STUDIES OF THE LYMPHATICS OF THE LIVER AND THE UPTAKE OF INTERSTITIAL FLUID FROM THE SPACE OF DISSE

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TEXT

By

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TO MY FAMILY

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INTRODUCTION

THE AIM OF THE PROJECT

It is now generally agreed that lymphatic capillaries are absent from the interior of the classical hepatic lobule and that endothelially-lined lymphatic vessels are first encountered in the periportal connective tissue. It is also well known that the liver is the largest single site of lymph production in the body and that much of this lymph is formed as interstitial fluid in the perisinusoidal spaces (of Disse). However, the pathway followed by fluid from these spaces into true lymphatic vessels is not known. Indeed our knowledge of the morphology of the hepatic lymph system as a whole is still incomplete.

The last thirty years or so have seen what has been described as a "renaissance of morphology", associated in particular with the development of the techniques of electron microscopy.

The primary aim of this study was to investigate the route followed by interstitial fluid from its site of formation in the space of Disse to true lymphatics in the portal tract. This led on to the study of three secondary objectives:

1. The intrinsic lymphatics of the liver and their development;
2. The extrinsic lymphatics of the liver and their draining lymph nodes;
3. The incidence and distribution of plasma cells in the liver.

A. A GENERAL ACCOUNT OF THE FORMATION OF INTERSTITIAL FLUID AND LYMPH

Interstitial fluid and lymph are formed at the level of the microcirculation, and it is important to keep in mind that vessels of the microcirculation are an integral part of the tissue they supply and that the structure of their wall is adapted to the local requirements of the area which they serve. Therefore the brief account that follows deals with the general principles of the formation and circulation of interstitial fluid and its uptake by lymphatic vessels.

Ludwig (1858) was the first to conclude that the chief factor in the formation of lymph was the pressure of the blood in the capillaries and that lymph was essentially the fluid part of blood filtered through vessel walls into the tissues. He stressed that osmosis was taking place between the tissue fluids and blood.

On the other hand, Heidenhain (1891) experimented with lymph flow from the limb of anaesthetized dogs and the thoracic duct. He obstructed the inferior vena cava and found an increase in the quantity and in the protein content of lymph taken from the thoracic duct. He also injected two substances in the blood. The first was a crystalloid, and he found some time later that the lymph contained a greater percentage of the crystalloid than the plasma. The other injected substance was a commercial

peptone and watery extracts of dried leeches or cray fish. The result was lowering of blood pressure but increased lymph flow and concentration. In the light of these experiments, Heidenhain concluded that lymph must be regarded as a "secretion" rather than as a "transudation" and felt that this result was irreconcilable with those of Ludwig.

Starling (1894, 1896, 1898) repeated and elaborated on Heidenhain's experiments, and came to the conclusion that, in fact, they supported the filtration theory. Starling interpreted the increase in thoracic duct lymph flow after obstruction of the vena cava as due to increased flow from the liver rather than from the intestine. He clearly demonstrated the principle of rapid movement of small molecules across the capillary wall by diffusion in either direction, and the slower filtration from blood to interstitial fluid, depending on the permeability of the capillary wall to proteins and the pressures, hydrostatic and osmotic, existing at the wall at the time. He established the principle, now known by his name, that filtration depends upon the balance between hydrostatic and osmotic forces acting across the capillary wall and upon the physical properties of the capillary membrane. Filtration of water and small molecules occurred when capillary hydrostatic pressure exceeded the sum of the colloid osmotic pressure of the plasma plus the interstitial fluid pressure. As filtration occurred at the arterial end of blood capillaries, the loss of water from

the blood plasma increased the concentration of plasma proteins, resulting in an increase in colloid osmotic pressure. Extravasation of fluid also caused a fall in capillary hydrostatic pressure, which at some point became less than the total of colloid osmotic pressure plus the interstitial tissue pressure, and as a result, tissue fluid entered the capillary, i.e. absorption occurred at the venous end of blood capillaries. Fluid not absorbed by the capillary was removed by the lymphatic system. Starling's principle is applicable to whole organs and on a short time basis. Starling himself did not provide any equation for the forces which he described, but subsequent workers have more than made good this deficiency. For example, Foldi (1977) used the following equations:

Filtration: $F = K_A A_A (P_A - P_T - \pi_{PL} + \pi_T)$

Reabsorption: $R = K_V A_V (P_V - P_T - \pi_{PL} + \pi_T)$

P_A & P_V - hydrostatic pressure in arterial & venous capillary limbs

P_T - hydrostatic pressure in the tissues of interstitial space

π_{PL} - colloid osmotic pressure of plasma

π_T - colloid osmotic pressure of tissue fluid

K_A & K_V - the permeability of arterial & venous capillary limbs for water

A_A & A_V - the surface area of the arterial and venous capillary limbs

According to Simpson (1981) the real implications of

Starling's law are mainly two:

1. That the capillary wall is permeable in both directions to water and small solutes and molecules as large as albumin (Field & Drinker, 1931).
2. That capillary wall permeability is limited to molecules up to the size of albumin under normal conditions.

In mammals, the osmotic pressure of the plasma proteins was found to be of the order of 21 - 25 mm Hg, depending mainly on the concentration of albumin and globulins. More detailed measurements in single capillaries were made by Landis (1925-6, 1930a,b) who found on average, a gradient of pressure from 30 mm Hg at the arteriolar end of the capillary to 12 mm Hg at the venular end. He also showed that filtration or absorption was the result of the algebraic sum of the colloid osmotic pressure and the capillary hydrostatic pressure. Direct and indirect measurements of tissue fluid pressure were made by Landis and others and were shown to be of the order of 0.5 to 5.0 cm H_2O. This pressure most likely resulted from proteins which had leaked from capillaries. Starling was aware of this but he thought leakage was very small. Drinker (1942) measured the extent of this leaked protein and showed that the protein content of lymph in different parts of the body varies. For any particular tissue, the lymph and the interstitial fluid were of approximately the same composition.

Studies on the movement of fluid, micro- and

macromolecules across the capillary wall raised the need of looking for the morphological basis of these events.

Palade & Bruns (1968) designated the three layers which constitute the capillary wall as follows:

1. The endothelium
2. The basement membrane and pericytes
3. The adventitia

Karnovsky (1970) stressed the fact that there is both structural and functional heterogeneity in capillaries and that in general, variations in capillary permeability can be related to capillary structure.

Substances traverse the endothelium in many different ways. They may traverse directly through the cellular membrane and cytoplasmic matrix (especially water and presumably certain other small molecules, especially the lipid soluble molecules). Curry et al (1976) estimated that about 10% of the total flow of water passed in this way, and about 90% via intercellular junctions, vesicles and vacuoles and fenestrae. Which is most important depends on the nature of the substance, the state of the endothelium and the local conditions.

