are clearly visible (arrows). The space between the blood vessel and the epithelial cells (Ep) is filled with cells including small lymphocytes (Ly), macrophages (Ma), and interdigitating-reticulum cells (Idc).

Per - pericyte, End - vascular endothelium.

x 1,400 Rat 4 Bl. 5

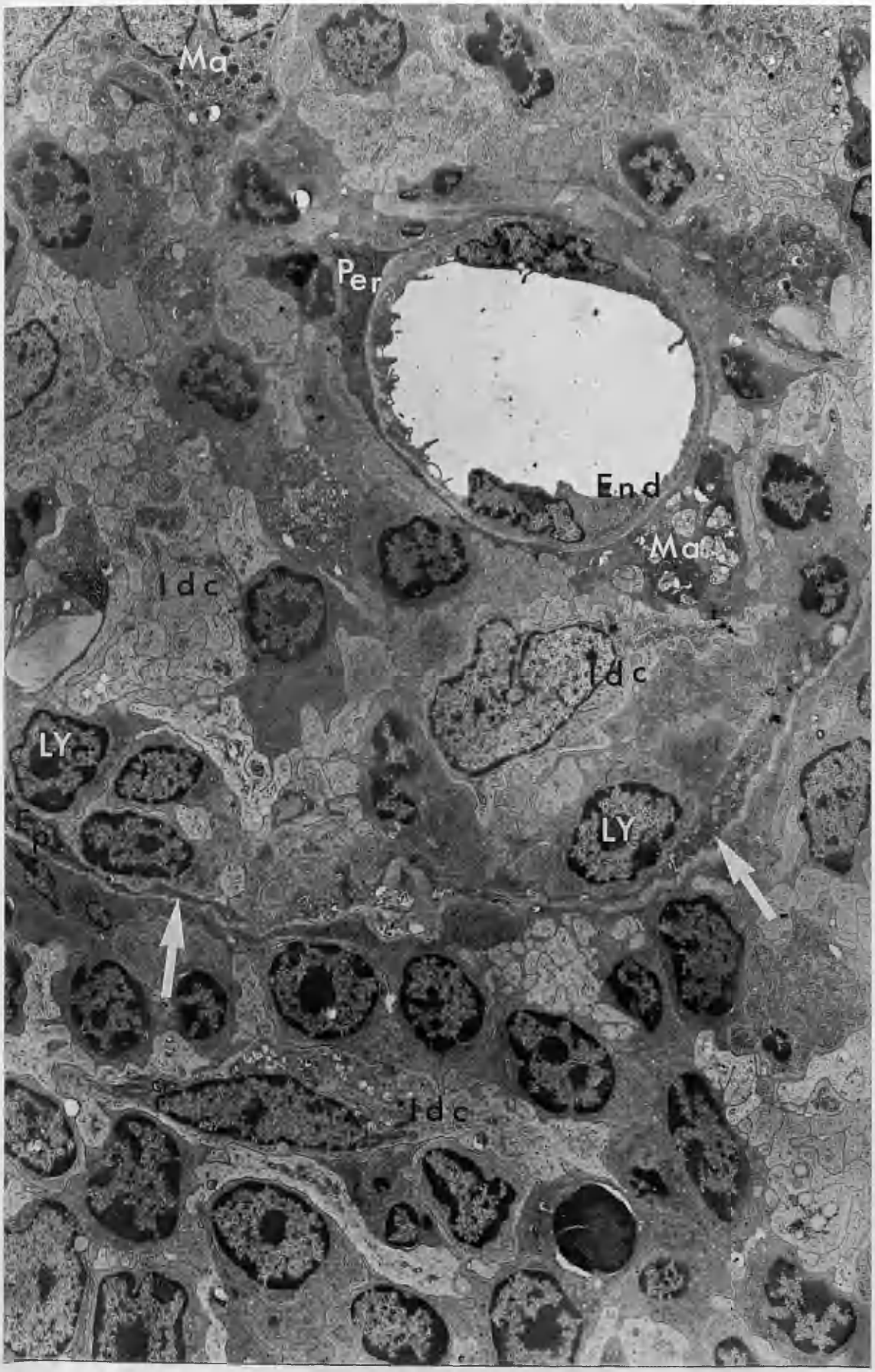

Ma
Per
End
Ma
Idc
Idc
LY
LY
Idc

Fig. 50

A branching blood capillary in the medullary parenchyma of the thymus. The perivascular space (Pvs) is partially surrounded by well-defined epithelial cell processes (arrows). Where the capillary is not surrounded by epithelial-reticular cells, it is in contact with lymphocytes (Ly) and interdigitating reticulum cells (Idc). End - endothelium.

x 3,500 Rat 7 Bl. 11

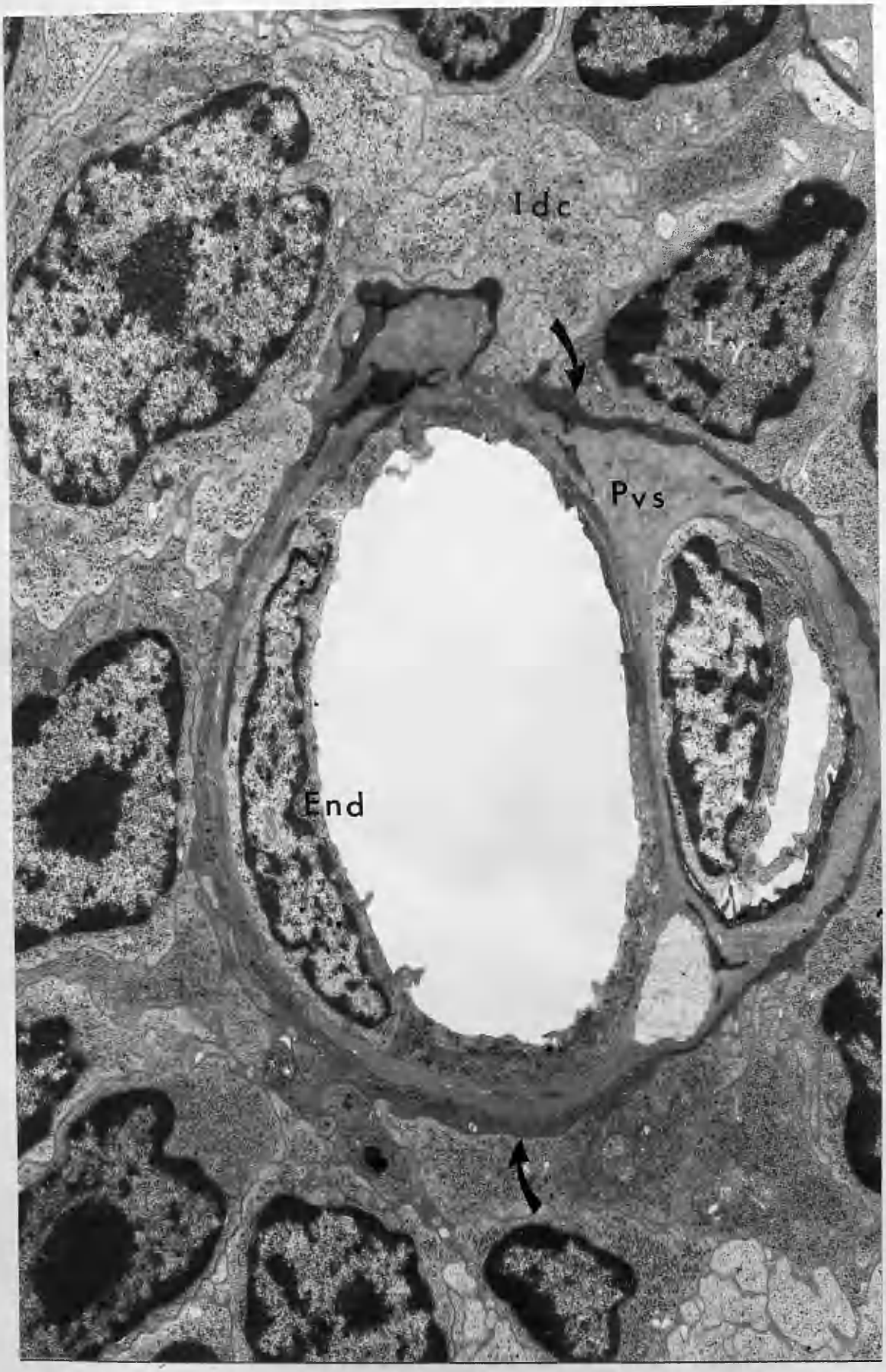

Idc
Ly
Pvs
End

Fig. 51

Electron micrograph of a medullary venule and part of the medullary parenchyma which shows similar features to Fig. 49. The perivascular space is well delineated by the basal laminae of epithelial-reticular cells (arrows). Small lymphocytes (Ly) and interdigitating cells (Idc) are seen in the space.

Ep - epithelial-reticular cell, Pyk - pyknotic lymphocyte.

x 1,400 Rat 1 Bl. 2

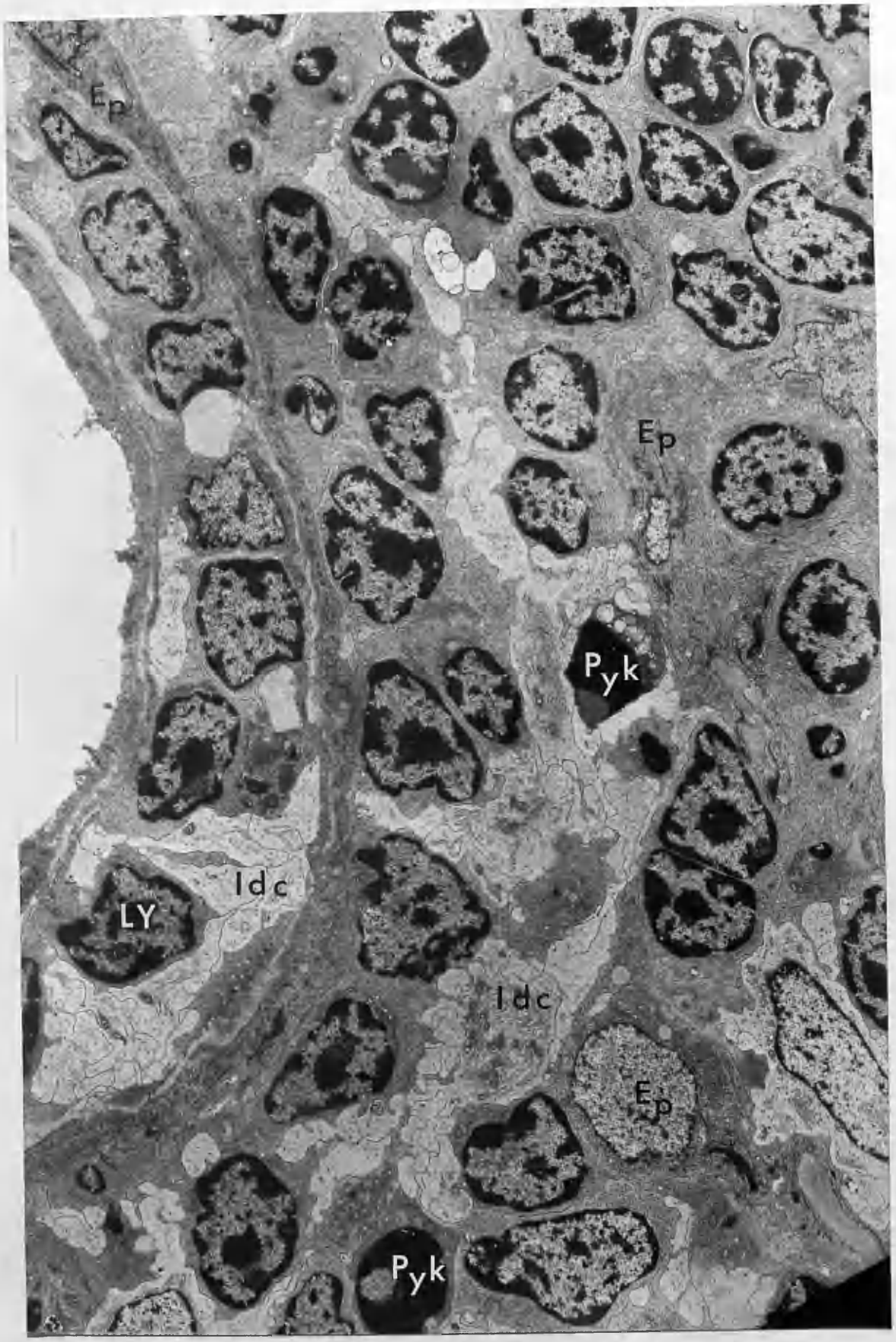

Ep
Ep
Pyk
LY
Idc
Idc
Ep
Pyk

Fig. 52

A reduced electron microscopic montage of a medullary arteriole (Art) and a capillary (Cap). Both are partially surrounded by epithelial basal lamina (arrows).

Ep - epithelial-reticular cell, Ma - macrophage, End - endothelial cell, Sm - smooth muscle cell, Per - pericyte, Rbc - red blood cell.

Rat 7 Bl. 11

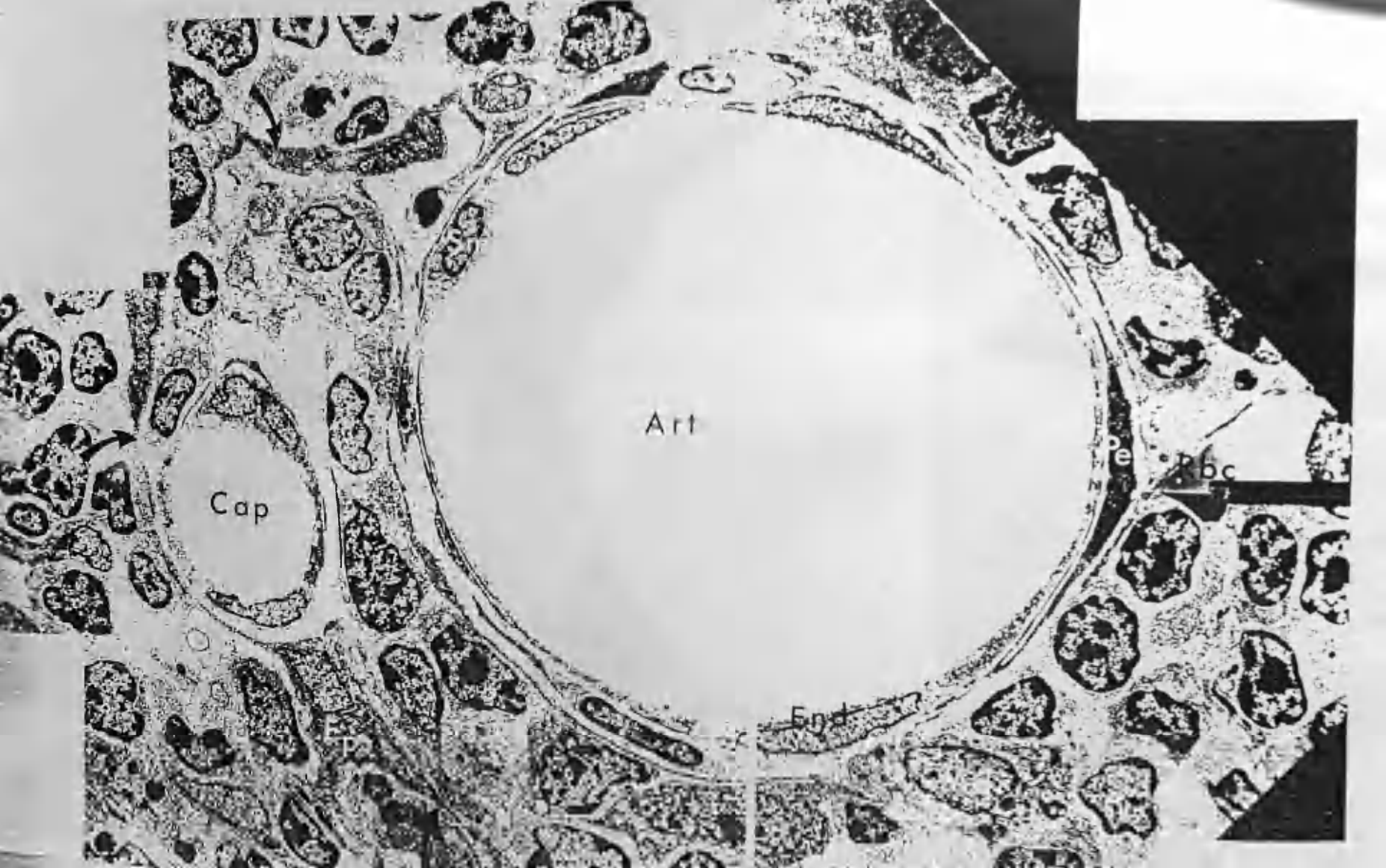

Art
Cap
Rbc
Endt
E
P

Fig. 53

A higher magnification of the wall of a medullary arteriole (Art) shown in Fig. 52. The lumen is lined by endothelium (End) containing the usual cytoplasmic organelles. Smooth muscle cells (Sm). Adventitia consists mainly of collagen fibres (Cf).

x 5,600 Rat 7 Bl. 11

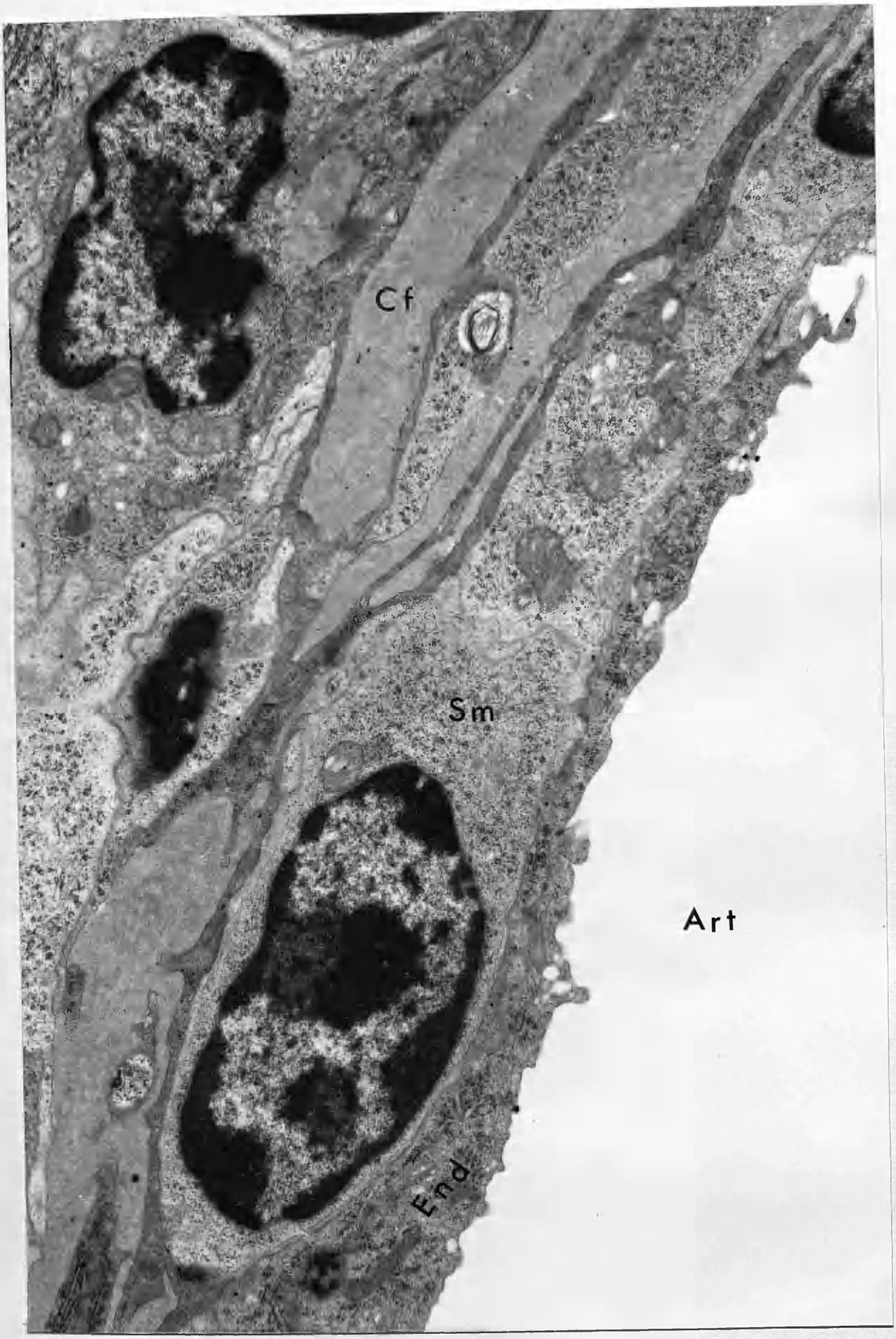

Cf
Sm
Art
End

Fig. 54

An example of another medullary blood capillary with similar features as described in Figs. 47 & 48. Both epithelial (long arrows) and endothelial (small arrows) basal laminae are clearly visible. The perivascular space (Pvs) contains only collagen fibres (Cf) and processes of pericytes (Per). Note the electron-dense areas (arrow heads) in contrast to the rest of the endothelial lining (End) which is electron-lucent.

Ly - lymphocyte, Idc - interdigitating-reticulum cell.

x 5,600 Rat 7 Bl. 11

End
Ly
Ly
Fdc

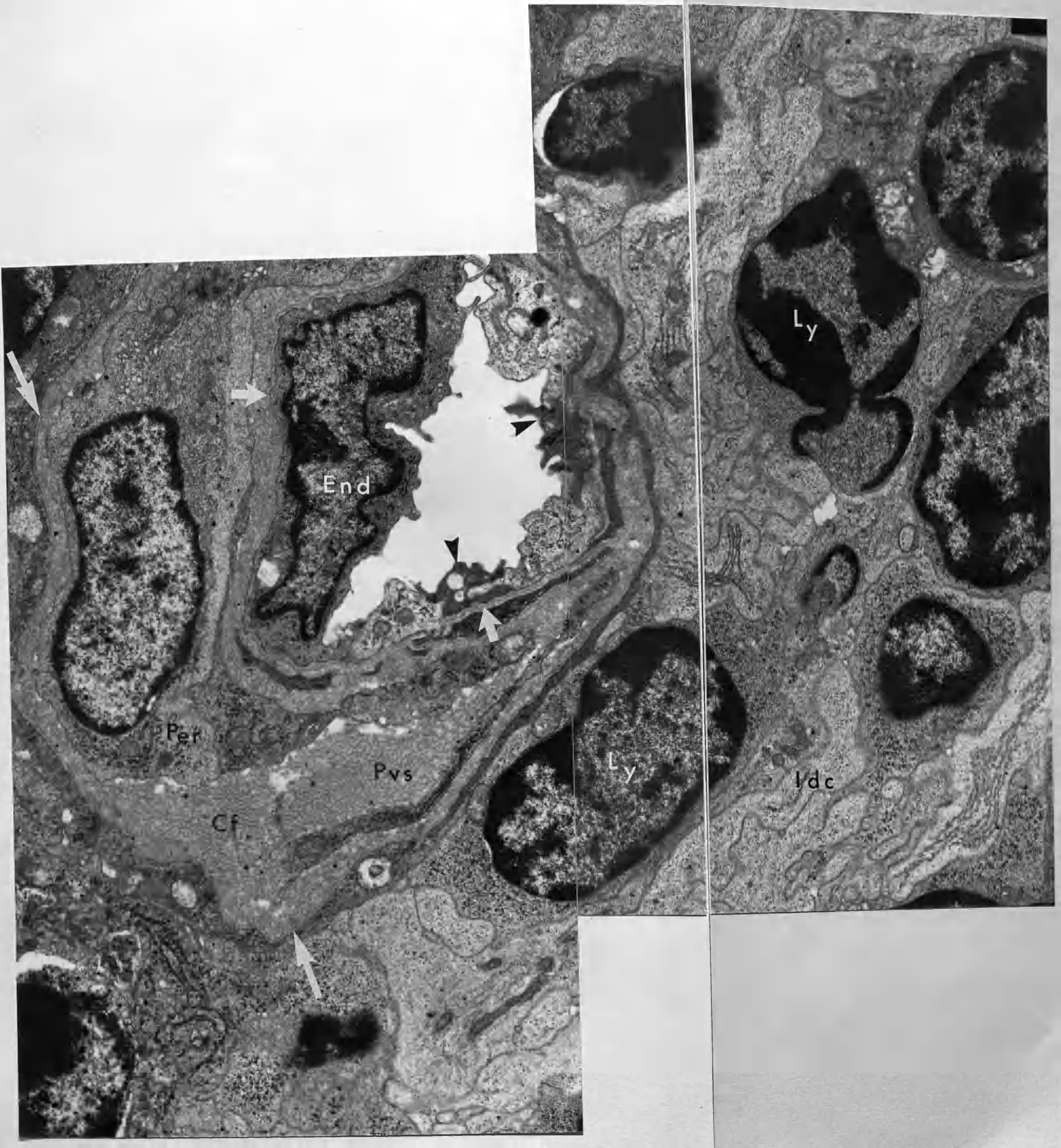

End
Per
Pvs
Cf
Ly
Ly
Idc

Fig. 55

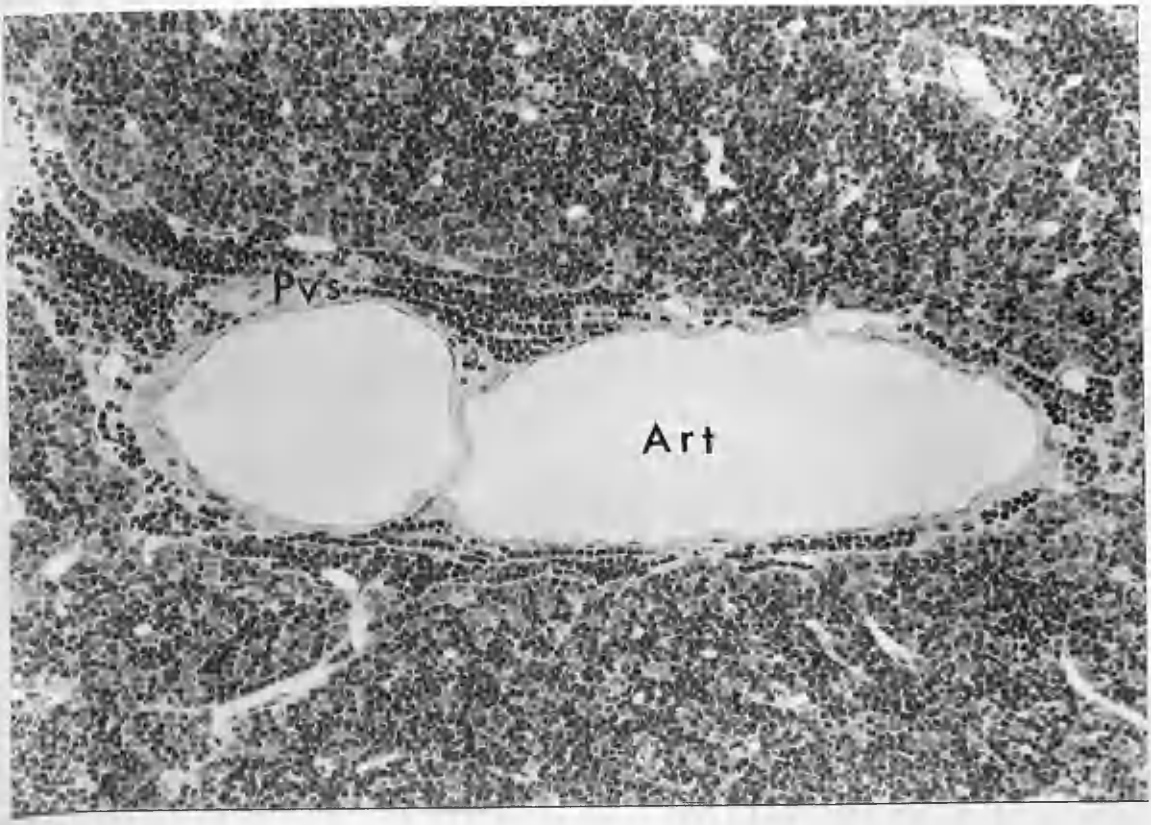
A low power photomicrograph of a secondary septal (intralobular) arteriole (Ar) surrounded by a perivascular space (Pvs) packed with several rows of cells, predominantly small lymphocytes. These perivascular lymphocytes appear smaller and stained more deeply than parenchymatous lymphocytes; presumably the characteristic of maturity.

x 250 Rat 8 H & E

Fig. 56

A higher magnification of some cells found in the perivascular space described in Fig. 55. Note the presence of many plasma cells (arrows) most of which are mature as indicated by the prominent cytoplasmic vacuoles (representing a well-developed Golgi apparatus).

x 1,250 Rat 8 H & E


Pvs
Art

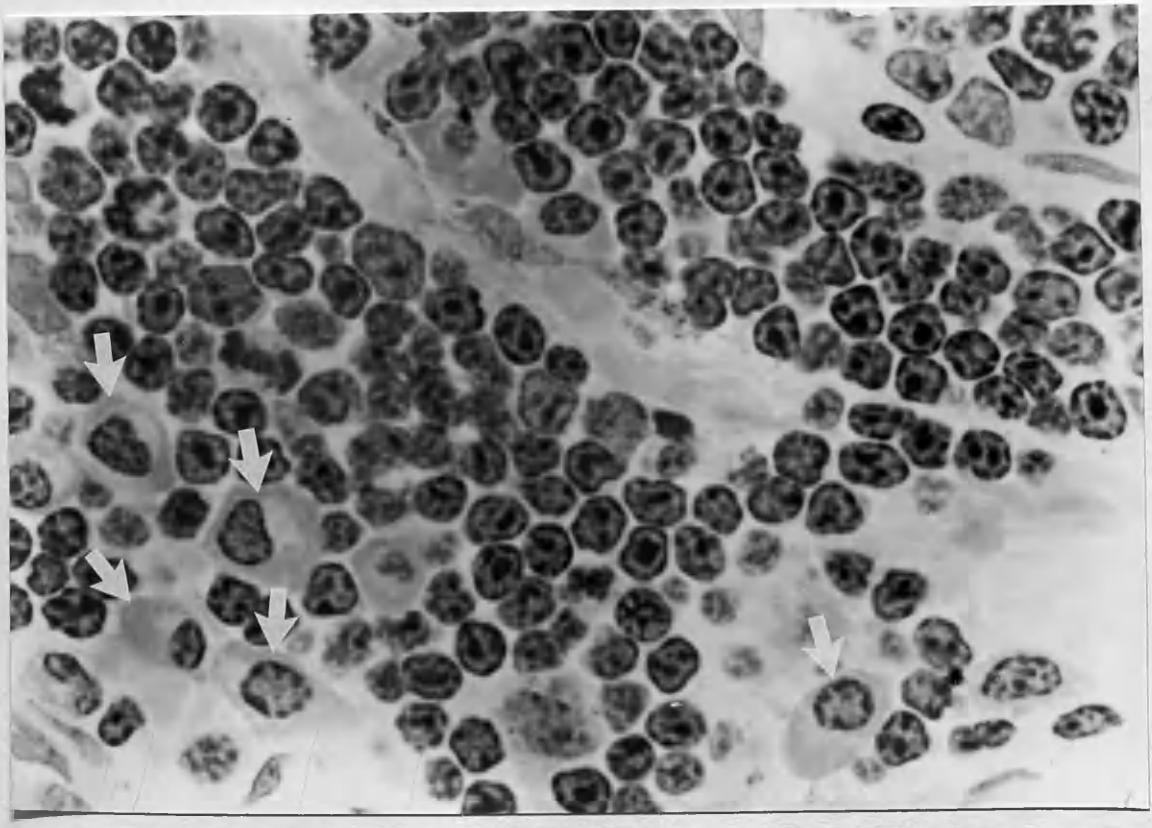


Fig. 57

High magnification of the same perivascular space in Fig. 55.

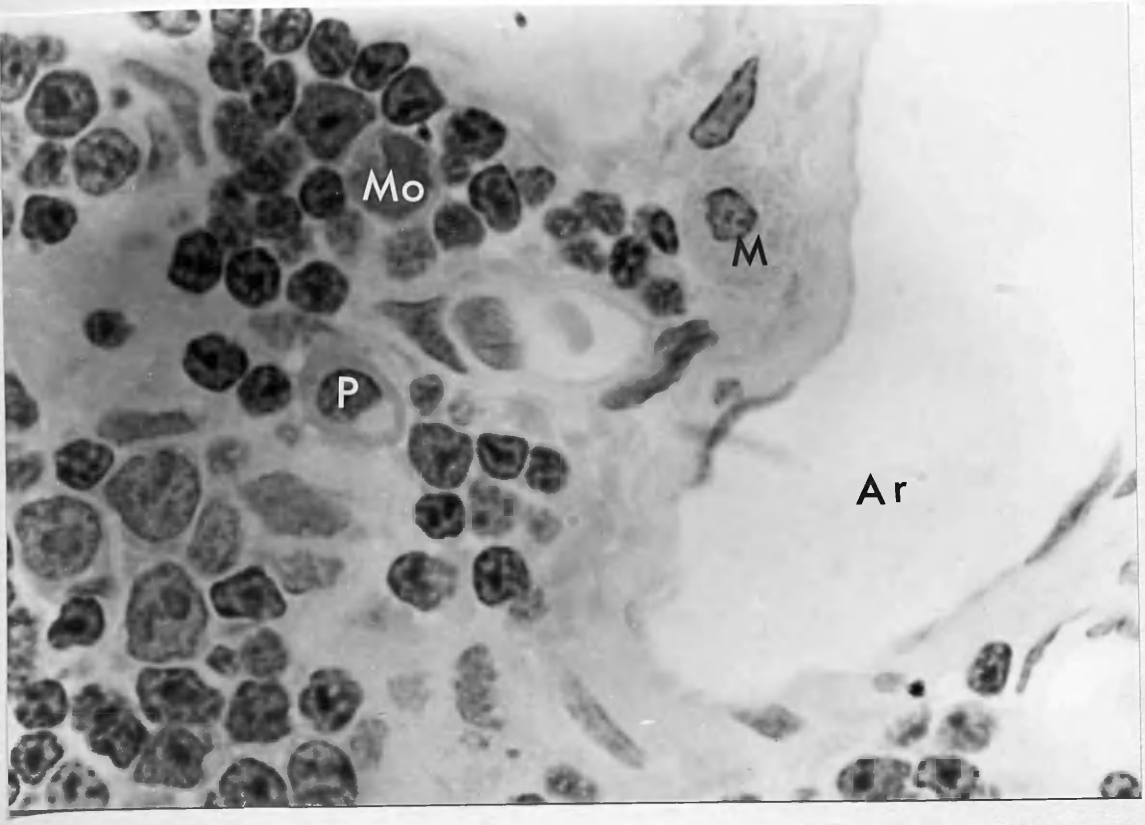
P - plasma cell, M - mast cell, Mo - monocyte, Ar - Arteriole.

x 1,250 Rat 8 H & E

Fig. 58

A cross-sectional view of a small intralobular arteriole (Ar) surrounded by a proportionally small perivascular space containing one row of small lymphocytes.

x 400 Rat 4 H & E


Mo
M
P
Ar

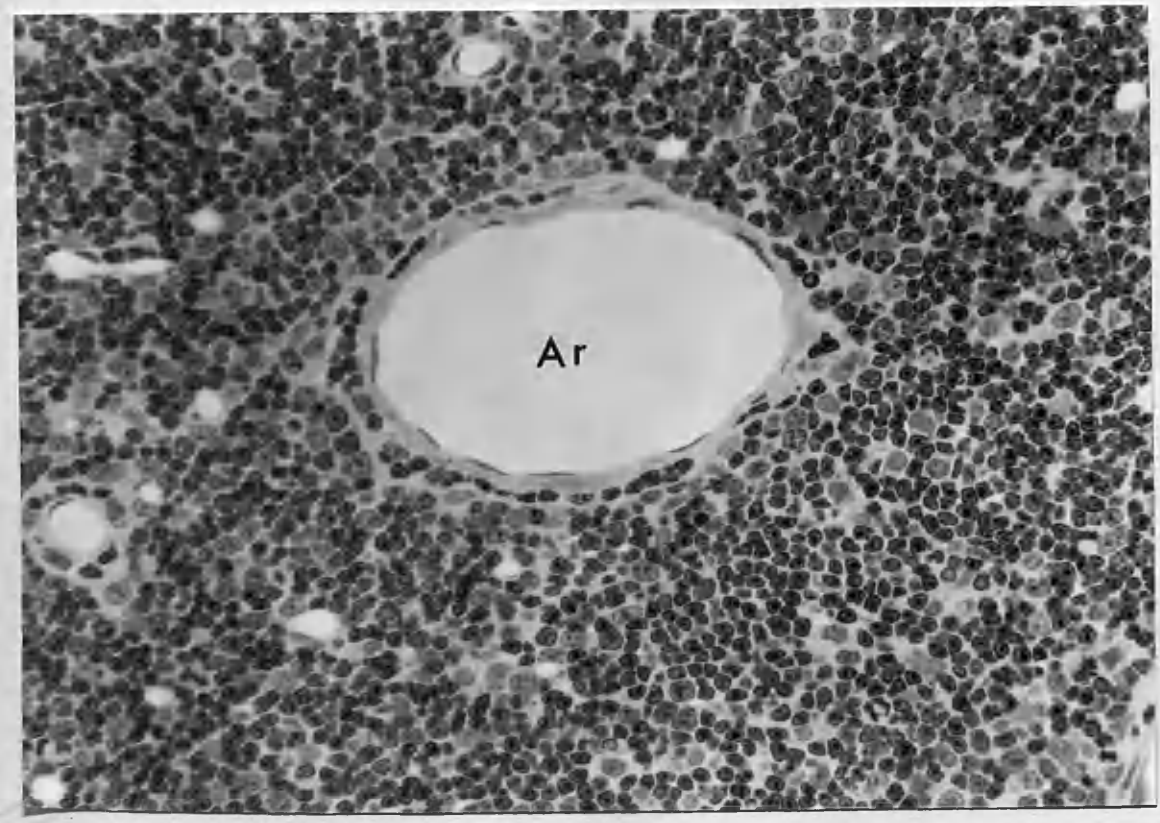

Ar

Fig. 59

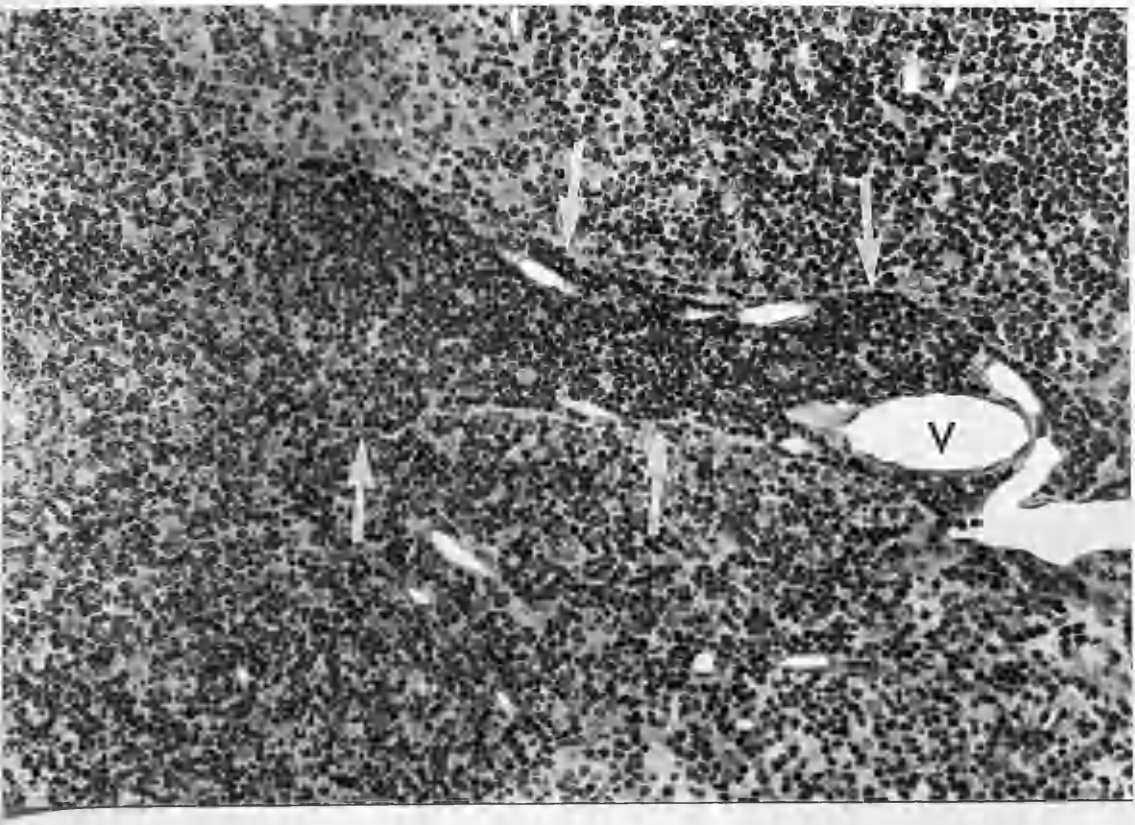
A low power photomicrograph of a venule (V) surrounded by a space filled with cells, most of which are small darkly-stained lymphocytes. Note the prominent epithelial boundary (arrows) which separates the space from the rest of the thymic parenchyma.

x 320 Rat 6 Bl. 7 Azur II

Fig. 60

A medullary venule (V) completely surrounded by epithelial basal lamina (arrows). The perivascular space (Pvs) contains a moderate number of cells, most of which are small lymphocytes.

x 280 Rat 1 Bl. 2 Azur II


V

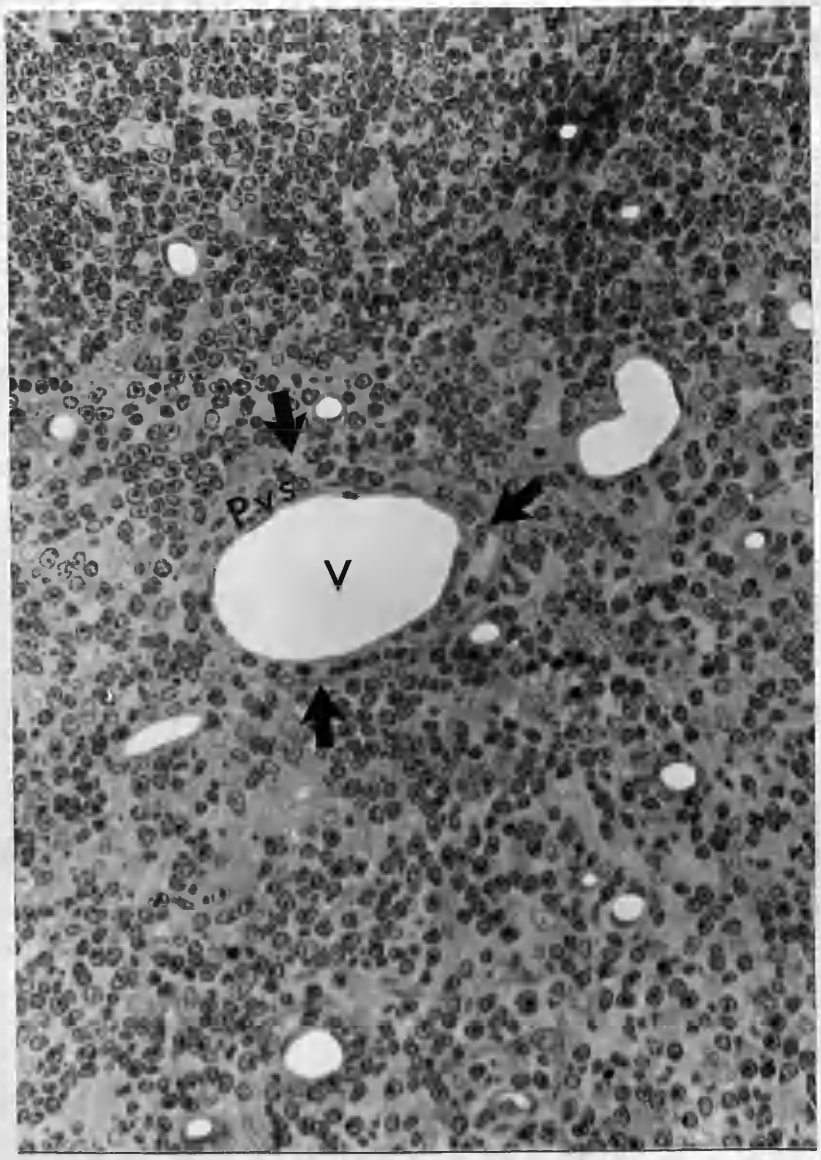

PVS
V

Fig. 61

A low power electron micrograph of an intralobular arteriole (Ar) with a portion of its wide perivascular space (Pvs). Many small lymphocytes (Ly), a red blood cell (Rbc) and a macrophage (Ma) are seen in the space. Epithelial-reticular cells (Ep) with their prominent basal lamina are clearly demonstrated (arrows). Note the branching character of the epithelial-reticulum forming a meshwork, within which lymphoid cells lie in the interstices (between arrows).