Capillaries can be put into three categories:

1. Continuous capillaries are those with a continuous lining of endothelial cells, such as are seen typically in skin and skeletal muscle; they are associated with a relatively slow rate of movement of water and small solutes across their wall. In these capillaries small molecules

traverse the walls faster than larger ones (Renkin & Curry, 1978). The general opinion seems to be that, in this type of capillary, small molecules pass through parts of the junctions between the cells and the larger ones pass much more slowly via the vesicles. Landis & Pappenheimer (1963) on the basis of experiments on perfused skeletal muscle capillaries of the hind limbs of cats and dogs, postulated the existence of water-filled pores or channels which were cylindrical, uniform and about 70 Å in diameter. Other geometrical forms were possible. They occupied a very minute fraction of the capillary surface. Small molecules exchanged rapidly across these pores. At molecular weights above about 10,000, there was a high degree of sieving and molecules of molecular weight greater than 90,000 were virtually excluded from passing through the pores. This was called the "small pore system".

A second system of larger pores of 120-350 Å was postulated by Grotte (1956). The ratio of the large pore system to the small one was estimated to be 1 to 34,000.

Bruns & Palade (1968b) have proposed vesicular transport as an alternative mechanism for transporting large molecules across the endothelium. There is strong evidence that tracers of large molecules such as ferritin travel across the endothelial cells via intracellular vesicles, since some vesicles contained tracer material when used; however, many vesicles did not. Casley-Smith (1963), Shea & Karnovsky (1966) suggested that the vesicles move randomly about the cells by Brownian motion. Palade

and Bruns (1968) illustrated in chemically fixed material that the processes of fusion and fission of vesicles was only found in 1.5% of the vesicles but increased to 60% when freezing was used to fix the cells. Palade et al (1979) suggested that vesicles, by concomitant fusion (followed by fission) generate transendothelial channels that act as mass-carriers of fluid and solutes across the endothelium. However, some other workers have failed to confirm these findings. It may be that the presence of tracers in channels is seen when immersion fixation is used (i.e. when fixation is slow). The role of vesicles in transendothelial transport remains debatable.

2. Fenestrated capillaries. These are found in the gut, in endocrine and exocrine glands and much of the urinary system, and only occasionally elsewhere. Sizes and numbers of the fenestrae have been measured in the jejunum by Casley-Smith et al (1975c) and by Simionescu et al (1972). The holes completely traversed the very thin part of the endothelium and were some 50 nm in diameter. Some, but not all, had diaphragms across them. Fenestrae were particularly numerous on the venous side of the microcirculation. When fenestrae were frequent, they greatly increased the permeability to macromolecules, but only slightly increased it to small molecules (Casley-Smith, 1980a; Renkin, 1977).

3. Sinusoidal capillaries or discontinuous capillaries. This type of capillary has an endothelium with large gaps

(100 - 1000 Å) and the basement membrane is discontinuous. It can be found in the liver, bone marrow and spleen.

The basement membrane

The other barrier in capillary permeability is provided by the basement membranes. They generally define the limits or boundaries of tissue spaces. They form a structural framework which isolates epithelia (including endothelium and mesothelium), muscle, nerve, and fat from the tissue space. Within this space lie the formed elements of the connective tissue (Low, 1961; Batting & Low, 1961; Low and Burkel, 1965). As an expression of this pattern, the endothelium of the blood vascular system is separated from the surrounding tissues by its basement membrane. This is especially the case for capillaries. Similarly the parenchyma of epithelial organs is separated from the tissue spaces by its own basement membrane.

The basement membrane varies in its composition and appearance according to location, but some of it underlies almost all epithelia. Some of the substance of the basement membrane is synthesized by the epithelial cells themselves, although its production involves some interaction with the underlying connective tissue. Basement membranes are generally made of two parts. The first is an amorphous sheet, closely applied to the cells, and containing type IV collagen. This is the part that is common to all epithelial tissues; it is not seen with the light microscope; electron microscopists commonly call it

the basal lamina. Below the basal lamina, in some tissues, is the reticular lamina which varies in its composition and remains poorly characterized. This part can be visualized by light microscopy when stained by P.A.S. or silver stains (Kefalides, 1979).

The function of the basement membrane is not well understood. It probably serves as a medium for transfer of cells such as those in the intestinal lining. It may have a role in stabilizing tissue shapes, since its removal during embryogenesis causes developing salivary glands to lose their characteristic structure. In the kidney (Heine, 1986), the special basement membrane of the glomerulus is thought to function as a selective permeability barrier contributing to the process of ultrafiltration of plasma to form urine. The absence of basement membrane in certain malignant tumours suggests that it may be important in growth control (Huang, 1978). Metastasis of cancer cells is considered to begin when the basement membrane is breached.

How do solutes of various sizes or even cells cross the basement membrane?

Simpson (1980a) proposed that all basement membranes function because of their properties as biological thixotropes, i.e., they undergo localized gel-sol-gel transformation under the influence of pressure alone. Menefee et al (1964) studied the passage of aggregated globin through the basement membrane of glomeruli which

could best be interpreted on the basis that the basement membrane had the properties of a thixotropic gel. Palade (1961) in a study using ferritin as a tracer in nephrotic rats, found the tracer molecules were embedded in the basement membrane "without channels ahead or trails behind", and he got the impression that the marker moved through a yielding gel creating a channel as it moved.

One would imagine that water and micromolecules would pass easily through these localized gel-sol-gel areas. However, large molecules would need to cause localized deformation of the lattice of the basement membrane in order to traverse it. This would be possible only if pulsatile intracapillary pressure was adequate to initiate deformation. Basement membrane thixotropy makes explicable the passage through basement membranes of objects ranging in size from water molecules to motile cells and parasites (Simpson, 1981).

The extracellular space

Next to the basement membrane comes the extracellular space, which is occupied by a continuous chain network which may or may not be homogenous by distribution. This network is made of connective tissue polysaccharides which have a molecular weight of the order of millions (Laurent, 1966a). These polysaccharides exist in a complex lattice form and may form a porous plug which may even exhibit marked resistance to water flow, as demonstrated by Day (1950) and Hedbys & Mishima (1962).

Laurent (1970) reviewed the concept regarding the

physiological role of high molecular weight connective tissue polysaccharides. Among the possible functions was the "exclusion phenomenon" meaning that two molecules cannot occupy the same space at the same time, so polysaccharides will exclude other macromolecules to a considerable degree from space in a solution, and the larger the molecule, the less space will be available for it in a three dimensional chain network. This network will act as a filter and retard the movements of large molecules more than of the small ones. The polysaccharide compartments will act as a sieving barrier (or membrane) which can regulate the transport of various biological macromolecules. In tissues where tissue fluid turn over rapidly, it may be that the fluid is transported via some sort of low resistant pathways or channels.

At this point, two additional factors affecting the production of interstitial fluid and its uptake from the tissues must be stressed.

1. Macromolecules may also enter the interstitium from the cells of the tissues, i.e. the capillary filtrate is not the only source of such molecules. For example, chylomicrons and lipoproteins in the intestinal mucosa during fat absorption; immuno-globulin synthesized in lymph nodes and other lymphoid tissues or absorbed from the gut of certain newborn animals, and enzymes liberated from injured cells. All are then transported to the blood stream by way of the lymphatic vessels.

2. Lymphatic vessels are not the only, or even the major, pathway for the absorption of proteins from tissues. The venous system plays a significant role in this process especially when the smaller proteins such as albumin are injected into various tissues (Szabo et al, 1973). However, the structure of the walls of the lymphatic capillaries makes then preferential channels since macromolecules, particles, baceteria and certain cells readily pass through the intercellular junctions to enter the lymph.

The movement of interstitial fluid into the lymphatic

If there is a positive pressure gradient across the lymphatic capillary wall from the interstitial fluid to the lymph, protein and other macromolecules would move freely across this barrier. Many factors facilitate this process.

1. There is little or even no basement membrane supporting the lymphatic endothelium.
2. The intercelllular junctions are often partially open or at least openable. This is of overwhelming importance in the entry into lymphatics of molecules of various sizes (Palay & Karlin, 1959a; French et al, 1960; Casley-Smith, 1964a, 1965; Cotran, 1956; Morris & Sass, 1966).
3. Within lymphatic endothelium, vesicles have been observed to contain intravenously injected markers, but their relative importance in the formation of lymph is quite controversial.

4. Endothelial cells may also to some extent be phagocytic, but phagocytosis seems to play little, if any, part in the normal absorption of macromolecules from the tissue fluid by the lymphatic capillaries.

Guyton (1963, 1965, 1966a,b) and Scholander et al (1968) suggested that, in the skin, the pressure of interstitial fluid is negative. If this were true it would obviously make it difficult to explain the filling of lymphatics. However, these authors gave no information about the pressure within the lymphatic capillaries of the skin.

B. THE SITE OF FORMATION OF HEPATIC INTERSTITIAL FLUID

The initial formation of interstitial fluid in the liver lobule is conditioned by the fine structure of the sinusoidal wall, with its unusual combination of a fenestrated endothelium and the absence of a basement membrane. This allows free escape of blood plasma from the sinusoid into the space of Disse, where it comes into intimate contact with the hepatocyte muralium (Burkel and Low, 1966). Another factor which affects interstitial fluid formation is the fact that, in the liver, the filtering head of pressure within the sinusoid is as low as 5-10cm of water (Yoffey & Courtice, 1970).

1. The endothelial cells lining the sinusoid show large numbers of fenestrations, which vary greatly in size and arrangement. Some are very small (0.1 μm in diameter) and arranged in clusters, forming what are called "sieve plates"; others are much larger (~ 1.0μm - 2μm) and are subdivided by slender strands of cytoplasm. Motta and Porter (1974) studied these cells by scanning electron microscopy in perfused young adult albino rats and showed that fenestrations occur in the thin part of the cytoplasm. Lines of fusion between adjacent or overlapping endothelial cells were not identified. Small pits were present in the surface of the larger arms of the endothelium and most prominently in the surface of the central mass. These pits may represent openings into cortical vesicles, which were numerous in those cells. They could not determine whether

the small pits were incipient perforations. Discontinuous fenestrations have been found in most species of mammals studied, e.g. mouse, rat, dog, rabbit and man. There are, however, significant species differences in the degree of endothelial discontinuity: Wood (1963) observed that in the calf there are no fenestrations, while Kuhn and Oliver (1965) showed a similar type of sinusoid in the liver of the goat.

These fenestrations exert some sort of selectivity on the filtration of molecules, depending on the size of the molecules and the size of fenestrations, as explained earlier.

Burkel and Low (1966) and Burkel (1970) described in the rat three structurally distinct zones in the endothelium of the liver sinusoids of rats perfused with 3% glutaraldehyde. Each zone was sharply demarcated.

i) A zone at the periphery of the classical lobule, having the appearance of an extension of the portal vein branch endothelium, which had a continuous basement. This peripheral zone was very small.

ii) An intermediate zone, making 90% or more of the sinusoid, in which the endothelium was discontinuous.

iii) A third, central zone, which had a basement membrane and was continuous with the endothelium of the central vein.

In the liver, the absence of basement membrane from both the sinusoidal lining and the adjacent hepatic parenchyma, along with the presence of a tissue space (of

Disse) occupied by a variety of structures, results in an unusual relationship between the vascular system, parenchyma and tissue spaces. It is, however, best understood as a variety of similar relationships found elsewhere in the body (Burkel & Low, 1966), and incompleteness of the basement membrane does characterize certain other slow-flowing vascular channels, e.g. Weiss (1961) described similar findings in the vascular sinuses of the bone marrow in rabbits.

2. Disse's space (perisinusoidal space)

This is the narrow perivascular space between the wall of the liver sinusoid and the liver parenchymal cells. It contains some bundles of reticular microfibrils and occasional peri-sinusoidal cells.

Disse (1890) claimed the existence of pericapillary (sinusoid) cavities within the liver acini. These cavities possessed, according to Disse, a wall of their own, a membrane composed of a homogenous ground substance and incorporated fibrils of variable thickness. The membrane was covered by stellate cells. The fibres extended between the hepatic trabeculae and connected the capillary sheaths. It was this blood-capillary sheath that became the base of the stroma of the hepatic lobule. The perivascular space, now generally known as "Disse's space", was, according to Disse, not bounded by the trabeculae of the liver cells, but by a membrane connected with the cells through the intercellular substance. Disse suggested that the space

between trabeculae and blood capillaries could be filled with dyes injected into the efferent lymphatics. This led him to the conclusion that a close connection existed between this space and the lymphatics. He thought that the sheath surrounding the capillaries opened into larger lymphatics on the periphery of the hepatic lobules.

Disse's findings started a lengthy debate: was it an artifact? If not, was it a part of the lymphatic system or not? The resolving power of the light microscope was not sufficient to answer these questions.

Fawcett (1955) using the electron microscope was among the first to demonstrate microvilli along the sinusoidal border of the hepatic cells and to give a clear idea of the much disputed perisinusoidal space of Disse.

Along with the numerous hepatocyte microvilli, reticular fibres and Ito cells are also present in Disse's space. Ito (1951) observed cells containing lipid droplets in the human liver lobules. These cells were located in the space of Disse and were surrounded by reticular fibres. Ito presumed that lipid droplets in these cells were derived from the blood stream and designated the cells as "fat storing cells". Satsuki et al (1956) injected various kinds of fat emulsions and cod-liver oil intravenously and found that these cells failed to show an increase in lipid droplets in their cytoplasm. On the other hand, these lipid droplets increased remarkably after administering glucose and insulin (Kano, 1952; Sunaga, 1954a).