Idc - interdigitating-reticulum cell.

x 1,400 Rat 6 Bl. 7

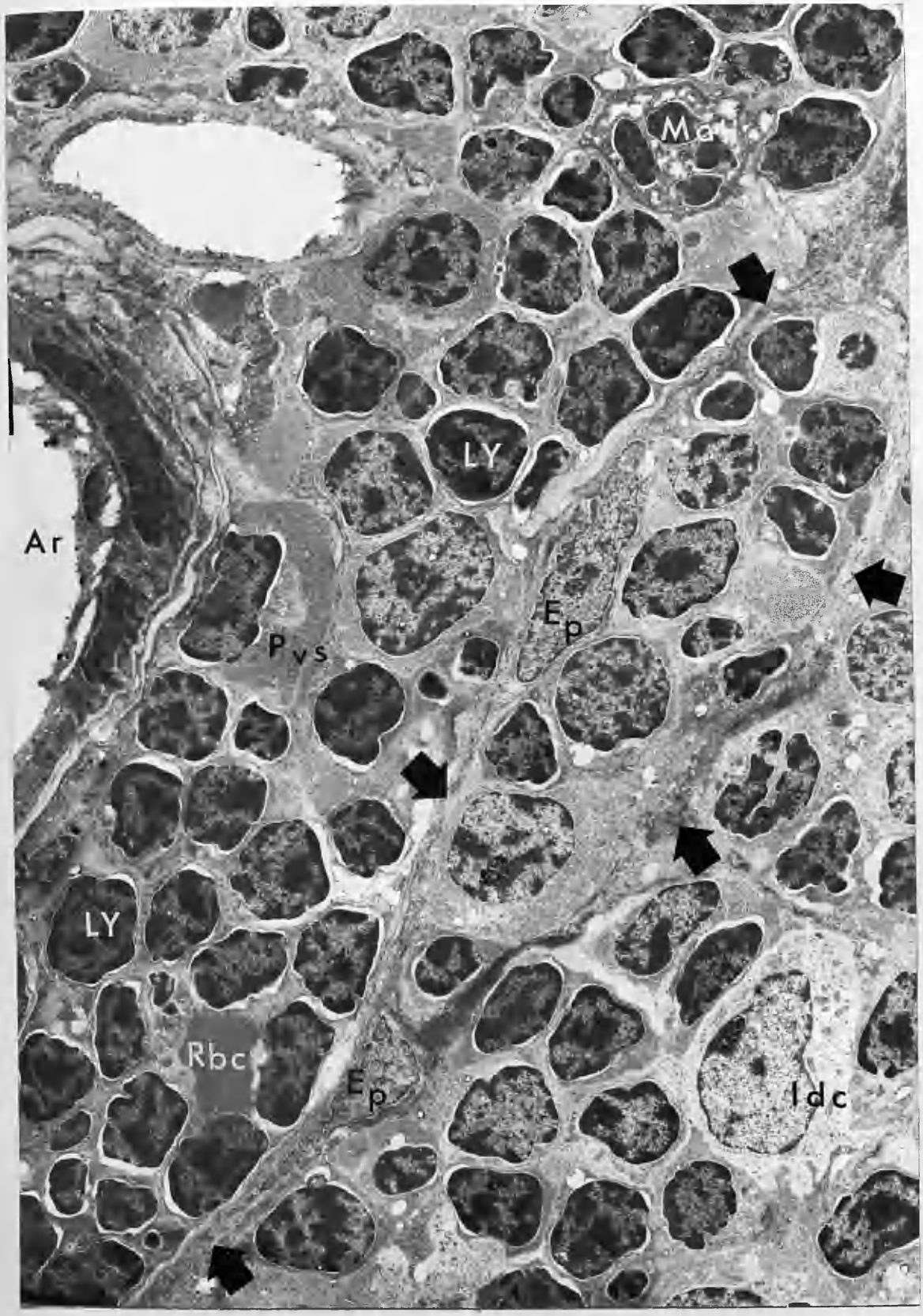

Ma
LY
Ar
Pvs
Ep
LY
Rbc
Ep
Idc

Fig. 62

An electron microscopic photograph of a cortico-medullary venule (V) with its clearly visible perivascular space (Pvs).

Ly - small lymphocyte, Ep - epithelial-reticular cell, P - plasma cell, End - endothelium, Per - pericyte.

x 1,400 Rat 5 Bl. 1

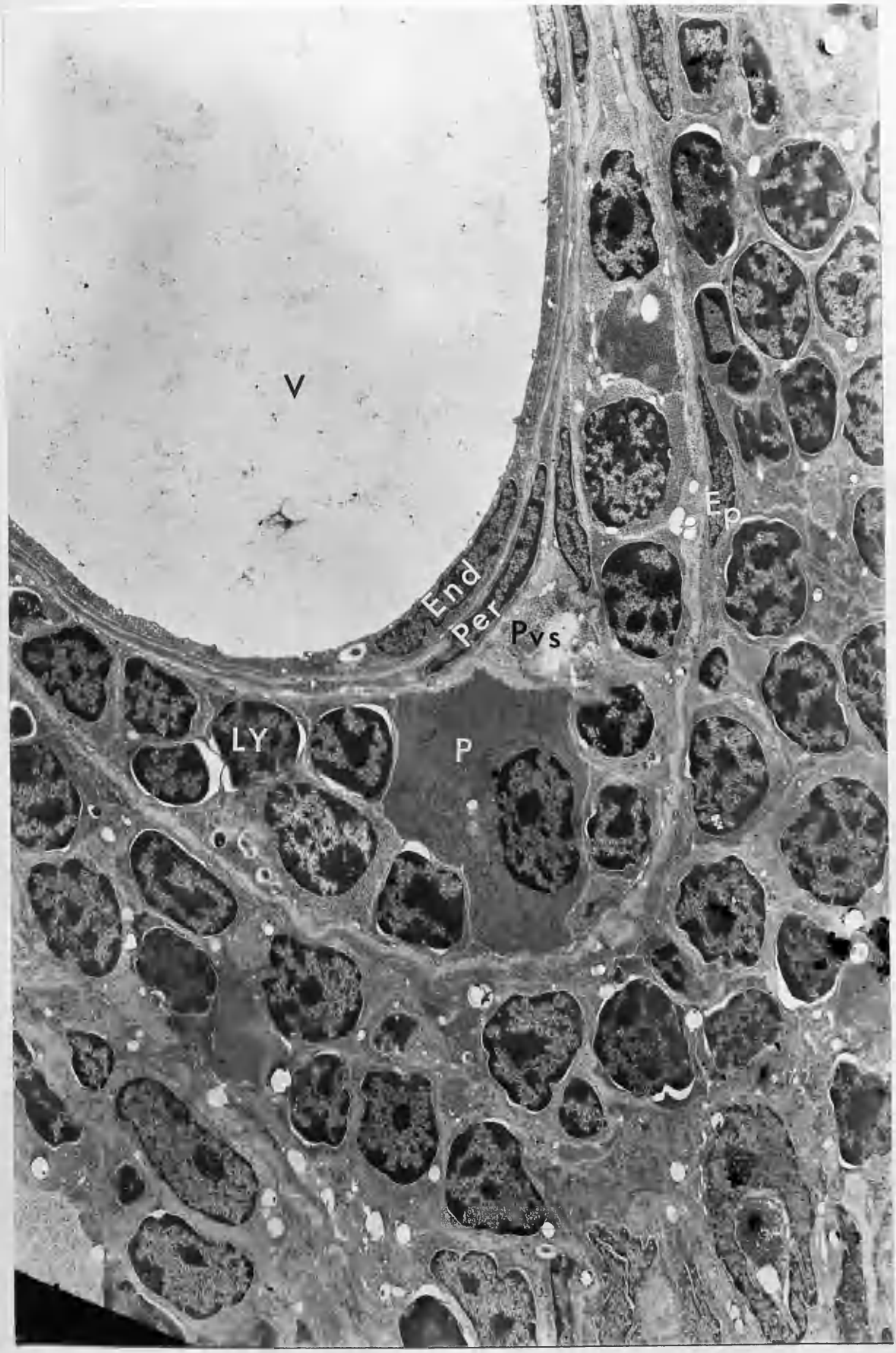

V
End
Per
Pvs
Ep
LY
P

Fig. 63

Electron micrograph of an arteriole (A) accompanied by a venule (V); both are located at the cortico-medullary junction of the thymus. It shows similar features to Fig. 62.

Pvs - perivascular space, End - endothelium, Sm - smooth muscle cell, Per - pericyte, P - plasma cell, Ly - small lymphocyte, Ep - epithelial-reticular cell, Cap - blood capillary, (arrows) - epithelial basal lamina.

x 1,400 Rat 5 Bl. 9

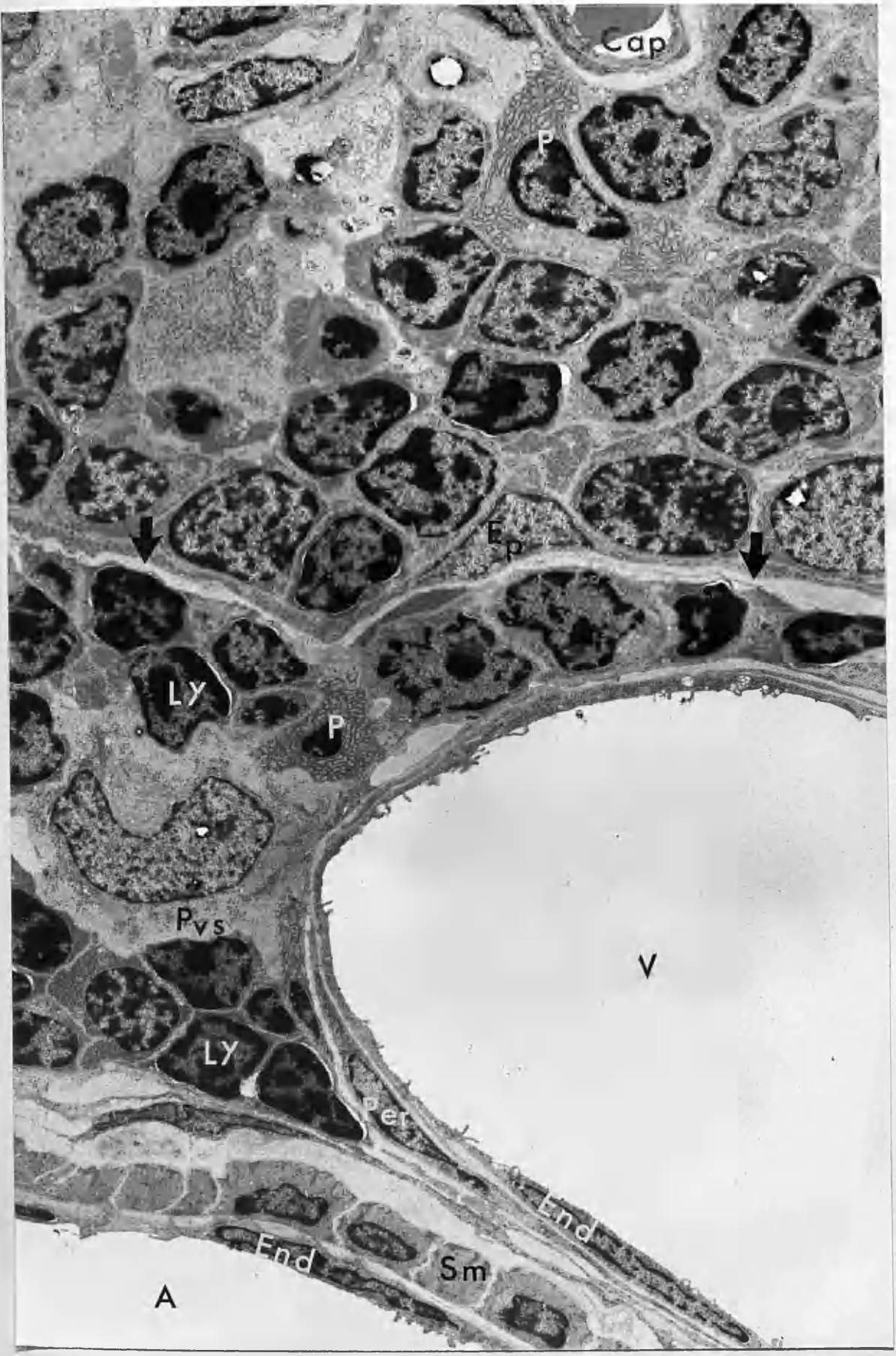

Cap
P
Ep
LY
P
Pvs
V
LY
Per
End
End
Sm
A

Fig. 64

An electron microscopic montage of a perivascular space marked B in Fig. 88. The space (Pvs) is separated from the thymic parenchyma by a clearly visible basal lamina (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.

x 1,400 Rat 1 Bl. 10

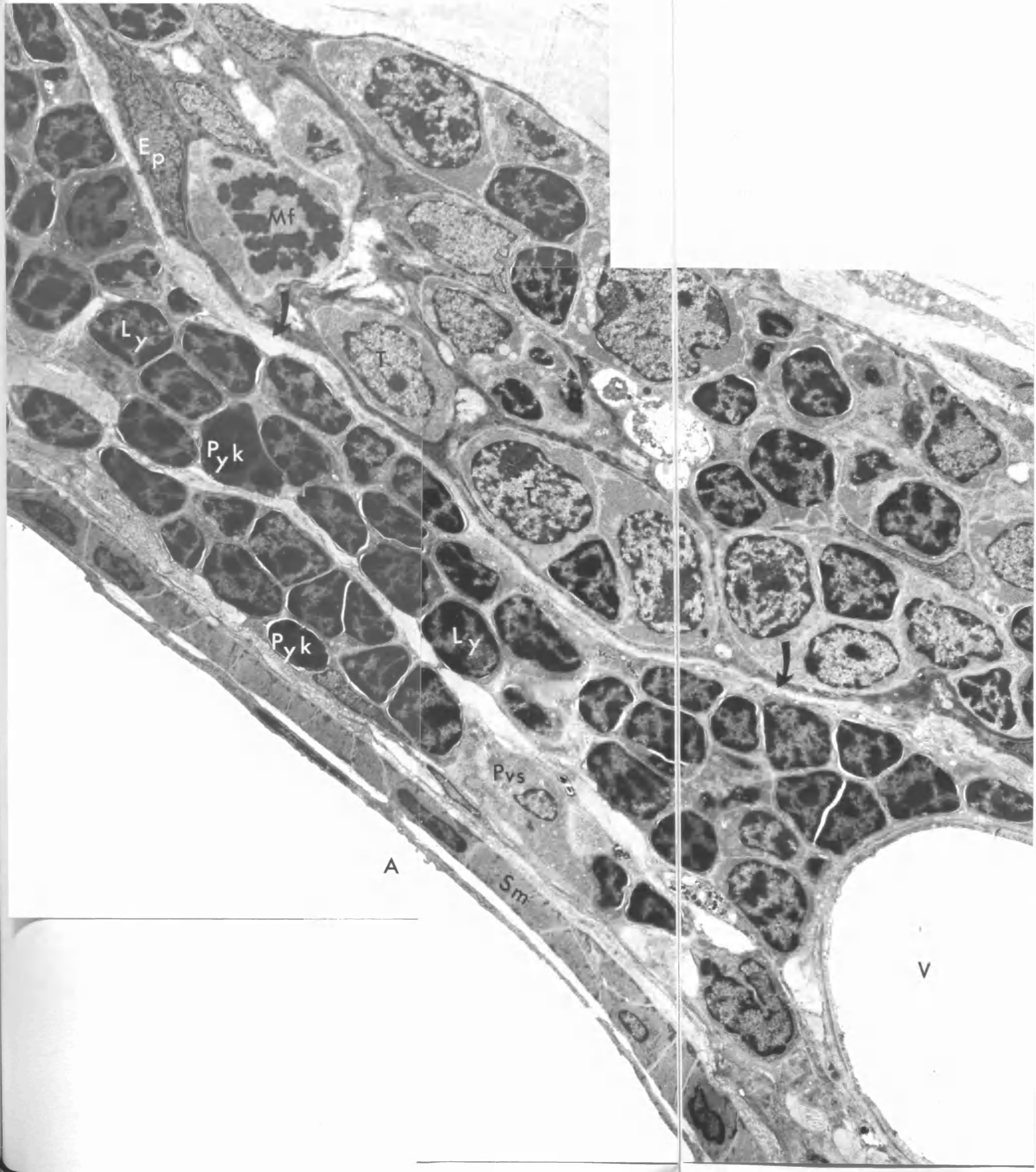

Ep
Mf
Ly
T
Pyk
Pyk
Ly
Pvs
Sm
A
V

Fig. 66

Electron micrograph of an intralobular arteriole (A) and its accompanying venule (V) with part of their perivascular space (Pvs). The space is clearly outlined by basal lamina (arrows) of epithelial-reticular cells (Ep). It contains collagen fibres (Cf), a mast cell (M) and a fibroblast (F).

End - endothelial cell, Sm - smooth muscle cell, Per - pericyte, Ly - lymphocyte, P - plasma cell, Mf - mitotic figure, (arrowheads) - endothelial cytoplasmic projections, (asterisks)- endothelial pinocytotic vesicles.

x 2,100 Rat 6 Bl. 13

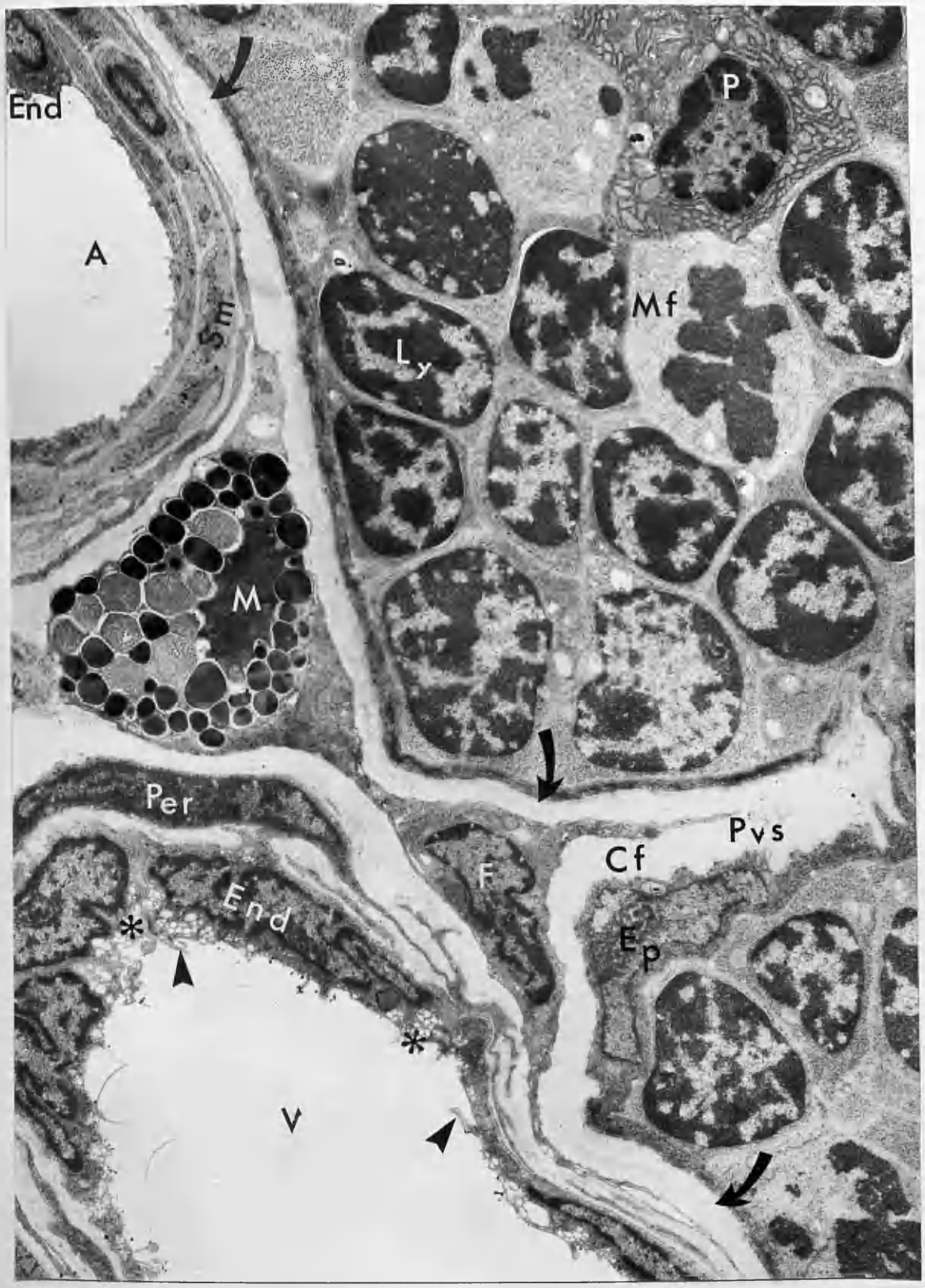

End
A
Sm
P
Mf
Ly
M
Per
Pvs
Cf
F
End
Ep
V

Fig. 67

Part of a thymic lobule. The darker cortex (Co) surrounds a paler medulla (Me). This photograph shows the course and distribution of arterial blood supply from connective tissue septum to the thymic medulla.

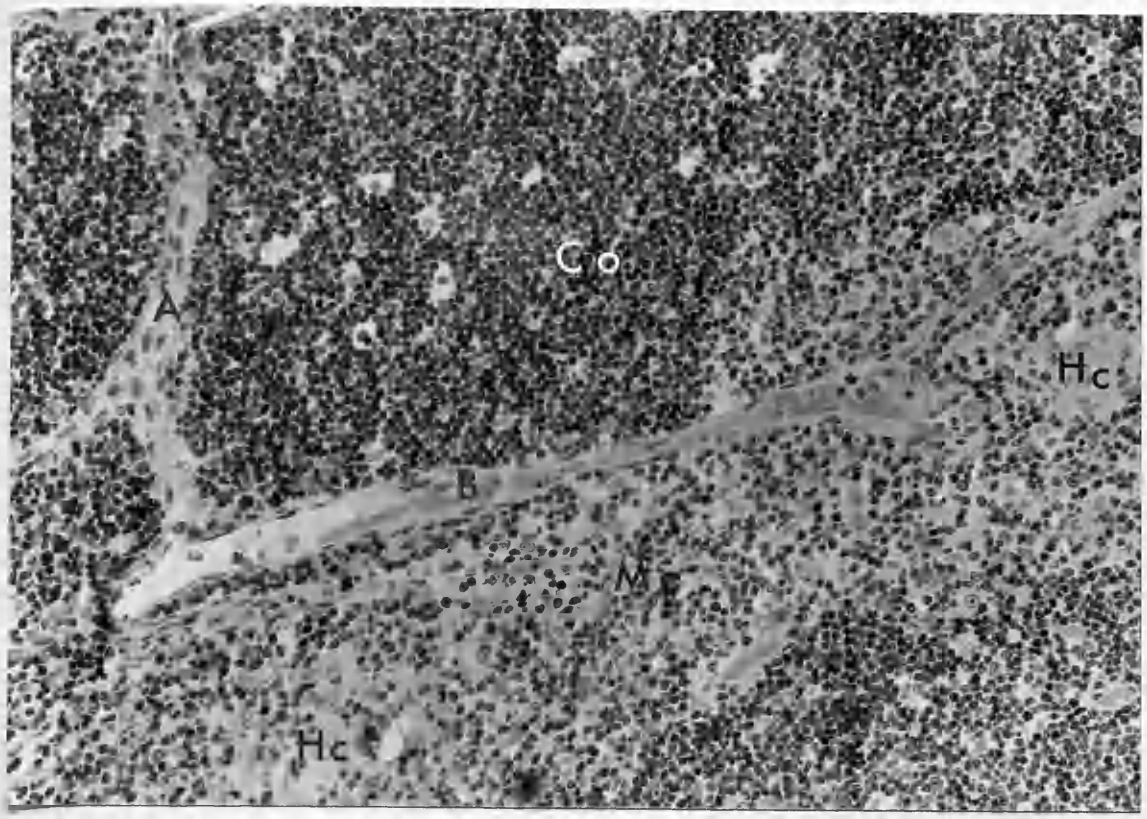
Hc - Hassall's corpuscle
A - Intralobular (secondary septal) artery
B - medullary artery

x 200 Rat 2 H & E

Fig. 68

A cross-section through thymic medulla. Numerous erythrocytes lie freely in the reticulo-epithelial meshwork among lymphocytes. Note that this collection of erythrocytes is only confined to the medulla, particularly around its blood vessels (Bv).

x 400 Rat 3 H & E


Co
A
Hc
B
M
Hc

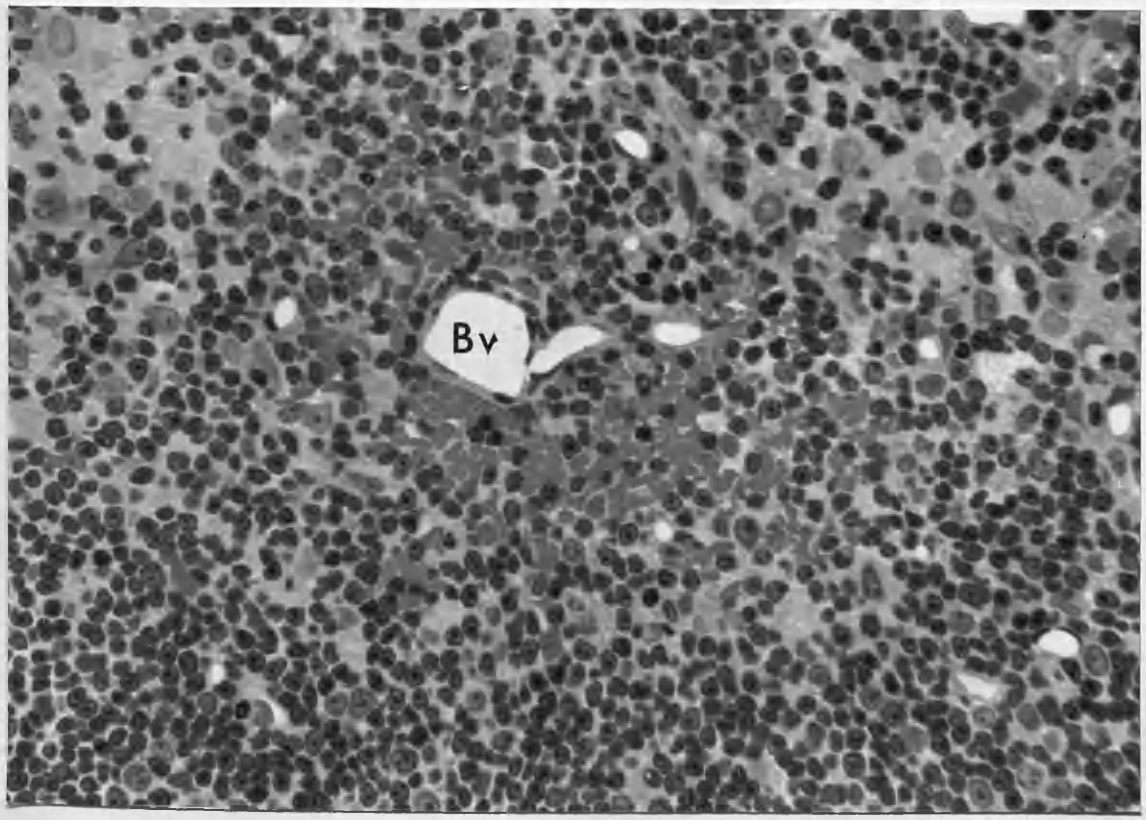

Bv
Hc

Fig. 69

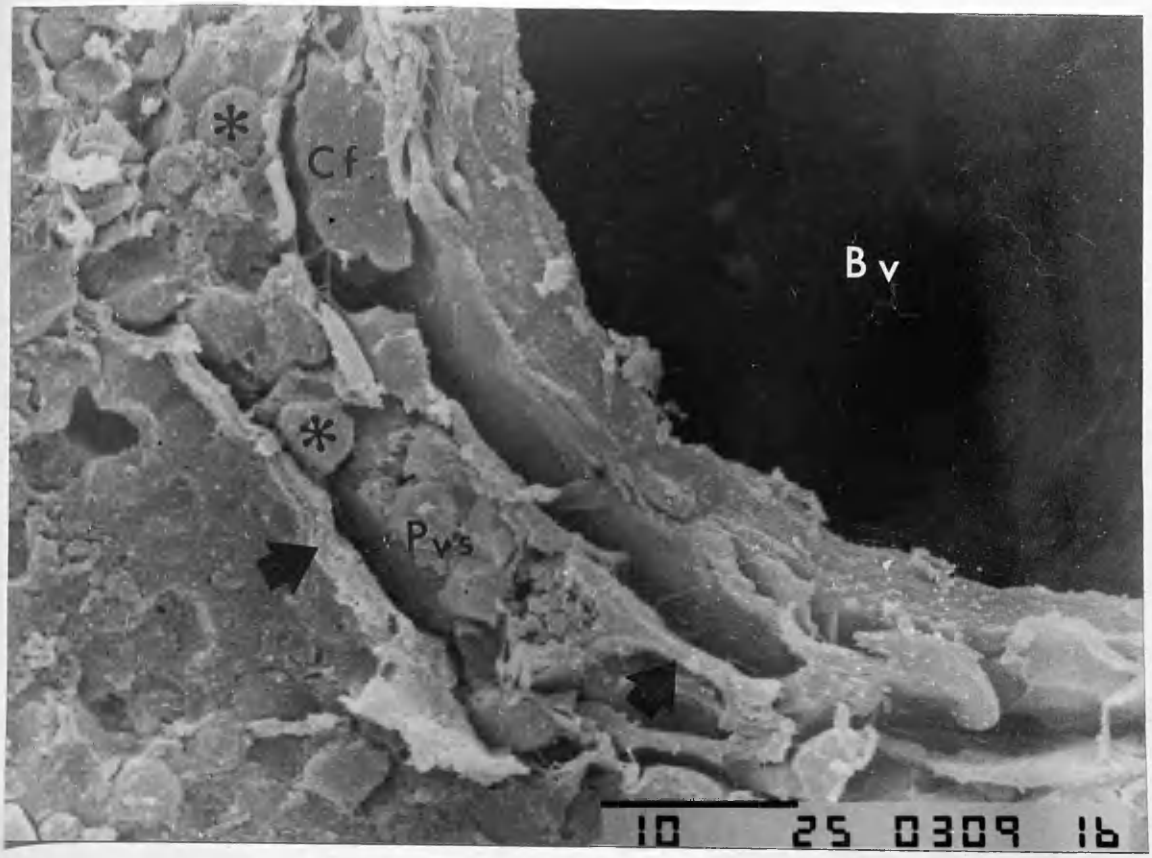
A scanning electron micrograph of part of a thymic blood vessel (Bv) and its perivascular space (Pvs). The vessel is surrounded by two concentric epithelial basal laminae (arrows). The space contains collagen fibres (Cf) and lymphocytes (asterisk).

Rat 9

Fig. 70

A low power photomicrograph of a small lymphatic vessel located in the capsule of the thymus (arrow).

x 320 Rat 8 Bl. 12 H & E


Cf
Bv
Pv's
10 25 0309 16

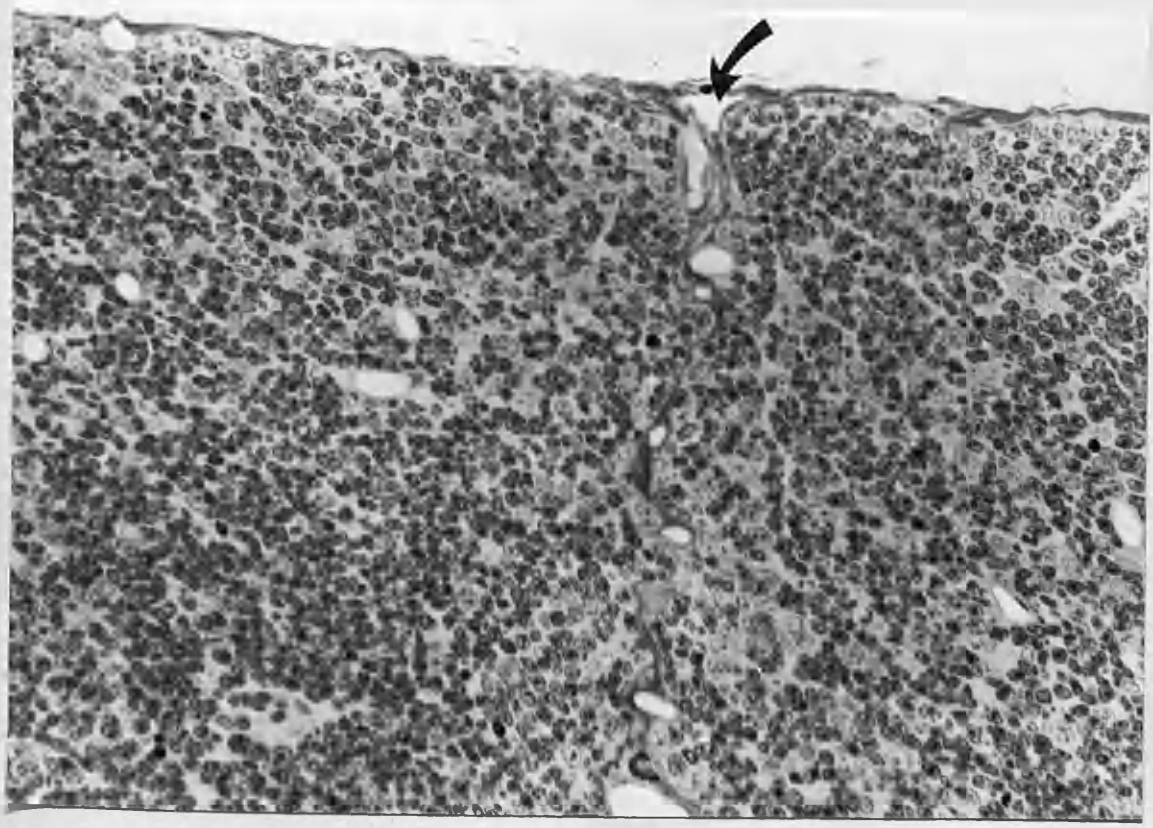


Fig. 71

This electron micrograph demonstrates the fine structure of a portion of the capsular lymph vessel shown in Fig. 70.

End - flattened endothelial wall lacking a basal lamina, J1 - overlapping endothelial cell junction, J2 - interdigitating endothelial cell junction, Cf - collagen fibres, F - fibroblast, L - lymphatic lumen containing precipitated lymph, (arrows) - anchoring filaments.

x 7,000 Rat 6 Bl. 12

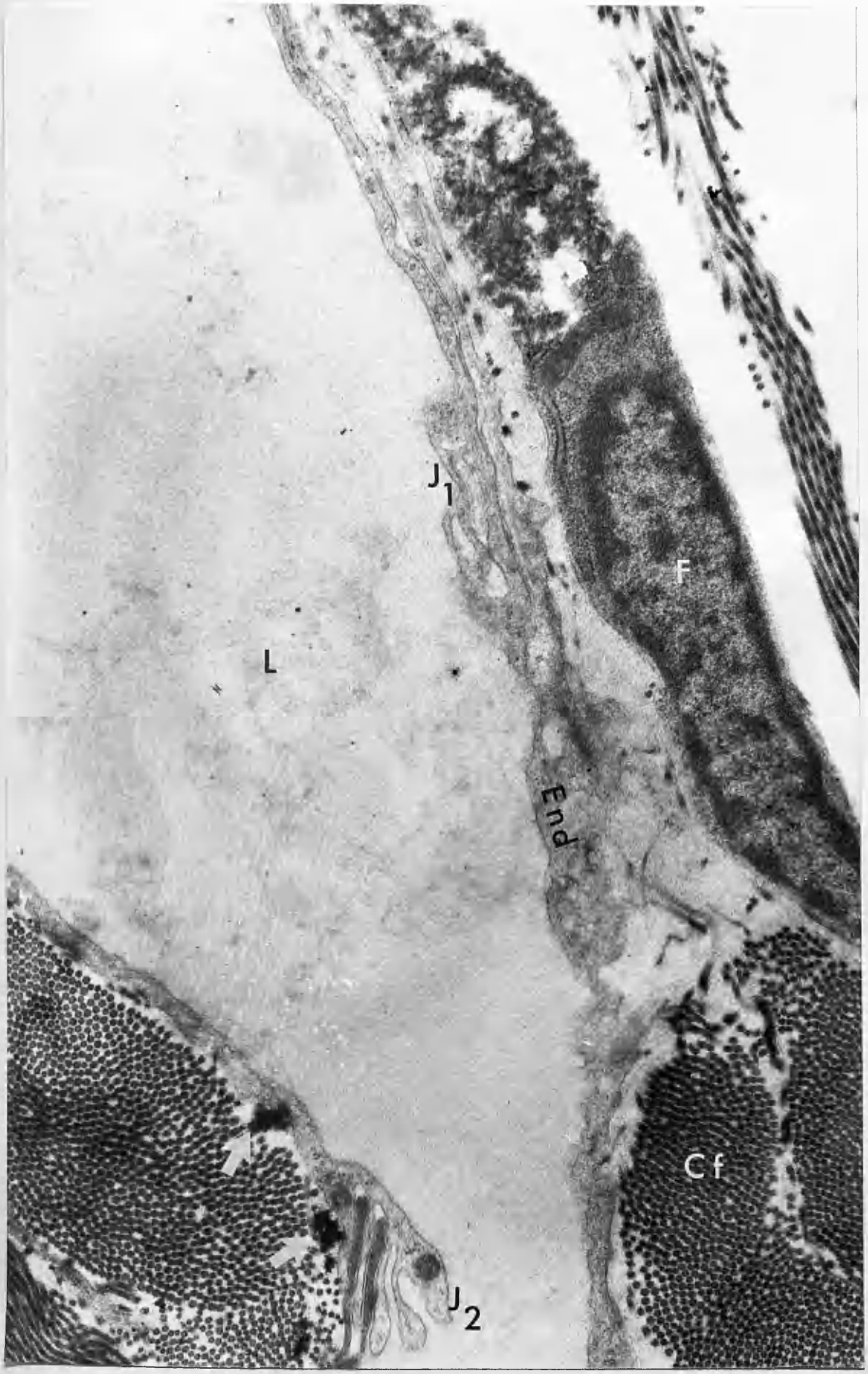

J1
F
L
End
Cf
J2

Fig. 72

A section of the thymus gland, showing an artery (Ha), a vein (Hv) and a lymphatic (Hl) in the hilar region. An artery (A) and a vein (V) are also present in the outer end of a septum, which also contains several sectional profiles of the lymphatic (short arrows).
INSET: See Fig. 73

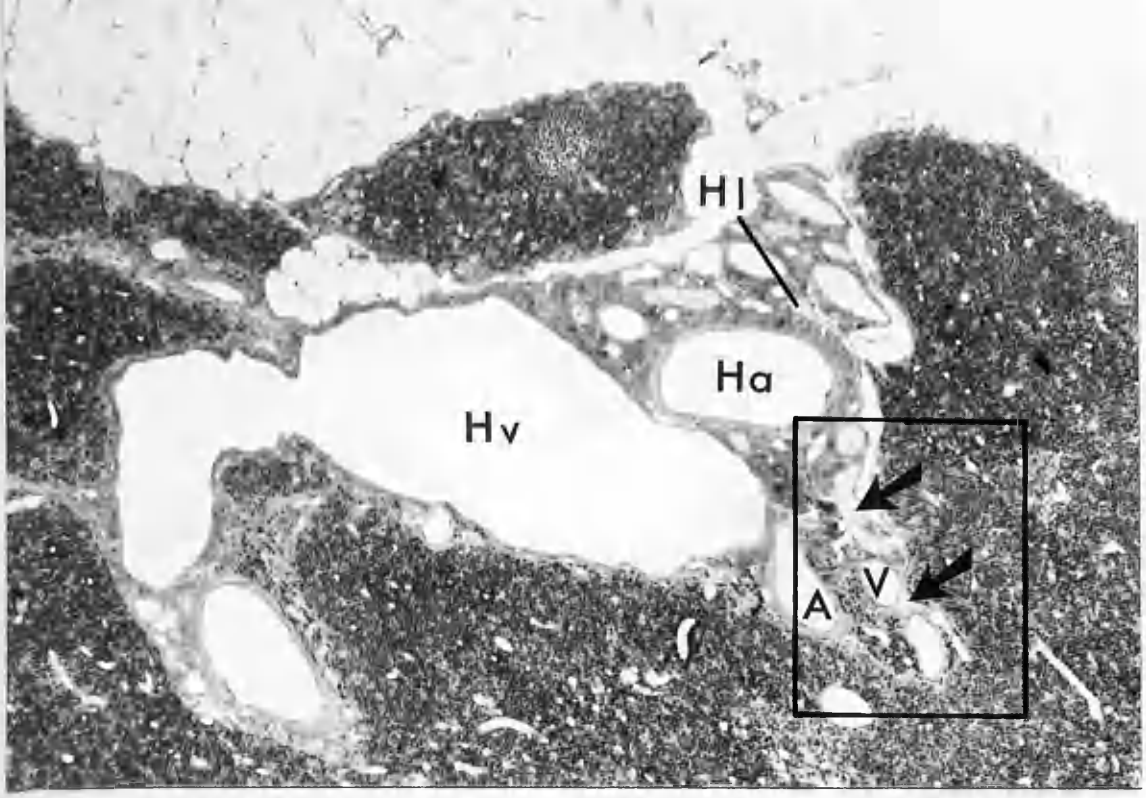
x 80 Rat 4 Bl. 15 H & E

Fig. 73

High power view of the area outlined in Fig. 72. It shows a sectional profile of lymphatic (L) which contains a bi-cuspid valve (arrow).