The electron microscopic studies of Yamagishi (1959),

Ito and Shibasaki (1968) and others have demonstrated the presence of a cell type in the space of Disse, (reviewed by Wake (1980)), whose possible connection with the intralobular connective tissue framework was considered by Schmidt (1960), Aterman (1963) and Wood (1963). It is now widely viewed as the "adventitial connective tissue cell" of the liver lobule (Schnack et al, 1967), responsible for the maintenance of the normal connective tissue, and for the production of excessive connective tissue, in the liver. Kent et al (1976) actually described them as lipocytes transformed into fibroblasts.

The space of Disse is found not only between the sinusoidal endothelium and the hepatocytes; it also extends into the space between adjacent liver cells (Motta & Porter, 1974). The full extent of this expansion of the space has not perhaps been fully appreciated from earlier observations on liver ultrastructure. Scanning electron microscopy has shown the space to be larger and as having more extensive ramifications than is generally evident from studies using the transmission electron microscope. Those inter-hepatocyte extensions, earlier recognized by Steiner 1961, are limited by the junctional complexes of the bile canaliculi, but they extend elsewhere in all directions, clearly to connect adjacent sub-endothelial spaces of Disse. The space of Disse, as originally described, now appears to be only a part of an extensive labyrinth of intercellular channels within which plasma filtrate moves

towards the peri-portal lymphatics.

Should the space of Disse be regarded as a part of the hepatic lymphatic system?

Although Elias (1949) considered it as a "virtual lymphatic lacuna", the modern consensus is that it is an interstitial slit, cavity, or space from which no fluid can get into lymphatic capillaries without first passing through their endothelial wall. It is also generally agreed that it is the place of origin of most of the hepatic lymph.

C. TISSUE FLUID Vs LYMPH

Among early studies of the lymphatic system, Sabin (1916) emphasized the importance of distinguishing between "tissue fluid" (outside vessels) and "lymph" contained in lymphatics.

Drinker (1931) on the other hand, revived the older concept of the interchangability of the terms "tissue fluid" and "lymph". While admitting the existence of anatomically closed lymphatics, he considered that the terminal lymphatics are so delicate as to be continually ruptured and hence physiologically open, and that the content of lymphatics draining any given region of the body is a "cross-section" of the tissue fluid there present. Following the extensive studies of lymphatics in vivo by the Clarks (1933) the concept of a closed system of lymphatic vessels came to be generally accepted.

Szabo and Magyer (1978) observed in the rabbit's hind limb that in the tissue fluid complex there are at least two distinct compartments. The first connects the blood capillaries and the lymphatics and forms the pathway taken by the fluid and protein leaving the blood capillaries adjacent to lymphatics, in other words, it constitutes the tissue fluid, some of which is going to be lymph. This lymph reflects the state of transcapillary exchange. The second compartment is the true tissue fluid. It contains the extravascular plasma protein pool and the protein molecules released from the cells, i.e. the fluid bathing

the parenchyma. It would seem, however, that the tissue fluid that is going to become lymph is in dynamic equilibrium with the second compartment and largely reflects its status.

In the liver, the first compartment is represented by the portal tract area where blood capillaries and lymphatics co-exist, and the second represents fluid in Disse's space. Courtice (1971) studying barriers within the tissue fluid compartments suggested that, in lobular organs, such as the liver, with discontinuous capillaries, there is no effective barrier to the movement of tissue fluid from the centre of the lobule to its periphery. Wisse (1970) studied the microcirculation of rat's liver by vital microscopy and found that large cells such as leukocytes, when passing through narrow sinusoids, move more slowly and indent the sinusoidal endothelium, compressing Disse's space as if massaging the sinusoidal endothelium and thus pushing the fluid in Disse's space towards the periphery.

Although lymph can be readily collected from collecting lymphatics for analysis, its composition may be constantly changed by redilutions as it travels through. Sampling of fluid from lymphatic capillaries is very difficult [see e.g. Hargens & Zweifach (1976) and Rutili & Hagander (1982), reviewed by Casley-Smith (1982)]. Because of these technical problems the relationship between lymph and tissue fluid has been much debated.

Taylor et al (1973) showed that fluid collected from

capsules implanted in rabbits' ears, lymph and interstitial fluid were all three characterized by identical protein concentration. Brace et al (1976) maintained the view that lymphoprotein concentration does not always reflect that of the interstitial fluid from which it is derived. Rutili and Arfos (1977) sampled, in the same area where interstitial fluid had been collected, lymph from lymphatics 30 - 100μm in diameter. All of the fluids were found to have a similar protein concentration.

Studies performed by Witte (1965-1975) by means of vital microscopy in the exposed mesentery of the rat are of the utmost importance. Fluorescein-isothiocyanate was bound to plasma proteins in vitro and after chromatography, injected intravenously. By means of fluorimetry the escape of the labelled material from the blood vessels into the interstitium and subsequently its penetration into the initial lymphatics could be visualized. Shortly after the injection of the labelled proteins its concentration in the initial lymphatics rose above that in the interstitium. By in vivo ultramicrospectrometry, the protein content of the perivascular connective tissue in the exposed rat mesentery could be measured. The concentration was higher near the wall of the blood vessels than that at a distance of 20-30 μm. The reason for this may be the existence of various elements in the interstitium, such as the complex lattice of the polysaccharides (mentioned earlier), which exerts some, maybe uneven, resistance to the propagation of various molecules. This result clearly demonstrates that

it is unwise to speak about an average protein concentration in interstitial fluid and about an average interstitial colloid osmotic pressure, unless it is borne in mind that each of these can vary quite considerably within even a small area.

Ruszynak, Foldi and Szabo (1967) injected the fluorescent dyes, thiazone red and acridine yellow, into the mesenteric lymphatics of cats, and observed that some of these dyes escaped into the interstitium again. These results supported Hudack and McMaster (1932) who injected dyes such as pontamine sky blue into the ears of mice and studied the uptake into, and the escape of substances from, the lymphatics. Although injecting lymphatics is much criticised, these experiments may highlight the fact that molecules can get out of lymphatics as well as they can get in, which makes the debate of tissue fluid versus lymph even more complicated.

Haddy (1972) and associates found that, in the dog's forelimb, the lymphoprotein concentration reflected the protein concentration in the interstitial fluid. Earlier Jacobsson and Kjellmer (1964) found exactly the opposite and showed that lymph does not give a true picture of the mean protein content of tissue fluid.

Courtice (1971) showed that the protein content of interstitial fluid is fairly near the values obtained from lymph from the same region.

In certain conditions and in localized areas such as wounds, infections or burns (Clark & Clark, 1933) in which

there is an increased circulation through the blood vessels and increased passage of fluid through the vessel wall followed by an accumulation of free fluid in the interstitium, the lymphatic capillaries may be damaged, so that equality of interstitial fluid and lymph may be actually true. Clark and Clark (1933) found some minute tears in lymphatic capillaries in such injured or inflamed areas which remained open for days and in which there was not only a free passage back and forth of fluid between the vessel lumen and the exterior, but also of formed elements such as red cells and fat globules. However, it is equally clear that regardless of what may be true of the permeability of lymphatic capillaries to the various chemical elements present in the tissues outside, the assumption that "lymph is tissue fluid" cannot be generally true in a physical sense, for not only do the lymphatic capillaries normally form a closed system, separated from the tissues everywhere by an intact endothelial wall, but under normal circumstances all the evidence appears to indicate that there is no free fluid present in the tissue spaces, except when oedema develops.