A - artery, V - vein, N - nerve bundle.

x 320 Rat 4 Bl. 15 H & E


Hl
Ha
Hv
A
V

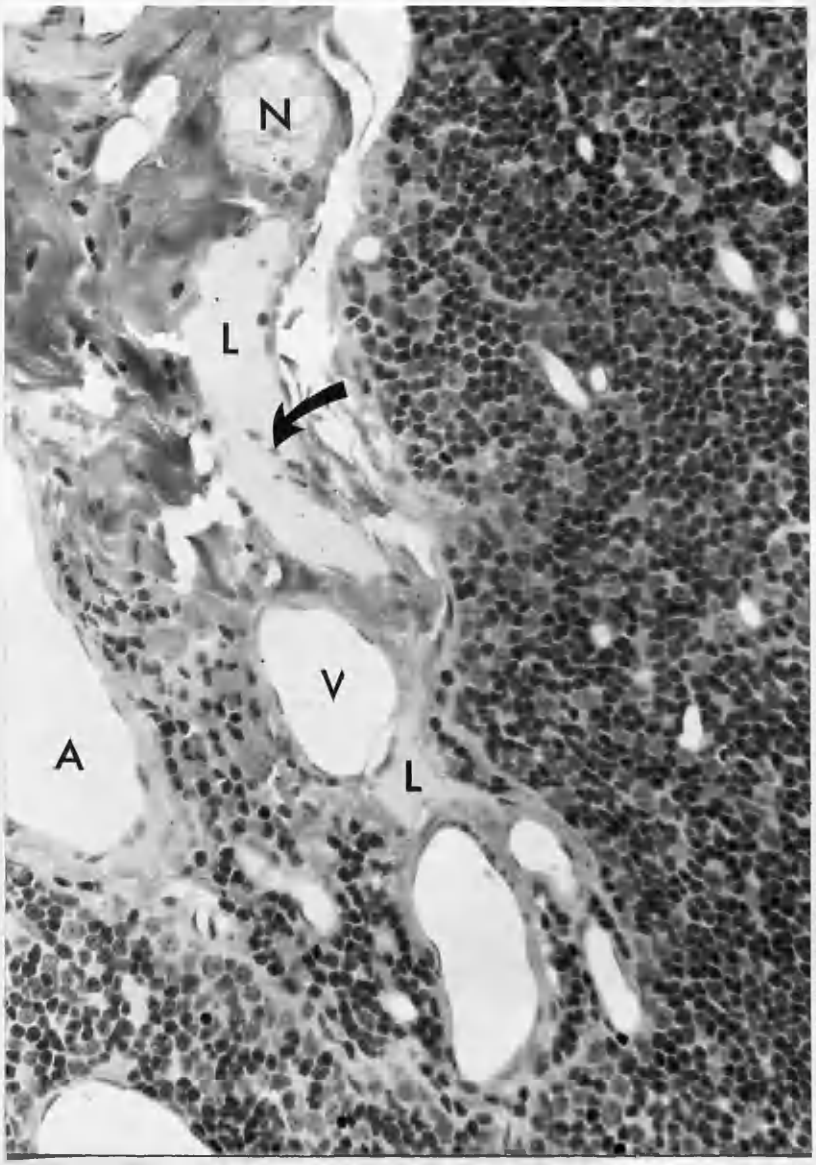

N
L
V
A
L

Fig. 74

A high power view of a hilar lymphatic (Hl) containing many small lymphocytes (darkly-stained) and a few erythrocytes (lightly-stained). Ha - hilar artery, N - nerve bundle.

x 400 Rat 5 Bl. 4 H & E

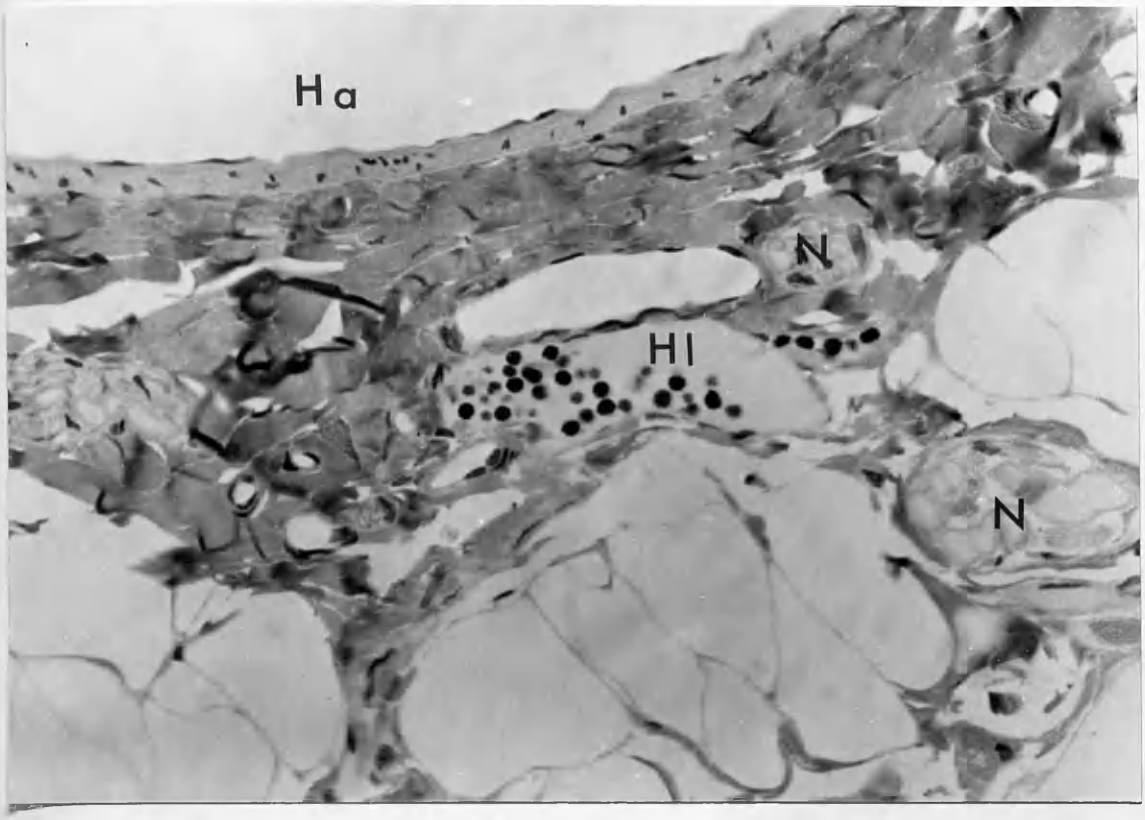

Ha
N
Hl
N

Fig. 75

A cross-section through a primary connective tissue septum of the thymus. Following vascular perfusion, blood vessels appear empty and distended and have a well defined wall. Lymphatic vessels (L) contain precipitated lymph and appear gray, with an irregular outline. The nuclei of the endothelial cells, lining the lymphatic, are visible. Two plasma cells (arrows) are seen close to the lymphatic wall.

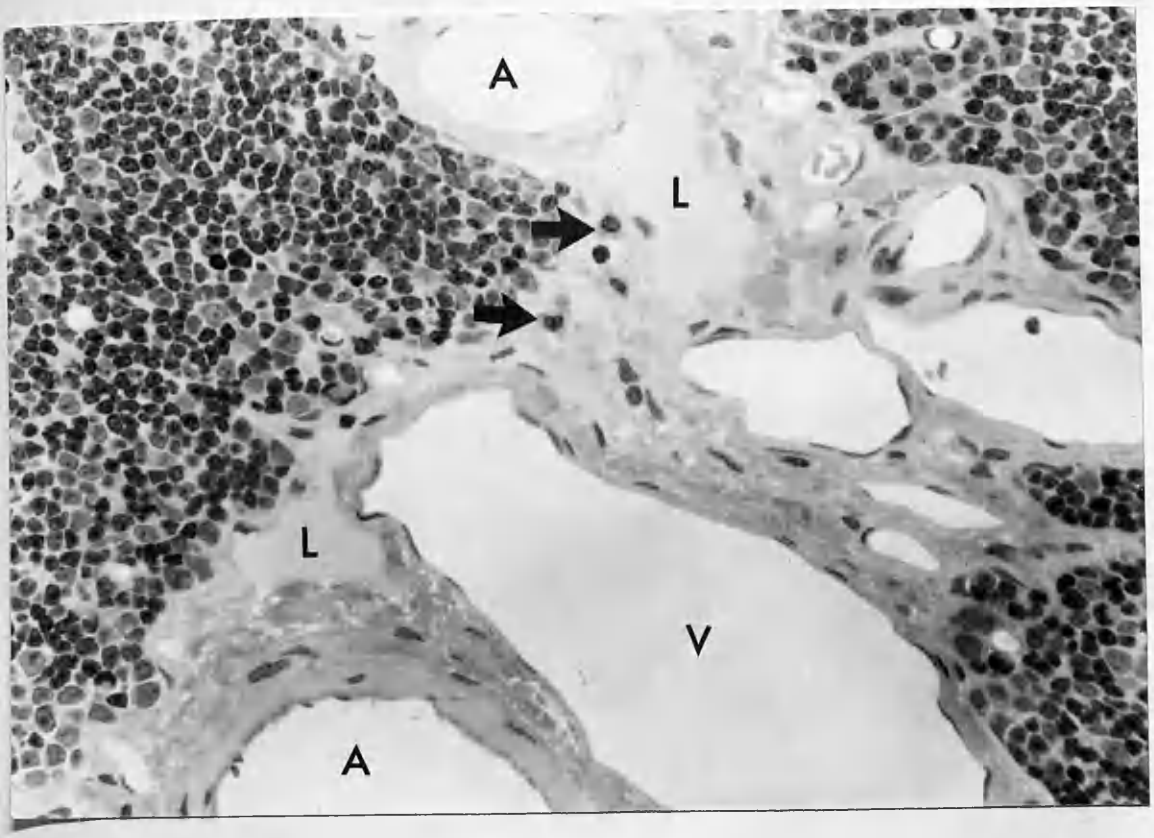
A - artery, V - vein.

x 400 Rat 1 Bl. 2 H & E

Fig. 76

A primary septal (interlobular) lymphatic (L) present close to a vein (V). It contains a few small lymphocytes. This lymphatic was selected for further ultra-thin sectioning in order to study its fine structure (See Figs. 84, 85 & 86).

x 400 Rat 6 Bl. 13 H & E


A
L
L
V
A

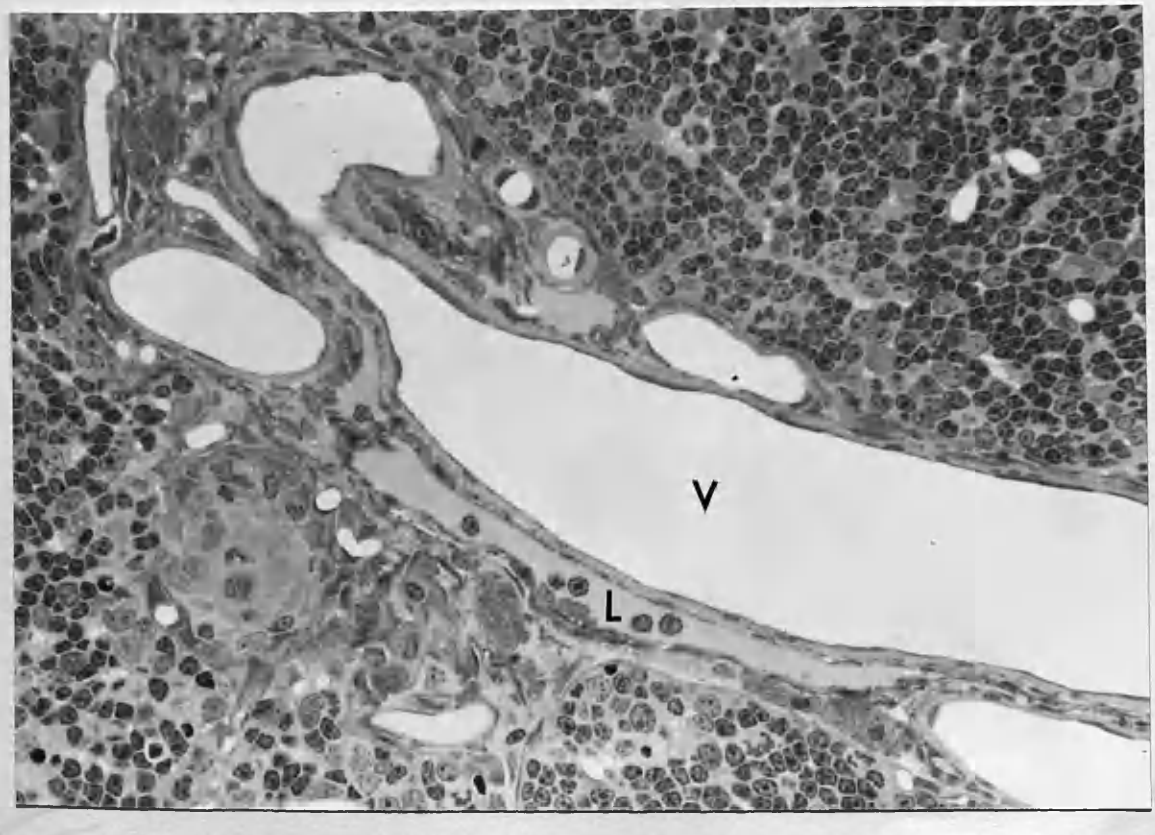

V
L

Fig. 77

A montage of a longitudinal section of a primary septal artery (A). Throughout its length it is accompanied by several sectional profiles of a lymphatic (long arrows). Numerous small lymphocytes and a few macrophages (short arrows) can be seen in the lymphatic.

Rat 4 Bl. 5 H & E


A

Fig. 78

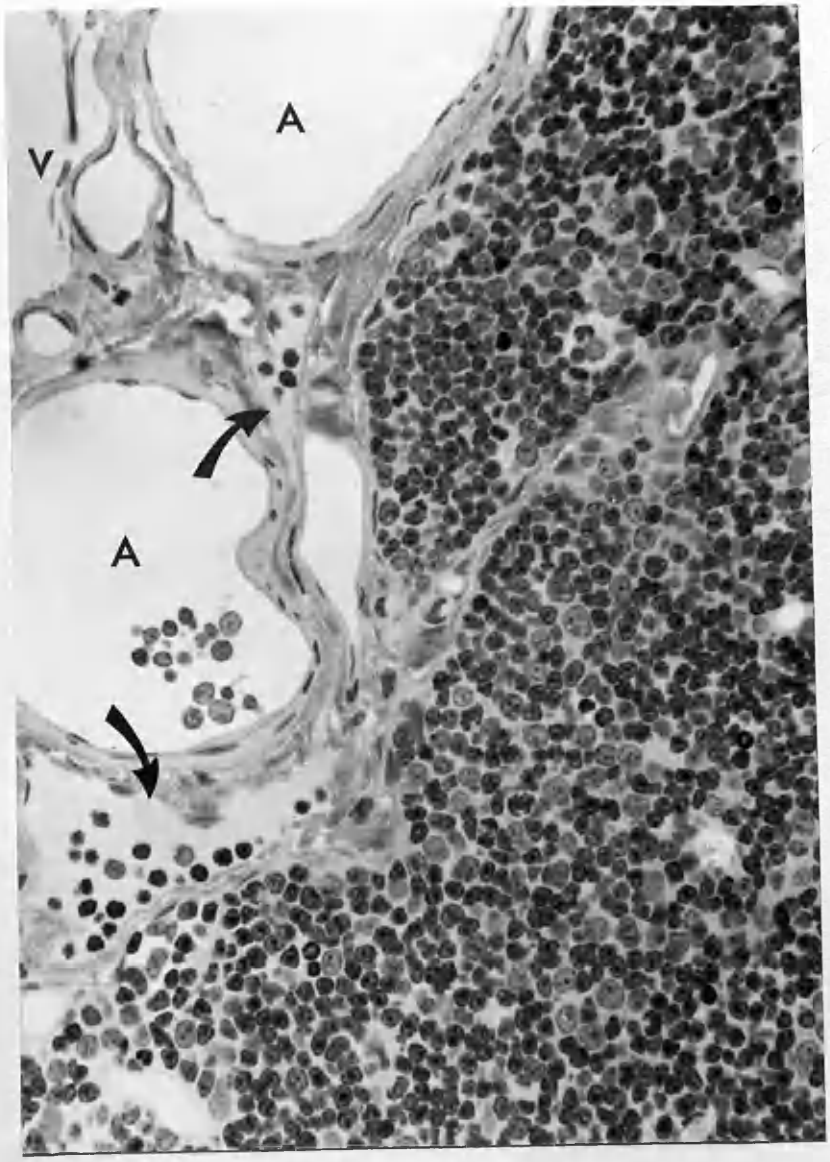
A high power photomicrograph showing a primary septal artery (A) accompanied by a vein (V). Two sectioned profiles of a lymphatic (arrows) are present close to the artery. They contain many small darkly-stained lymphocytes.

x 400 Rat 2 H & E

Fig. 79

This shows two profiles of a secondary septal (intralobular) artery (A) with a lymphatic vessel (L) in between. The lymphatic contains a few small lymphocytes, having the same size and density of staining as perivascular lymphocytes. Note the perivascular space (Pvs) is clearly outlined by epithelial basal lamina (arrows).

x 400 Rat 7 Bl. 8 H & E


V
A
A

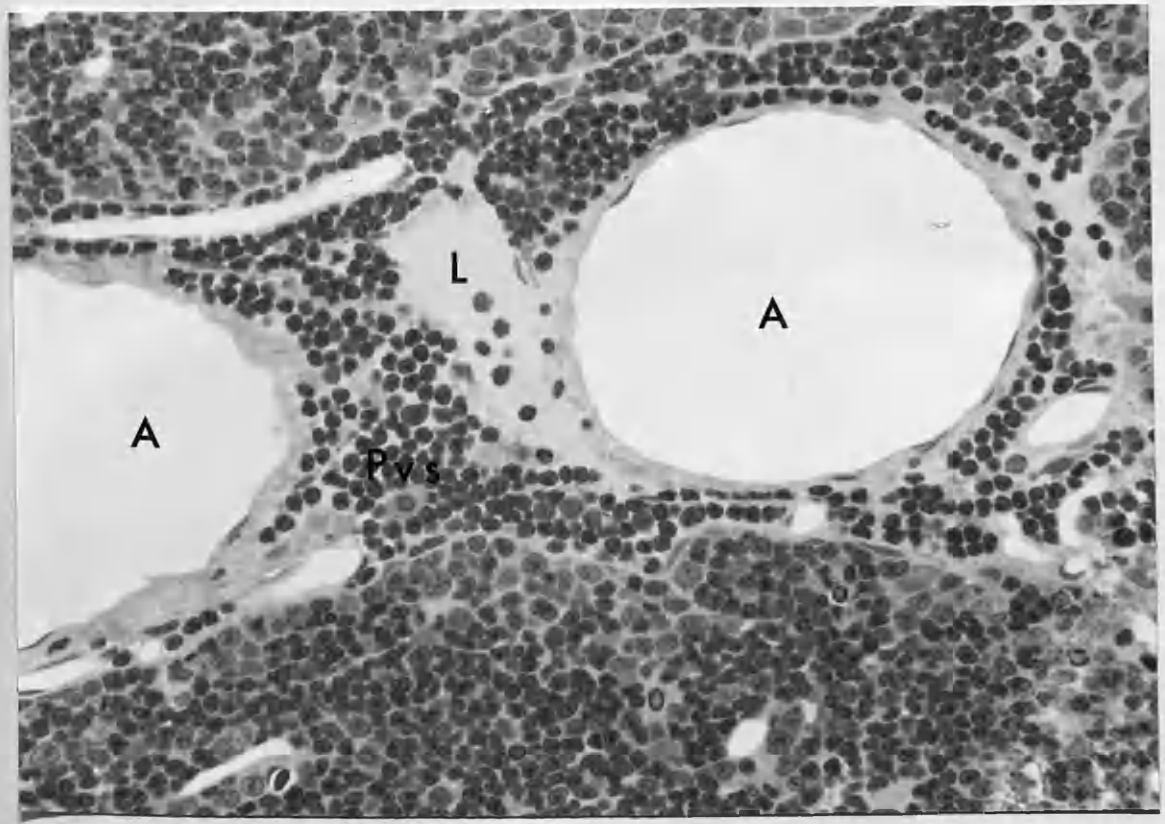

L
A
A
Pvs

Fig. 80

Another example of a secondary septal artery (A) with similar features to Fig. 79. The lymph vessel (arrows) is filled with small lymphocytes.

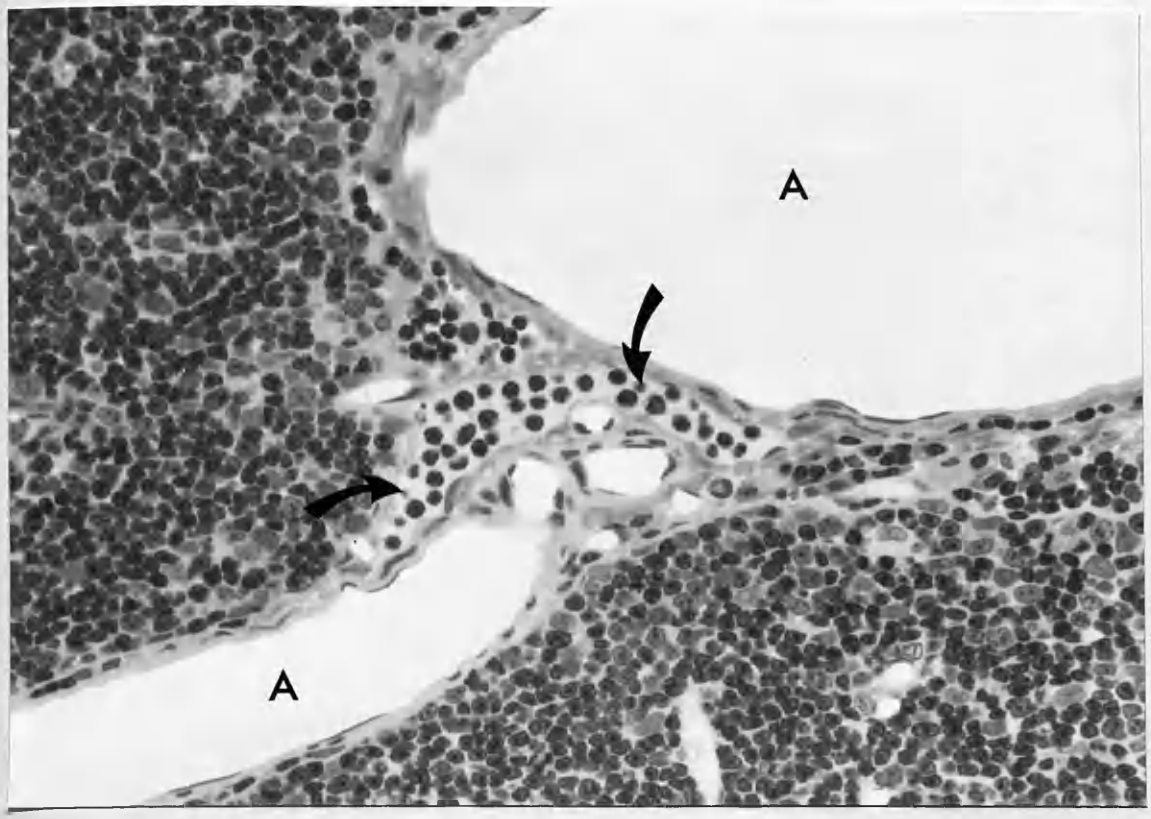
x 400 Rat 6 Bl. 12 H & E

Fig. 81

Two secondary septa approaching cortico-medullary junction.

A - artery, V - vein, L - lymph vessel.

x 250 Rat 6 Bl. 13 H & E


A
A

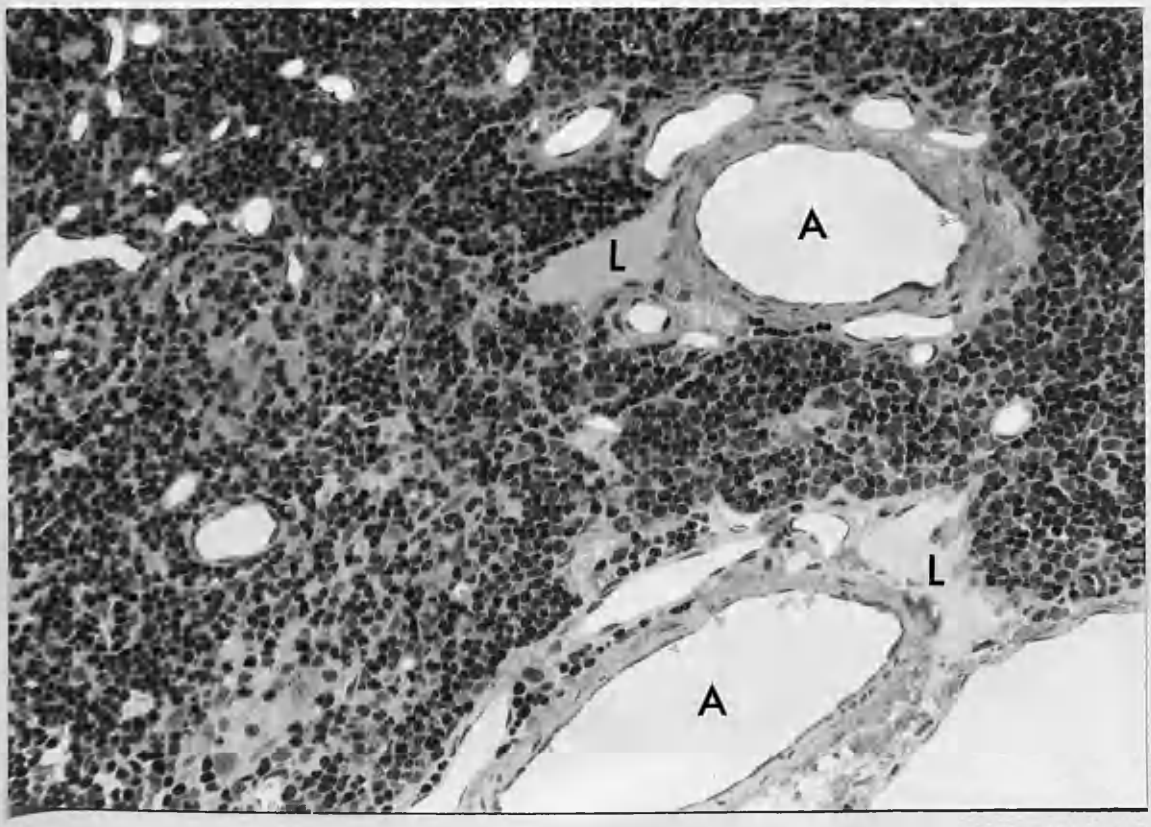

A
L
L
A

Fig. 82

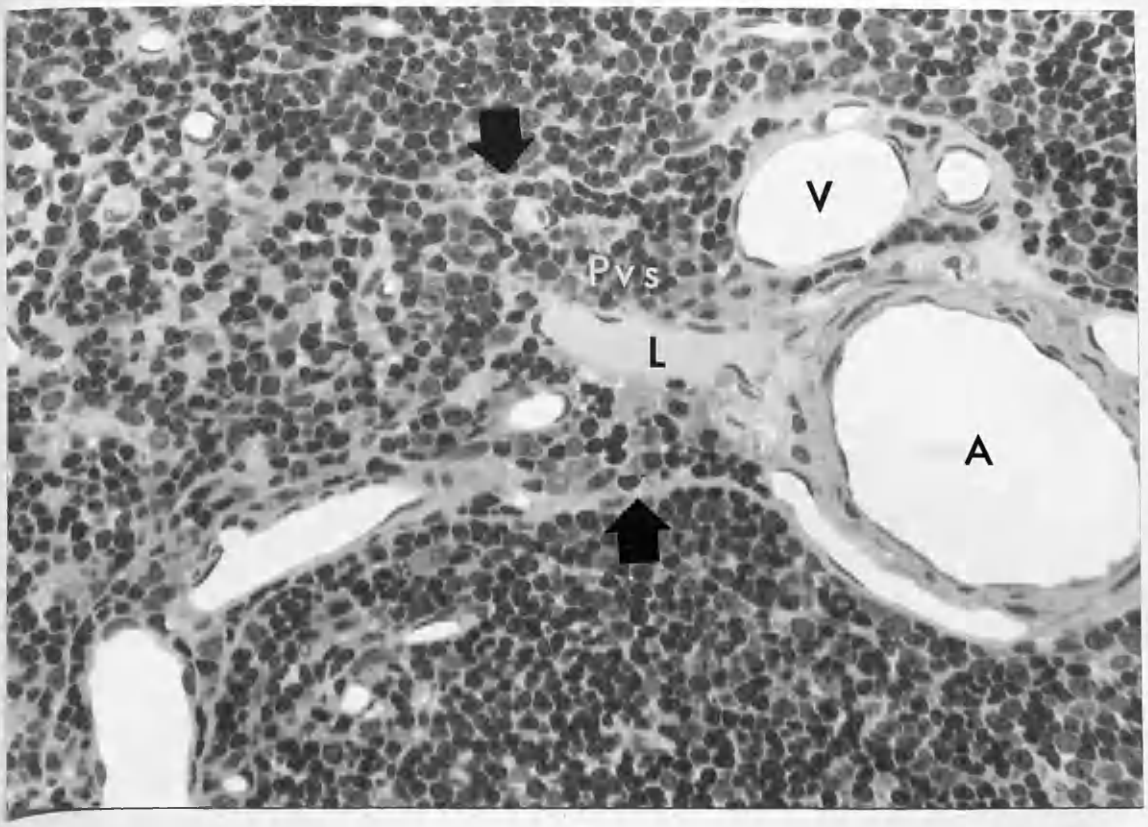
The artery (A) accompanied by vein (V) and lymph vessel (L) are located at the cortico-medullary junction. Note that, as the perivascular space (Pvs) approaches the medulla its epithelial basal lamina (arrows) become continuous with the reticulo-epithelial framework of the medulla.

x 400 Rat 6 Bl. 13 H & E

Fig. 83

This shows a lymph vessel (L) located in the outer medulla. It contains many small lymphocytes. Note that at this power, the lymphatic endothelial wall is barely visible.

x 320 Rat 4 Bl. 15 H & E


V
Pvs
L
A

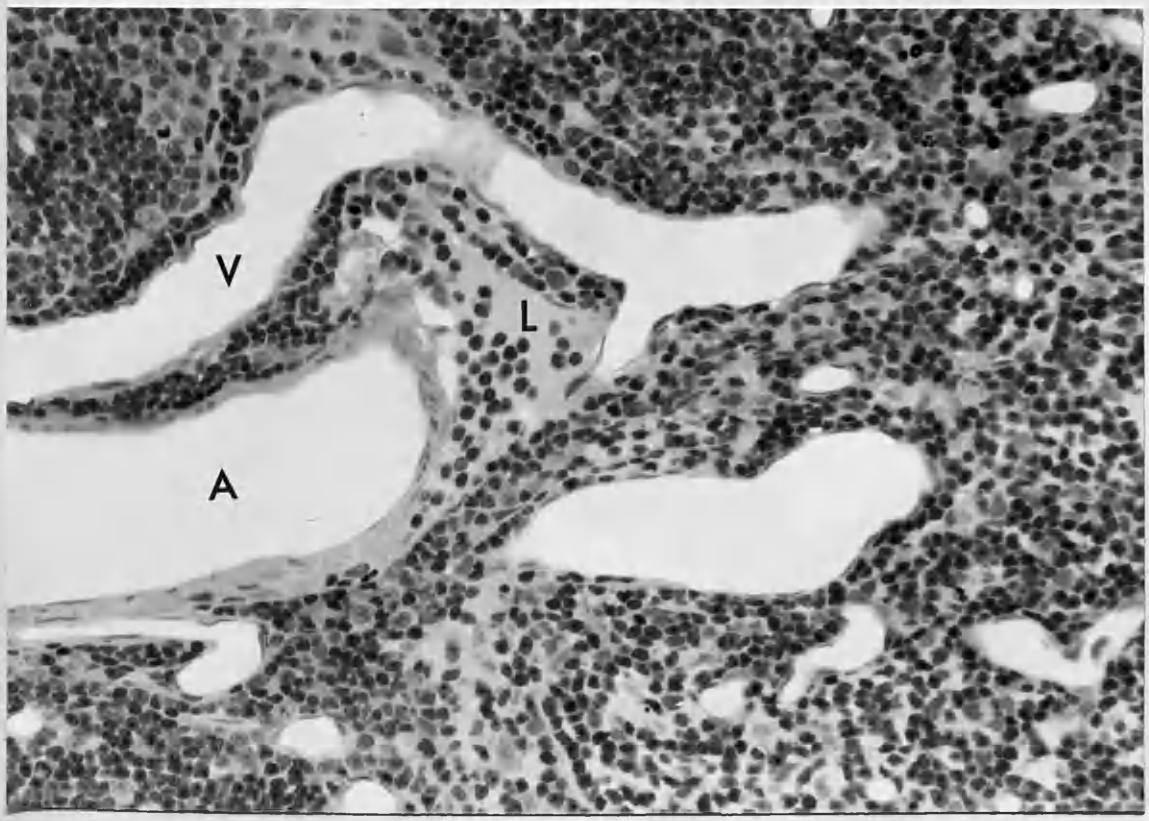

V
L
A

Fig. 84

An electron-microscopic montage of a collecting lymphatic vessel (L) shown in Fig. 76. The vessel is located in a primary septum, close to an empty vein (V). It contains several darkly-stained small lymphocytes (Ly) with microvilli present on their outer cell surface.

End 1 - endothelial lining of vein
End 2 - endothelial cell of lymphatic
Sm - smooth muscle cell
F - fibroblast or mesenchymal-like cell
Cf - collagen fibres
Ep - epithelial-reticular cell
(arrows) - cytoplasmic processes of epithelial cell and its basal lamina
P - plasma cell
T - parenchymatous lymphocyte
INSET: See Fig. 86

Rat 6 Bl. 13

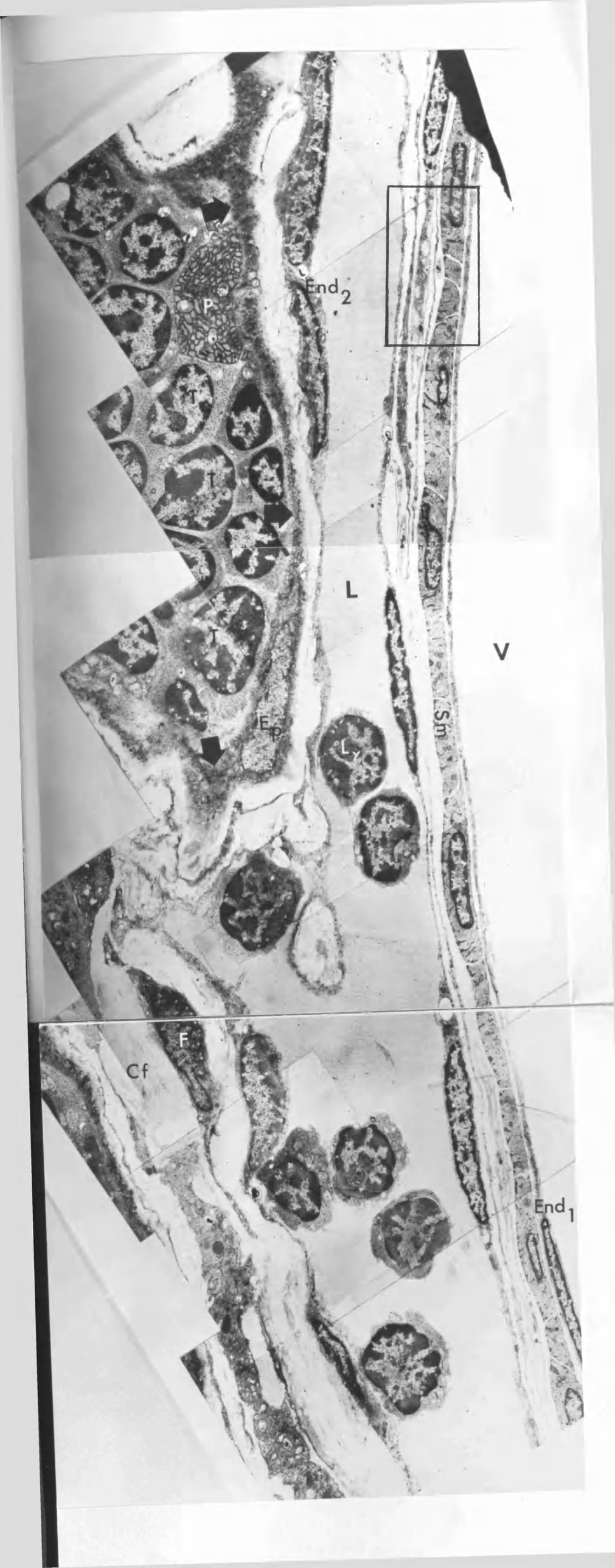

P
T
T
T
End 2
L
V
Sm
Ep
Ly
F
Cf
End 1

Fig. 85

An enlargement of part of the lymphatic vessel (L) shown in Fig. 84.

End 1 - endothelial lining of vein (V)
End 2 - lymphatic endothelial wall showing many pinocytotic vesicles and overlapping intercellular junctions (J)
Sm - smooth muscle cell
F - fibroblast
Cf - collagen fibres
Ep - epithelial-reticular cell
(arrows) - prominent epithelial basal lamina
P - plasma cell
Ly - circulating lymphocyte present in lymphatic vessel
T - parenchymatous lymphocyte
(asterisk) - precipitated lymph

x 2,100 Rat 6 Bl. 13

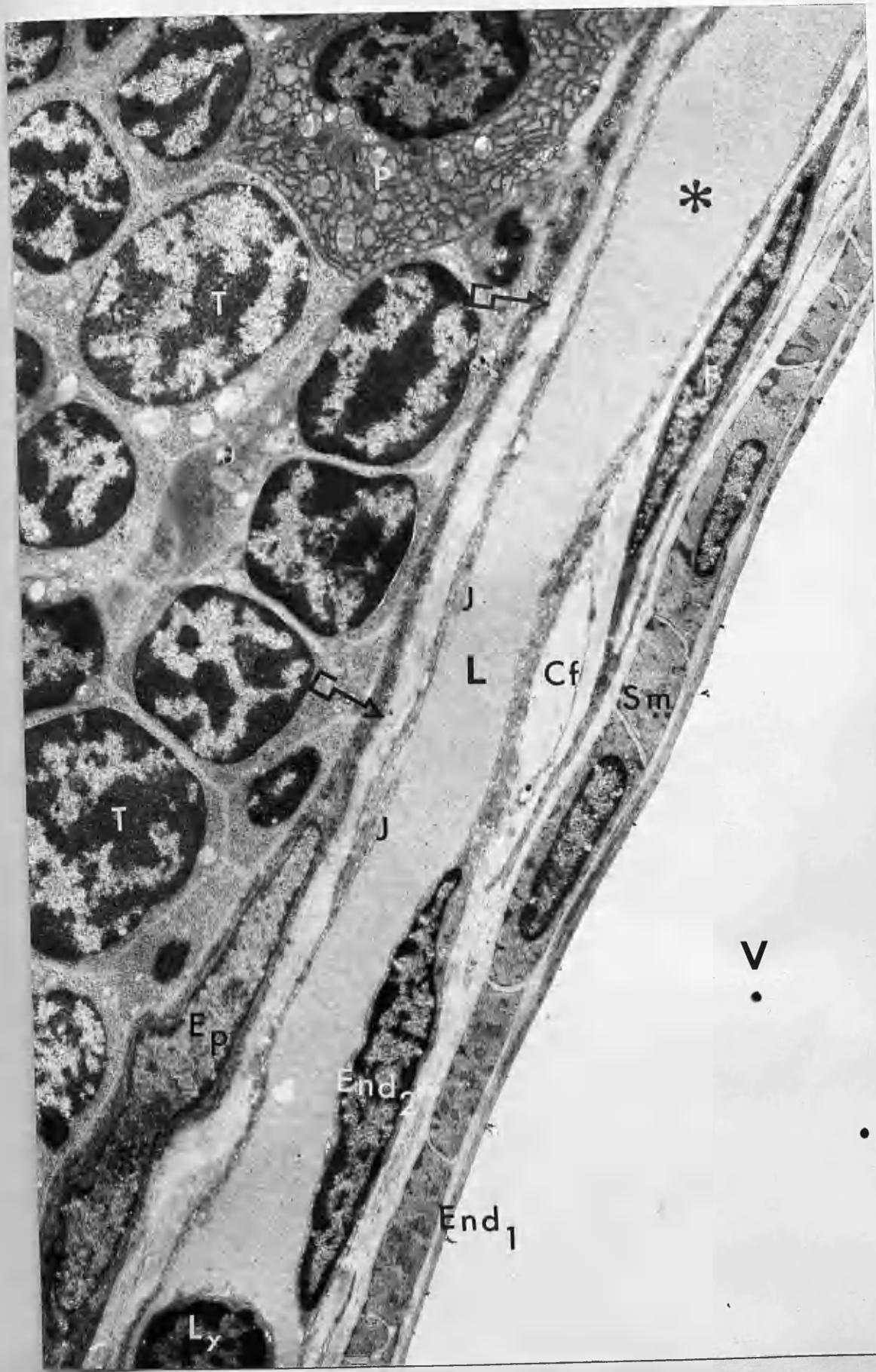

P
*
T
J
L
Cf
Sm
T
J
V
Ep
End2
End1
Ly

Fig. 86

A further enlargement of the area outlined in Fig. 84. Endothelial lining (End 1), of vein (V) is surrounded by a layer of well developed smooth muscle (Sm); both are bounded by a prominent and continuous basement membrane (Bm = arrowheads). The endothelial wall (End 2) of the lymphatic (L) is more flattened, contains numerous pinocytotic vesicles, and has a discontinuous basal lamina (arrows).

F - processes of fibroblasts
Cf - collagen fibres

x 10,000 Rat 6 Bl. 13

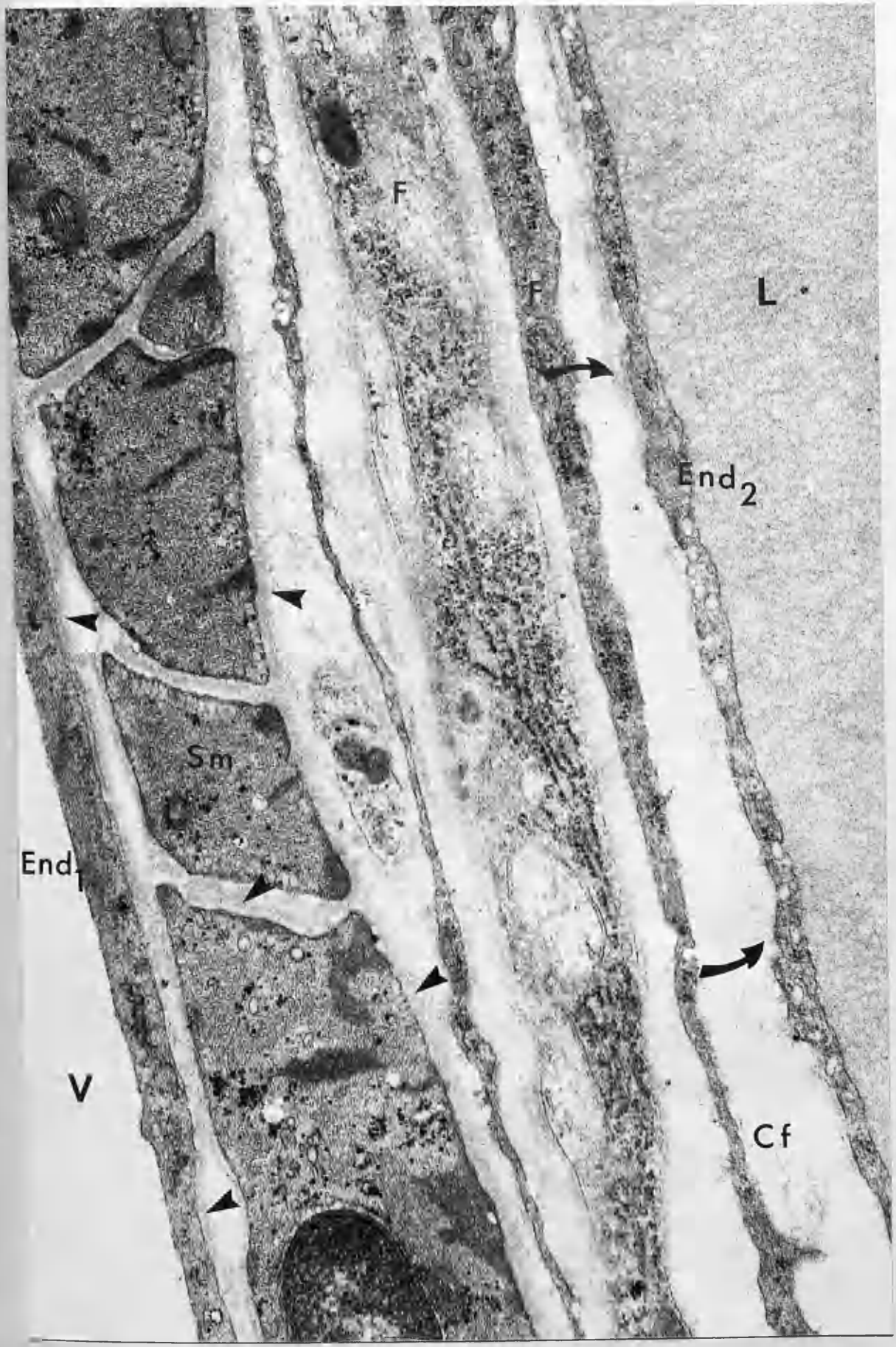

F
F
L
End2
Sm
End1
V
Cf

Fig. 87

Electron micrograph showing a portion of a lymphatic capillary (Ly Cap), a vein (V), and a branching venule in an interlobular septum. Note the content of precipitated lymph, the attenuated endothelial wall and the absence of cell pericytes around the lymphatic capillary. The basal lamina is also absent, although this cannot be seen clearly at this magnification. Two small lymphocytes are seen inside the lymph capillary. This animal received an IV injection of india ink, before it was killed 7 days later. Even after this long period, carbon particles (C) are only seen in lumina of vein and venule, adherent to their endothelial lining. There is no trace of particles to be found in the perivascular space (Pvs), nor in thymic parenchyma.

N - myelinated and unmyelinated nerve fibres
M - mast cell
Cf - collagen fibres
(arrows) - epithelial cellular processes and basal lamina
T - parenchymatous lymphocyte

x 1,400 Rat 12

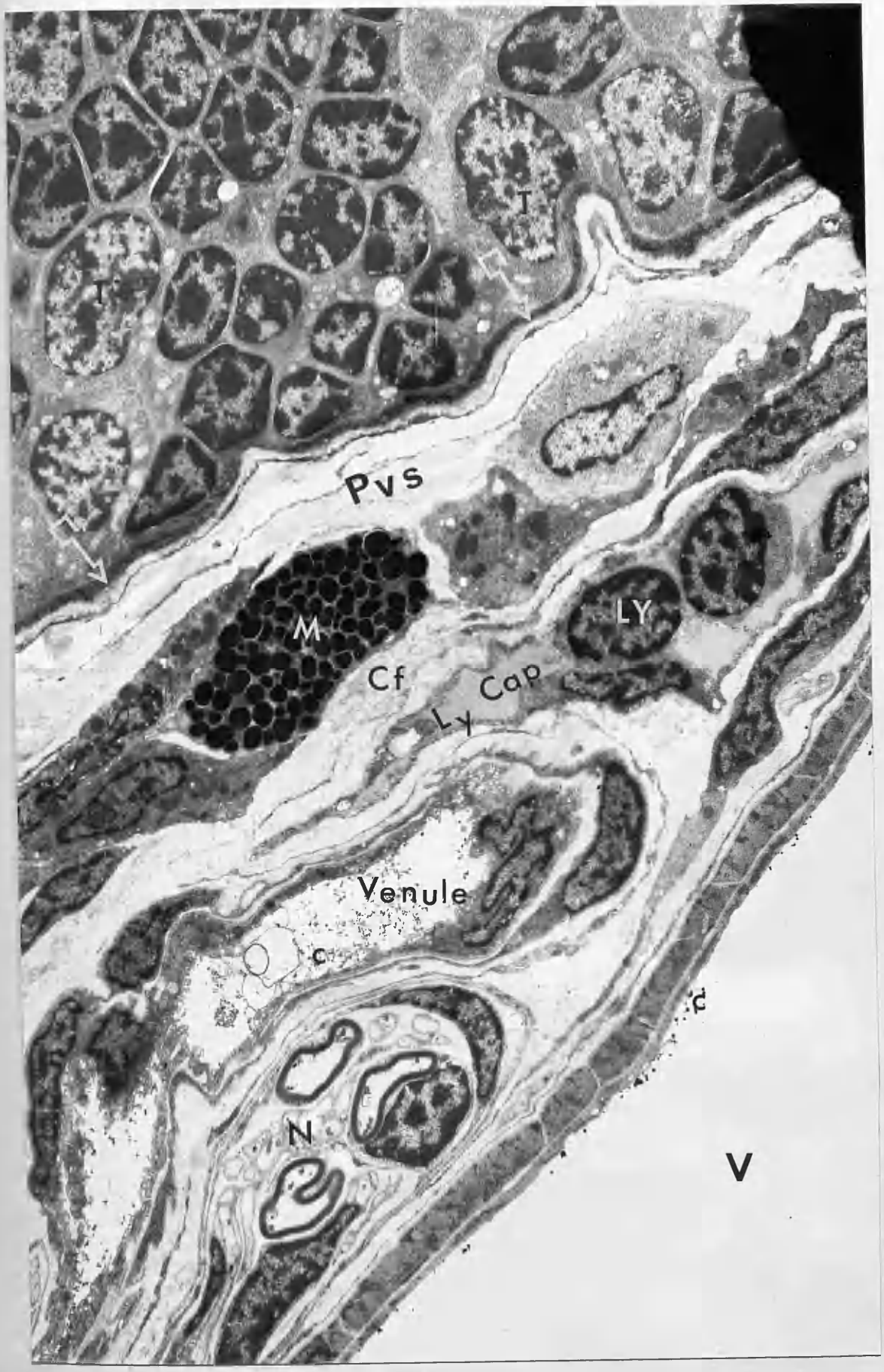

T
T
T
Pvs
M
LY
Cf
Cap
Ly
Venule
c
c
N
V

Fig. 88

This photomicrograph shows a branching interlobular vein (V) and a portion of thymic parenchyma. The areas marked A, B, and C are perivascular spaces magnified in Figs. 91, 64 and 89 respectively. An unusual collection of lymphocytes, enclosed by prominent epithelial basal lamina (arrows), is clearly visible in the parenchyma; dark pyknotic lymphocytes and macrophages with a foamy appearance (arrow heads) are also seen.

x 175 Rat 6 Bl. 10 H & E

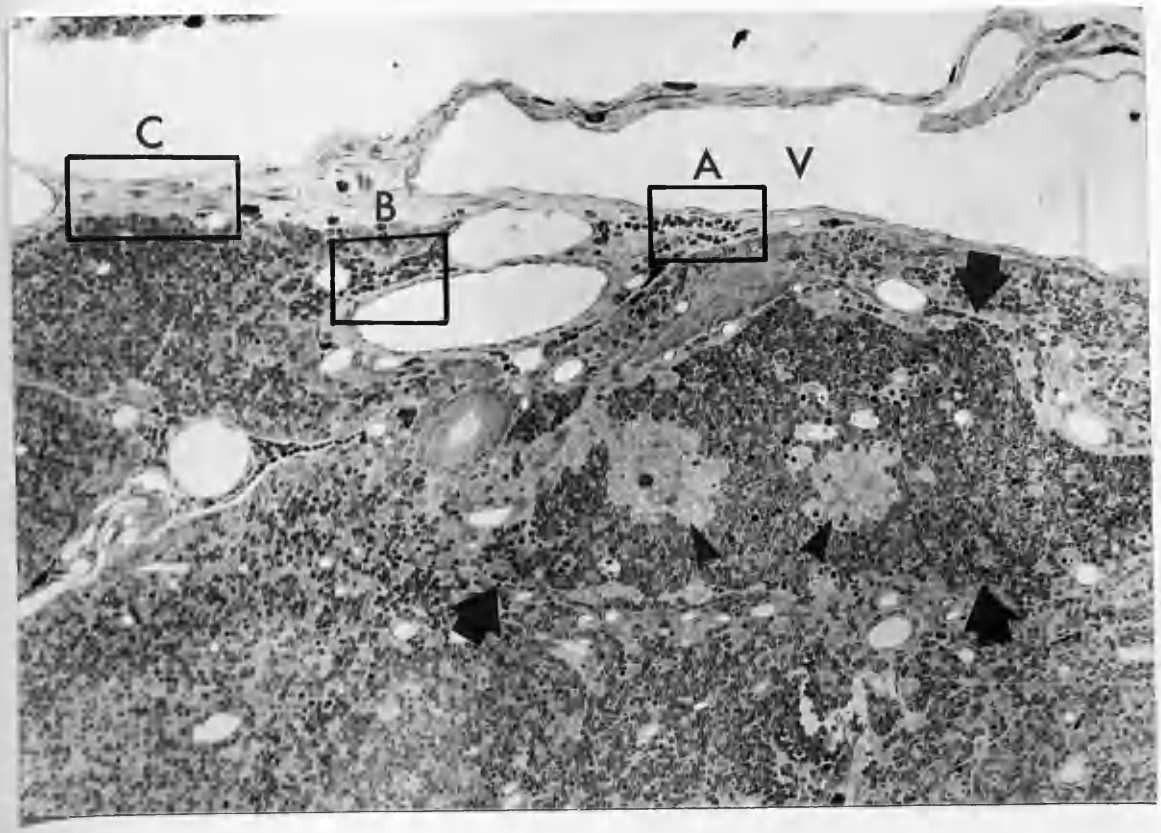

C
B
A
V

Fig. 89

A reduced electron microscopic montage of the area marked (C) in Fig. 88. It shows a perivascular space (Pvs) containing a lymphatic vessel (L), a nerve bundle (N) and many cytoplasmic processes of fibroblasts (F) or mesenchymal-like cells. These processes partially enclose irregular "pre-lymphatic" spaces (Pl) containing interstitial fluid, collagen fibres (Cf) and many cells. They become in continuium with endothelial cells of the lymphatic.

Ep - epithelial-reticular cells
E - eosinophil
M - mast cell
Ly - small lymphocyte
Cap - blood capillary
T - parenchymatous lymphocyte
INSET: See Fig. 90

Rat 6 Bl. 10

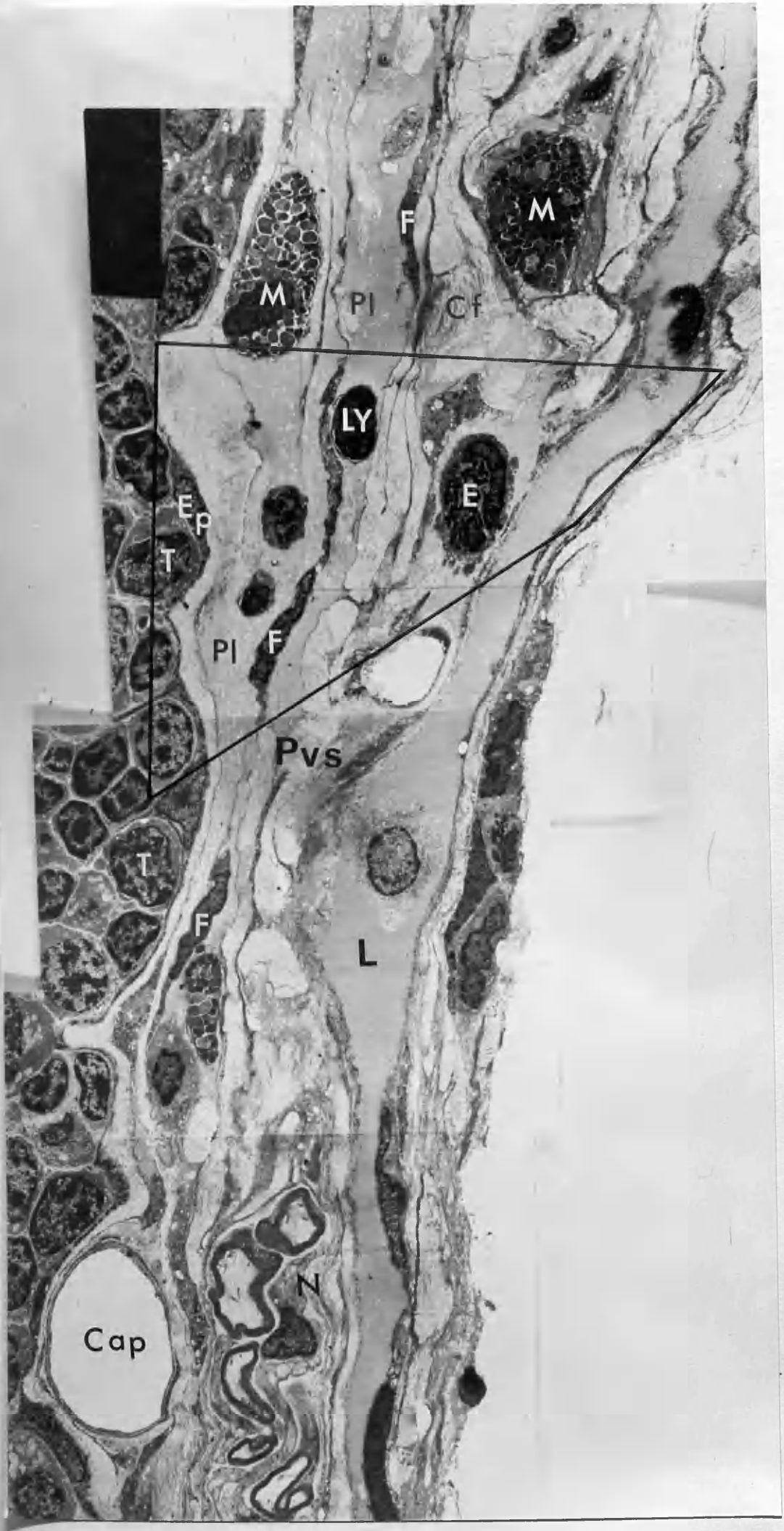

M
F
M
Pl
Cf
LY
E
Ep
T
Pl
F
Pvs
T
F
L
N
Cap

Fig. 90

High power electron micrograph of area outlined in Fig. 89.

L - lymphatic vessel
Pvs - perivascular space
Pl - prelymphatic channel
F - fibroblast
Cf - collagen fibres
E - eosinophil
Ep - epithelial reticular cell
Ly - small lymphocyte in (Pvs)
T - parenchymatous lymphocyte

x 2,100 Rat 6 Bl. 10

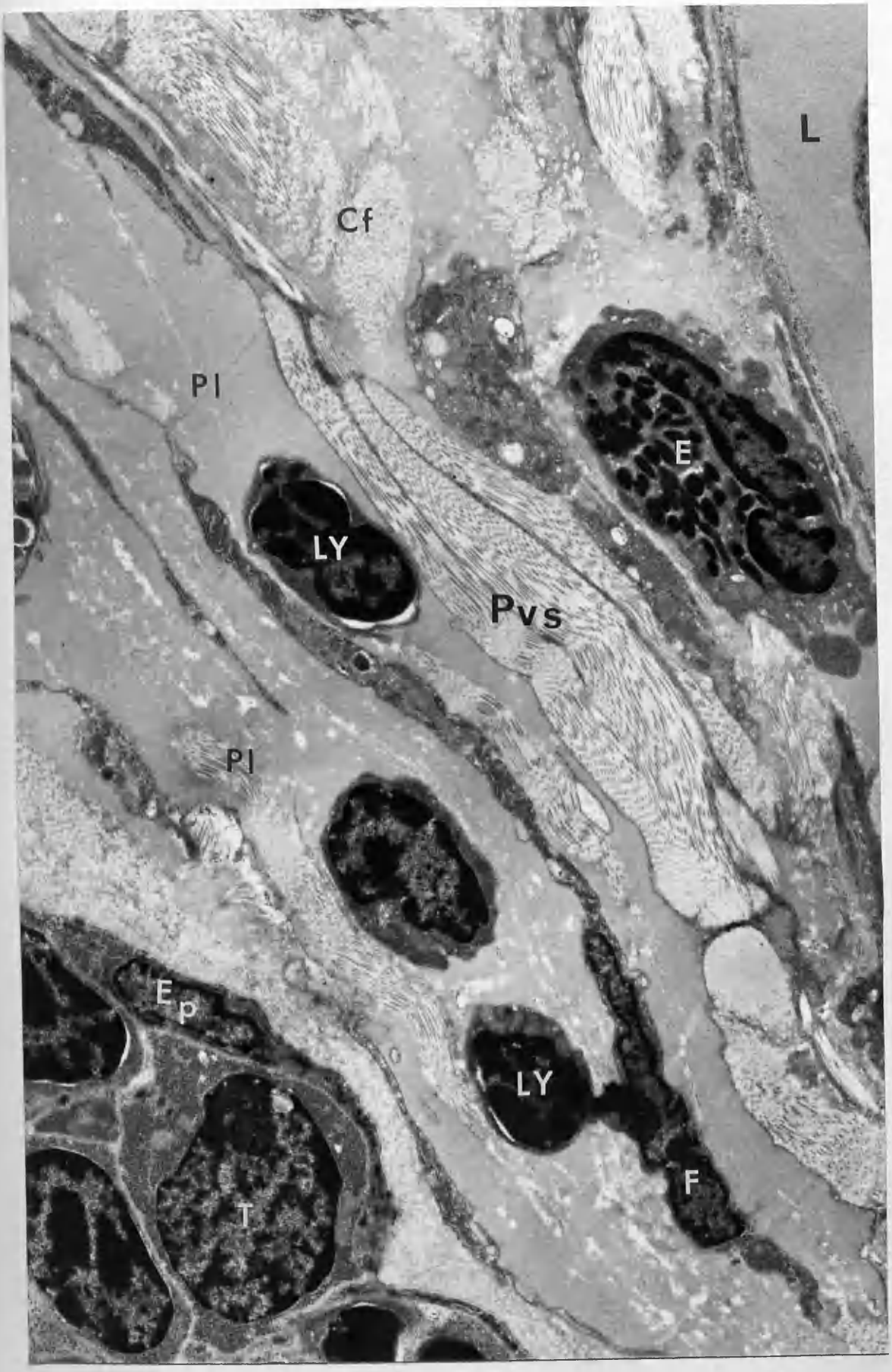

L
Cf
Pl
E
LY
Pvs
Pl
Ep
LY
F
T

Fig. 91

Electron micrograph of the area marked (A) in Fig. 88. It shows a perivascular space (Pvs) containing many small and distinct dark lymphocytes (Ly). These lymphocytes are surrounded by processes of mesenchymal cells forming "prelymphatic" spaces (Pl).

Ep - epithelial-reticular cell
V - vein
T - parenchymatous lymphocyte

x 1,400 Rat 6 Bl. 10

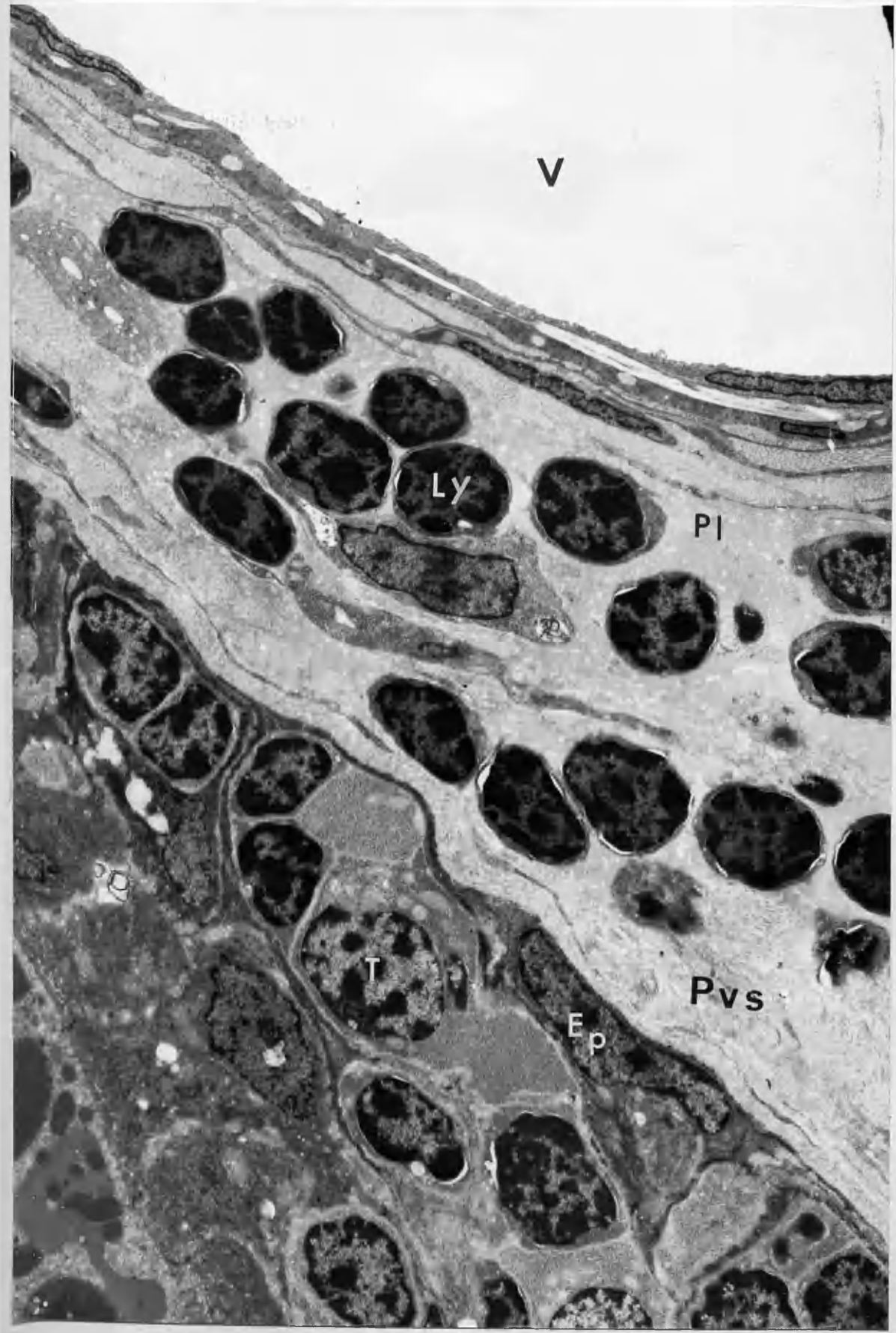

V
Ly
Pl
T
Pvs
Ep

Fig. 92

This is a further example of a perivascular space (Pvs) containing many mesenchymal-cell processes. Some processes partially enclose collagen fibres (Cf); others contribute to the wall of an irregular loculated channel (Pl) containing small lymphocytes and precipitated protein.

Cap - blood capillary
T - parenchymatous lymphocyte
Pl - pre-lymphatic

x 2,100 Rat 5 Bl. 1

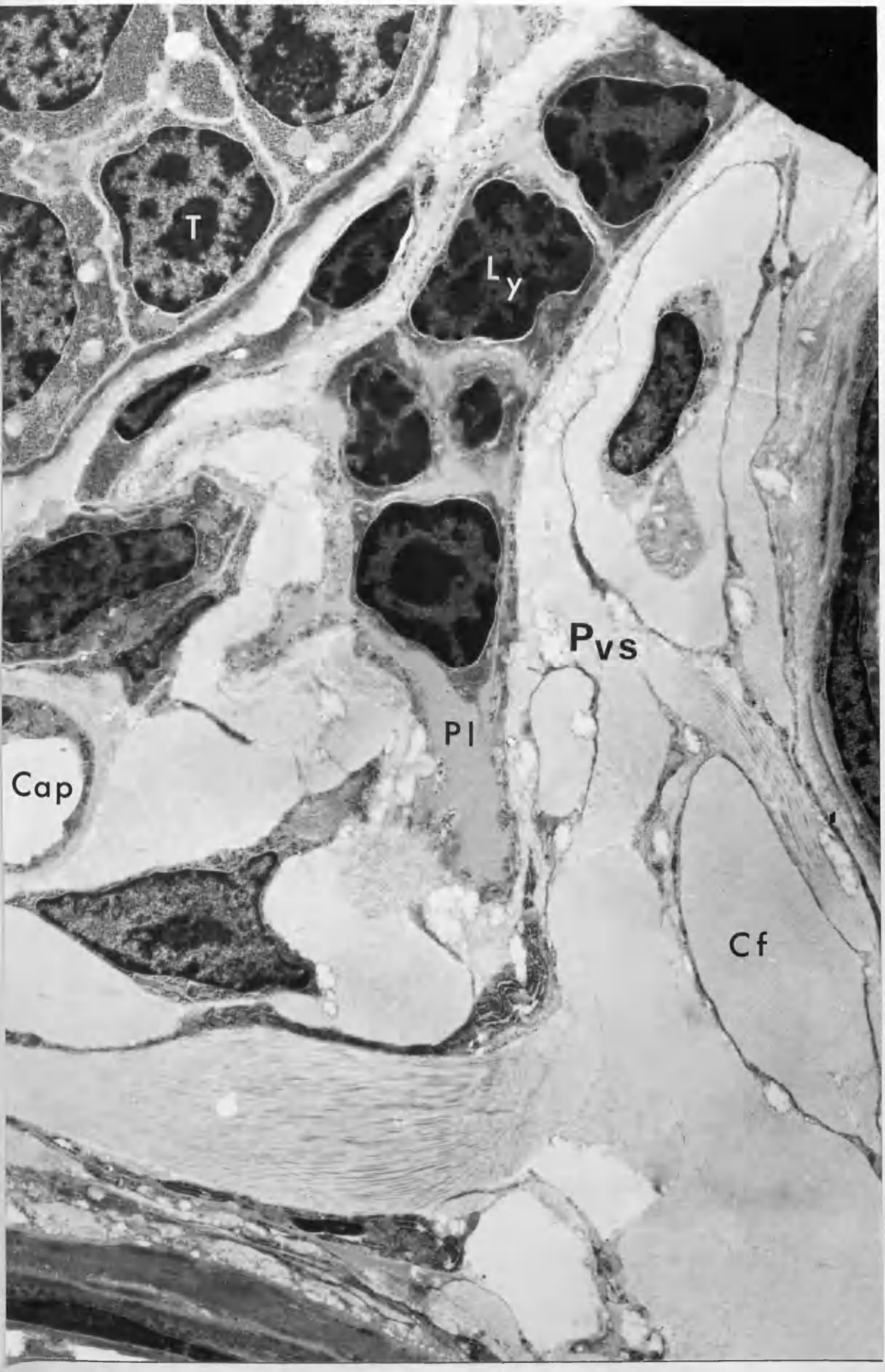
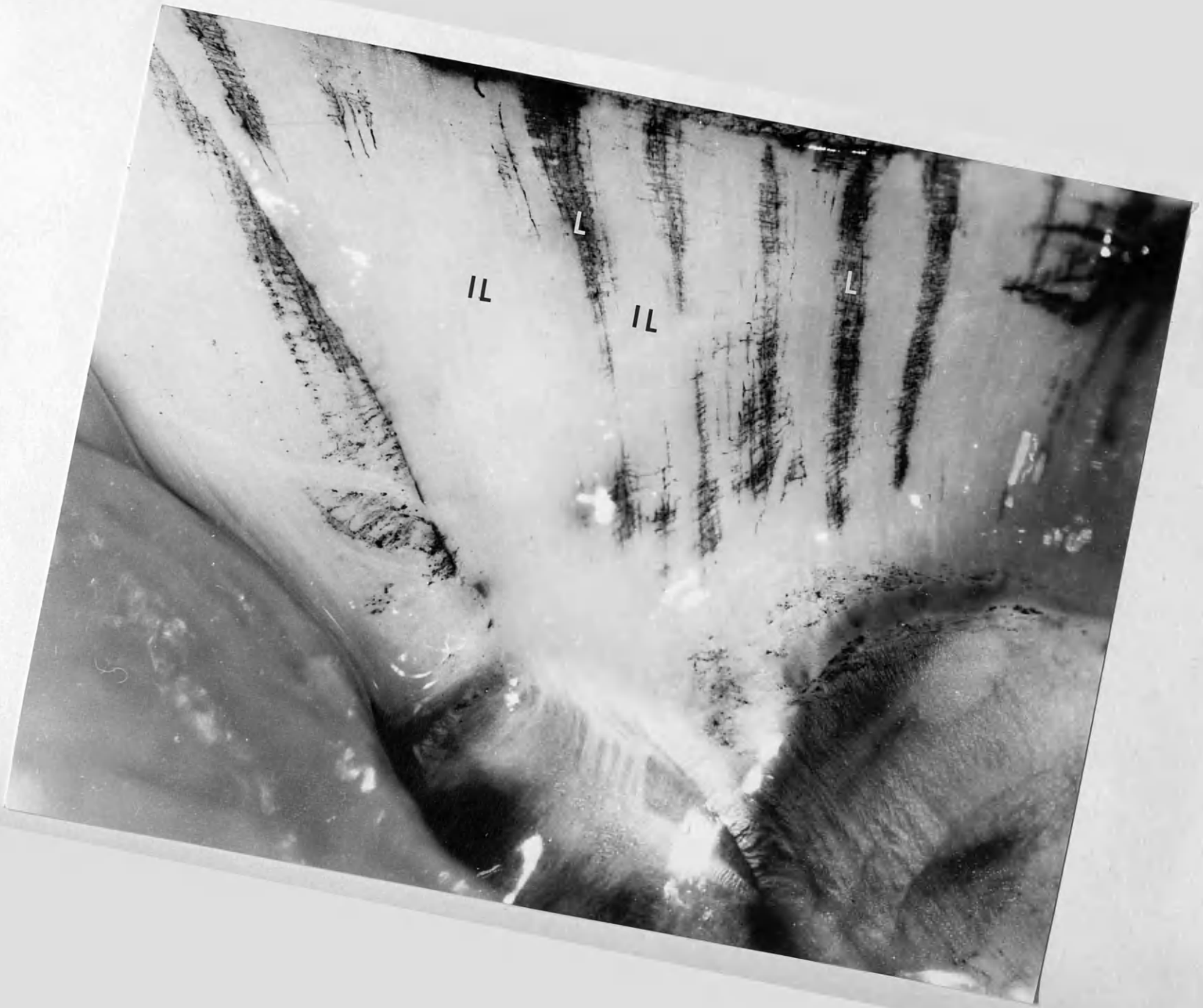

T
Ly
Pvs
Pl
Cap
Cf

Fig. 93

Low power photomicrograph of peritoneal surface of the left diaphragm. It shows ink-filled subperitoneal lymphatic lacunae (L). Note that they run parallel to muscle fibres, and are sharply delineated from inter-lacunar areas (Il).

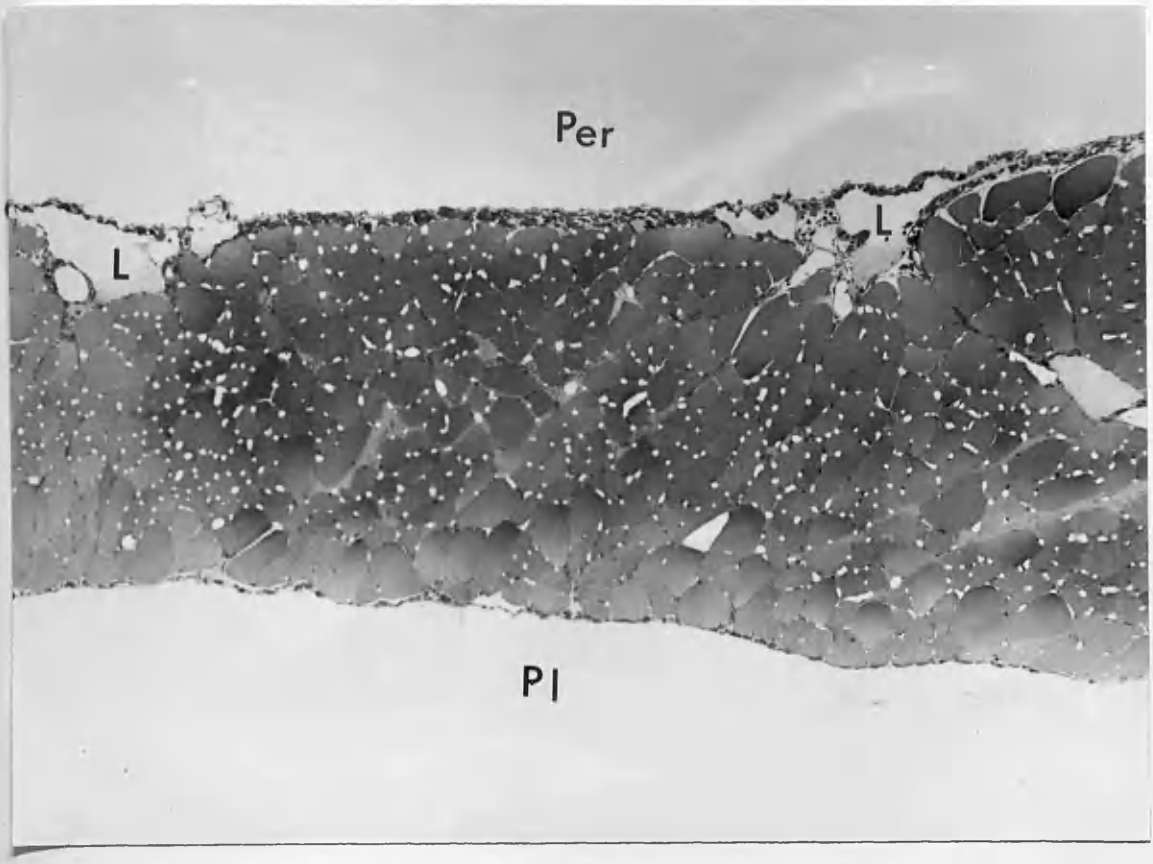
x 8 Adult Rat, 3 minutes after IP injection of ink.

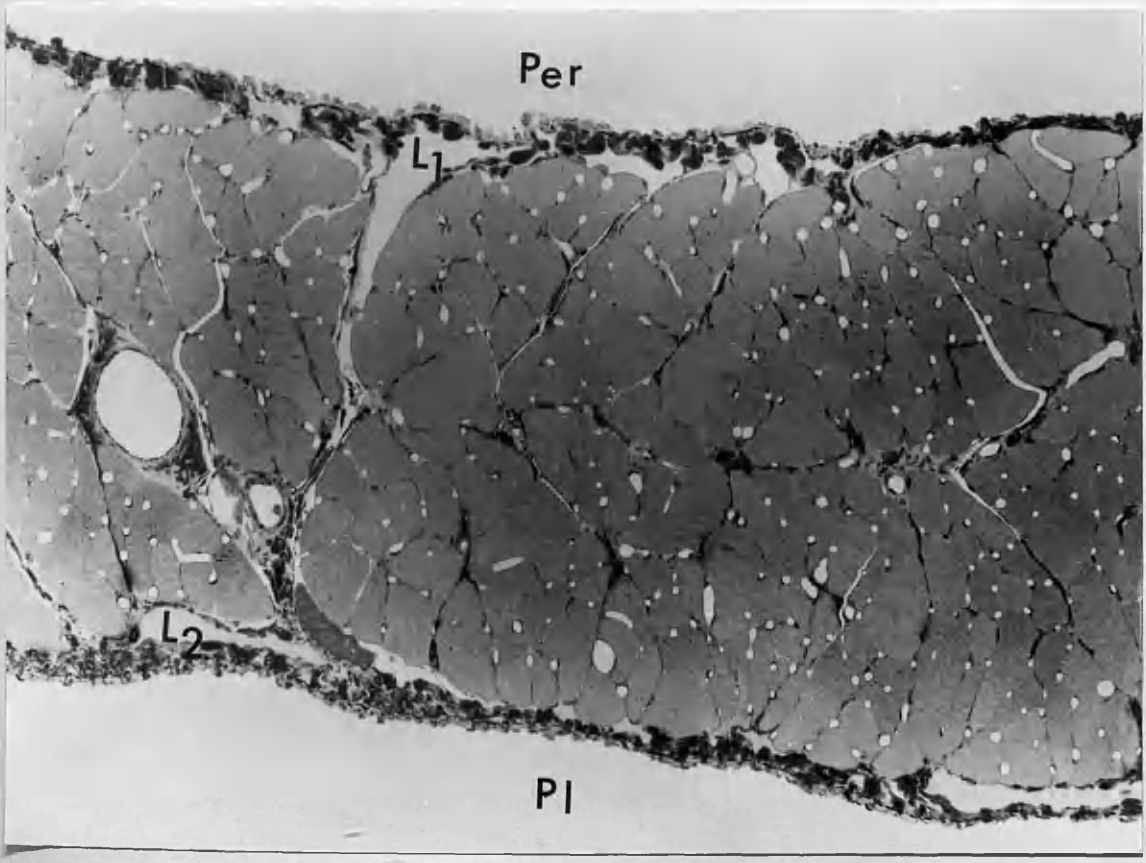

IL
L
IL
L

Figs. 94 & 95

Low power photomicrographs of cross-section through the full thickness of the diaphragm. Note that the peritoneal surface faces upward (Per) and the pleural surface faces downward (Pl) and the skeletal muscle fibres in between. Fig. 94 shows two separate subperitoneal lymphatic lacunae (L). In Fig. 95 one subperitoneal lymphatic lacuna (L1) is seen continuous with subpleural lymphatic plexus (L2).

x 65, x 150 Adult rats, 3 & 5 minutes after IP injection of ink. H & E


Per
L
L
Pl


Per
L1
L2
Pl

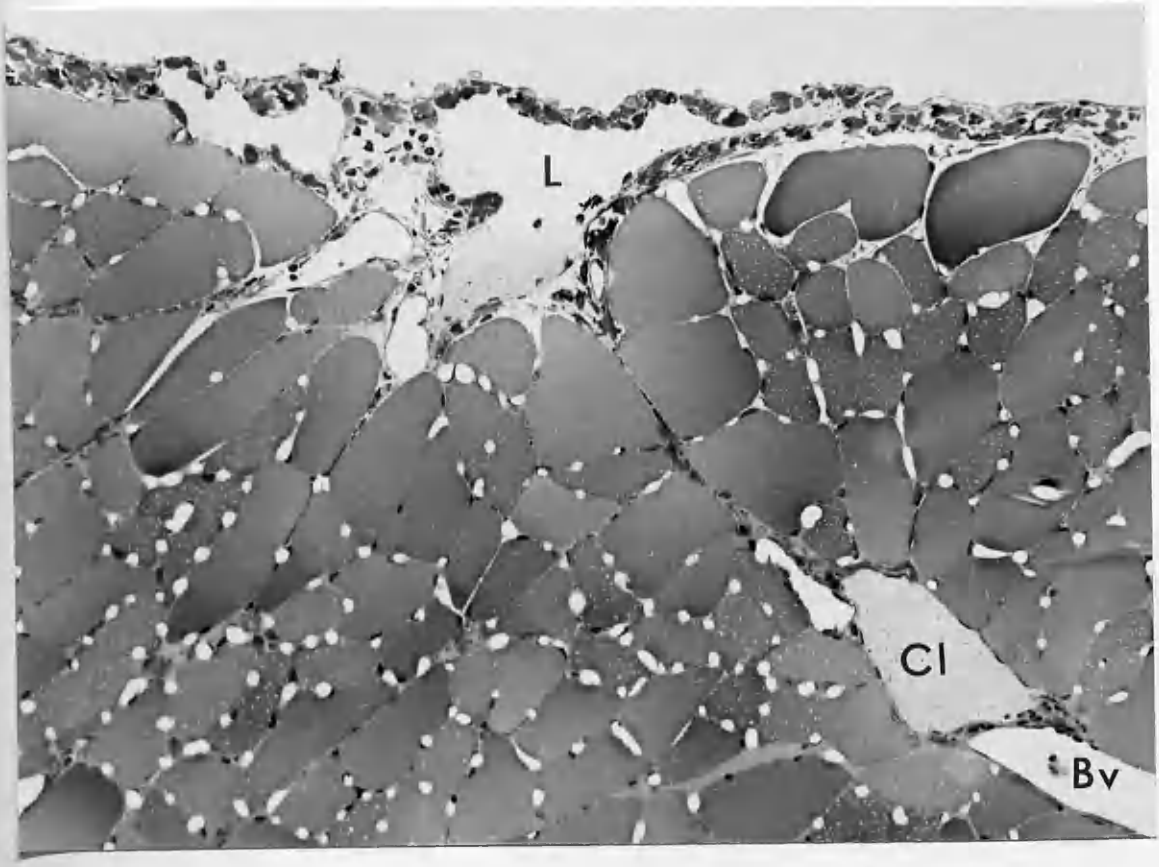
Figs. 96 & 97

Two medium-power photomicrographs showing subperitoneal lymphatic lacunae (L) anastomosing with deep collecting lymphatics (Cl) of the diaphragm. Note:

1) The large size of lymphatic lacunae compared with blood capillaries
2) Their content of stained lymph proteins and ink particles
3) Blood vessels (Bv), which are empty due to vascular perfusion
4) The presence of valves (arrowheads) in collecting lymphatics.

N - nerve fibres.

x 150, x 150 Adult rats, 5 minutes after IP injection of ink. H & E


L
Cl
Bv

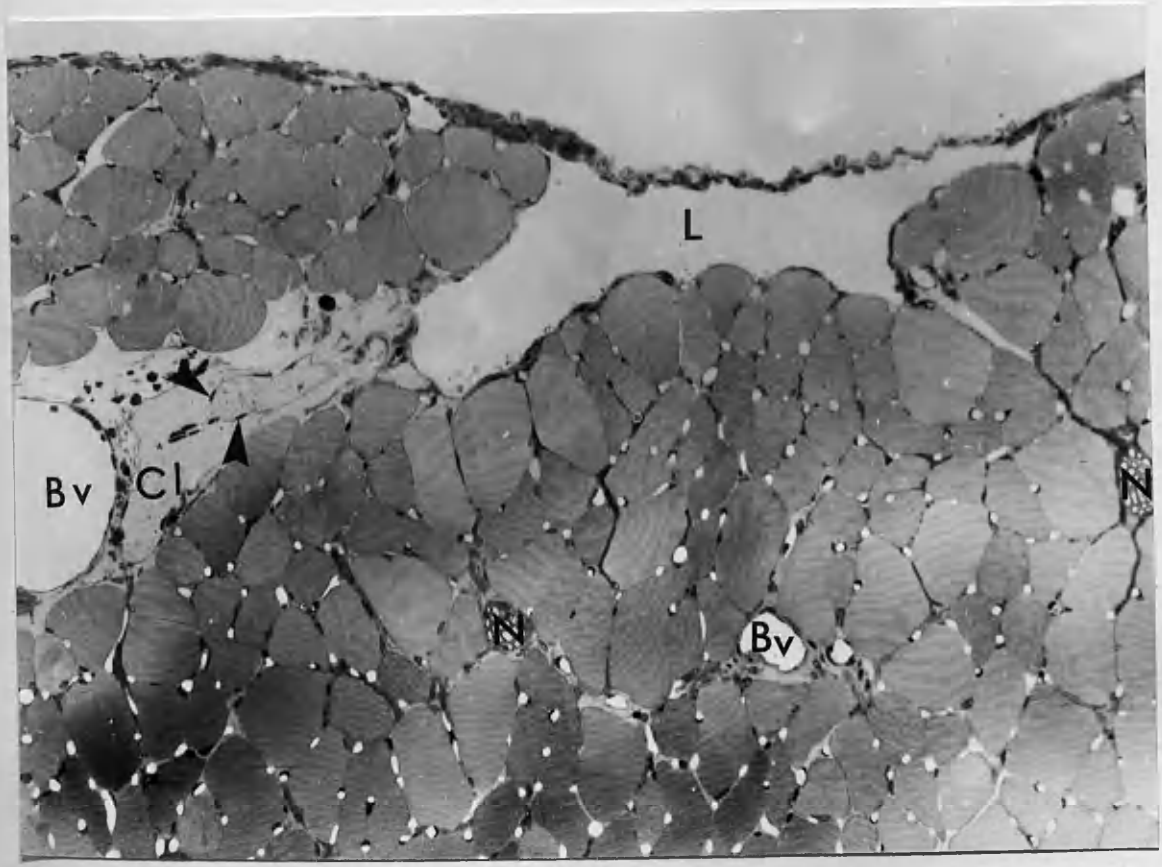
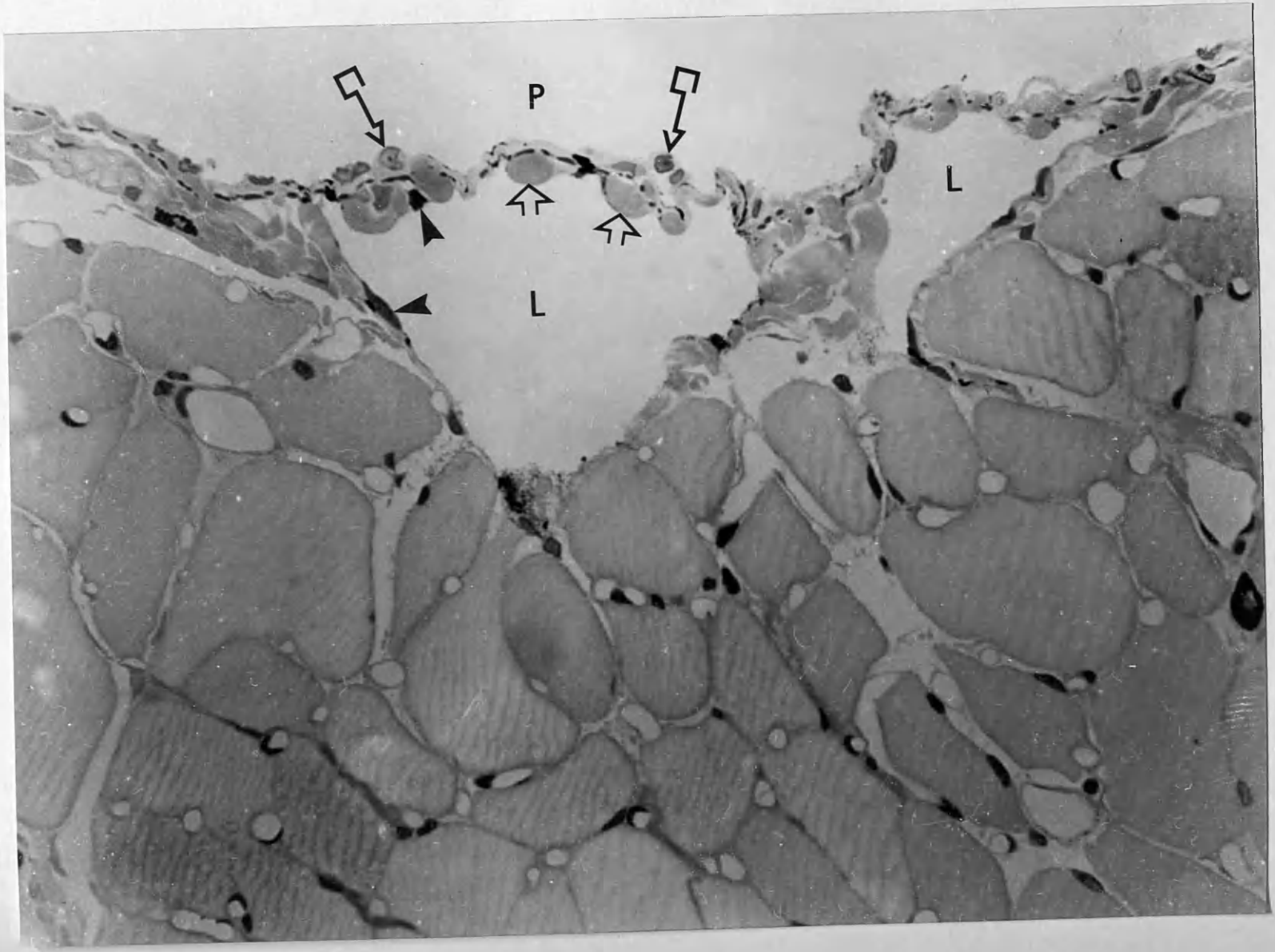

L
Bv
Cl
N
N
Bv

Fig. 98

A high power view of another diaphragmatic subperitoneal lymphatic lacuna (L). The lacunar lumen is separated from the peritoneal cavity (P) by a very thin wall, the roof of the lacuna. This consists of a specialised mesothelium (long arrows), a fenestrated elastic membrane represented by a darkly stained interrupted line, a layer of collagenous bundles (short arrows), and the lymphatic endothelial cells (arrowheads).