In the liver, the relationship between interstitial fluid and lymph is even more complex. Not only because of the fenestrated endothelium, the absence of basement membrane in the sinusoidal and hepatocytes, but also because of the complex fluid dynamics of the fluid in the space of Disse.

D. FLOW AND COMPOSITION OF HEPATIC LYMPH

The flow of lymph from the liver has been determined in both anaesthetized and conscious animals. The flow is spontaneous, depending on the changes in pressure due to respiration, as well as the contractility of the collecting lymphatics when they possess smooth muscle in their wall (Yoffey & Courtice, 1970) (described later on).

The focal point of the majority of studies of hepatic lymph is the protein moiety. Starling (1894, 1909) collected lymph from the large lymphatic vessels in the lesser omentum and found that it contained nearly as much protein as did the circulating plasma.

Many workers have studied the thoracic duct lymph in contrast to the few who have reported on hepatic lymph. It is suggested that liver lymph differs from lymph elsewhere in that there appears to be an equivalence of its protein and lipid moieties to those of plasma itself,and this is no strange conclusion if one recalls the nature of its site of origin previously described.

Rate of flow:

Smith et al (1970) who cannulated the largest lymph trunk at the porta hepatis after tying the rest in conscious Marino ewes and rams, found that the rate of flow was 1.0-3.0 ml per hour. Friedman et al (1956) found that in rats it was 0.08-0.1ml per 12 hours and at the end of 12 hours about 1.5ml of lymph was collected, with considerable individual variations (range of 0.45-

3.5ml/12hrs). Popper and Schaffner (1957) found that in the normal non-anaesthetized dog, hepatic lymph amounted to nearly 0.2ml/min, and that in the anaesthetized dog the rate of flow increased. In rats they found that the lymph output was around 5ml/day, the exact amount depending on the state of hydration and digestion. Courtice (1960) found that in rabbits the normal average lymph flow is 0.7ml/hr which is somewhat less than the flow of lymph in the cat (Morris, 1956b) and in the dog. The rate of flow was found by Courtice (1960) to increase in cases of hypercholesterolaemia and after biliary obstruction. It appears that different species have different rates of flow under different circumstances.

It is generally agreed that the liver has a large lymph output, perhaps greater than that of any other organ. Thus, it may contribute up to 20% of the total thoracic duct lymph, and in starvation it may be up to 50% [Popper & Schaffner (1957)]. Friedman et al (1956) found that the maximal pressure observed of rat hepatic lymph was 26cm of lymph.

Protein content:

It is calculated that the total proteins of hepatic lymph is about one third less than that of the plasma. Smith et al (1970) found that the ratio of total lymph proteins to total plasma proteins in case of the liver equals 0.76 $\pm$ 0.02. This makes the hepatic lymph the richest lymph in proteins, a fact which supports its origin principally, from fluid in Disse's space. Yet the evidence

indicates a certain degree of molecular sieving of the protein molecules according to their size. This molecular sieving has been further demonstrated for even larger molecules or complexes, the lipoproteins. This molecular sieving, in the case of the liver, can be explained on the basis of the presence of small endothelial fenestrations, large ones, and larger ones partitioned with strands of endothelial cytoplasm. So small molecules pass through any fenestrations and larger molecules pass only through larger ones.

Since the liver is also a major site of synthesis of plasma proteins, it is possible that some proteins entering the lymphatics via Disse's space are newly synthesized. The balance of evidence, however, suggests that plasma proteins newly formed in the liver mainly pass directly into the blood stream. This conclusion is based on experiments in which the thoracic duct was ligated or a thoracic duct fistula created. Cremer et al (1974) ligated the thoracic duct of rats and studied the biochemical analysis of the serum. They showed that the loss of protein from the thoracic duct was much greater than the decrease in total circulating plasma proteins.

Courtice (1960) found that the relative concentration of albumin was greater than that of globulin as a whole and of each of the individual globulin fractions. In normal animals, the albumin concentration was above 96% of that of plasma, whereas that of the globulin was 74% of that in the

plasma. Brinkhous and Walker (1941) examined in dogs the prothrombin concentration of the lymph in the thoracic duct, femoral lymphatics and the liver. Taking the prothrombin level of the blood plasma as 100% they established the following values: femoral lymph 7.6%, thoracic duct 50.7%, liver lymph 93.2%.

Glucose content:

In normal rats, the average glucose concentration of hepatic lymph is about one third greater than that in the plasma. In previously fasted rats, the average glucose concentration of hepatic lymph is about one fifth greater than that of plasma.

Cholesterol content:

The average concentration of cholesterol in hepatic lymph (33mg/100ml) is considerably less than that of plasma.

Total lipids (Friedman et al, 1956):

Little difference was observed between the concentration of lipid in lymph and plasma.

Cholate content:

It is doubtful if a true difference exists in the concentration of this substance in the two fluids (Friedman et al, 1956).

Urea content:

It is approximately the same as that of plasma.

Cells in hepatic lymph:

Smith et al (1970) calculated the cell count in

sheep's hepatic lymph to be 2000 - 6000 cells/mm^3, compared to 100 - 1000 cells/mm^3 in lymph collected from other tissues, with lymphocytes making 70-85%, macrophages 5-20% and others 5-10%. This high cell count indicated that there was an extensive traffic of cells through this organ (Lascelles and Morris, 1961). Heath, Lascelles and Morris (1962), taking the average cell content of peripheral liver lymph as 4×10^6 /ml estimated that 2×10^9 cells migrate from the liver each day, of which, 2×10^8 are macrophages. Many of these cells appear at some stage of their life history to be part of a population of phagocytic cells that are in contact with blood and able to take up particulate material from it. The most obvious cells in the liver with these capabilities are the Kupffer cells. It seems that having phagocytised particulate material from the blood stream in the liver sinusoids, many Kupffer cells vacate their position on the walls of the hepatic sinusoids and migrate into the hepatic lymph.

There are some reports suggesting that the liver may be the site of the preferential recirculation of lymphocytes. Fichtelius and Didenholm (1959), Fichtelius et al (1961) and Fichtelius and Groth (1963) labelled splenic lymph node and thymic cells with radioactive phosphorus ^{32}P and ^{3}H-thymidine and infused them intravenously, they found that there were higher concentrations of radio-activity in the hepatic lymph nodes than in other nodes. No evidence was presented that the labelled cells specifically came via the hepatic peripheral

lymph.

Horii et al (1950) measured the number of lymphocytes in the hepatic artery and portal vein of rabbits and found it to be higher than in the hepatic vein.