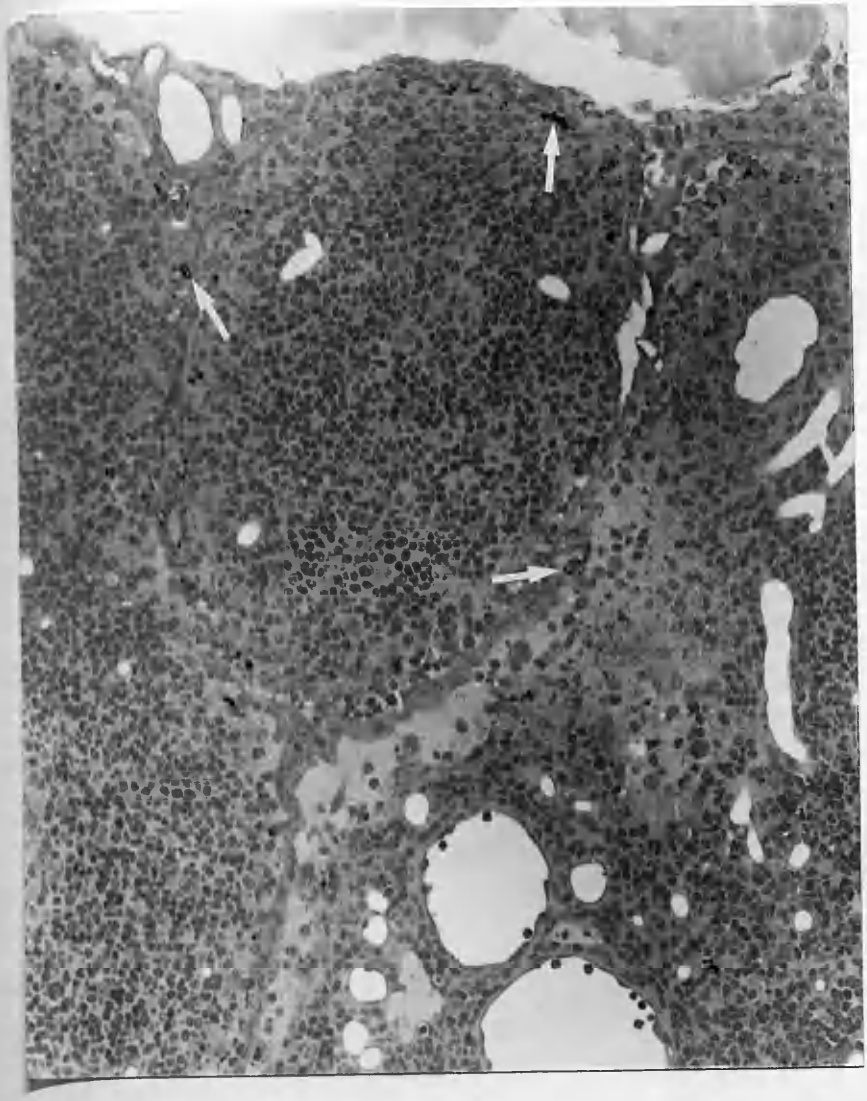
x 400 Young adult rat Azur II

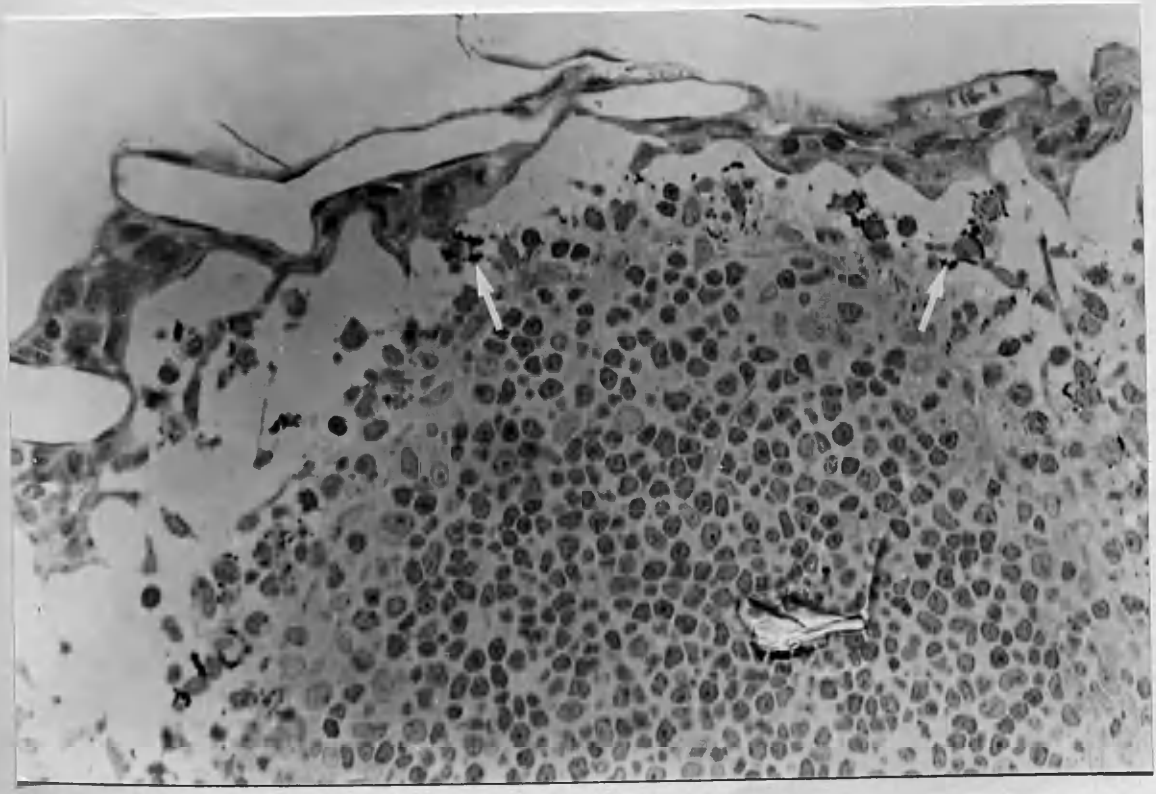

P
L
L

Figs. 99 & 100

Peripheral sinuses of parathymic nodes, 1 & 3 minutes after IP injection of ink. In spite of this short time interval, carbon particles (arrows) are already clearly seen in the subcapsular and intermediate sinuses.

x 150, x 400 Adult rats H & E

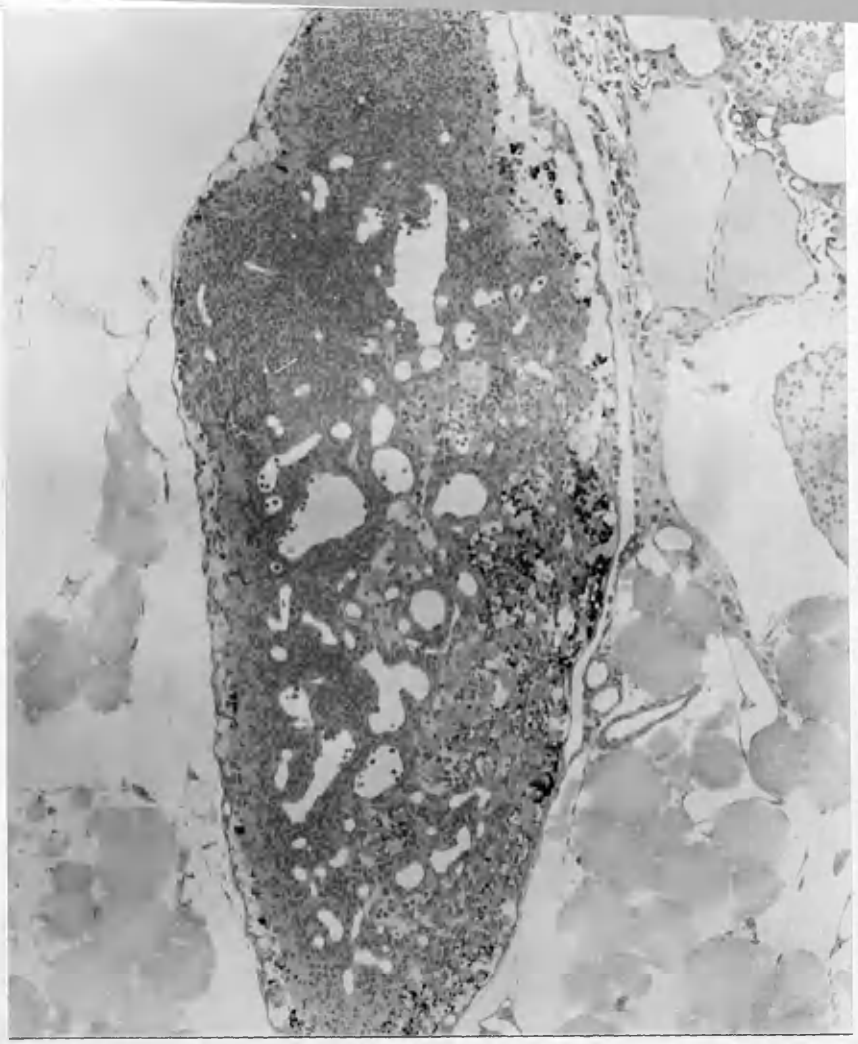




Figs. 101 & 102

Parathymic lymph nodes, 5 minutes after IP injection of ink. Fig. 101 shows abundant carbon particles, principally in the subcapsular sinus. Fig. 102 is a higher power view of the subcapsular sinus showing many ink-filled macrophages (arrows).

x 60, x 400 Adult rat H & E



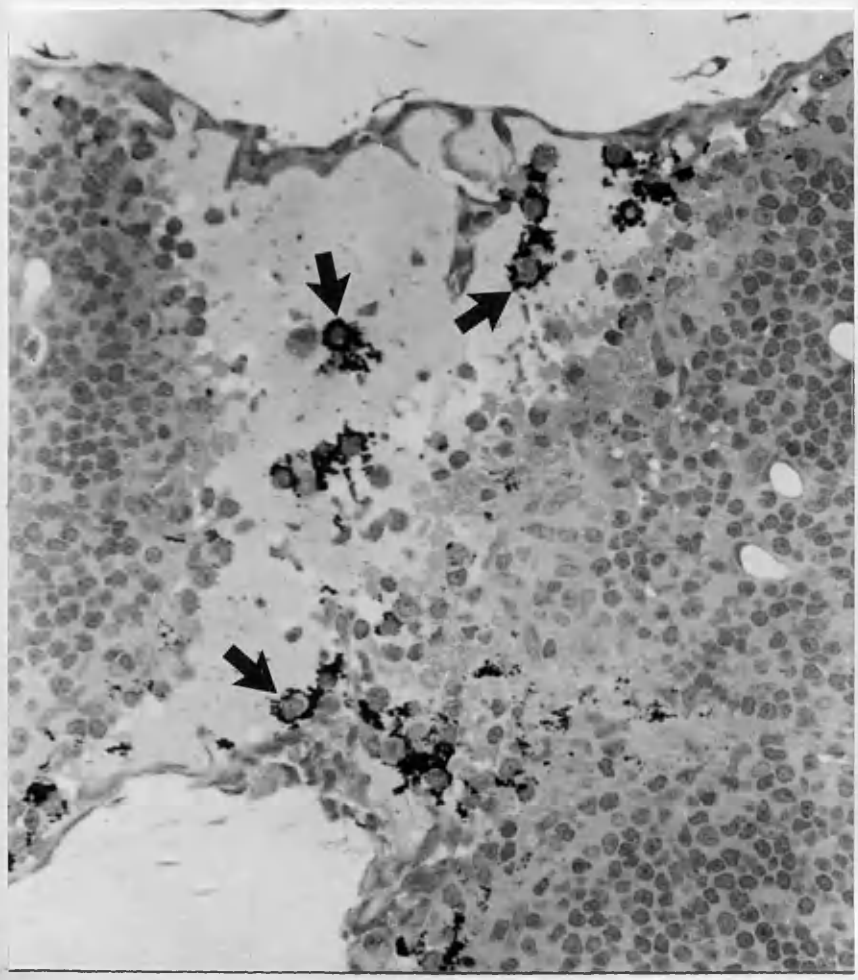


Fig. 103

A dissected rat, 15 minutes after IP injection of india ink. Ink was rapidly absorbed by subperitoneal lymphatic plexus of the diaphragm (arrows) and distributed, via several lymph trunks, to mediastinal and retroperitoneal lymph nodes.

x 5 Adult rat

Fig. 104

A higher magnification of the retroperitoneal lymph drainage route (arrows) of the left diaphragm of Fig. 103.

C - cisternal node, R - left renal node,
Cr - left crus of the diaphragm, K - left kidney

x 15 Adult rat

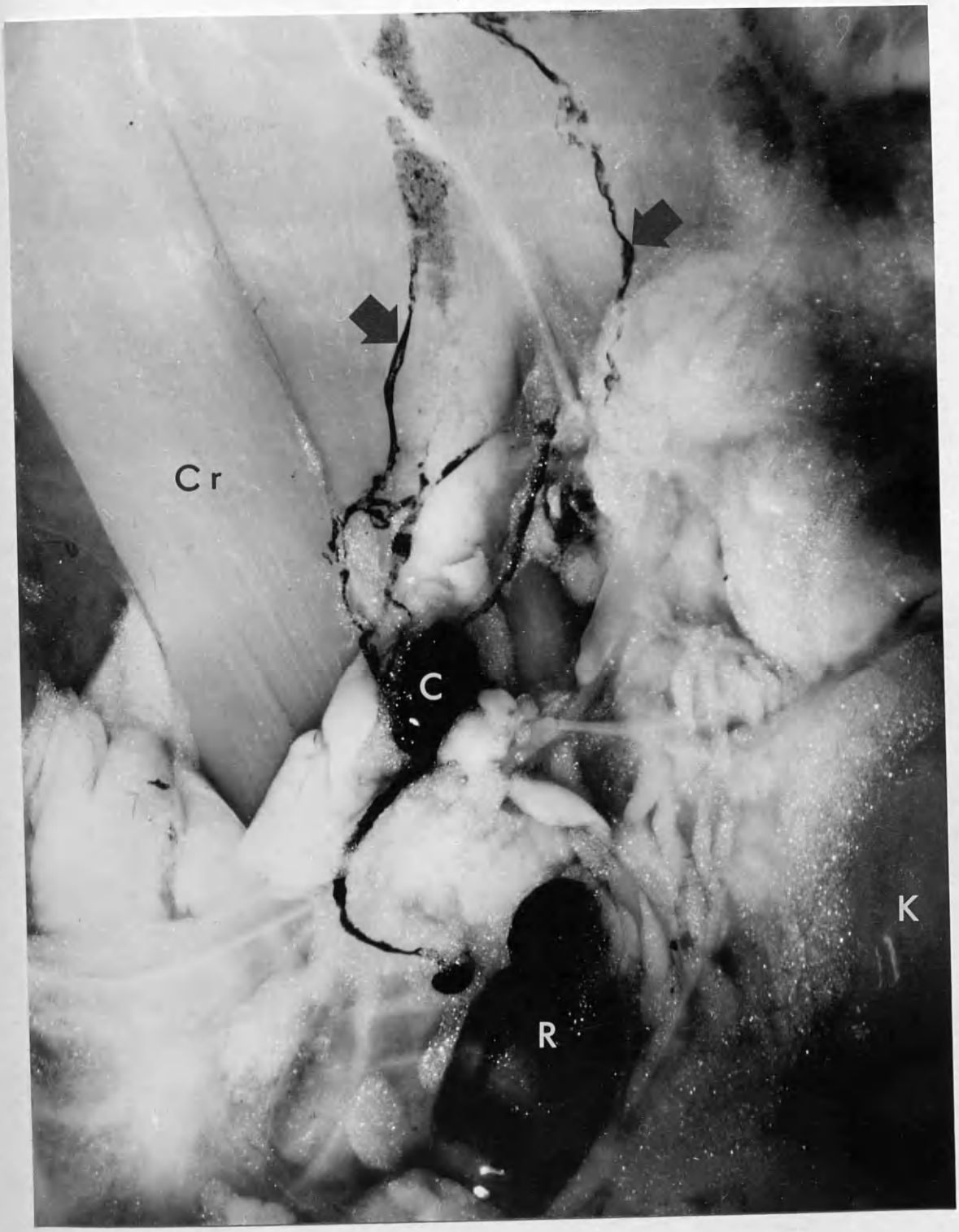

Cr
C
R
K

Fig. 105

Drawing of regional lymphatic drainage routes following IP injection of ink. The sternum and part of the rib cage had been reflected upward.

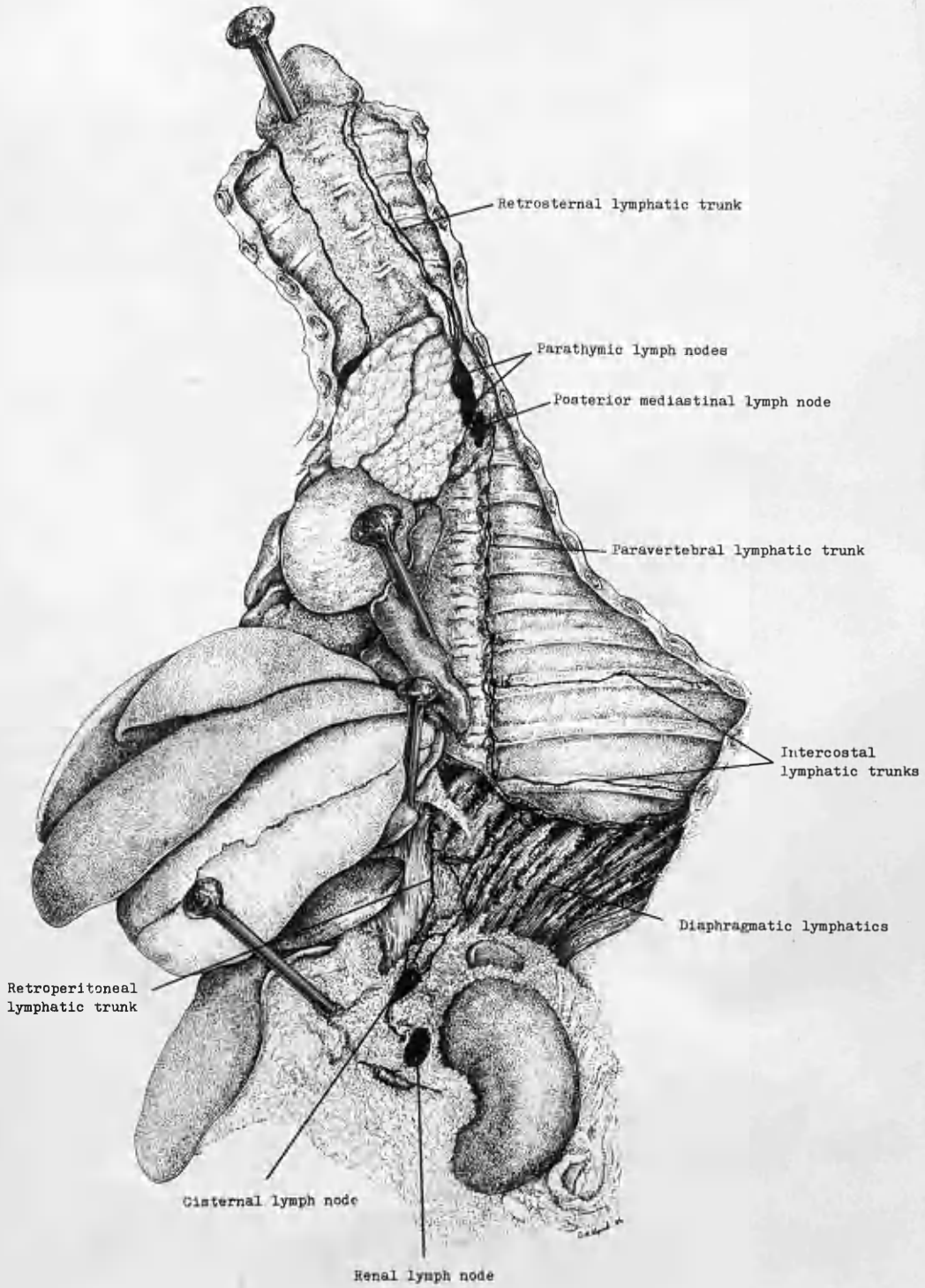

Retrosternal lymphatic trunk
Parathymic lymph nodes
Posterior mediastinal lymph node
Paravertebral lymphatic trunk
Intercostal lymphatic trunks
Diaphragmatic lymphatics
Retroperitoneal lymphatic trunk
Cisternal lymph node
Renal lymph node

Fig. 106

A diagram showing various lymphatic drainage routes of the diaphragm and their relative importance. The latter is judged on the basis of their size, frequency and intensity of the dye, as demonstrated by the key underneath.

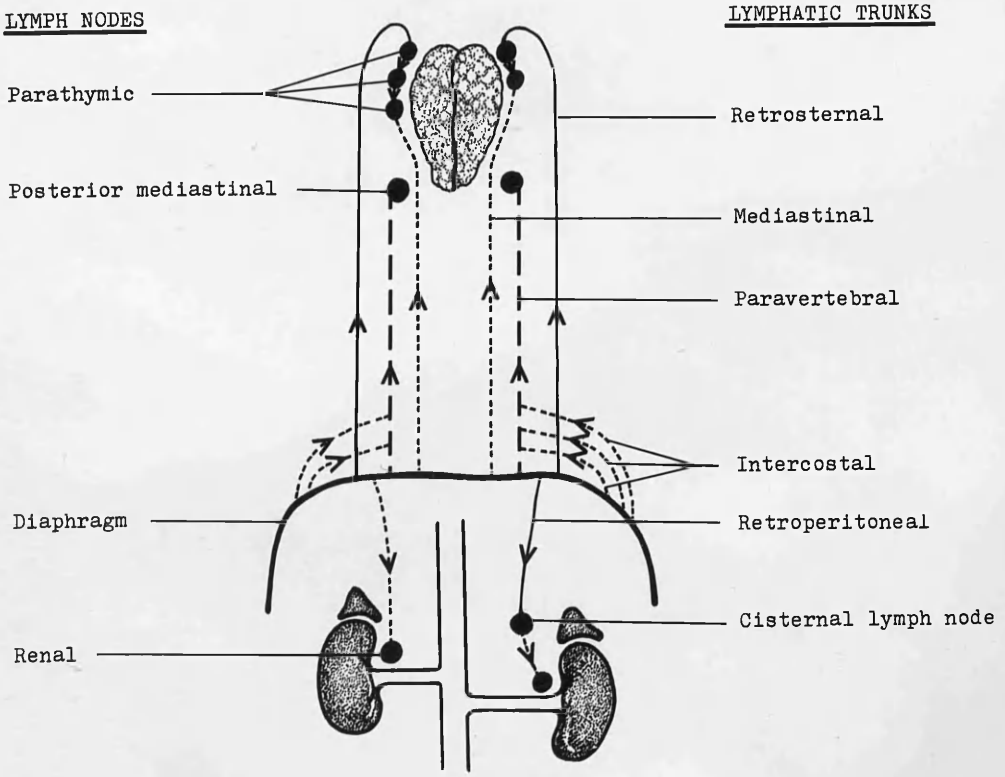
RIGHT

ANTERIOR VIEW

LEFT

LYMPH NODES

LYMPHATIC TRUNKS



——— CONSTANT

– — – USUAL

------- OCCASIONAL

Fig. 107

A diagram demonstrating the lymphatic drainage routes of the diaphragm and their relationship to one another and to regional nodes involved.

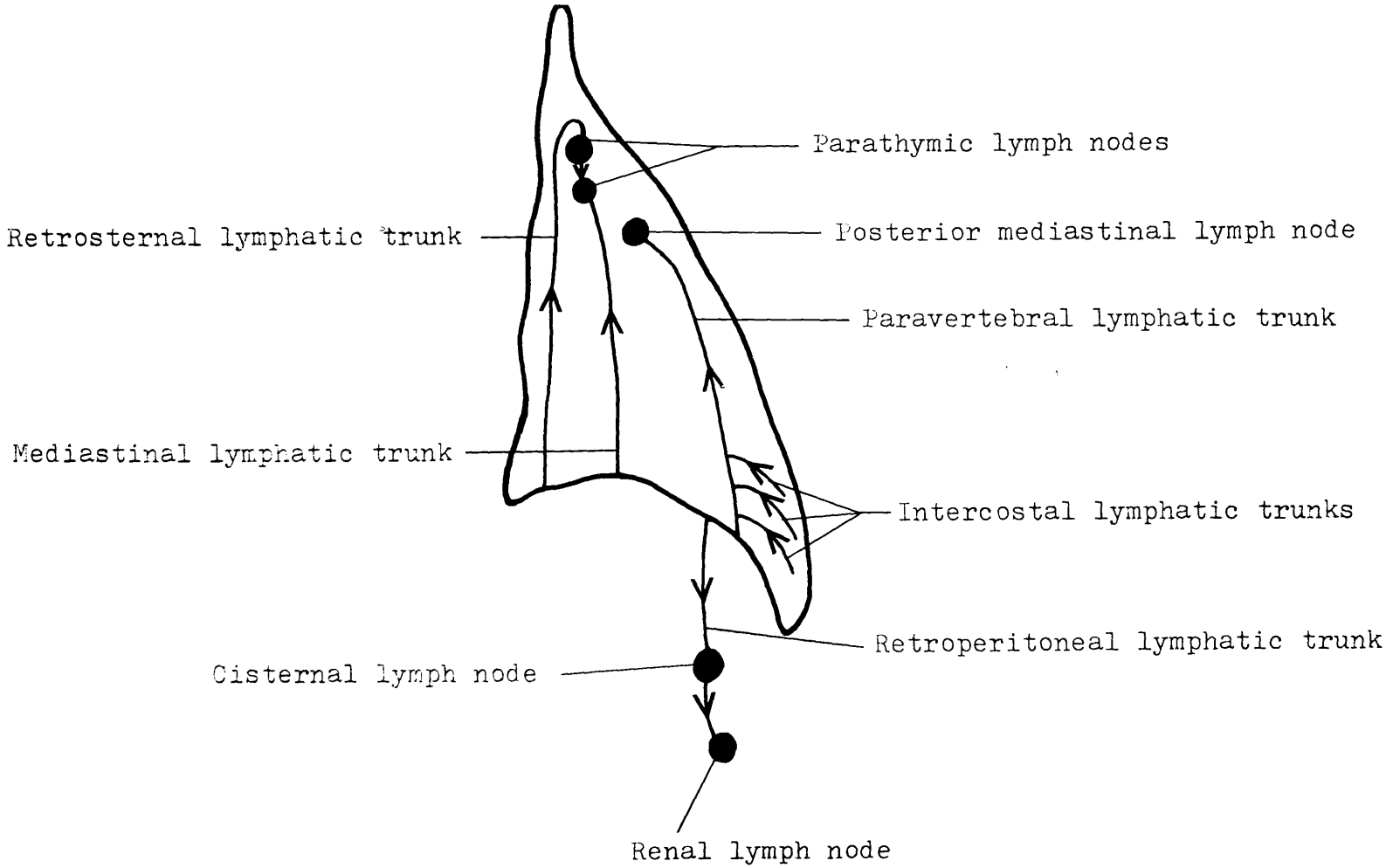
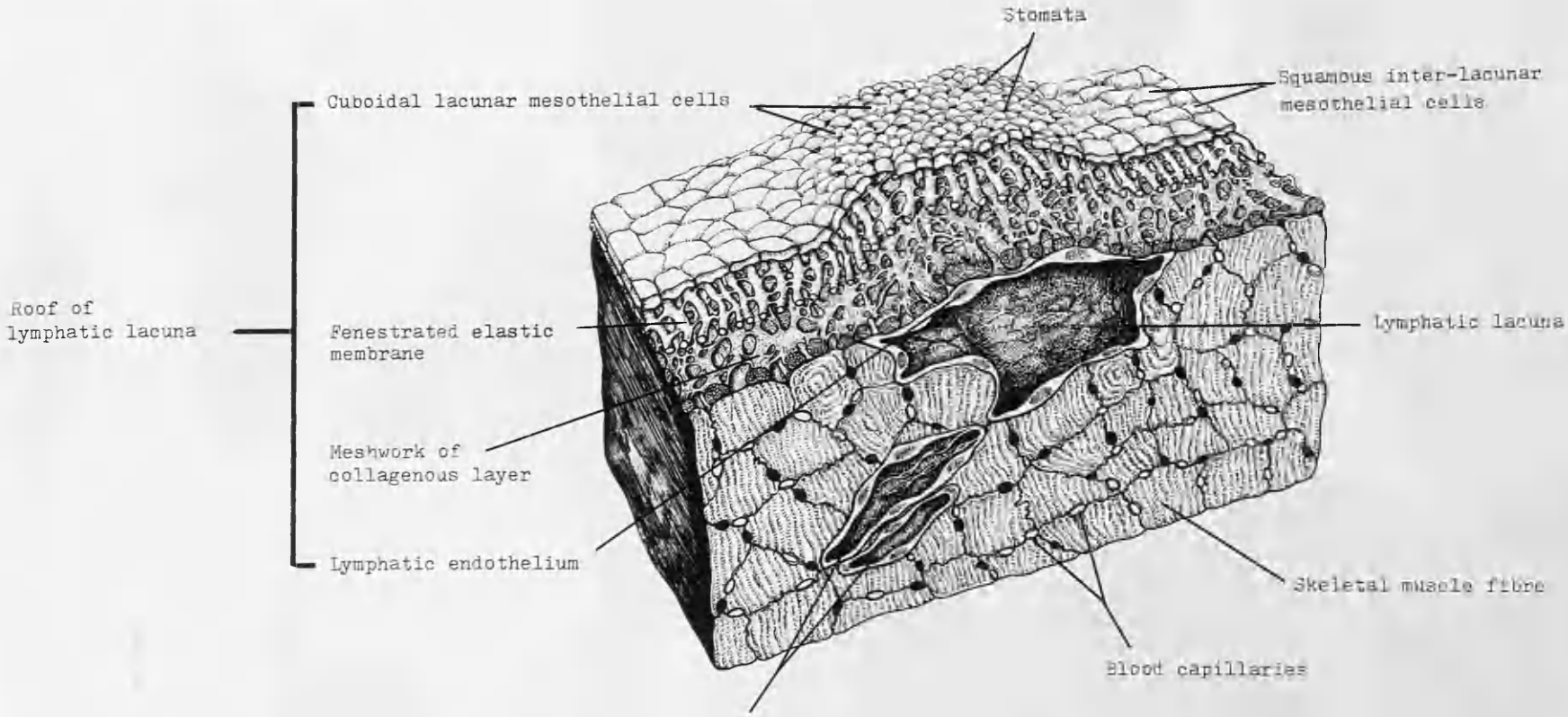

Parathymic lymph nodes
Retrosternal lymphatic trunk
Posterior mediastinal lymph node
Paravertebral lymphatic trunk
Mediastinal lymphatic trunk
Intercostal lymphatic trunks
Retroperitoneal lymphatic trunk
Cisternal lymph node
Renal lymph node

Fig. 108

This is a composite diagram of a cross-section through rat diaphragm. It shows the different components of the lymphatic lacunar roof. The peritoneal surface is uppermost.

"3-D IMPRESSION OF LYMPHATIC LACUNA IN RAT'S DIAPHRAGMATIC PERITONEUM"



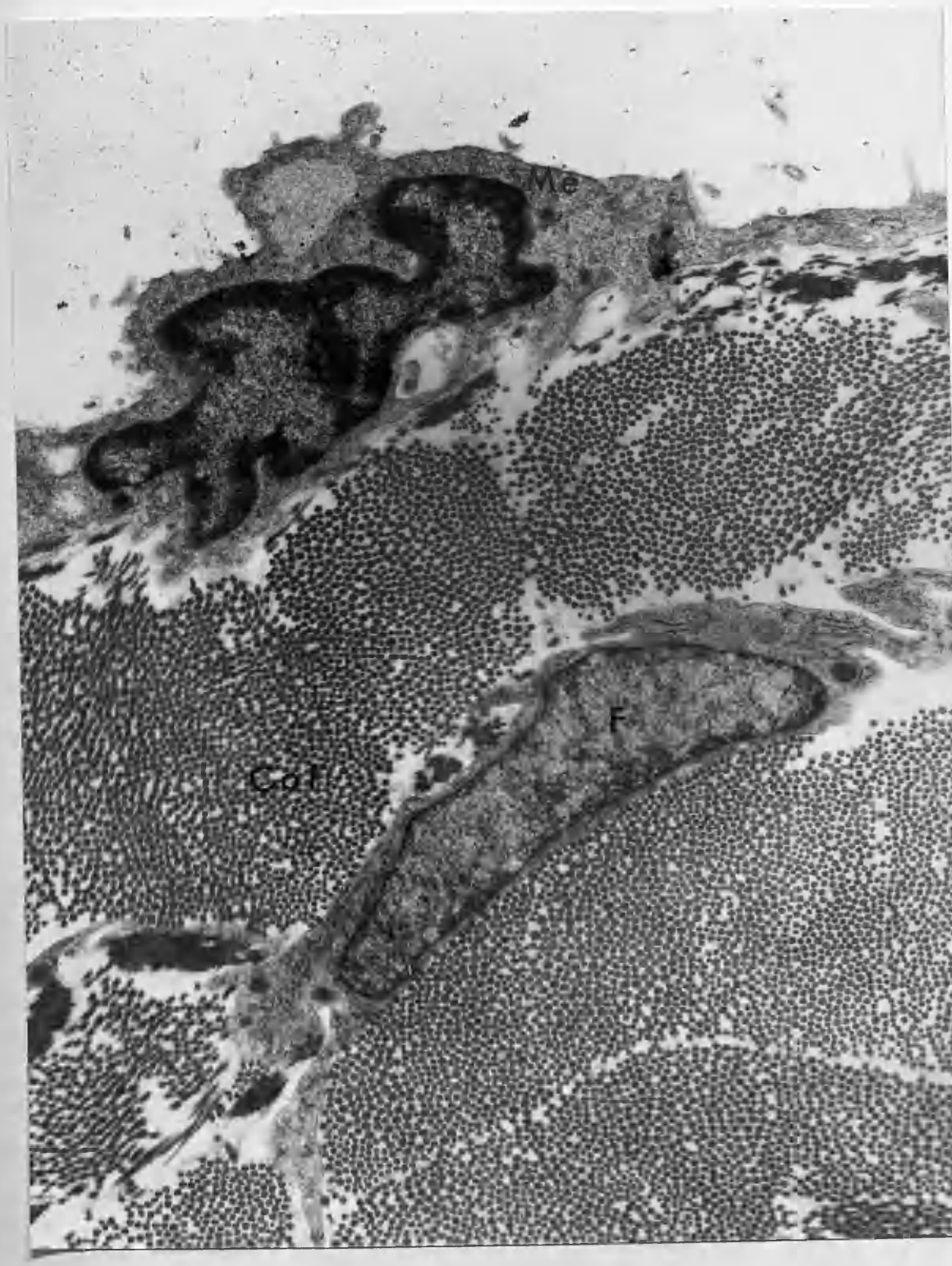
Intermuscular collecting lymphatic and blood vessel

Fig. 109

A TEM micrograph, taken from inter-lacunar area of the diaphragmatic peritoneum. It mainly shows the rather flattened mesothelial cell (Me), in contrast to specialised cuboidal cells of the lacunar areas as demonstrated in the following figures.

Col - bundles of collagen fibres.
F - fibroblast.

x 5,600 Adult rat

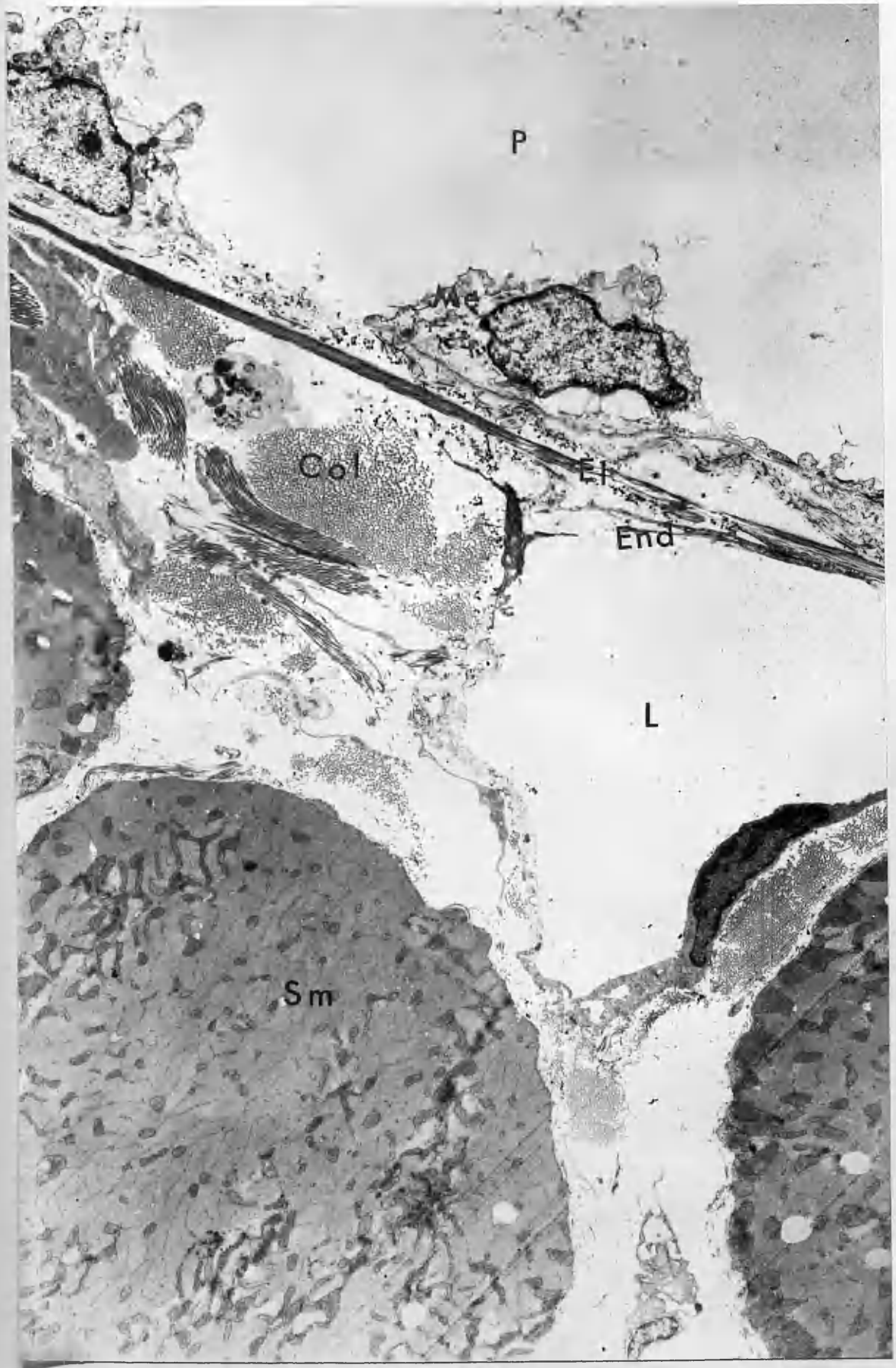

Me
F
Co F

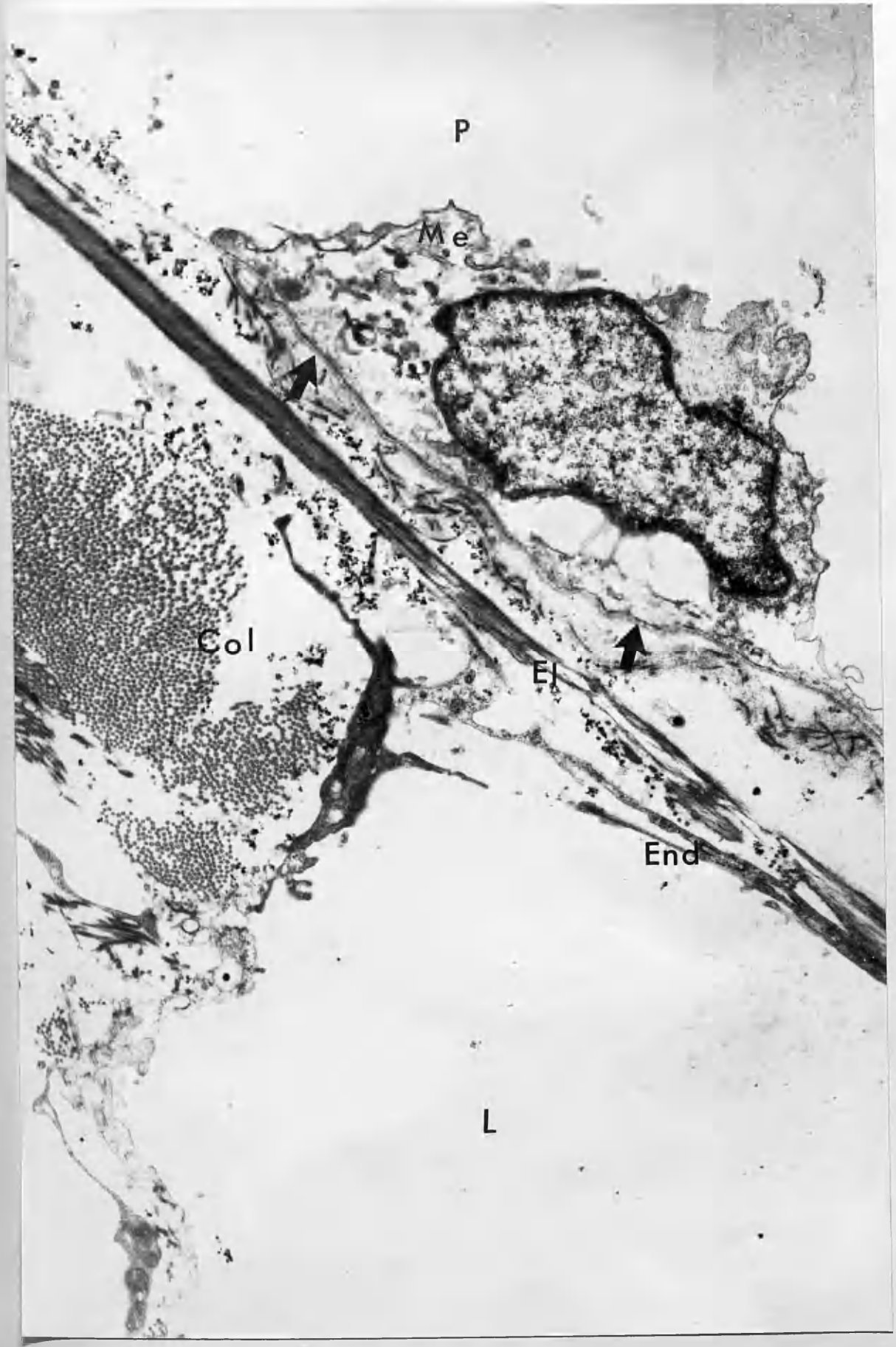
Figs. 110 & 111

These TEM micrographs show a subperitoneal lymphatic lacuna (L) with its overlying roof of cuboidal mesothelial cells (Me), a fenestrated elastic membrane (El), a thin band of collagenous tissue (Col), and lymphatic endothelial cells (End). Continuous mesothelial basement membrane is indicated by arrows in Fig. 111.