E. THE PORTAL CANALS

Portal canals are seen in sections of the liver as small triangular or round profiles at the "corners" of the classic liver lobules. Each portal canal consists of loose connective tissue and contains an interlobular vein, interlobular artery, interlobular bile duct, lymphatic vessels and non-myelinated nerve fibres. The frequently used term "portal triad" designates the three most prominent elements of the portal canal: the vein, artery, and bile ducts. "Portal tetrads" is to be preferred since it also includes the lymphatic vessels.

Between the portal canals and the perilobular limiting plate lies a space that contains hepatocytic microvilli, bundles of collagen and interstitial fluid. It was first described by Mall (1906) and is now known as the space of Mall. There has been much debate about two main features of Mall's space. First, whether it really exists as such under normal physiological conditions or whether it is merely a potential space. Secondly, whether it is a lymph space, as it was thought to be by Mall himself. Leak (1976) thought that any fluid from the space of Mall might drain into lymphatics. A commonly held view is that it belongs to the series of spaces through which the lymph passes before finally reaching the lymphatics.

The portal canals, also called interlobular spaces or portal tracts, consist of irregularly arranged collagenous connective tissue bundles between which some fine fibrillar

reticulum fibres are suspended. The denser connective tissue protects the vessels which are firmly anchored within the portal canal. Elastic fibres or membranes originating from the elastic lamella of the portal vessels are interwoven.

The structure of the portal canals shows species variations (Popper & Schaffner, 1957). In man the adventitial layers of arteries and veins seem to unite. The interlobular bile ducts are close to the vein or separated from it by lymphatic vessels. In the larger portal canals, many lymph vessels are found. The tissue of the portal canals has been estimated to form 4.6% of the total volume of the liver in the adult and 6% in children (Popper & Schaffner, 1957). In pigs it forms complete capsules around each lobule. This, however, is not necessarily associated with an increase of the total portal connective tissue of the portal canals, since the connective tissue in the pig is much thinner around the vessels. In man, narrow secondary trabeculae extend into the parenchyma from the portal canal. They contain the interlobular branches of the hepatic artery or precapillary arterioles.

The morphological division of the connective tissue framework of the liver into a capsule, the fibrous tissue of the portal canals and the intralobular network of fibres, happens to correspond to the order in which these components were discovered. The old observers, such as

Glisson, viewed the connective tissue framework of the liver more or less as one unit, a concept embraced by the original meaning of the term "Glisson's capsule". It was only after the introduction of silver stains by Oppel (1891), Maresch (1905, Wolf (1905) and after Mall (1888, 1891) and Siegfried (1892) had demonstrated some physical and chemical differences between them, that the three divisions of the connective tissue framework were separately recognised.

Between the connective tissue bundles of the portal canals, cells with large vesicular nuclei are found. Their cytoplasm may contain engulfed material. Some of these cells are fixed macrophages, others are wandering monocytes. Some lymphocytes and plasma cells may also be present, though whether they are normal constituents or not has been debated. These cellular elements are frequently arranged around the lymphatics. The incidence of accumulations of macrophages and lymphocytes varies in different individual rats, all apparently normal (Aterman, 1981). Accumulations become larger and more common with increasing age. All transitions may be encountered, from only a few cells, to large aggregations forming lymph follicles with germinal centres (Aterman, 1981), to irregular accumulations without any pattern and without any evidence of a significant disorder of the liver.

F. THE GENERAL STRUCTURE OF LYMPHATICS

The significance of the lymphatic system lies in the fact that excess proteins and fluid in the interstitium are capable of disturbing the normal Starling pattern of exchange between blood capillary wall and the surrounding tissue spaces. This process, if left unchecked, would lead to oedema formation. The constant loss of diffusible substances and plasma proteins from the blood capillaries in the interstitium, if allowed to continue would not only deplete the blood circulatory system of its plasma colloids, but would also disrupt the balance of forces responsible for the control of fluid movement and the exchange of gases, fluids and materials across the blood vascular system. Lymphatics subserve the body tissues by constantly removing the excess interstitial fluid and plasma proteins that are not removed at the venular limb of the blood vascular system. In this way the lymphatic system prevents a build up of these components within the interstitium, accomplishing the maintenance of fluid homeostasis for the various tissues of the body.

The beginnings of the lymphatic vessels are located near the site of cellular metabolism and also have a close topographic relationship with the blood capillaries and small venules (Leak, 1976). In this way, the drainage system is strategically placed and is anatomically constructed to permit a continuous and rapid removal of transient interstitial fluids, plasma proteins and cells.

The gross and microscopic structure of the lymphatic system is well covered in several general reviews, on which the following account is based, e.g. Mayerson (1963), Allen (1967), Rusznyak, Foldi and Szabo (1967), Foldi (1969), Kampmeier (1969), Yoffey and Courtice (1970), Courtice (1971), Casley-Smith (1973) and Courtice (1981).

The most peripheral elements of the system are innumerable, small, thin-walled vessels. They perform the primary function of the lymphatic system of removing material from the tissues. They progressively merge centrally to form the "collecting lymphatics", which perform the second function of the system of transporting the material to the blood. The peripheral lymphatics are variously named lymphatic capillaries, terminal lymphatics, small lymphatics or initial lymphatics. They are usually about 0.5mm long, 20-60μm in diameter. The size of the lumen of any individual lymphatic capillary changes considerably from time to time depending on the state of hydration of the tissue concerned. They are very irregular in shape, usually rather flattened, although varying throughout the initial lymph cycle (see later). The lining layer of endothelium is, at best, supported by a poorly developed basement membrane, making lymphatics easily distinguished from blood capillaries in most tissues of the body.

Passing centrally, the collecting lymphatics add other elements to their wall, such as internal elastic

lamina (which is usually incomplete and disappears in the thoracic duct), smooth muscle cells and connective tissue in general (Casley-Smith, 1969a; Schipp, 1967). There are many non-myelinated nerves (Morris, 1956b). The collecting -lymphatics have many centrally-directed valves which appear in sectional profile as truncated cones (Boussauw & Lauweryns, 1969). The smooth muscle and the valves play an important role in the intrinsic propulsion properties of the lymphatics, at least in some mammals.

The fine structure of the initial lymphatics has been reviewed by Majno (1965), Ottaviani and Azzali (1965), Viragh et al (1966), Leak and Burke (1968), Lauweryns and Boussauw (1969), Casley-Smith (1969a), Leak (1970), Yoffey and Courtice (1970), Dobbins and Rollins (1971), Kalima (1971), Courtice (1981).

The walls of lymphatic capillaries in all tissues and in all mammals are somewhat similar in their fine structure (Courtice, 1981).

The endothelial cell is thickest in the nuclear region, up to about 6μm, and elsewhere becomes attenuated, sometimes as little as 0.1μm.

The endothelial cells are irregular in shape with numerous projections on both their luminal and abluminal faces. These projections may extend deeply into the connective tissue. The basement membrane is much less developed, often greatly attenuated or even not visible at all. Endothelial cells of small lymphatic vessels are connected to the surrounding tissue by fibrils. These have

been suggested as early as 1876 by Gaskell and Starling (1898). Later they were confirmed by light microscopy by Clark and Clark (1921) and by McMaster and Hudack (1934). Leak and Burke (1966) detected them by electron microscopy in the lymphatic vessels of the ears of albino guinea pigs. They consist of numerous fine filaments (~ 60Å in diameter) that are attached to the abluminal face of lymphatic endothelium at areas of increased electron density, similar to hemi-desmosomes. The filaments extend for varying distances into the adjacent connective tissue, to which they anchor the lymphatic vessel. As the amount of tissue fluid increases, the tension on these filaments causes the wall of lymphatic vessels to be drawn apart instead of the vessel collapsing. This ensures that the vessels dilate, and fill with the surrounding fluid.

The overlapping endothelial intercellular junctions are the most important structures in the whole system, for upon them depend both the uptake of material and its retention (Casley-Smith, 1977; Leak, 1976); many of these junctions resemble those in the blood capillaries. Those with interdigitations probably help to hold the cells together (Majno, 1965). Others may be "tongue and groove" and edge to edge approximations. Sometimes there is a space between endothelial cells approximately 90Å in width, which appears to contain no intercellular cement substance. In some areas the cells may be more tightly joined together with "tight" junctions (zonulae occludentes) or desmosomes,

(zonulae adherentes). It seems, however, that most endothelial cells may be easily separated in places, either by an increased formation of tissue fluid or by movement, as in the diaphragmatic lymphatics. In this way open junctions are formed (French et al, 1960; Casley-Smith, 1964a,b). In blood vessels, open junctions are only seen in sinusoids and injured vessels, but in the lymphatic system they may occur in all initial lymphatics.

In quiescent regions, e.g. the pinna of the mouse ear, there are very few portions of the junctions open. These increase dramatically after injury, especially if oedema is present (Casley-Smith, 1967a, 1970a, 1972, 1973). They are also more frequent in active regions, particularly where there is much motion of tissues or frequent variations in tissue pressure, as in the intestinal villi. Dobbins and Rollins (1970) studied 254 sections through junctions in the intestinal lymphatics: 6 were open, 10 were closed, 89 were tight and 149 could not be seen clearly enough to be identified, i.e. about 6/105 (6%) were definitely shown to be open. Other workers using the same tissue (Casley-Smith, 1962; Palay & Karlin, 1959a; Papp et al, 1962) found greater numbers of open junctions. Dobbins and Rollins (1970) considered that such numbers were too small for the junctions to contribute significantly to lymphatic filling. Later on Casley-Smith (1975b), Elhay and Casley-Smith (1976) showed that they were more than sufficient. It should be pointed out that open junctions are not necessarily open all the time. They may be closed

during tissue compression, in the sense of two cells being forced close together.

Electron microscopic sections give a false impression of the actual nature of the junctions. It is only through the labour of serial sectioning that an impression is gained of how much individual junctions vary along their length. In places along their length, the complex junctions became simpler until they are just overlapping cells; then the zonulae disappear and this portion of the junction becomes open. After an interval the complexity of the junctions reappears and continues until the next open junction (Casley-Smith, 1977).

As lymphatics pass centrally, the junctions in the collecting lymphatics become less frequently open until they are all closed.

Small smooth (~ 70nm) vesicles are a prominent feature of the endothelium, but they may be less important than the junctions for lymphatic permeability. In the lymphatic endothelium they occupy 35% of the non-nuclear cytoplasmic volume, with about half of this being accounted for by their limiting membrane (Casley-Smith, 1969b).

The plasmalemma contains numerous invaginations (caveolae or pinocytotic vesicles) along both luminal and abluminal surfaces and form part of the vesicular system (Leak, 1984). They vary in depth from mild depressions to almost complete vesicles that merely touch the plasma membrane. It is believed that they represent the formation

or dissolution of endocytotic vesicles on the surface.

Phagocytic vesicles (0.1-5 μm) are found if there are particles to stimulate their formation. Their contents usually stay in the cells (Casley-Smith, 1977).

The nucleus is located in the thicker region of the endothelium and has a scalloped outline when the vessel is collapsed; however, in dilated lymphatic capillaries the nucleus has a smooth to elliptical contour.

The cytocentrum, which contains a pair of centrioles and a Golgi complex, is located in the perinuclear region.

The Golgi apparatus is a crescent shaped lamellar membranous structure, with numerous vesicles closely associated with its periphery. Two centrioles are occasionally observed in close association with the Golgi apparatus with one oriented perpendicular to the other. This arrangement is reminiscent of that for centrioles in other tissues.

The endoplasmic reticulum in the lymphatic endothelium is mainly represented by cisternae of the rough variety. In addition, ribosomes are scattered throughout the cytoplasm in single units and as polyribosomes. The small amount of endoplasmic reticulum perhaps indicates a slow or very limited turnover of protein in these cells.

Mitochondria occur throughout the perinuclear area and are also randomly distributed throughout the attenuated rim of the cytoplasm. They show no special features.

There is a small population of microtubules in the cytoplasm, usually in the vicinity of centrioles and

occasionally observed throughout other areas of the cytoplasm.

Cytoplasmic filaments are especially numerous in dermal lymphatics but also observed in lymphatic endothellium of other tissues (Casley-Smith, 1964) (Lauweryns and Baert, 1974).

G. THE CRITERIA FOR IDENTIFYING LYMPHATICS

It is difficult to distinguish initial lymphatic capillaries from blood capillaries by light microscopy. Both have an endothelium, and, in material fixed by immersion, both contain a plasma coagulum. The presence of erythrocytes does not positively exclude lymphatics, since some lymphatics, such as those draining the kidney (Hogg 1980, Hogg et al, 1982) and the spleen (Abbas, 1984) regularly contain erythrocytes. Lymphatics draining the liver were found by Samanta et al (1974) to contain erythrocytes in idiopathic portal hypertension. It may be even more difficult to distinguish very small initial lymphatics from spaces in the interstitium, particularly when stab injections of a dye or ink have been used in the attempt to identify lymphatics; ink filled spaces may closely mimic lymphatics.

Electron microscopy has enabled the recognition of a cycle of change in the initial lymphatics - filling, propulsion and emptying - and the fact that, at times, lymphatics may contain more protein than do the capillaries.

The difficulty of recognizing lymphatics, especially the initial ones, has frustrated many workers. While some such as Casley-Smith (1977) have maintained that the matter was fairly simple, that lymphatics could be recognized on the grounds of their size and contents provided that they can be seen at all, others such as Comparini and Bastianini

(1965) and Rusznyak et al (1967) have thought that the only way of identifying lymphatics with certainty is to use some form of experimental manipulation. This can be achieved by ligating big efferent lymphatics, which results in the enlargement of the small lymphatics (Rusznyak et al, 1967) or by microinjection of tracers into lymphatics. These methods have improved the recognition of lymphatic capillaries in some situations, although they have been criticized as being unphysiological.