P - peritoneal cavity, Sm - skeletal muscle.

x 1,400, x 2,800 Adult rat


P
Me
Col
El
End
L
Sm

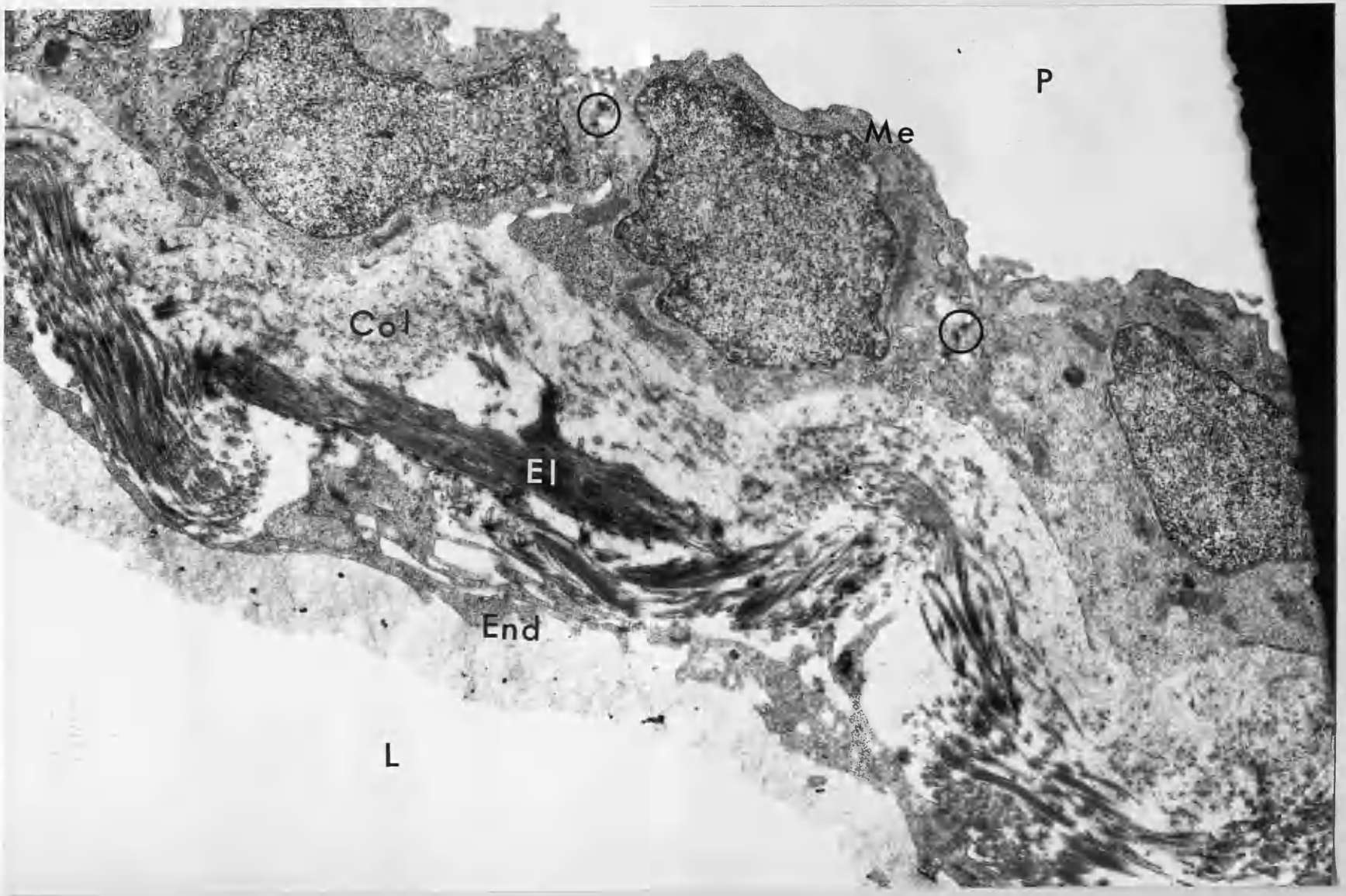

P
Me
Col
El
End
L

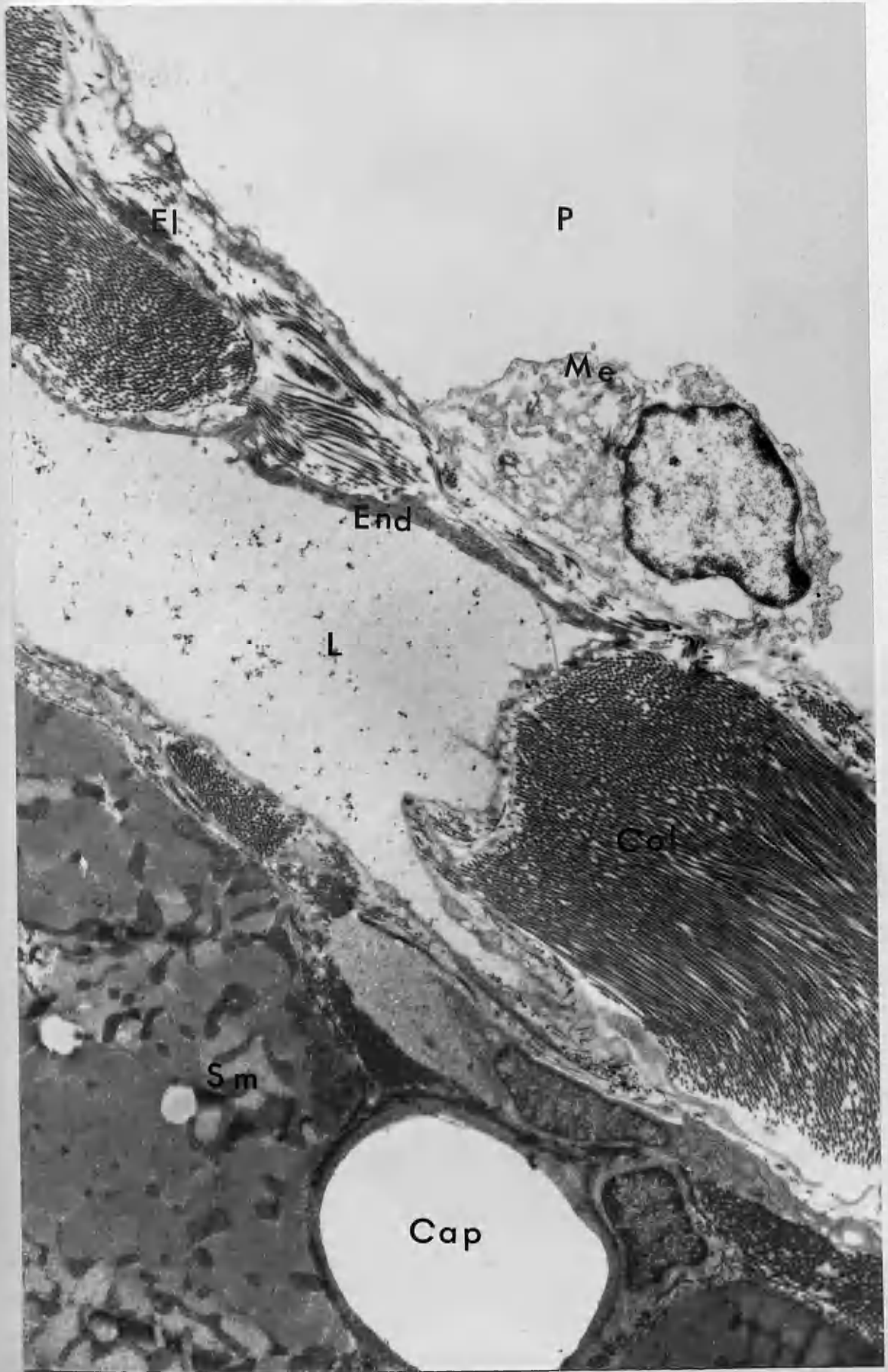
Figs. 112, 113 & 114

These TEM micrographs demonstrate the ultrastructure of the lacunar roof which consists of specialised cuboidal mesothelial cells (Me), a fenestrated layer of elastic tissue (El), bundles of collagenous fibres (Col) cut in different planes of section, and finally a lymphatic endothelial cell (End). Note the carbon particles and lymphoprotein precipitate in the lymphatic.

Crenated red blood cell (asterisk); encircled are desmosomal junctions between adjacent mesothelial cells; P - peritoneal cavity, Cap - blood capillary, Sm - skeletal muscle - F - fibroblast.

x 4,200, x 2,100, x 2,100 Adult rats


P
Me
Col
El
End
L


El
P
Me
End
L
Col
Sm
Cap

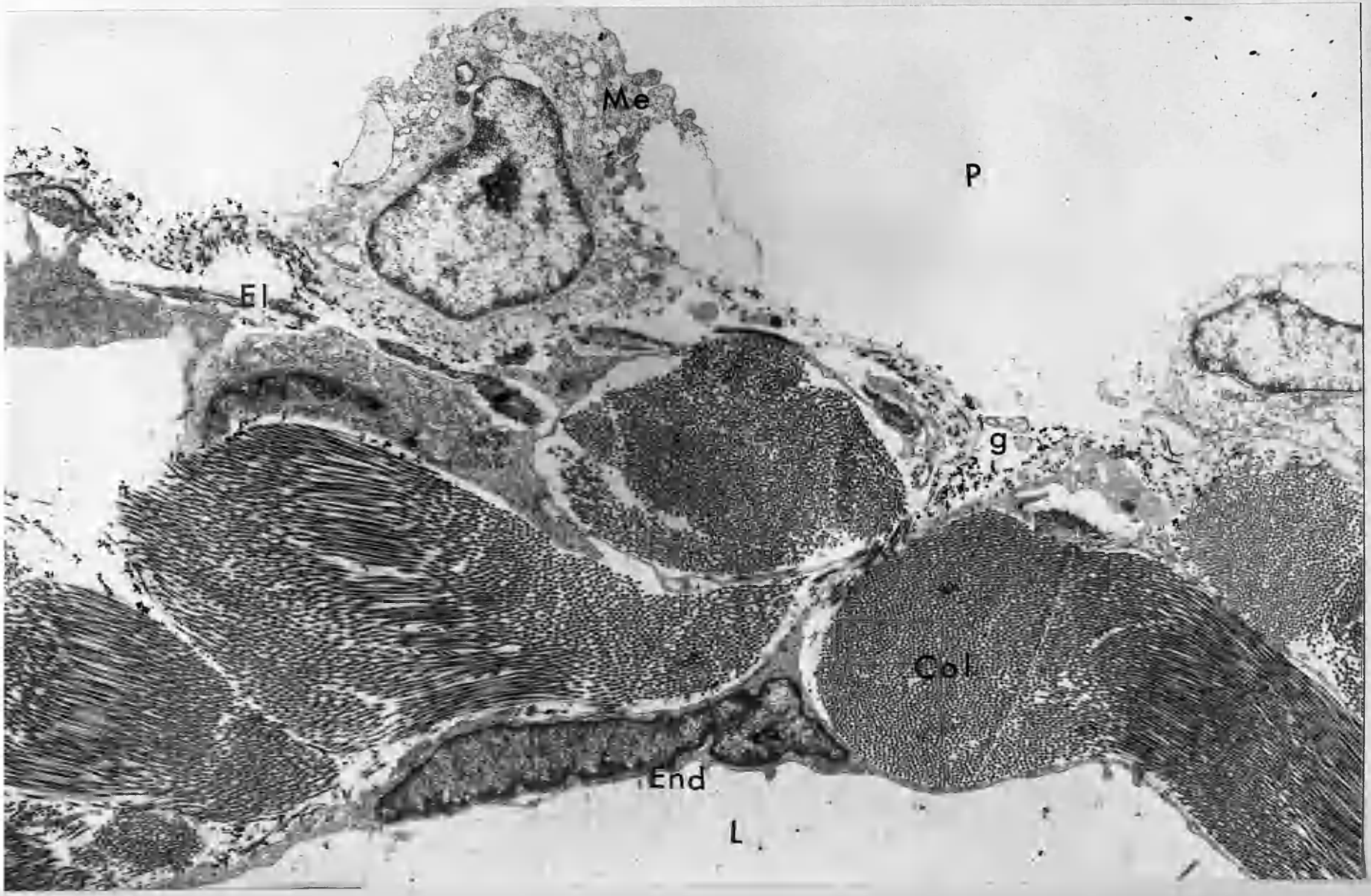

Me
P
El
Col
End
F
L
*
Sm

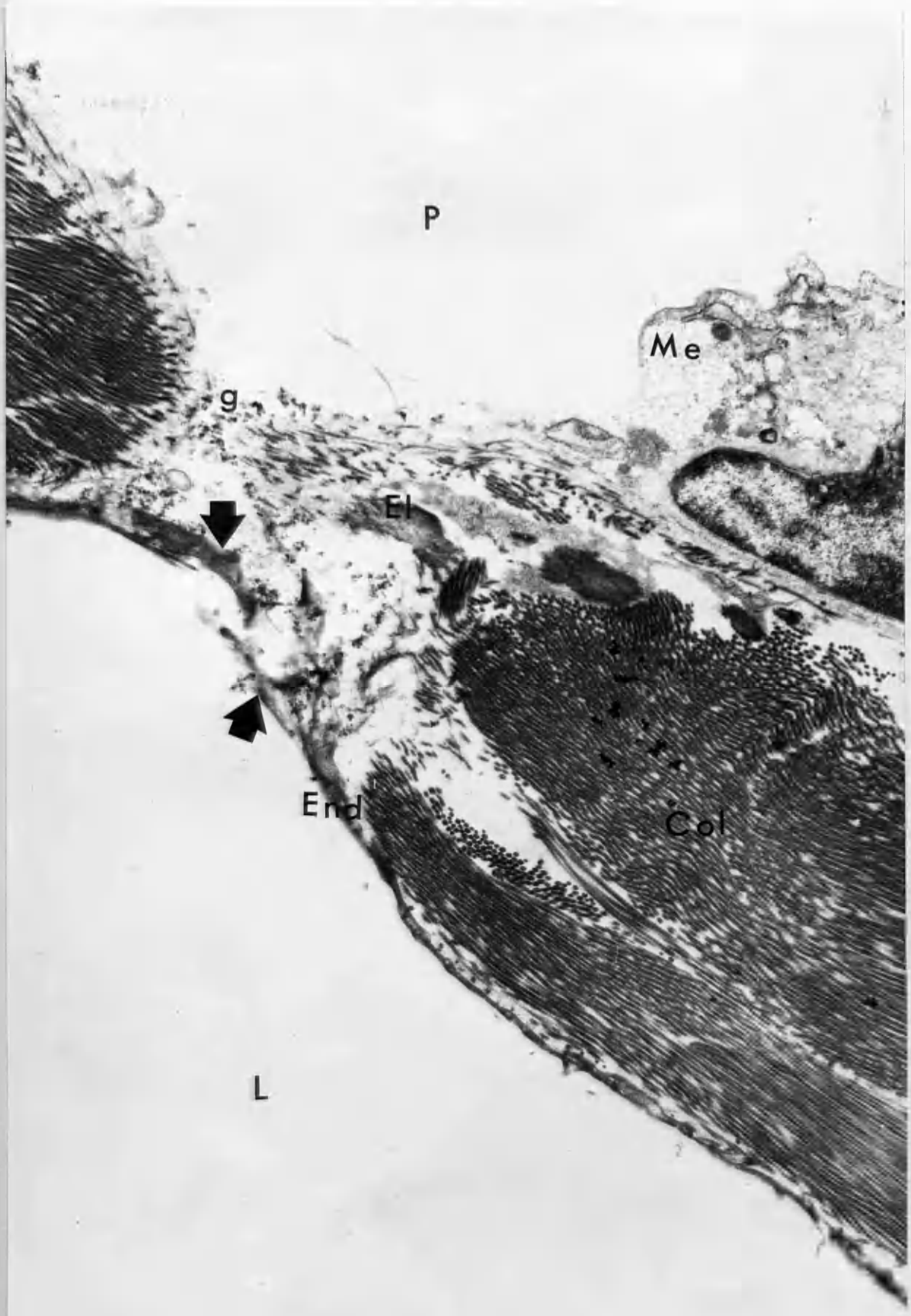
Figs. 115 & 116

Roof of diaphragmatic subperitoneal lymphatic lacuna (L), shortly after IP injection of carbon. The tracer is localised over mesothelial cell margins and within gaps (g) between adjacent cells. Note in Fig. 116 the valve-like flaps of lymphatic endothelial cells (arrows).

P - peritoneal cavity
End - lymphatic endothelium
Me - mesothelial cells
El - elastic tissue
Col - collagenous connective tissue

x 2,100, x 3,500 Young adult rats


Me
P
El
g
Col
End
L

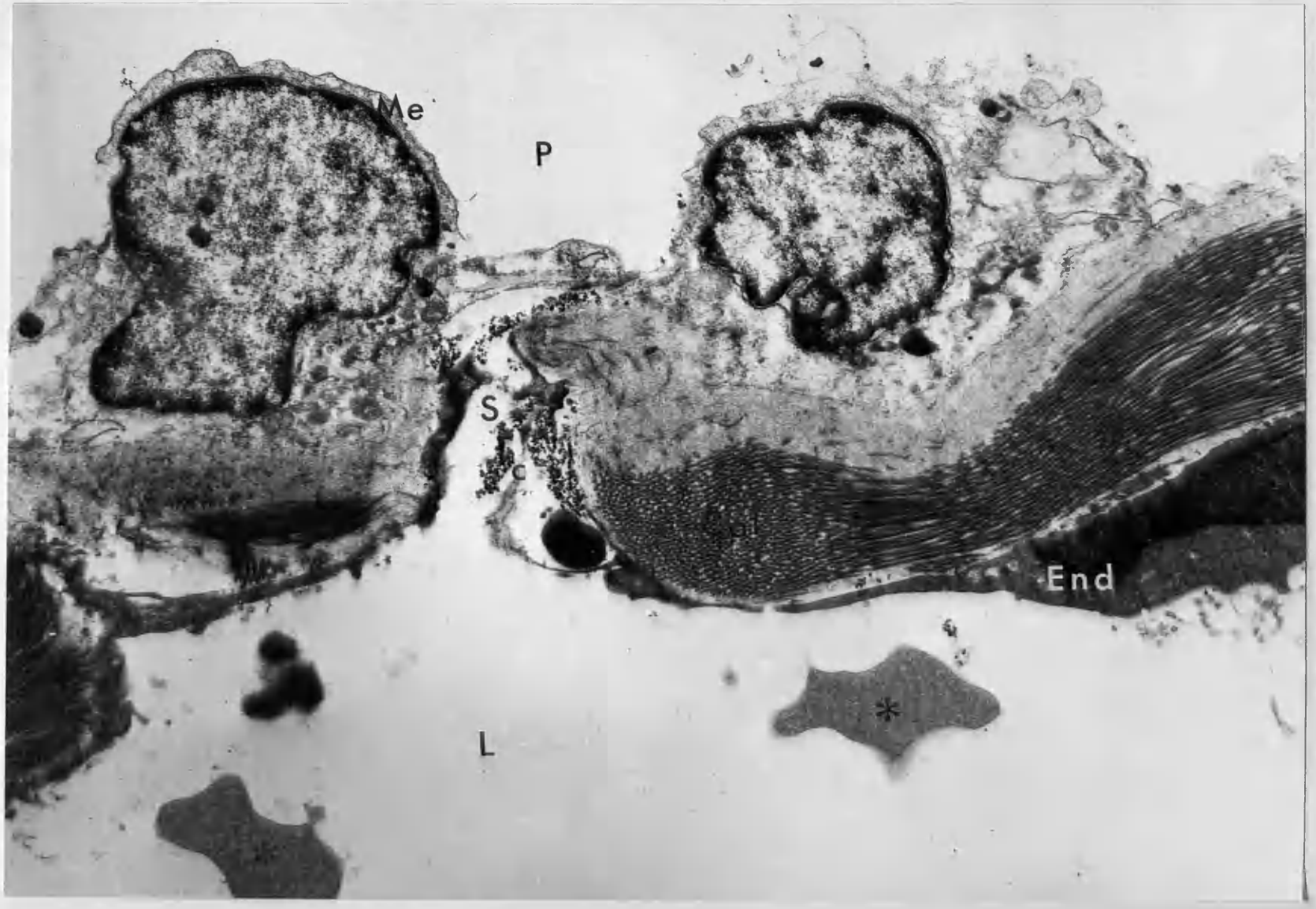

P
Me
g
El
End
Col
L

Figs. 117 & 118

TEM micrographs of a stomal orifice (S). Note that endothelial cells (End) extend from lymphatic lumen (L) into the peritoneal cavity (P) between bundles of collagenous fibres (Col). This provides a direct passageway between the peritoneal cavity and underlying lymphatic lacuna, through which carbon particles (C) and red blood cells (asterisk) pass freely and rapidly into the lymphatic lumen.

Me - peritoneal mesothelial cells.

x 4,200, x 10,000 Young adult rats


Me
P
S
c
End
L
*

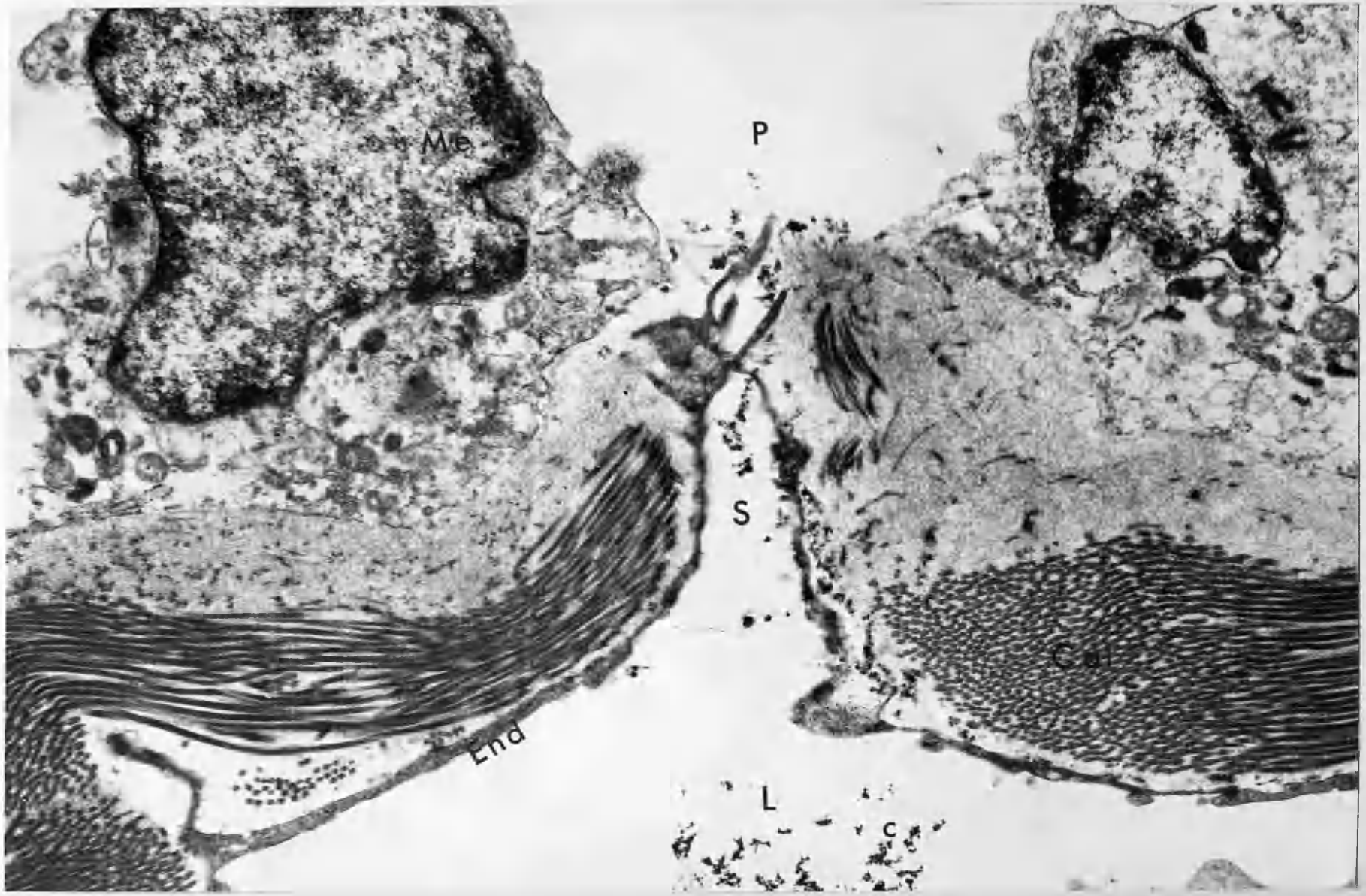

Me
P
S
Co
End
L
C

Fig. 119

This is a section through a stoma (S) containing abundant carbon particles (C).

P - peritoneal cavity, L - lymphatic lumen, Me - mesothelial cell.

x 5,600 Adult rat

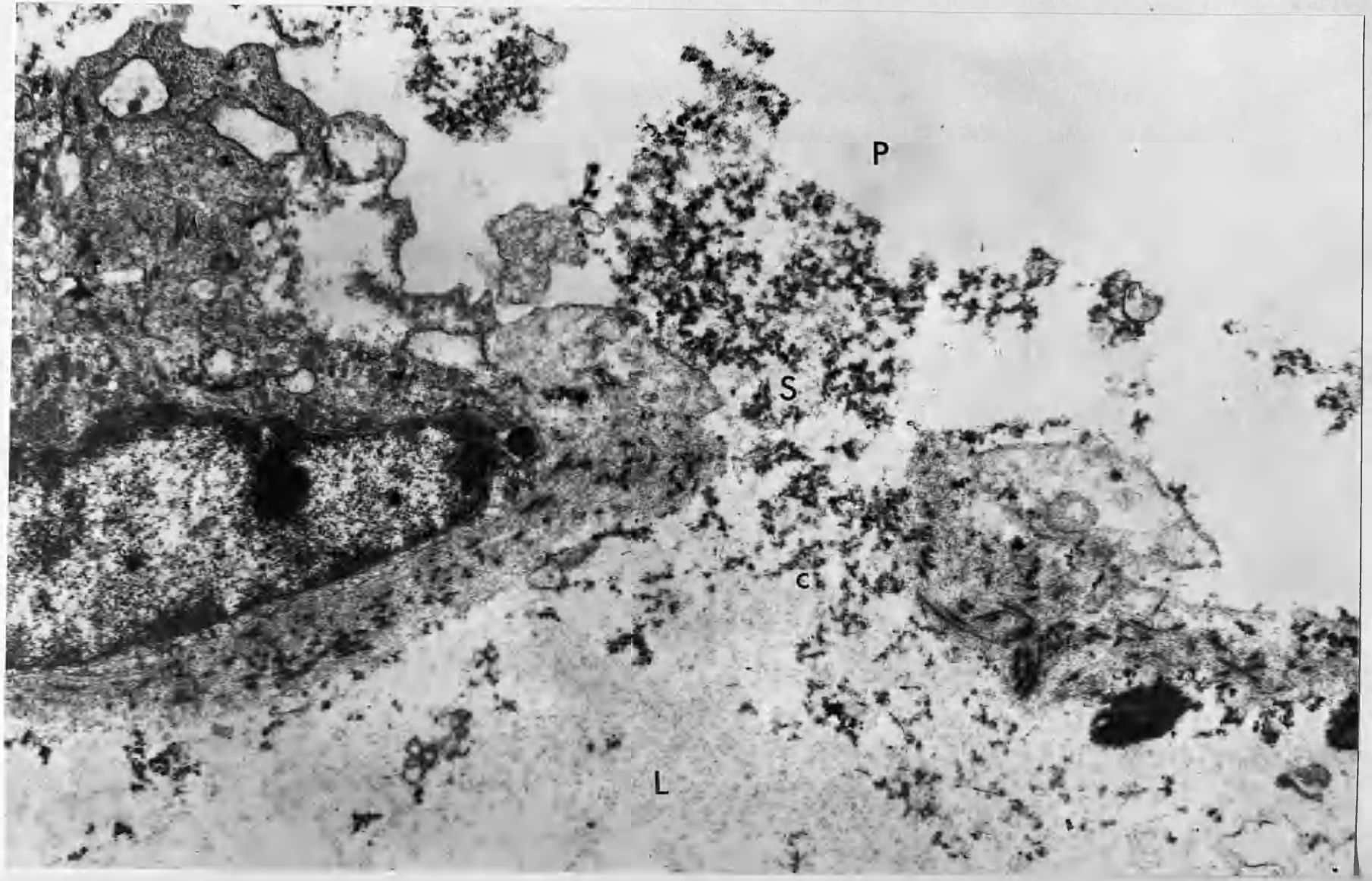
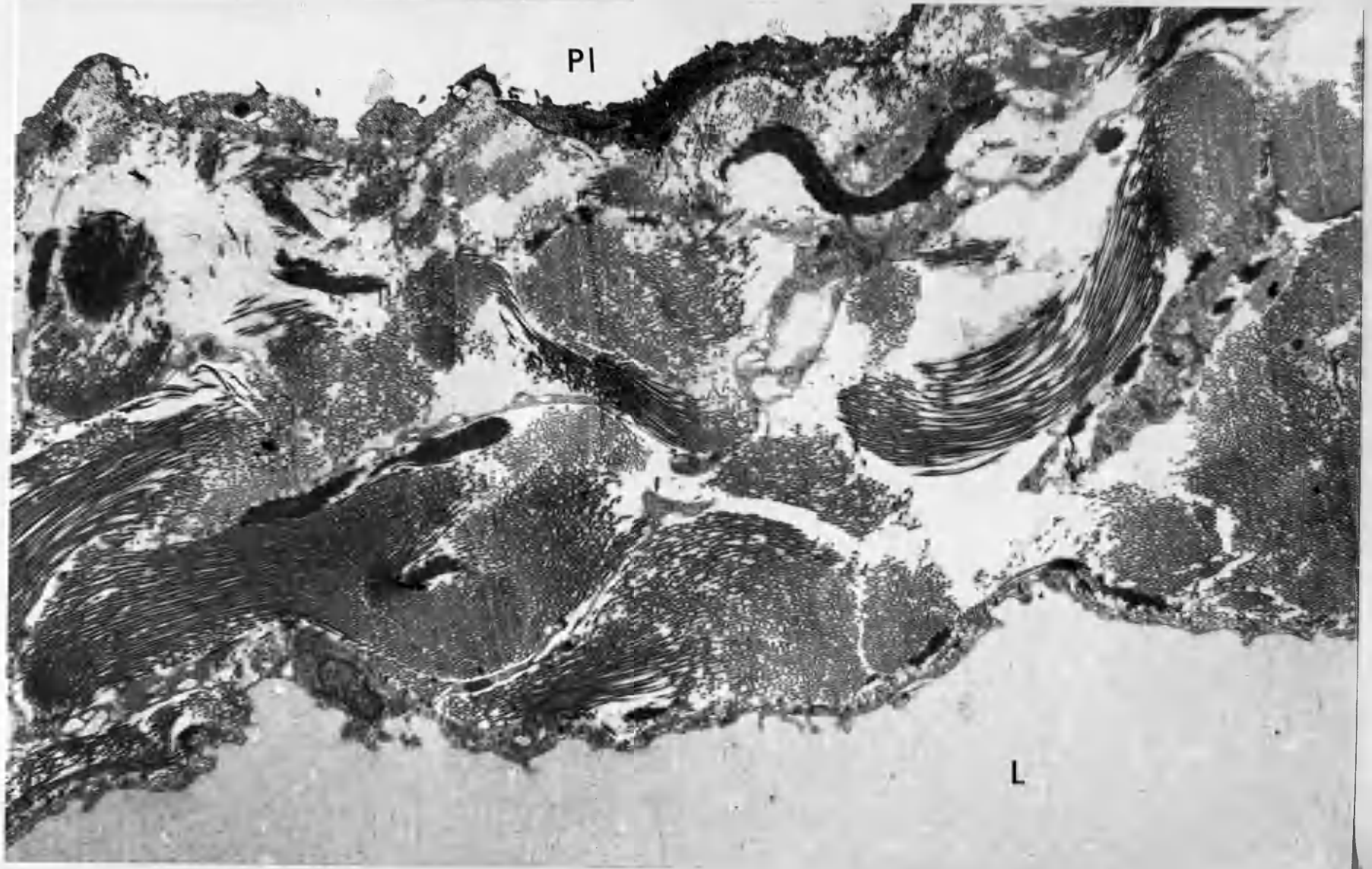

P
S
C
L

Fig. 120

A thin section of the diaphragm taken through the pleural surface (cf. Fig. 95). Note that the subpleural lymphatic plexus (L) is separated from the pleural cavity (Pl) by a rather thick layer of connective tissue.

x 2,100 Adult rat

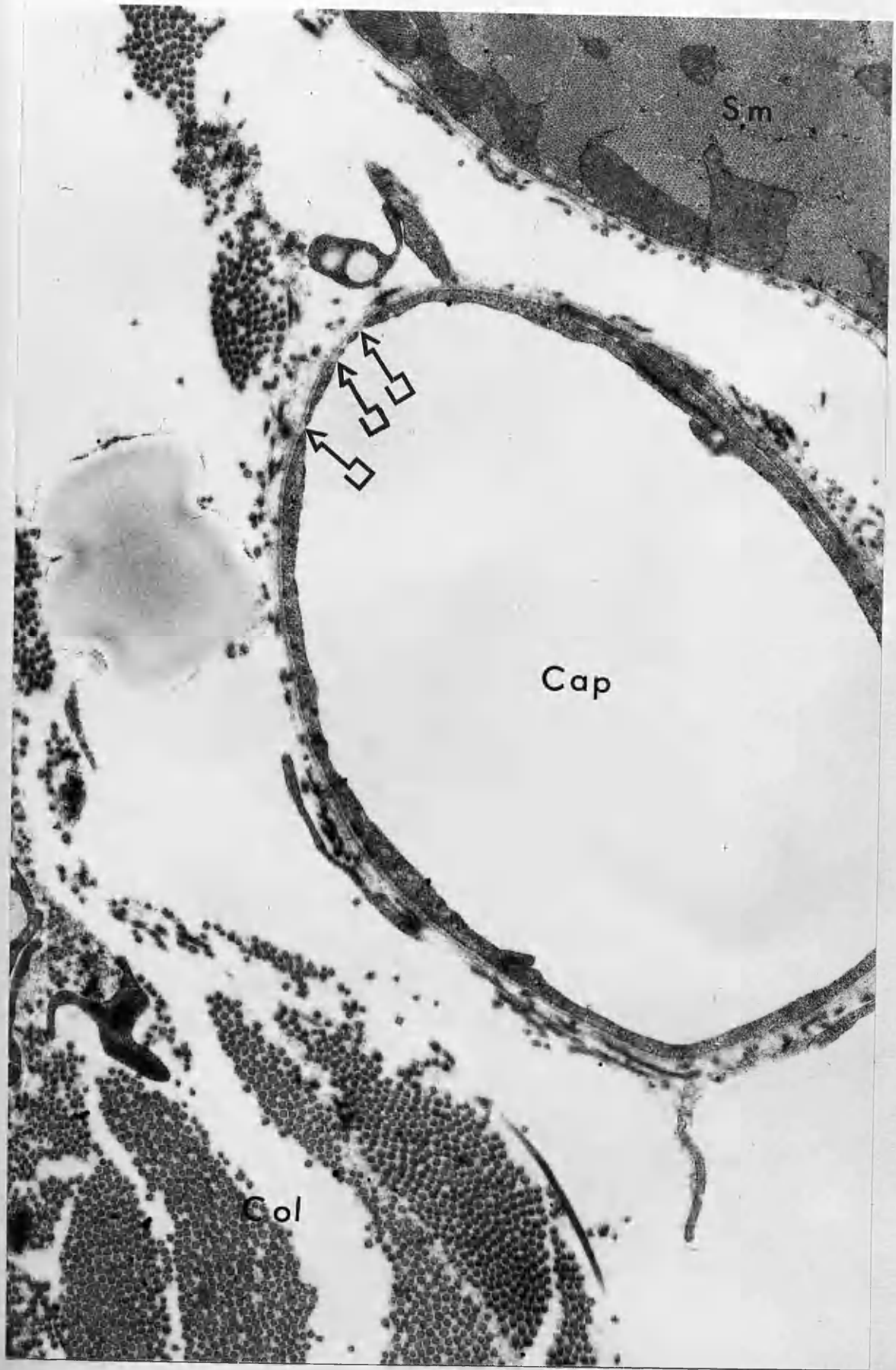

Pl
L

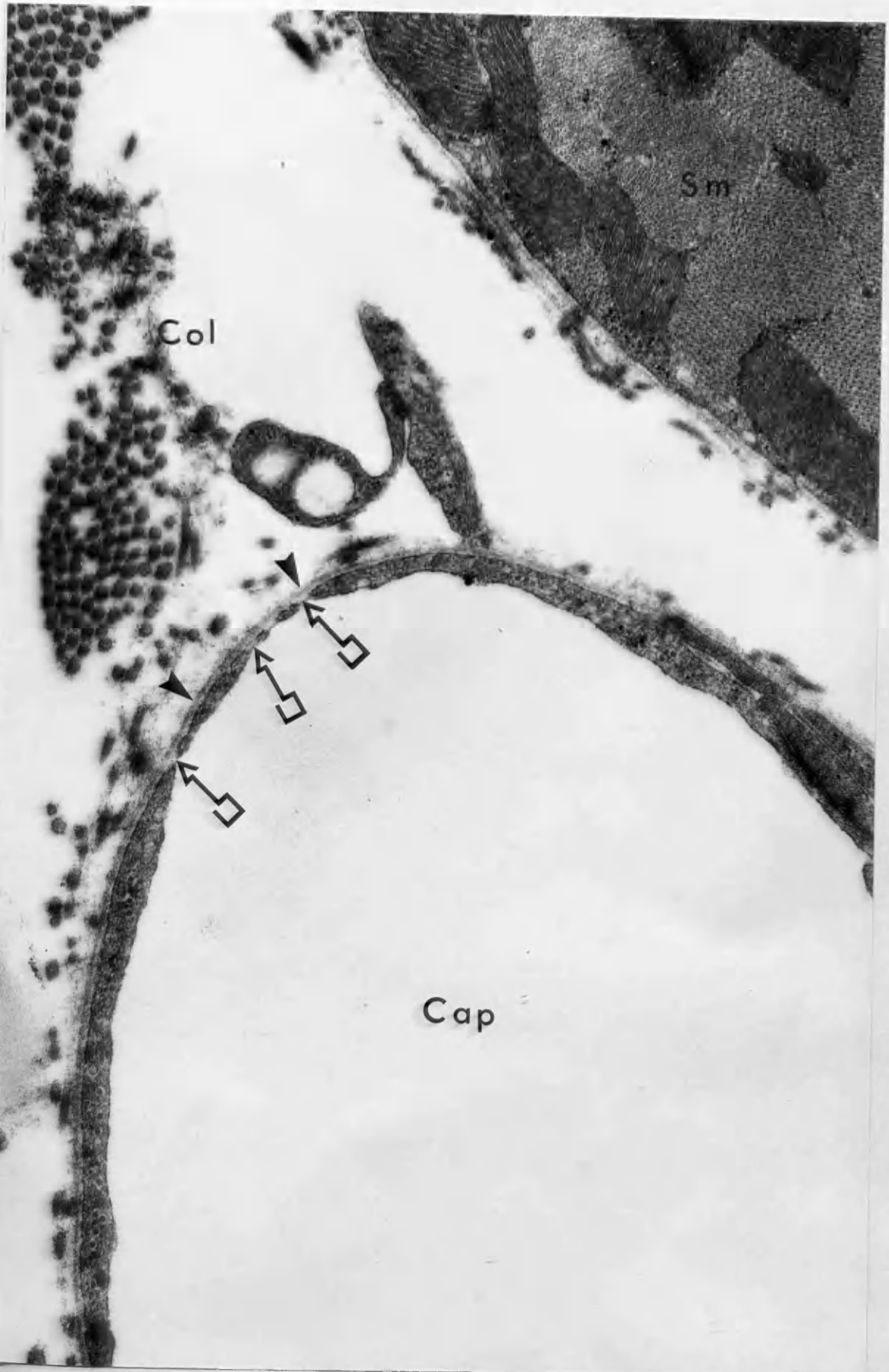
Figs. 121 & 122

A fenestrated blood capillary (Cap) located in intermuscular connective tissue of the diaphragm.
Note: 1) Its attenuated endothelium has several fenestrae (arrows) closed by a thin single-layered diaphragm.
2) The continuous endothelial basal lamina (arrowheads).

Col - collagenous fibres, Sm - skeletal muscle.

x 5,600, x 10,000 Adult rat


Sm
Cap
Col


Sm
Col
Cap

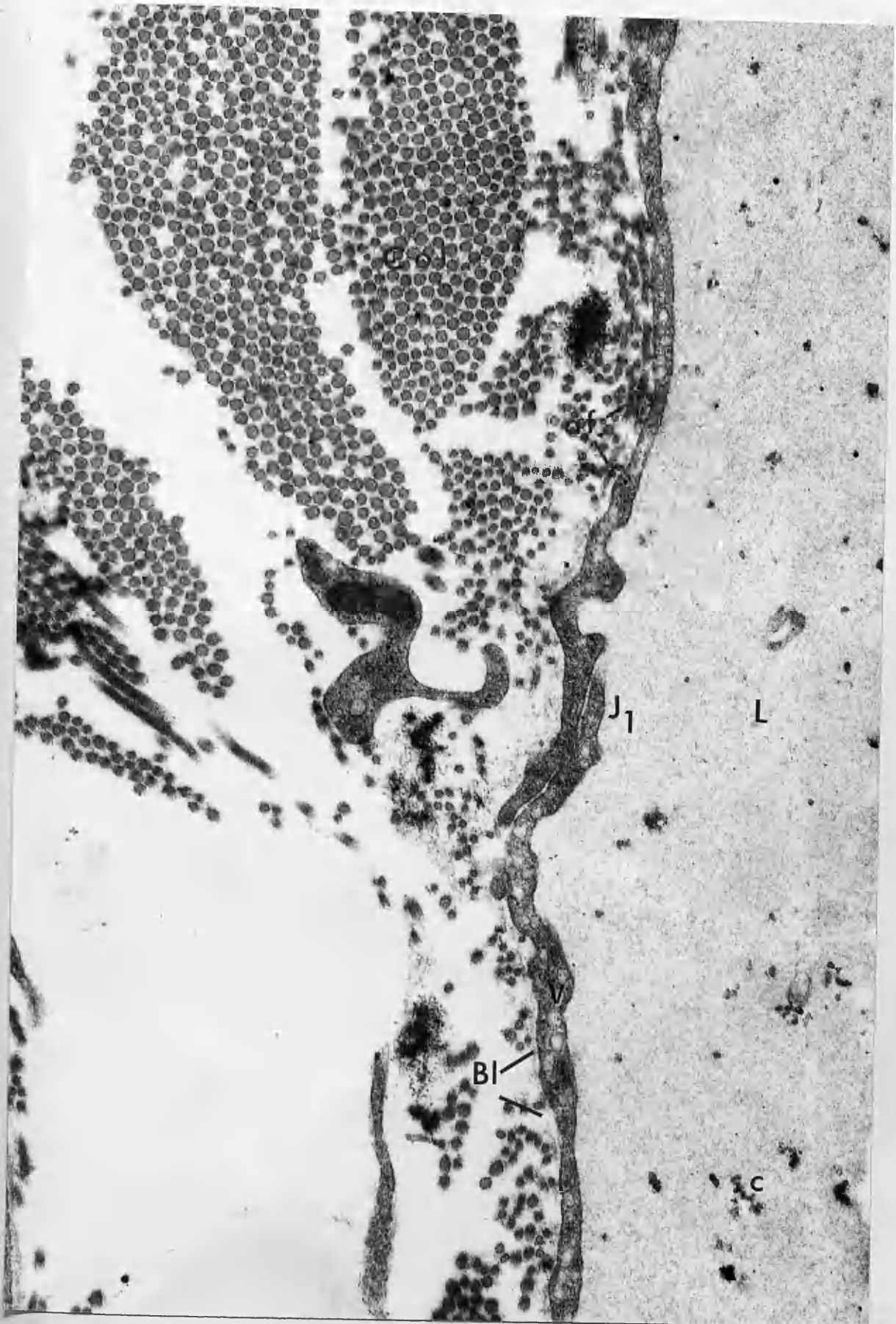
Figs. 123, 124 & 125

Survey TEM micrographs showing the structure of the lymphatic wall, and its relationship to the adjoining intermuscular connective tissue of the diaphragm. Note:

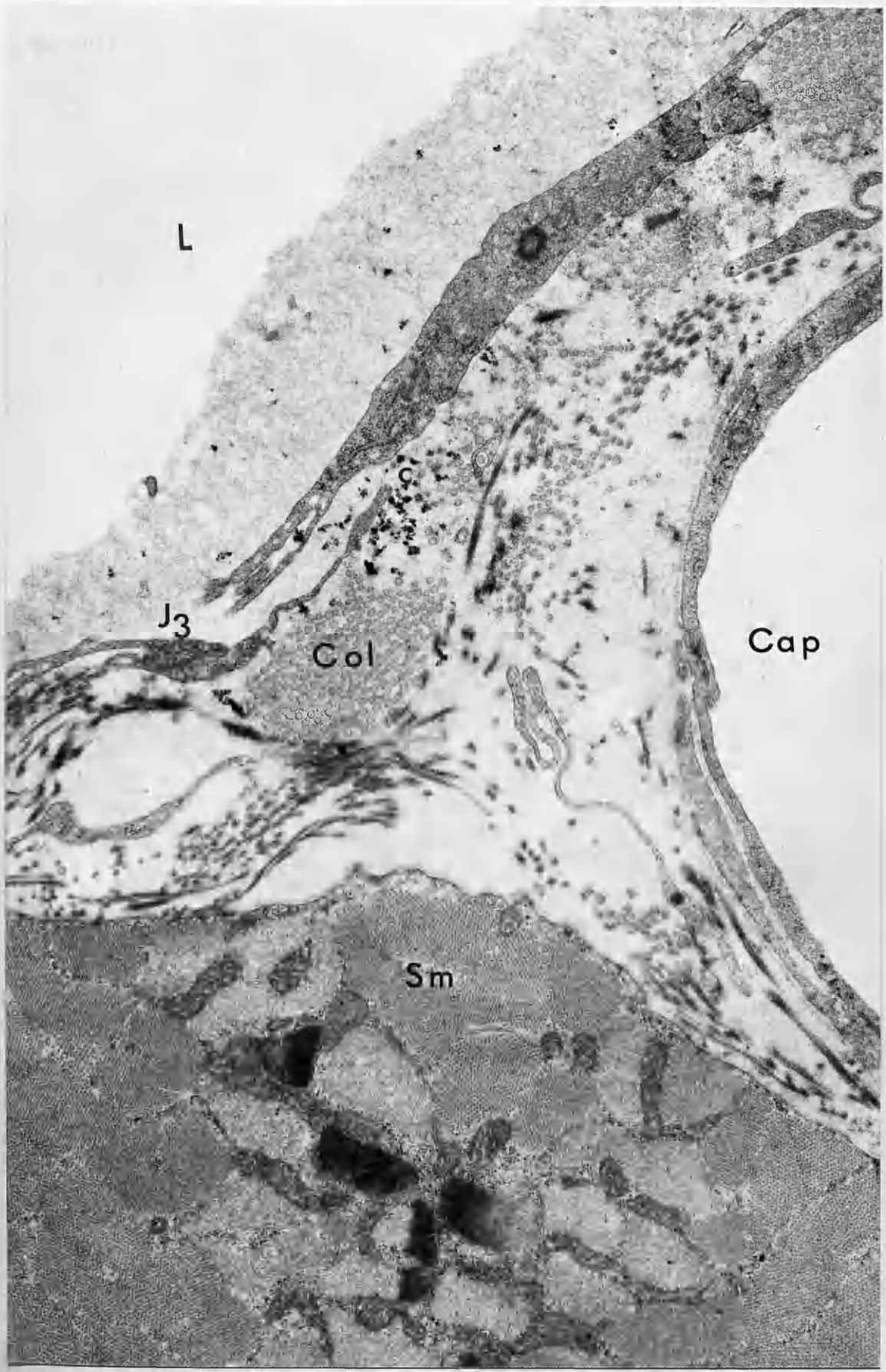
1) Irregular segments of the basement lamina (Bl)
2) Anchoring filaments (af) attached at areas of increased electron density and extending between the collagen fibres (Col).
3) Numerous micropinocytotic vesicles of various sizes (V) in endothelial cell cytoplasm.
4) Overlapping, interdigitating and patent intercellular junctions are shown at (J1), (J2) and (J3) respectively.
5) Carbon particles (C) in the lumina (L) and in some of the intercellular junctions (J3).

Sm - skeletal muscle, Cap - blood capillary.

x 10,000, x 10,000, x 5,600 Adult rat


Gl
Gf
J1
L
V
Bl
C

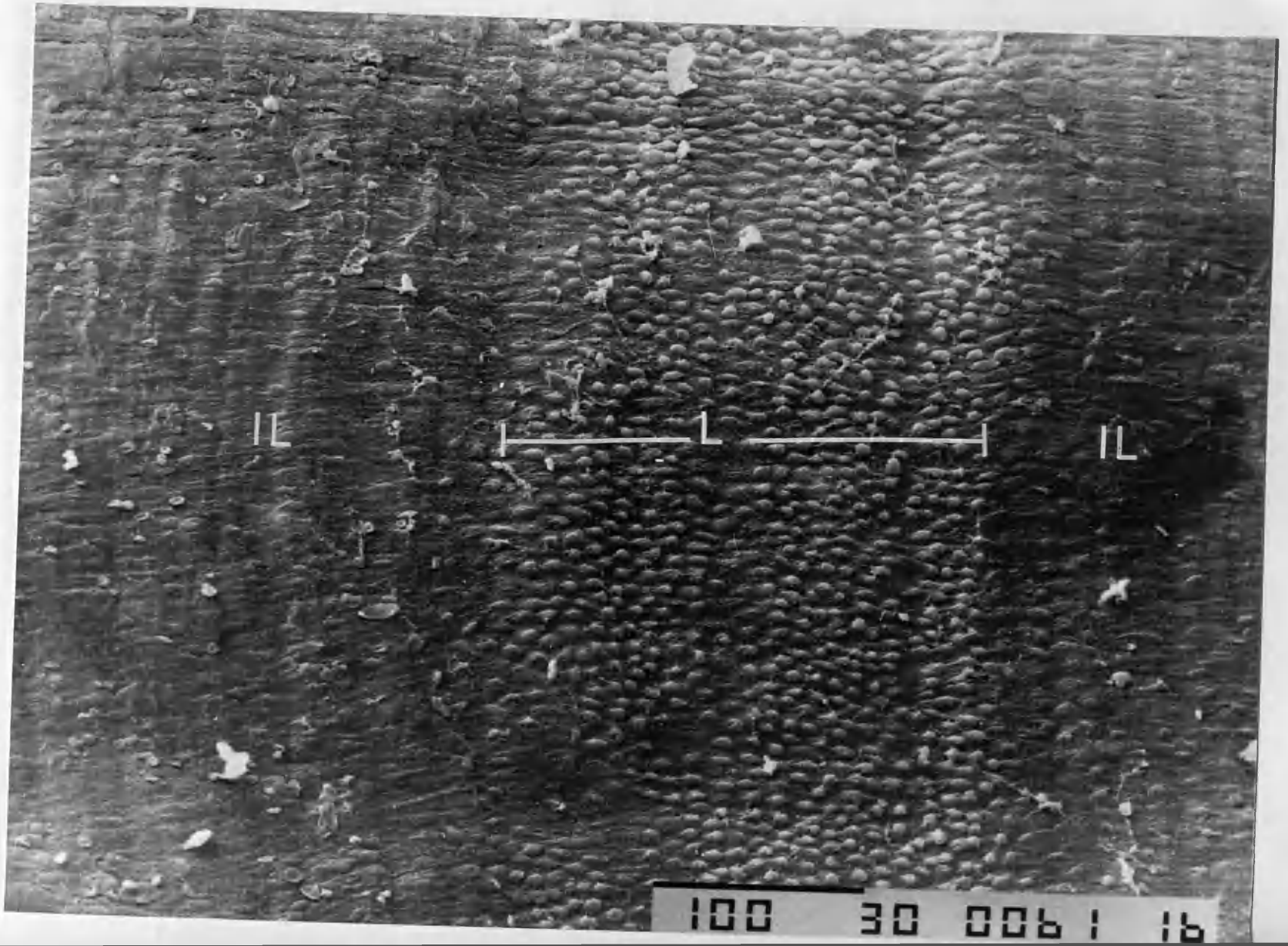

af
L
V
Col
J2
Sm


L
C
J_3
Col
Cap
Sm

Figs. 126 & 127

SEM of peritoneal surface of the diaphragm. Mesothelial cells overlying lacunar zones (L) are cuboidal in shape and have distinct boundaries, while cells over inter-lacunar zones (Il) are more flattened and individual cells cannot be easily distinguished. Note that the parallel bands of lacunar zones, observed here, correspond to the elongated black streaks shown in Figs. 93 & 138.

One week-old rat, fixed by immersion.


IE
L
IL
100 30 0061 16

21 1 000 00E 001

Fig. 128

Higher magnification of a lacunar zone. It illustrates the appearance of cuboidal cells covering the lacunar roof. Note:

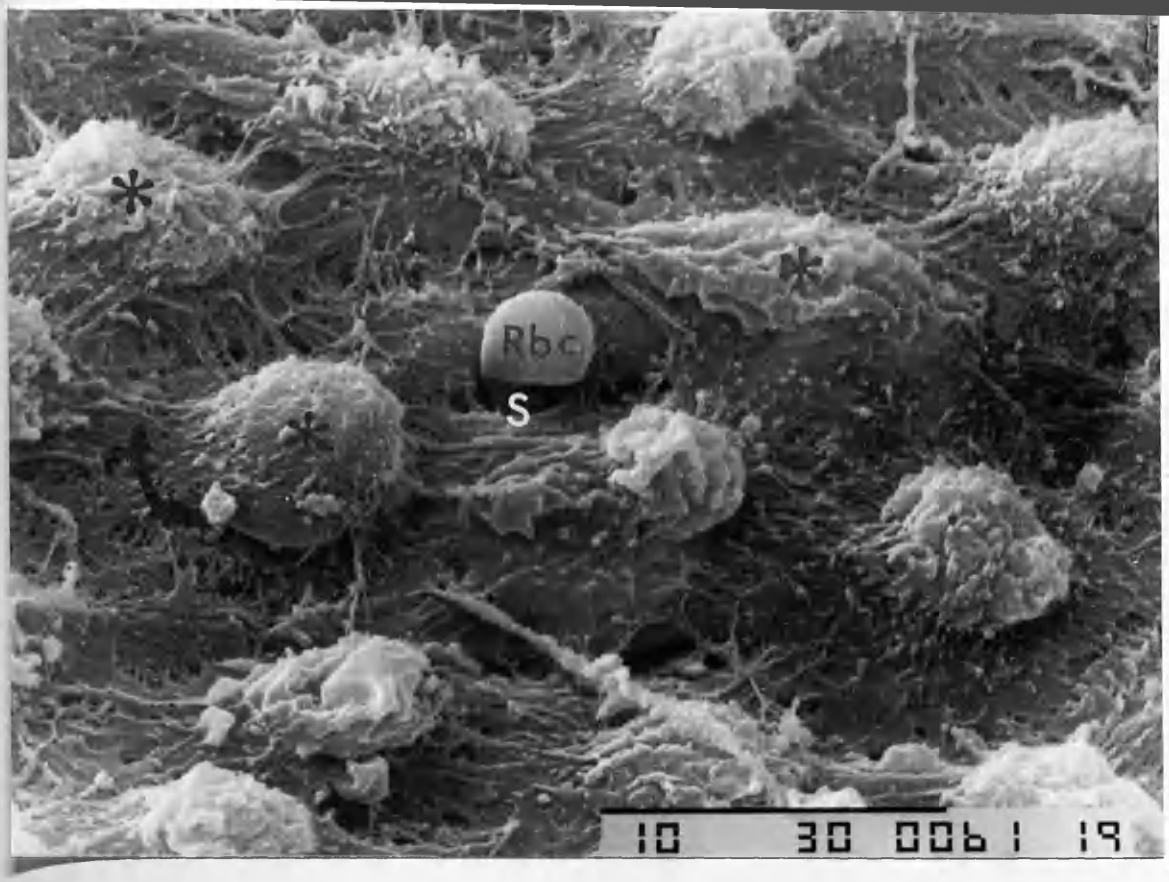
1) The nucleus of each individual cell is represented by a dome-shaped swelling in the central region (asterisks).
2) Cytoplasmic processes extending from lateral walls of adjacent central regions. These are interconnected to form a loose network on the surface of cell perimeters (arrows).
3) A stoma (S) between several mesothelial cells is shown with one red blood cell (Rbc) caught in passage.

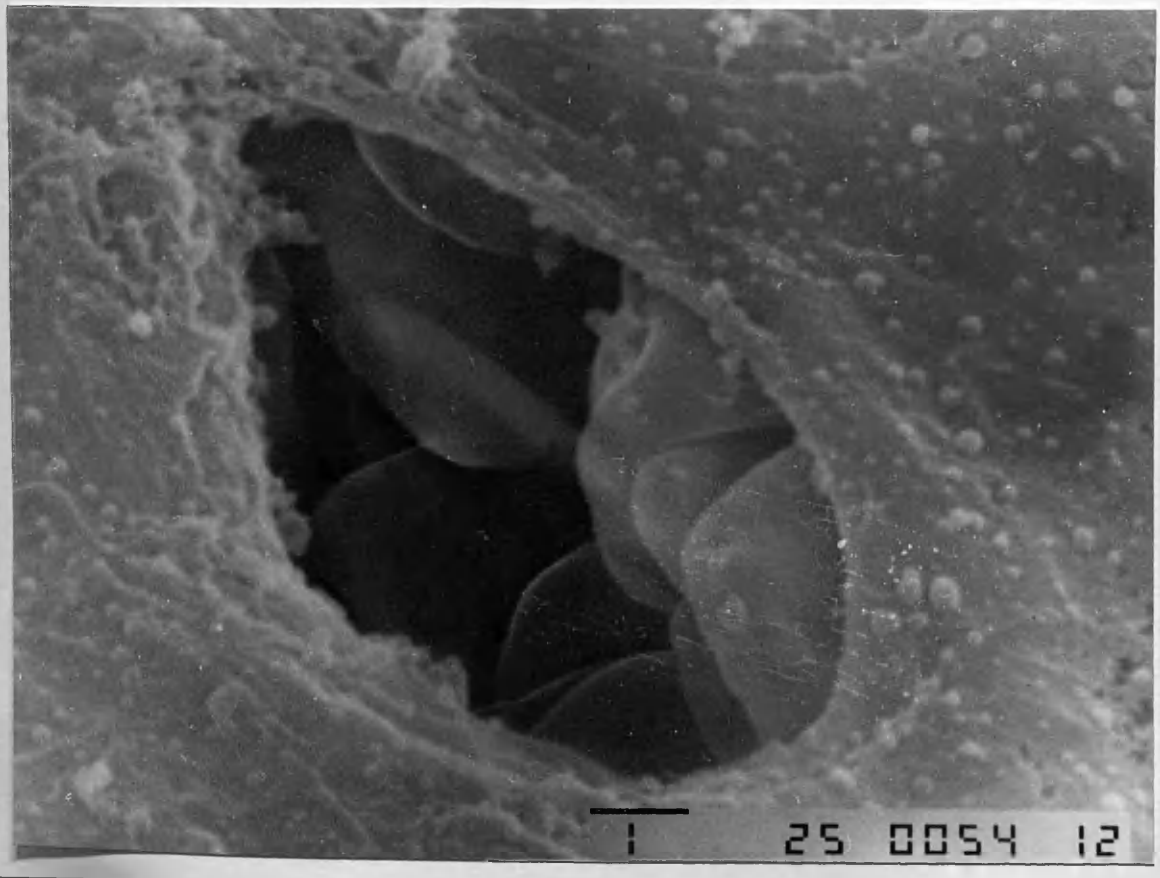
One week-old rat, fixed by immersion.

Fig. 129

In this SEM micrograph, several red blood cells are located within a stoma, presumably having already entered the lymphatic lumen.

Young adult rat, fixed by perfusion.

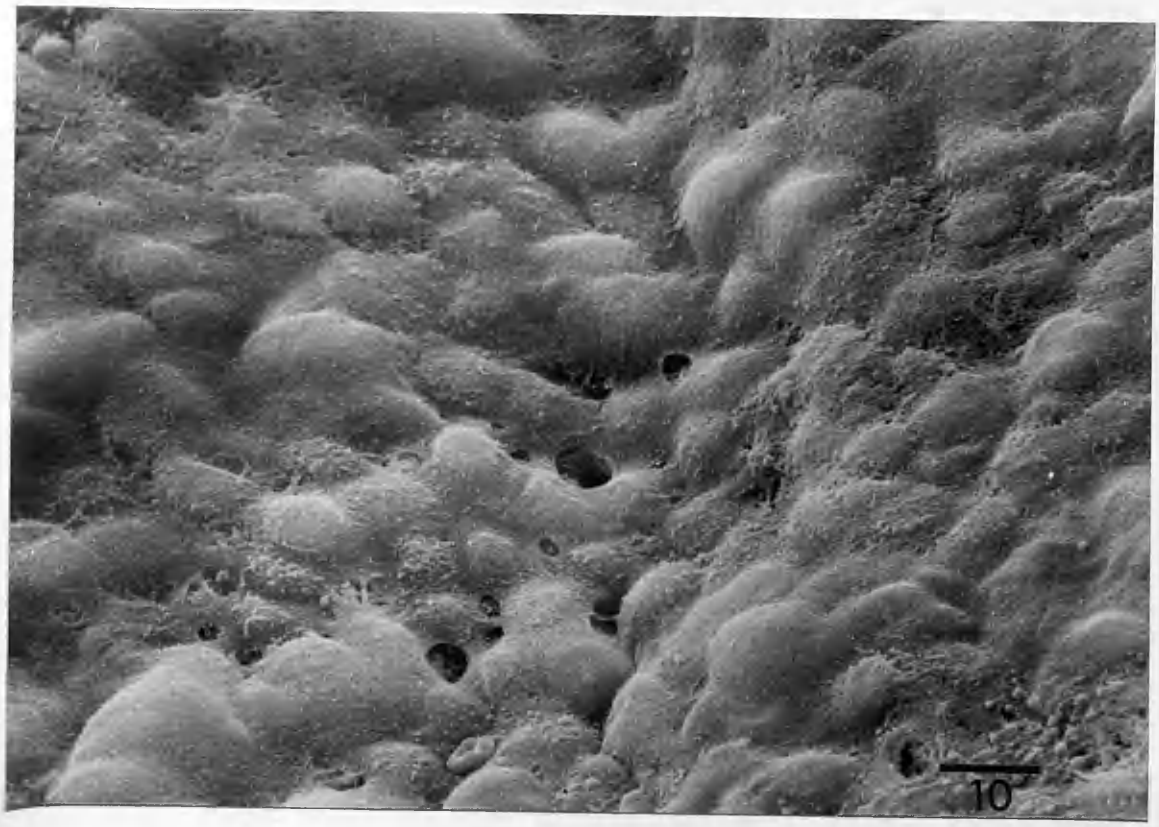

Rbc
S
10 30 0061 19


1 25 0054 12

Figs. 130 & 131

Lacunar roof of the diaphragmatic peritoneum. Numerous circular stomata are observed between lacunar mesothelial cells.

Young adult rat, fixed by perfusion.


10

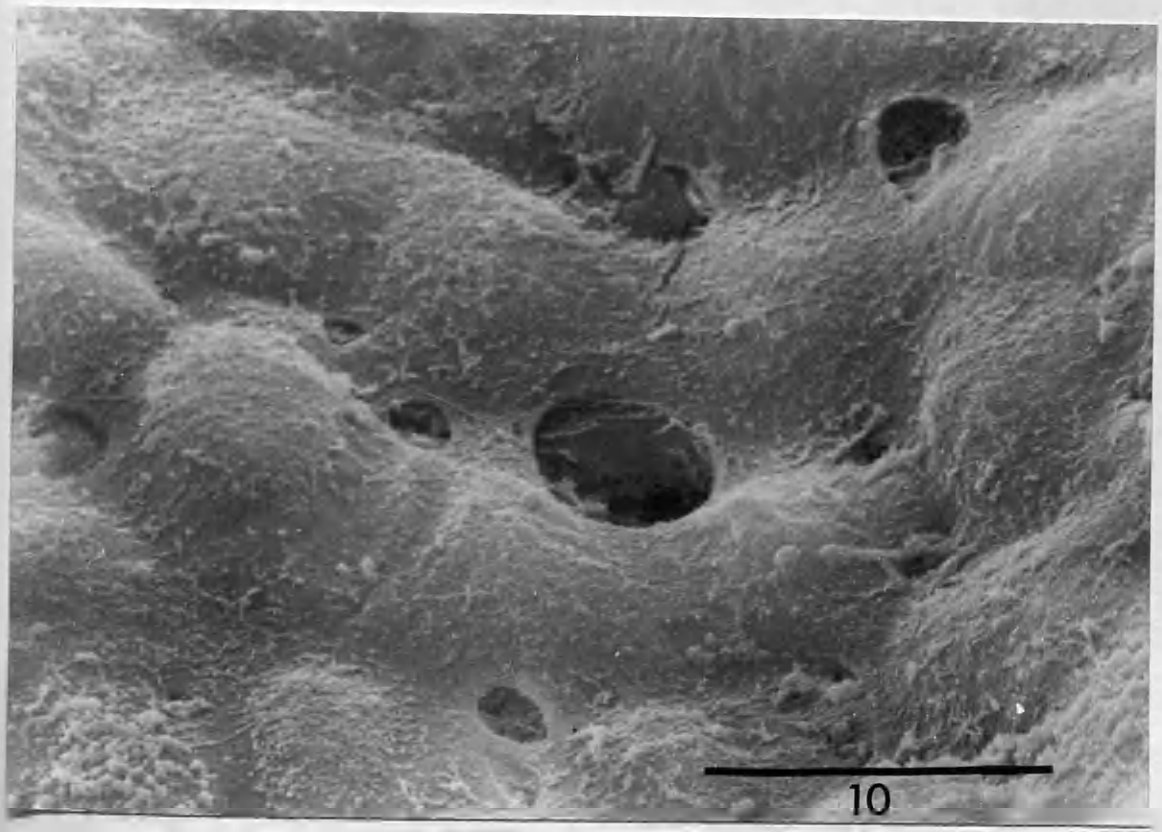

10

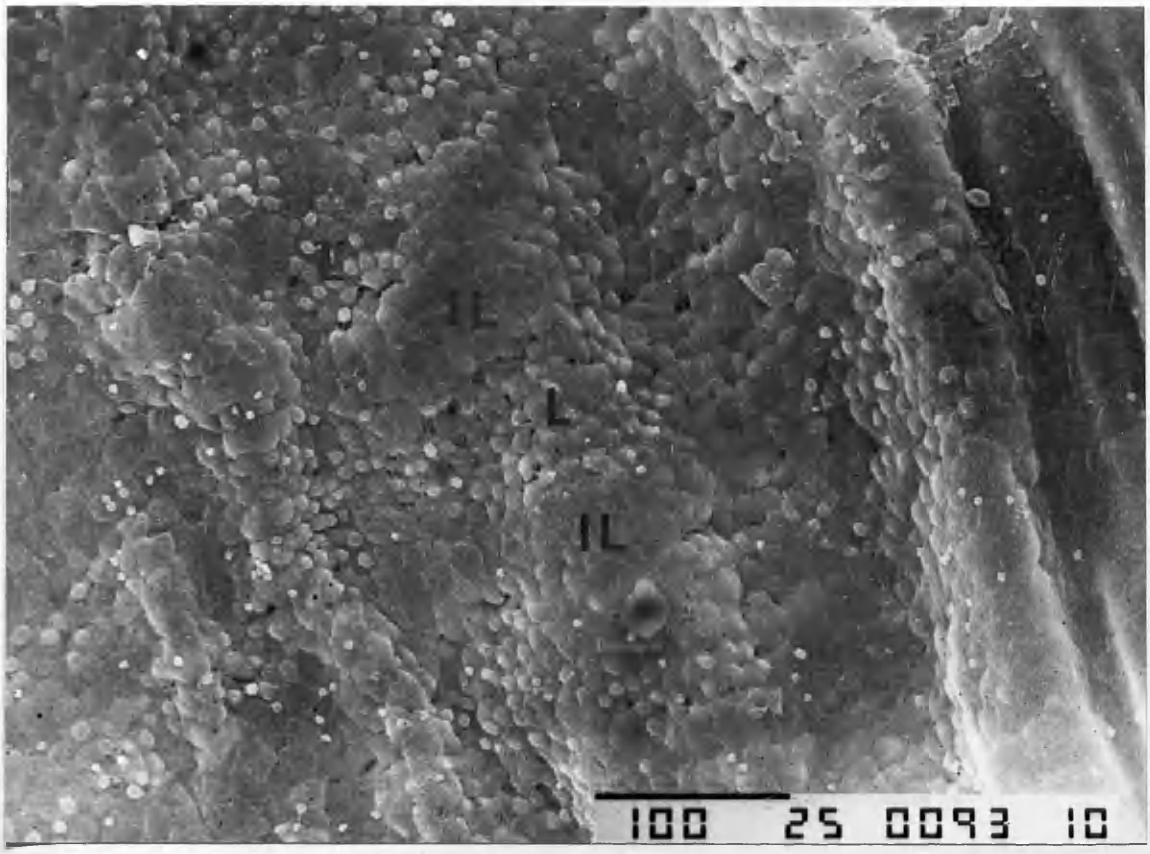
Fig. 132

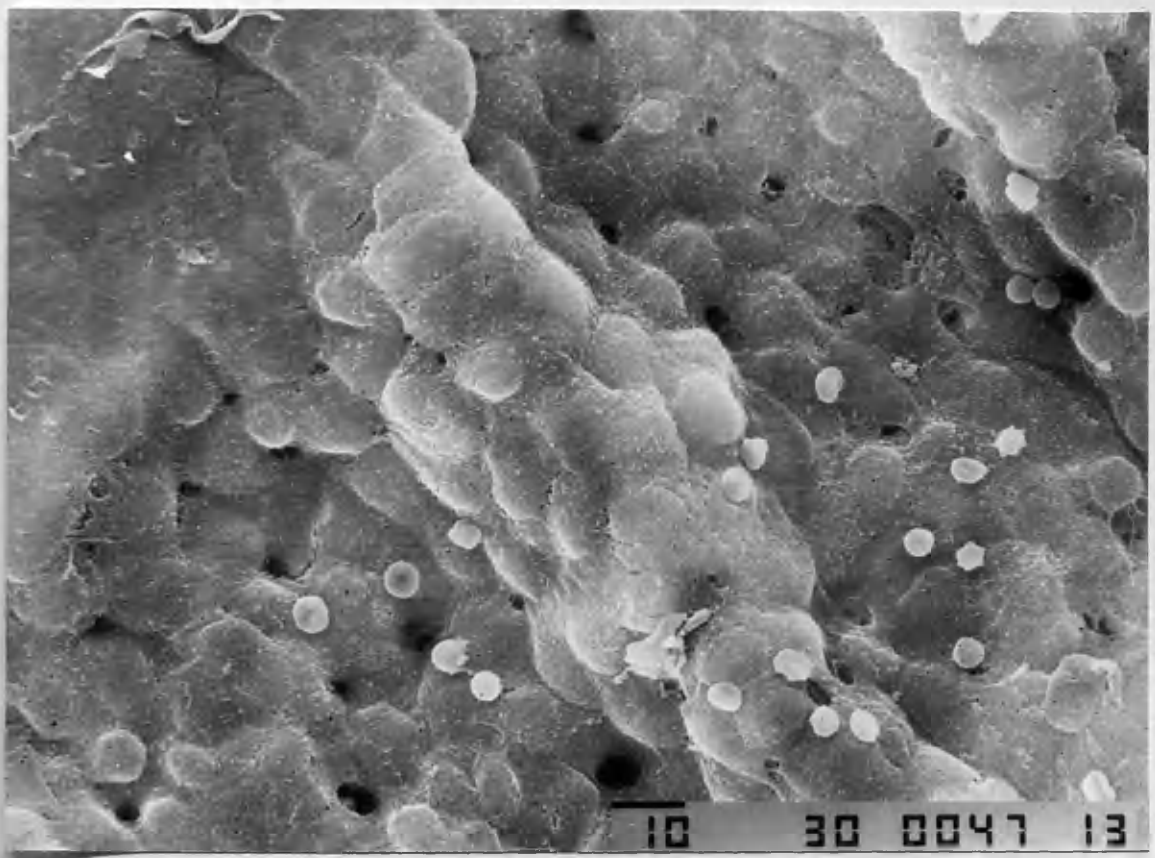
A low power SEM micrograph of peritoneal surface of the diaphragm. It shows, once again, the lacunar roof (L) and the more flattened cells of inter-lacunar areas (Il). Even at this low magnification, numerous stomata are observed between adjacent mesothelial cells of the lacunar roof.

6 week-old rat, fixed by perfusion.

Fig. 133

See following page.


IL
L
IL
100 25 0093 10


10 30 0047 13

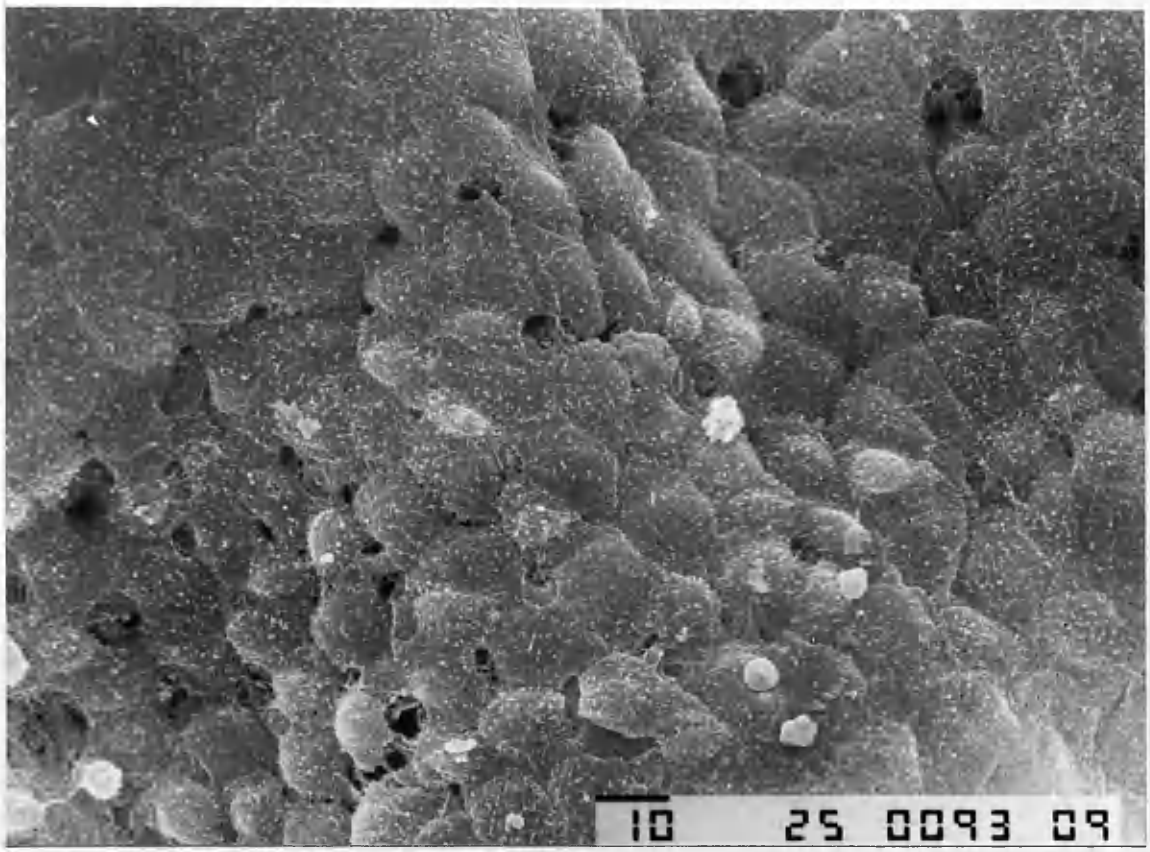
Figs. 133, 134, 135 & 136

All are enlargements of lacunar zones shown in Fig. 132. In many areas of the lacunar roof, patent stomata are seen, under low power (Figs. 133 & 134) and in more detail under high power (Figs. 135 & 136), between adjacent cuboidal mesothelial cells. These special openings are only found in the lacunar roof. Many appear to be deep channels extending beyond the underlying layer of connective tissue (Figs. 136 & 137). Note that a muscular contraction, probably due to the action of the fixative or the diaphragm has been fixed in the contraction-phase, causing folding of the diaphragmatic surface (Figs. 132 & 133).

6 week-old rat, fixed by perfusion.

Fig. 135

See above.


10 25 0093 09

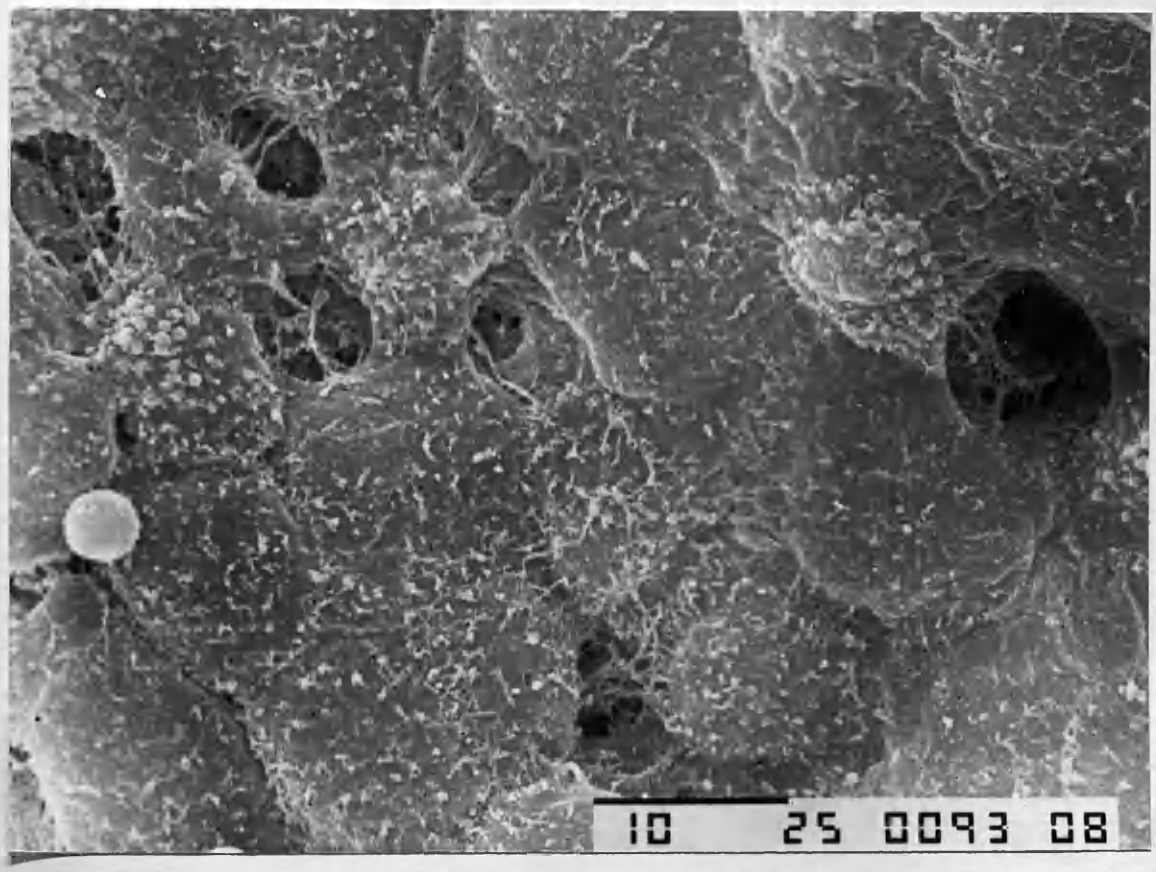

10 25 0093 08

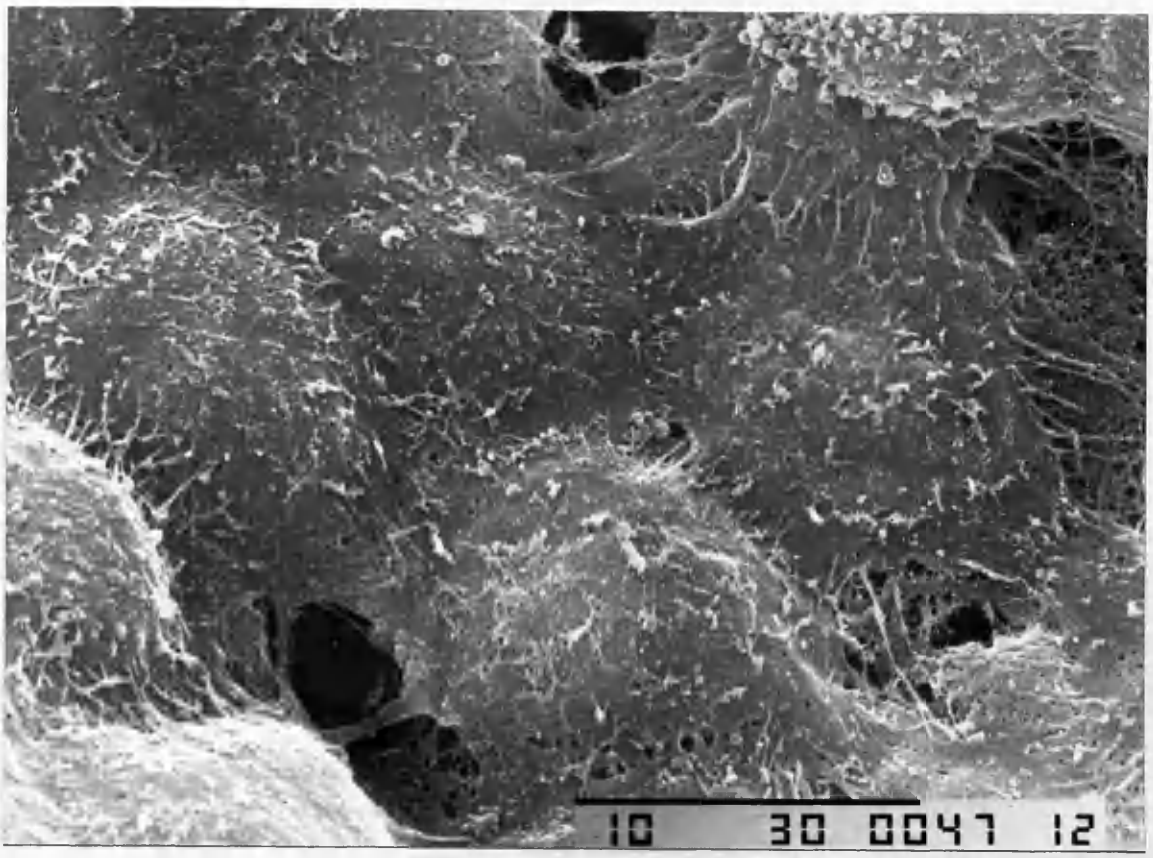
Fig. 136

See previous page.

Fig. 137

SEM micrograph showing the location of a stoma (S) at the junction of five cuboidal mesothelial cells (numbered 1-5).

6 week-old rat, fixed by perfusion.


10 30 0047 12

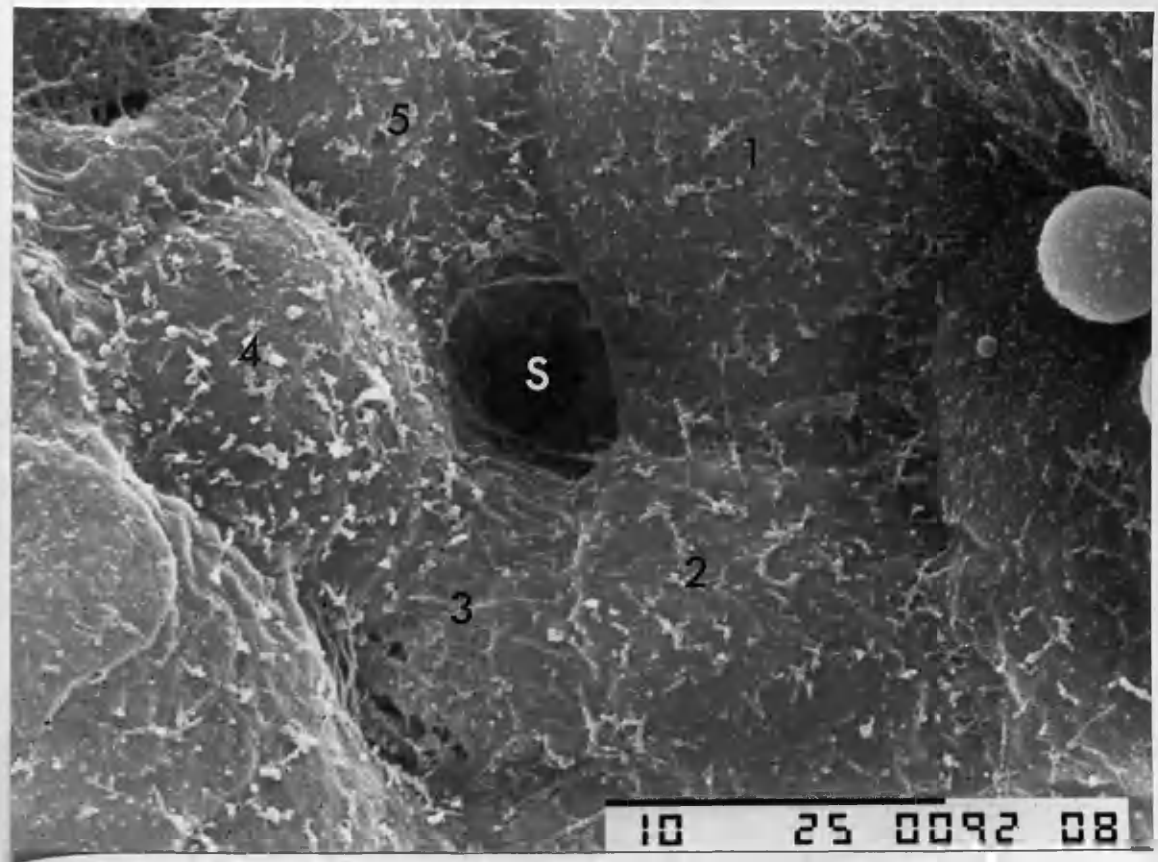

5
1
4
S
2
3
10 25 0092 08

Fig. 138

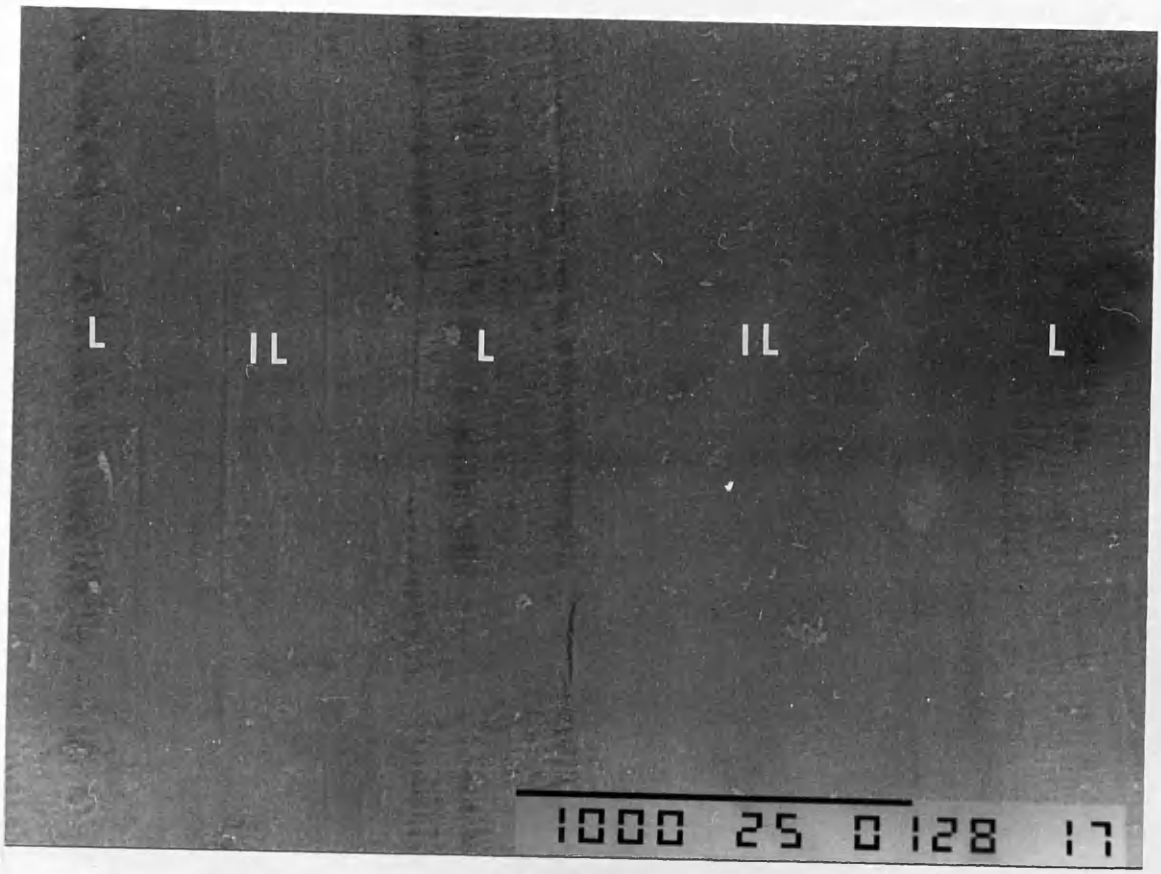
A low-power back-scattered SEM micrograph showing the peritoneal surface of the diaphragm, shortly after IP injection of carbon. The tracer has been rapidly absorbed by subperitoneal lymphatic lacunae (L) which appear as elongated bands, and are clearly distinguished from inter-lacunar zones (Il). Compare this view with Figs. 93, 126 and 127.

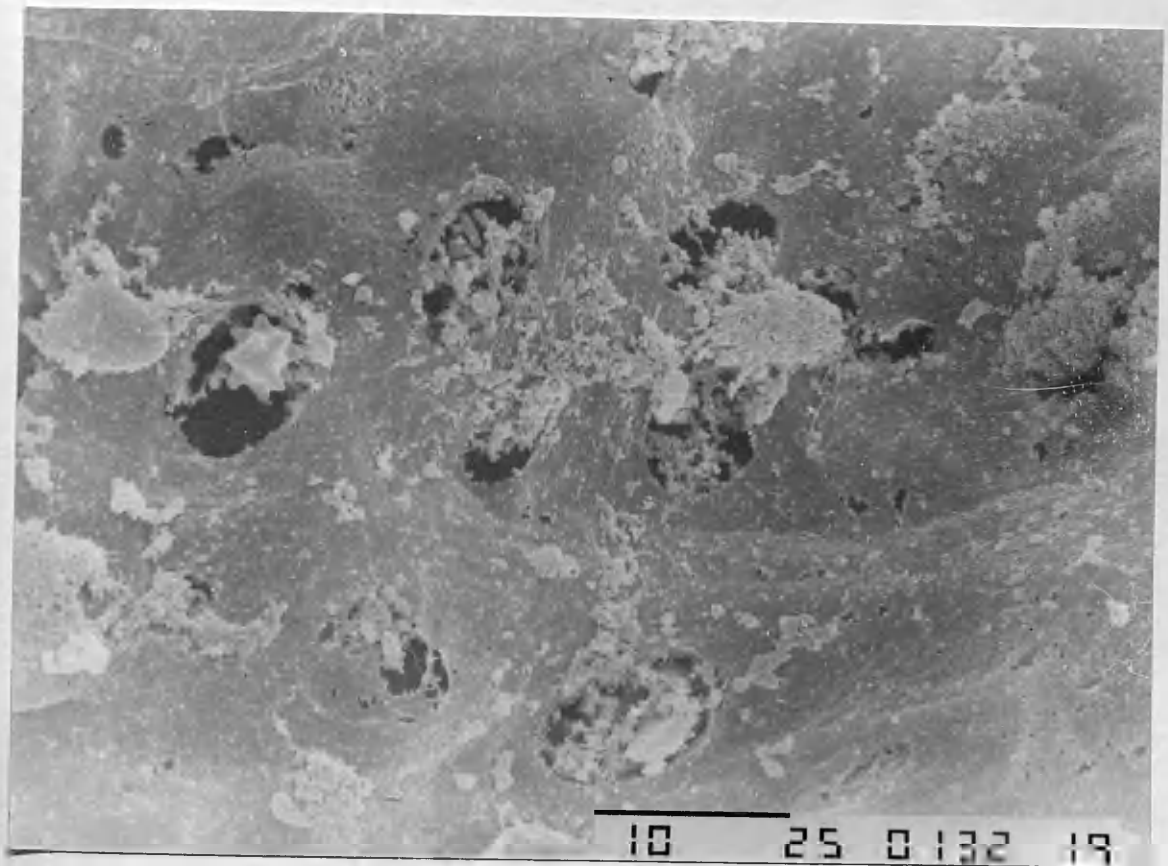
Adult rat, fixed by perfusion.

Fig. 139

This is a high-power view of part of the lacunar area shown in Fig. 138. It demonstrates the localisation of many carbon particles along the margins and within stomata between adjacent mesothelial cells.

Adult rat, fixed by perfusion.

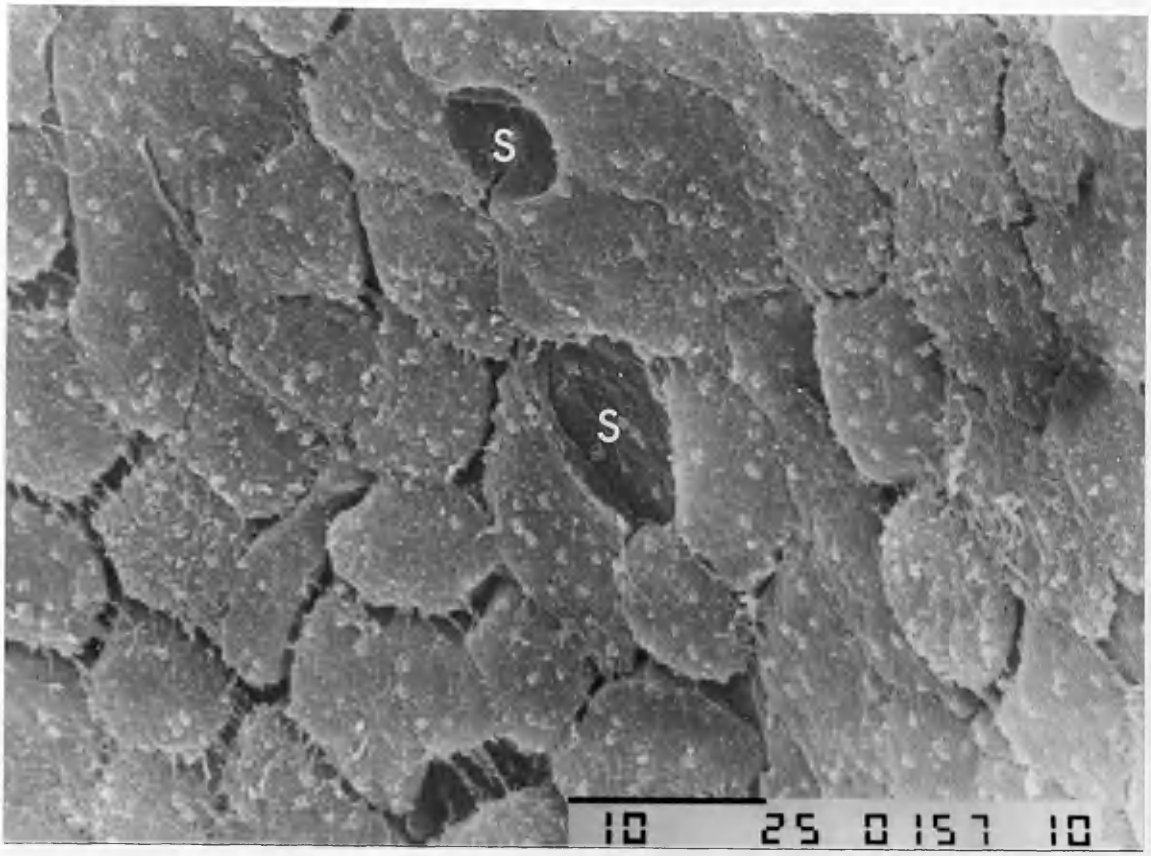

L
IL
L
IL
L
1000 25 0128 17

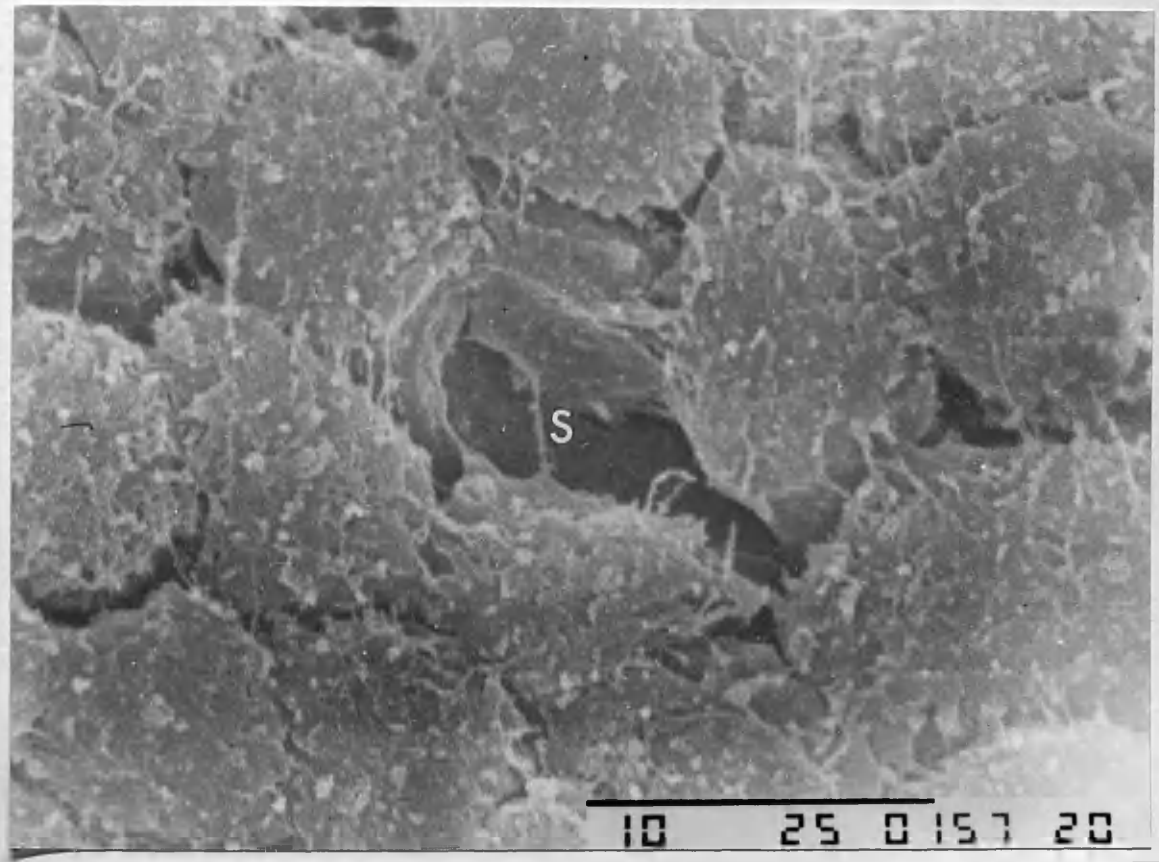

10 25 0132 19

Figs. 140 & 141

SEM micrographs showing the peritoneal surface of the diaphragm in a 20 day-old rat embryo. Even at this stage of development, stomata (S) are clearly present between adjacent mesothelial cells. These peritoneal stomata may appear early in development of the diaphragm, since they were also observed in 18 day-old rat embryo.

20 day-old rat embryo, fixed by immersion.

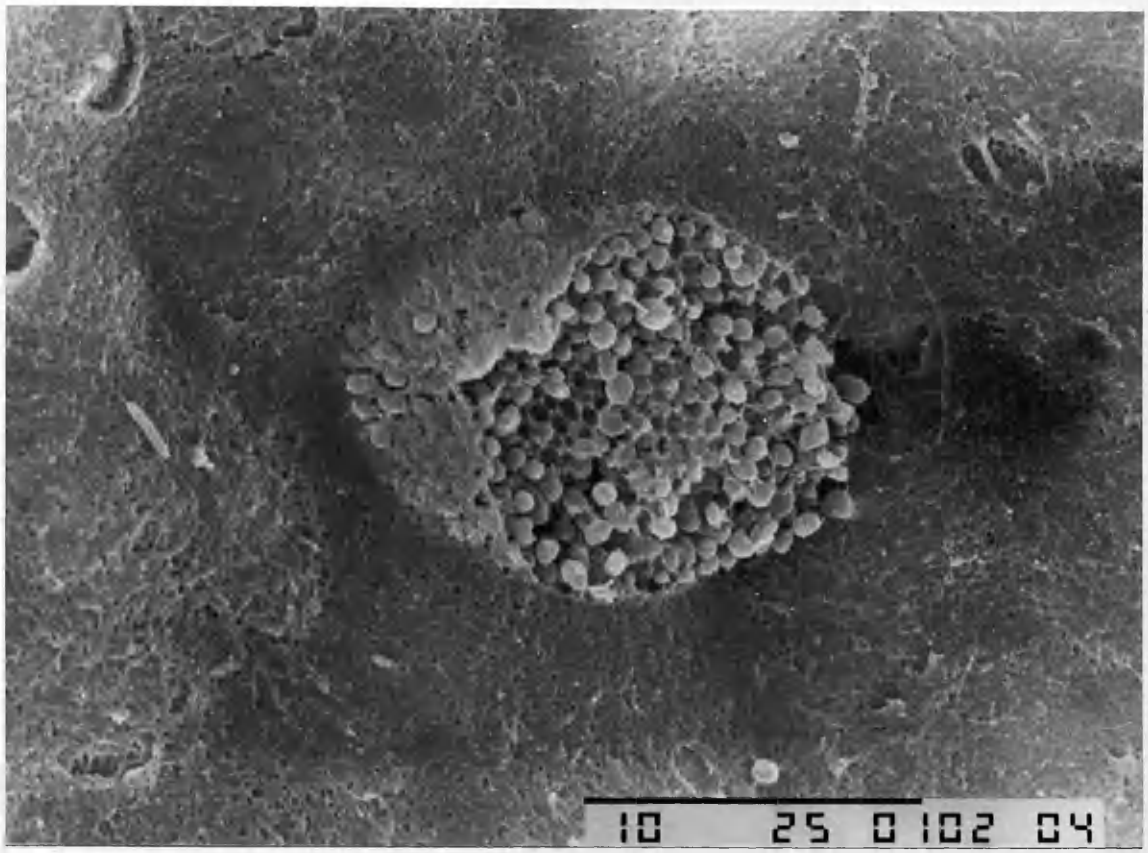

S
S
10 25 0157 10

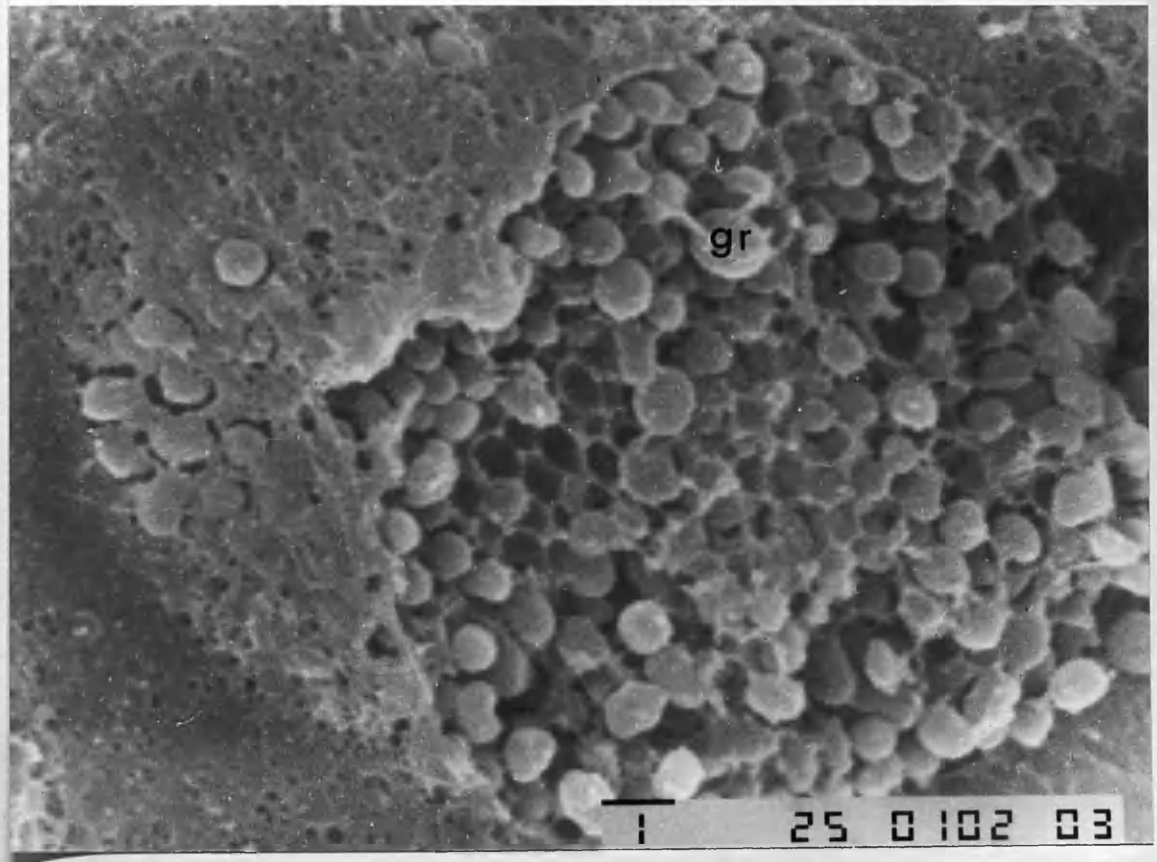

S
10 25 0157 20

Figs. 142 & 143

A typical mast cell located on the peritoneal surface of the diaphragm. The cell interior is partially exposed, so that the distribution of underlying cytoplasmic granules (gr) become apparent.

Adult rat, fixed by perfusion.

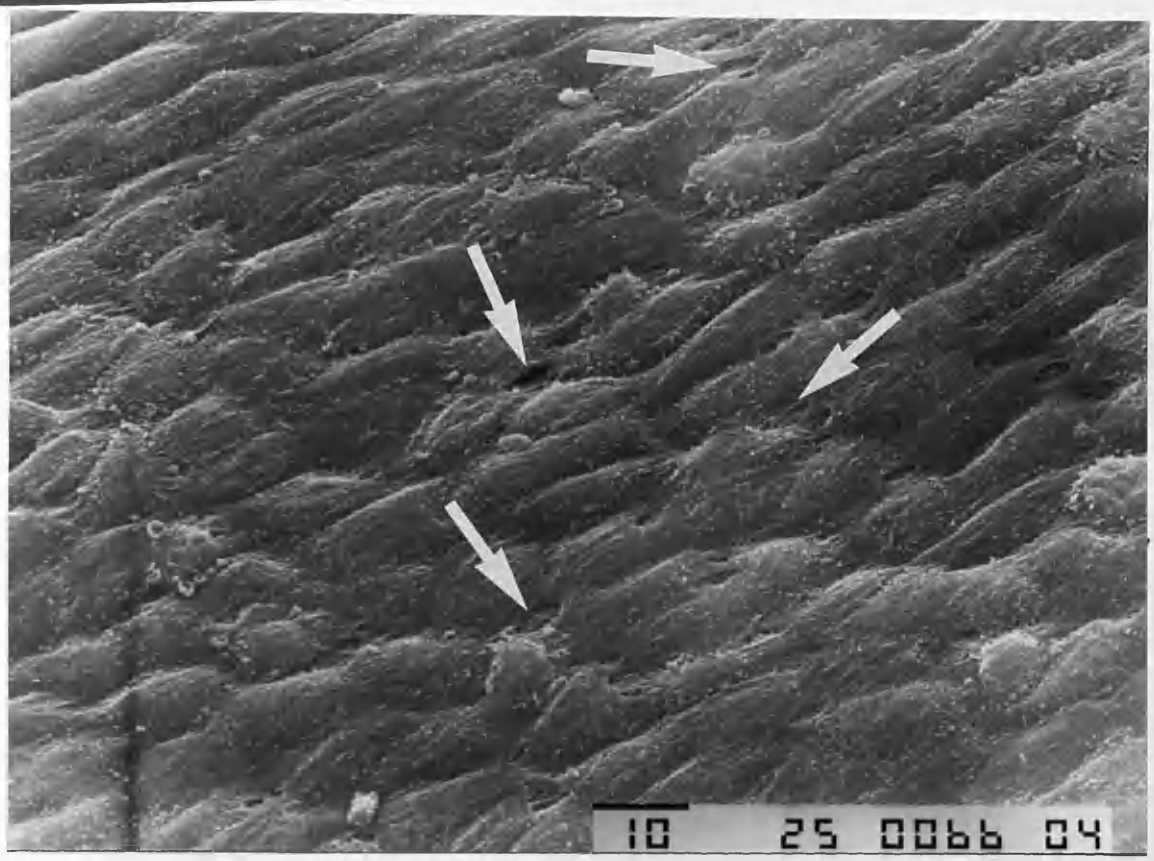

10 25 0102 04

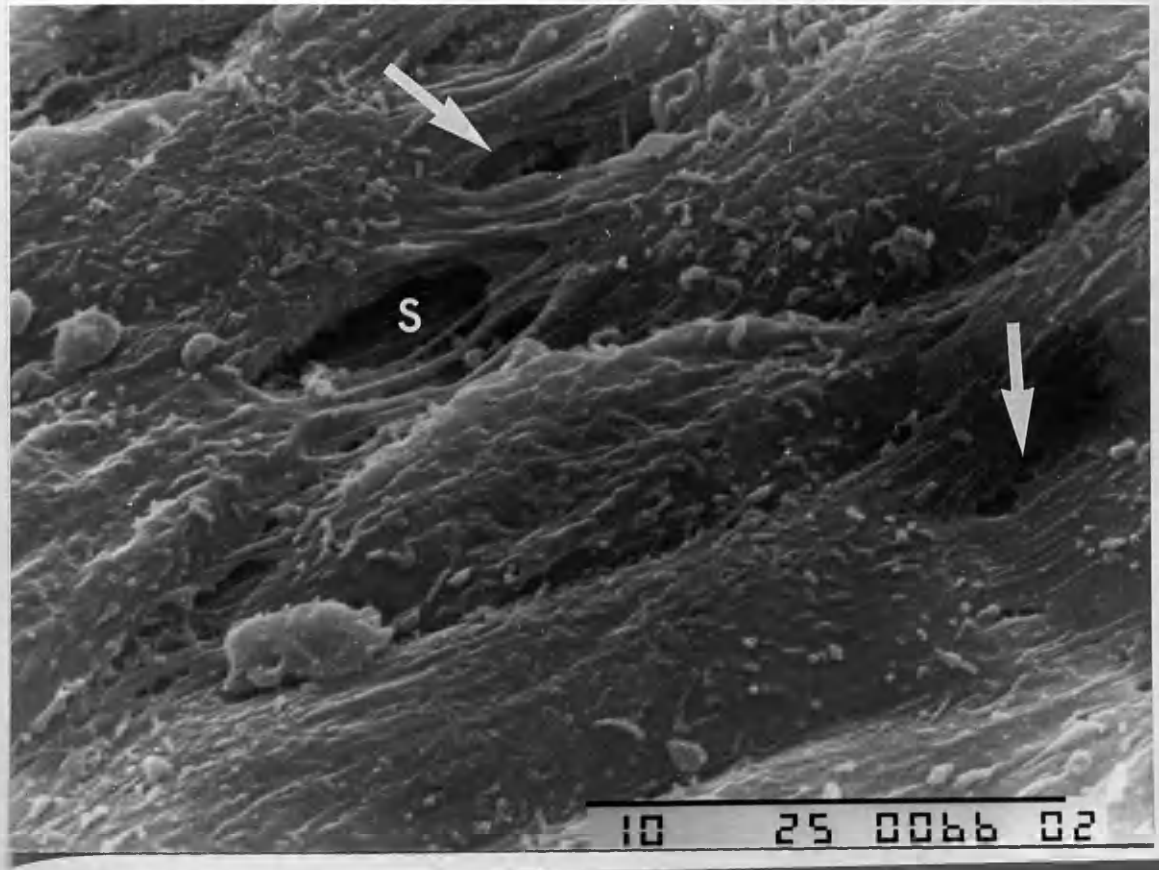

gr
1 25 0102 03

Figs. 144 & 145

These SEM micrographs show the pleural surface of the diaphragm. Note that stomata are also present (arrows), but in general they are fewer in number than those found on diaphragmatic peritoneum. These stomata are round, oval or slit-like in shape, and some show a bridge dividing the opening (S).

One week-old rat, fixed by immersion.

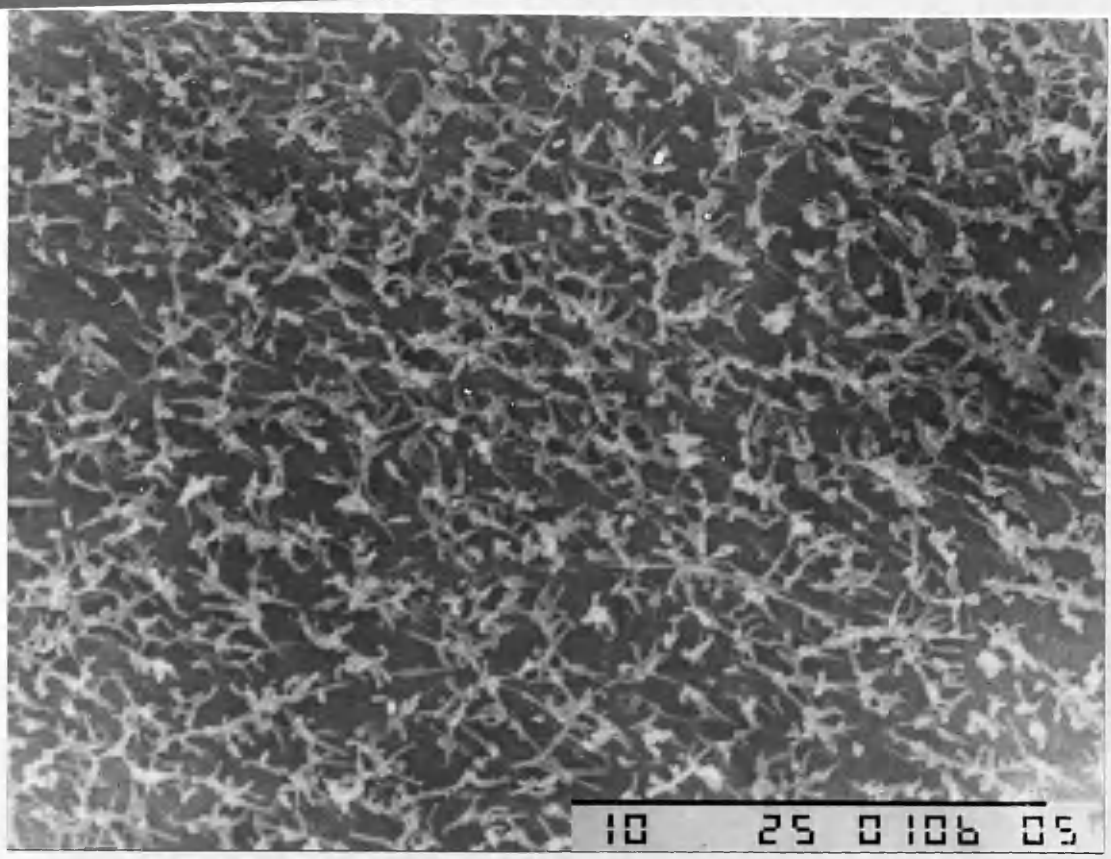

10 25 0066 04

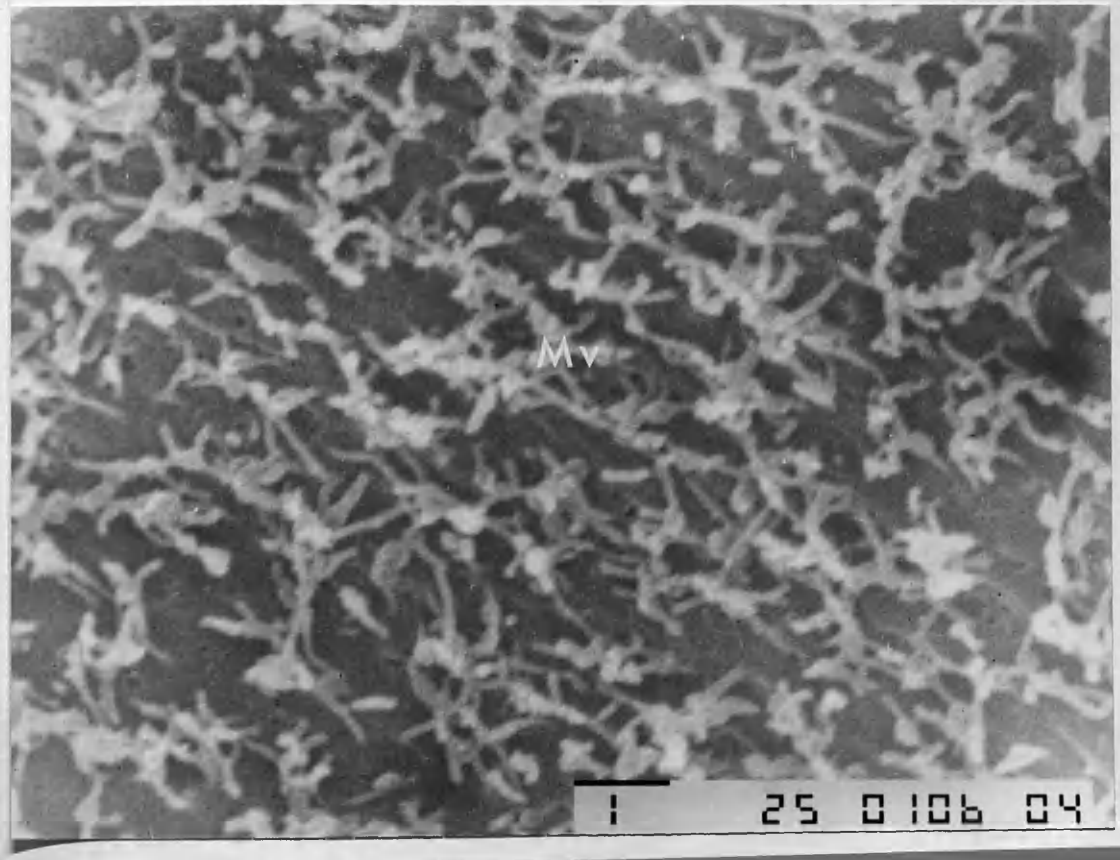

S
10 25 0066 02

Figs. 146 & 147

Parietal pleura lining the thoracic cavity. Note that mesothelial cells form a sheet of flattened cells with indistinct boundaries. These cells have numerous long microvilli (Mv) with a fluffy appearance.

Young adult rat, fixed by perfusion.

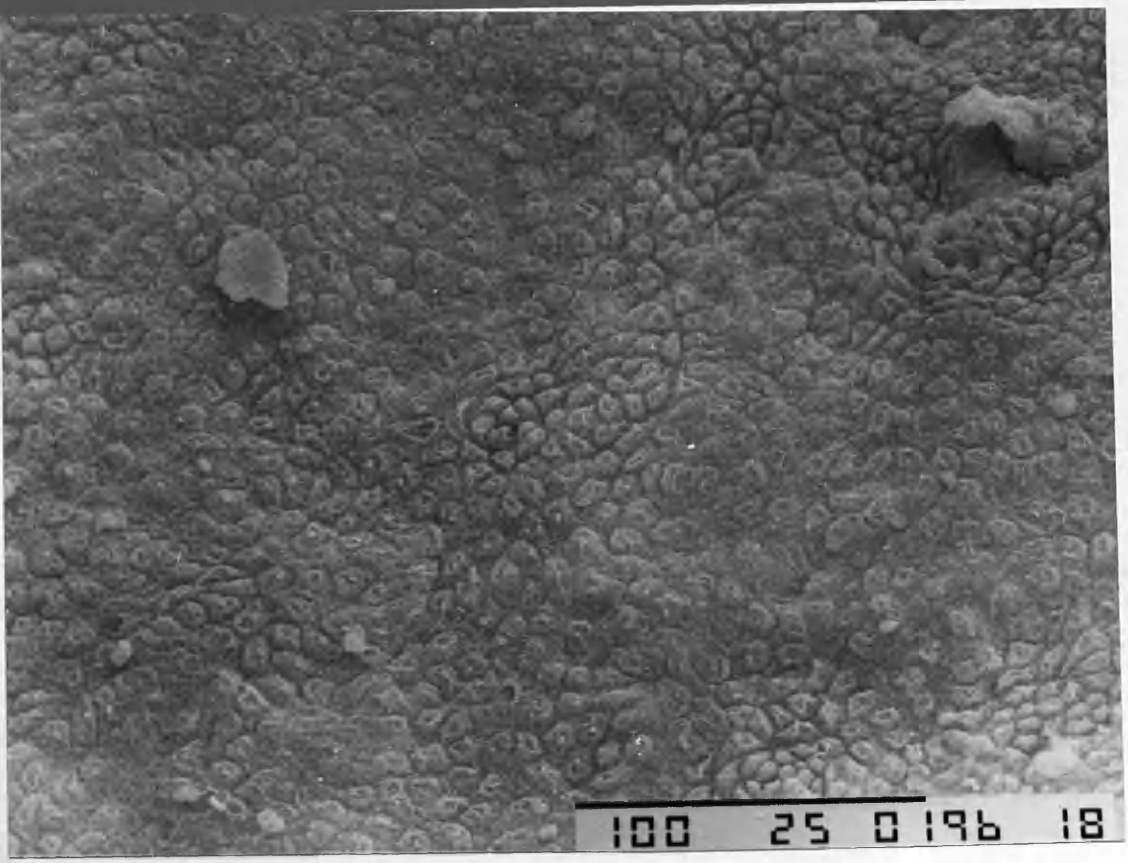

10 25 0106 05


Mv
1 25 0106 04

Figs. 148 & 149

Visceral pleura of lungs. The borders between individual squamous mesothelial cells are apparent (arrows). There are many short microvilli (Mv) on the apical cell surface. Note that stomata are not found in visceral, nor parietal pleurae (Figs. 146 & 147). (Fig. 149 reveals details of the pleural surface at higher magnification).

Adult rat, fixed by perfusion.

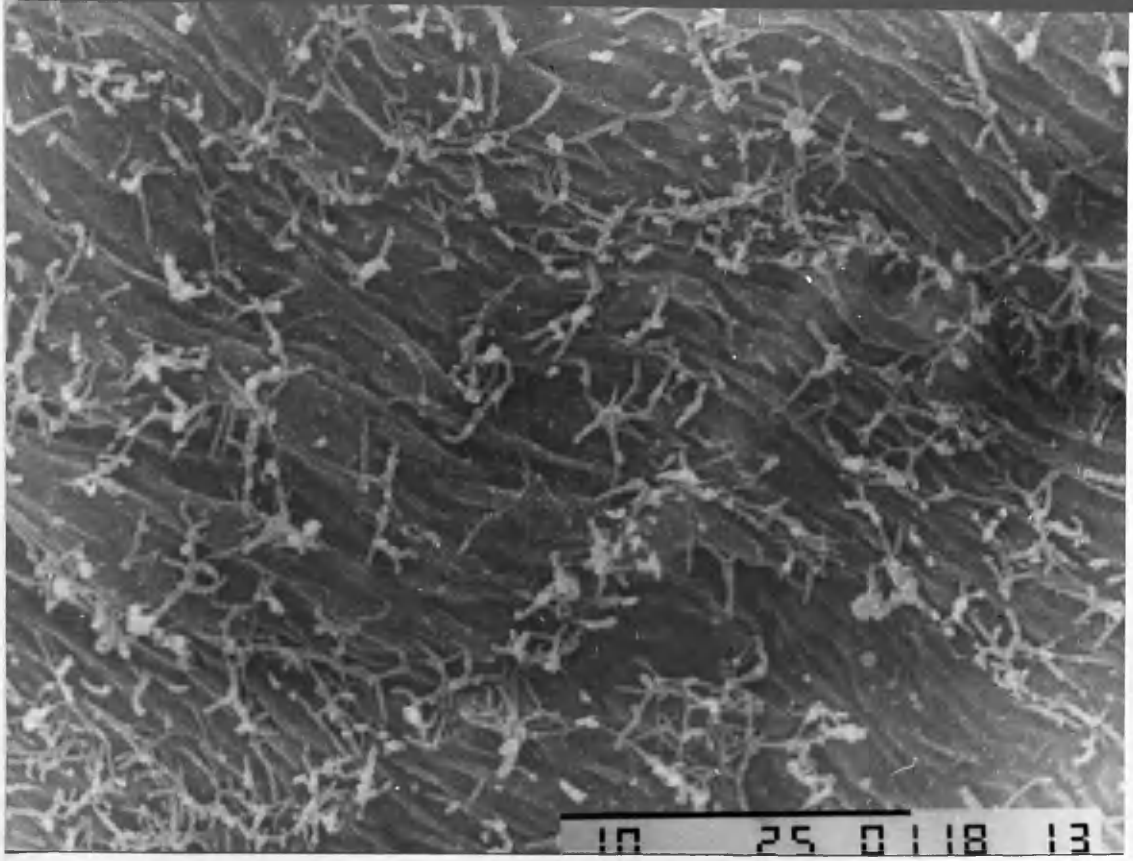

100 25 0196 18

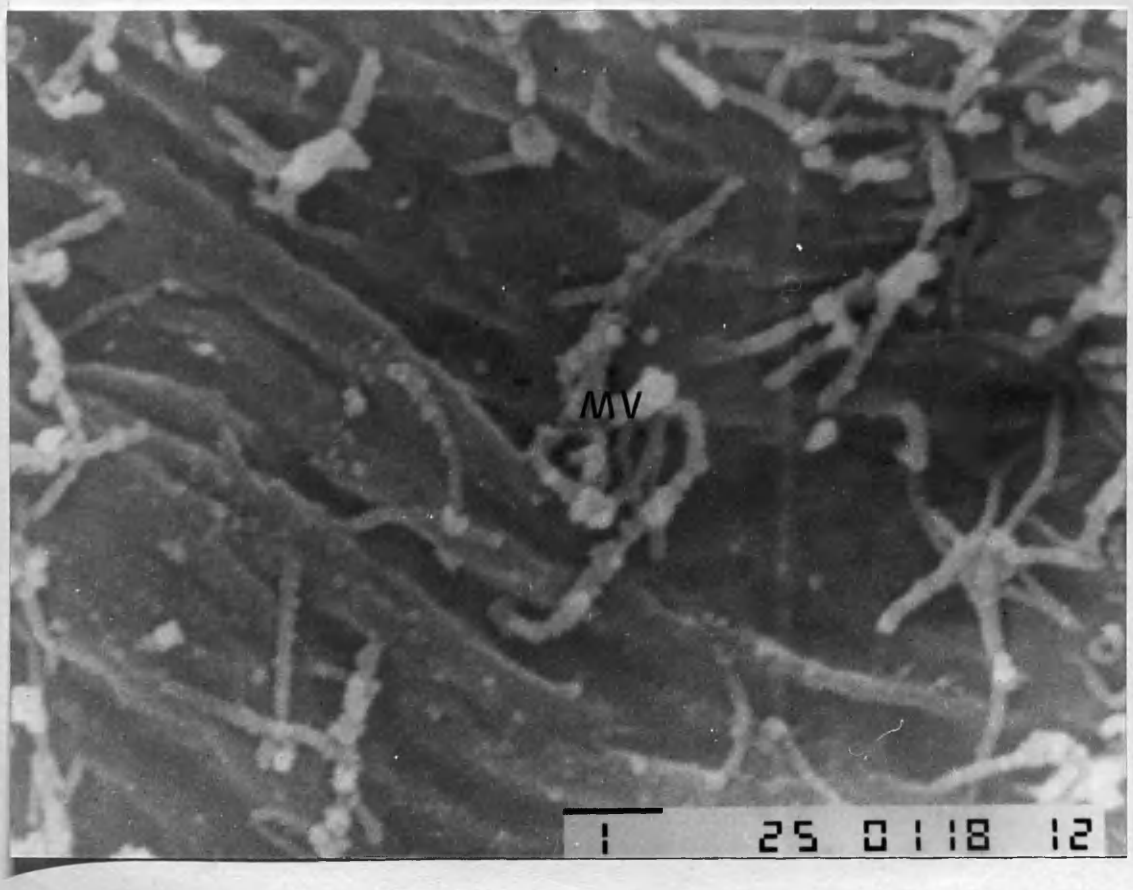

Mv
10 25 0196 16

Figs. 150 & 151

Parietal peritoneum of abdominal wall. Fig. 150 is a low magnification showing the rather flattened mesothelium. Fig. 151 is a higher magnification showing many long surface microvilli (Mv). Note that this surface appearance is similar to that of parietal pleura, but with fewer microvilli (c.f. with Figs. 146 & 147).

Adult rat, fixed by perfusion.

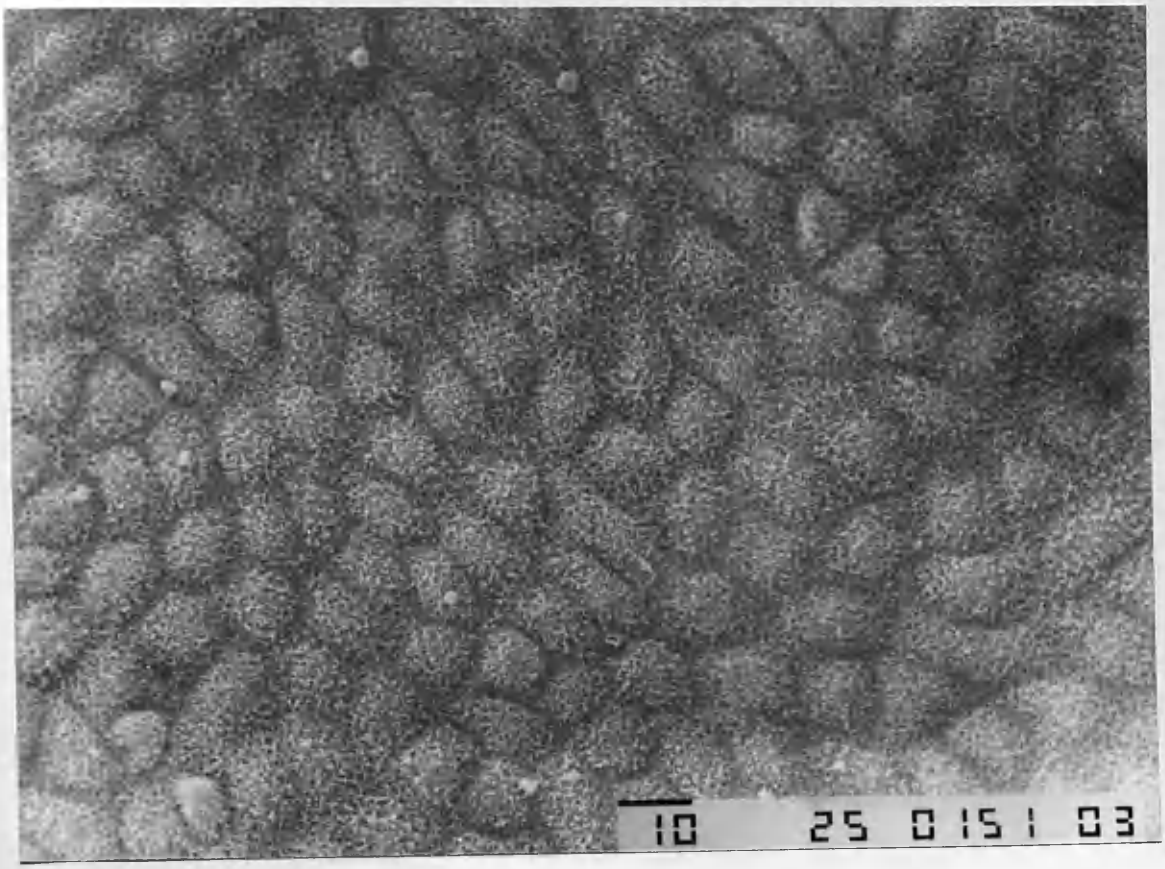

10 25 0118 13

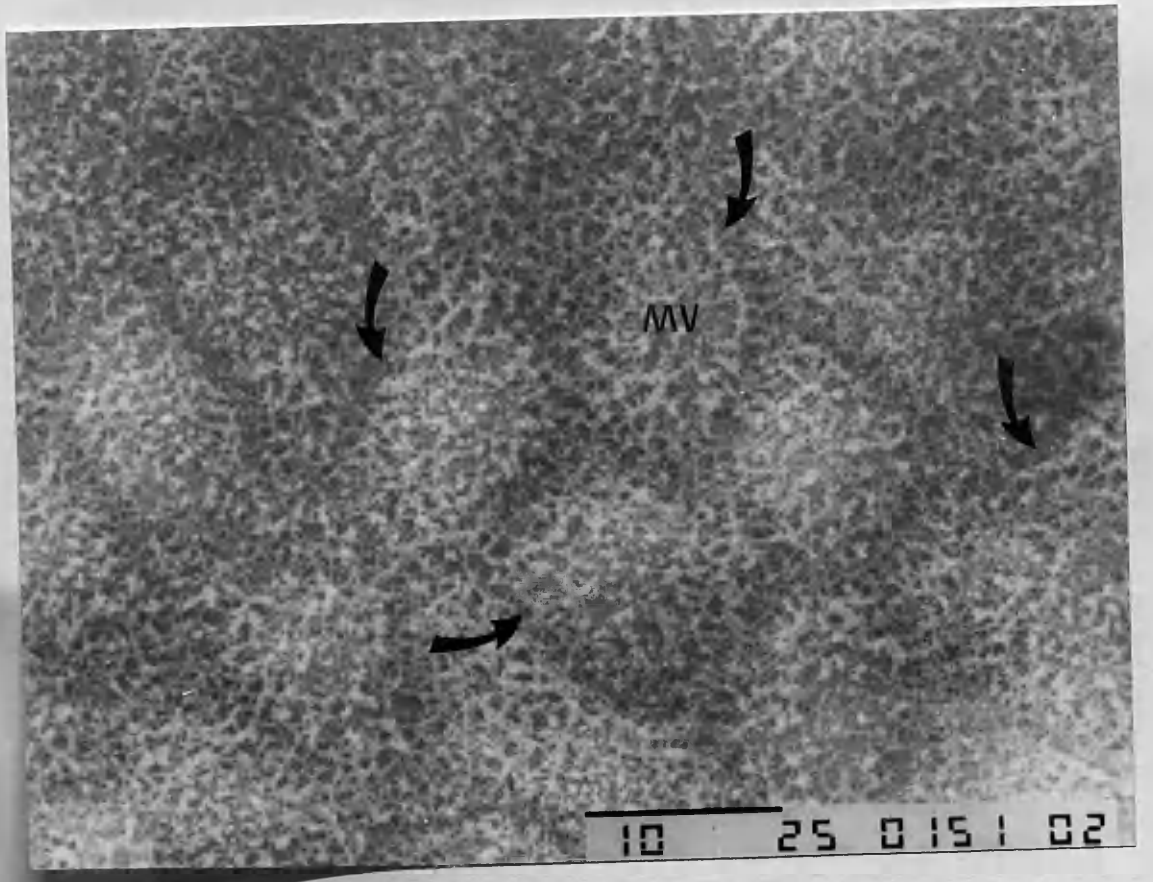

MV
1 25 0118 12

Figs. 152 & 153

Visceral peritoneum of spleen. Note that the borders of mesothelial cells are clearly visible (arrows). Short finger-like microvilli (Mv) extend from their free surface into the peritoneal cavity.

Adult rat, fixed by perfusion.


10
25 0 15 1 03


MV
10
25 0 15 1 02

Figs. 154 & 155

Visceral peritoneum of liver. In Fig. 154, the borders of individual mesothelial cells (Mc) are not as clearly visible. The density of microvilli varies on mesothelial surfaces of different organs. Note in Fig. 155 the presence of mast cells (asterisks) infiltrating mesothelial surface of the liver. Peritoneal stomata or openings, similar to that present in the diaphragm are not seen in visceral (Figs. 152, 153 & 154) nor in parietal peritoneum (Figs. 150 & 151).

Adult rat, fixed by perfusion.