Changing concepts of the criteria by which lymphatics are identified in E.M. material are illustrated in the following paragraphs which summarize the methods and criteria reported in some major papers.

Earlier electron microscopic studies of the lymphatics, e.g. those in the small intestine, fixed by immersion, (Palay & Karlin, 1959a), the diaphragm (French et al, 1960) and the glabrous skin of rat's penis (Fraley & Weiss, 1961) described lymphatic capillaries as extremely irregular in shape and lacking a continuous basement membrane.

Casley-Smith and Florey (1961) studied lymphatics in the ears of mice and guinea pigs, the diaphragm of mice and colons of rats by injecting 25% thorium dioxide, 5% pontamine blue and intraperitoneal injections of pelikan ink for lacunae of the diaphragmatic lymphatics. All specimens were fixed in immersion into Caulfield's solution, and the general morphological features distinguishing lymphatics were:

- usually much bigger than blood capillaries
- very irregular walls
- often rather collapsed
- the endothelium is usually slightly thicker but appears thinner because of their larger diameter
- they have no fenestrations
- paler and less electron dense
- many have edge-to-edge inter-cellular junctions, overlapping or sometimes, controversially, opened
- often there were luminal projections (blood vessels may have them too)
- the basement membrane may be tenuous or may be absent
- abluminal projections to which filaments were attached
- normally contained fewer red cells and less plasma proteins than the blood vessels

Apart from the absence of fenestrae, none of these criteria was absolute.

Leak and Burke (1966), using electron microscopy, studied the ultrastructure of lymphatic capillaries and the intimate association of numerous fine filaments with the abluminal endothelial wall in the guinea-pig's ear, after injecting them with shellac-free colloidal carbon and fixing them by immersion in 6.5% glutaraldehyde. They found that lymphatics in this area were characterized by the following:

- they have a wider lumen
- the absence of definite basement membrane

- the occurrence of numerous fine filaments along the abluminal endothelial surface
- intercellular junctions which may be overlapping or interdigitating
- many endothelial processes extend for varying distances into the lumen of the lymphatic capillaries
- numerous pinocytotic vesicles, ranging from 75 - 100Å in diameter appear along the luminal and abluminal surfaces and also throughout the cytoplasm
- larger vesicles 250 - 500Å in diameter with oval to irregular profiles were also observed within the cytoplasm

In samples injected with colloidal carbon many of the larger vesicles contain one to several carbon particles.

Fawcett et al (1969) studied the lymphatic vascular system of the interstitial tissue of the testis in guinea-pig and chinchilla by electron microscopy, after vascular perfusion of glutaraldehyde or acrolein. A physiological salt solution in a reservoir at a height of 130cm ran through a polythylene tube No. PE60 to the shaft of a 26-gauge needle slightly blunted on a hone. Then under deep ether anaesthesia the testis was delivered through a scrotal incision and a silk suture placed under the spermatic artery. The needle was introduced into the vessel and tied in with the sutures. The testis immediately blanched as the saline displaced the blood. As soon thereafter as practicable, the saline was replaced with the fixative. The perfusion was continued for 30 to

45minutes. The hardened testis was then cut into small pieces and immersed in the fixative for an additional 30 mins. After repeated rinsing in the buffer, the tissues were fixed in collidine or phosphate buffered 1% osmium tetroxide for one hour and then rapidly dehydrated through 50, 80, 95% and absolute ethanol, and embedded in Epon.

This method has been reported in detail because it constituted a most important step in improving the fixation of tissues, especially for use in electron microscopy. Moreover, washing the capillaries free of erythrocytes and plasma helped greatly in identifying the unwashed lymphatics, which remained filled with lymphoproteins precipitated by the fixative.

The criteria Fawcett adopted in identifying lymphatics were:

- lymhatics contained granular proteinous precipitate, while blood capillaries were empty
- a lumen, wider and more irregular than that of blood capillaries
- an endothelium with an extremely attenuated cytoplasm, except in the nuclear region
- a discontinuous basement membrane
- endothelial cell junctions generally lacking the so-called tight junctions that have been described for some blood capillaries. In the testis Fawcett et al (1969) demonstrated large gaps between endothelial cells of lymphatics

- anchoring filaments which terminate on the vessel wall and serve to connect the vessel wall to the adjoining connective tissue.

Fawcett's method and criteria have become the standard tool for the study of lymphatics.

It is important to emphasize again that the structure of lymphatics, as a part of the microcirculation, is an integral part of any particular tissue, and regional variations are to be expected, such as the wide open junctions in lymphatic sinusoids in the testis and the lacunae in the lymphatics of the diaphragm.

H. THE DEEP INTRAHEPATIC LYMPHATICS

Well defined lymphatics can be demonstrated by light microscopy in large portal areas of the liver, but not in smaller portal areas. Schatzki (1978) studied hepatic lymphatics in female Sprague-Dawley rats weighing about 350gm. The animals were anaesthetised with diethyl ether, supplemented by intraperitoneal injections of pentothal, the portal vein was perfused with 2% glutaraldehyde. The inferior vena cava was incised to create an outlet for the perfusate. The liver hardened immediately. The liver and porta hepatis were removed en bloc and kept in the fixative for 4 hours followed by overnight washing with 0.15 molar sodium cacodylate buffer pH 7.4. Areas of liver in continuity with the porta hepatis were trimmed, coated with 7% agar, chilled and cut into 225 μm slices. The slices were floated in buffer and trimmed under a dissecting microscope with a razor blade into blocks showing continuity of liver parenchyma and fibrous tissue of the porta hepatis. The blocks were then post fixed for 1 hour in 1% osmium tetroxide in water; dehydrated with increasing concentrations of alcohol, treated with propylene oxide and embedded in Epon. Sections cut with a diamond knife, double stained with uranyl acetate and lead citrate, mounted on uncoated grids and studied in an electron microscope.

Schatzki (1978) found that lymphatics in the liver were not identified away from the fibrous tissue of the

porta hepatis. "Cleft-like" spaces were noted at times in the space of Mall or surrounding hepatic veins. These might be mistaken for lymphatics by light microscopy, but they were not lined by endothelium and therefore did not represent lymphatics. Schatzki also found that in areas representing junctions of the portal canals and liver parenchyma numerous dilated channels with a very thin layer of endothelium were seen. These contrasted with the very much smaller blood capillaries lined by a much thicker endothelium. The lining endothelium of the rather dilated lymphatic vessels appeared quite thin, about 375nm. In general, no basement membrane was seen, but in a few areas there was granular basement membrane material. The endothelium, which generally abutted directly on collagen, contained many vesicles, some mitochondria, glycogen particles and occasional lysosomes; anchoring filaments were found to be scarce. Endothelial cells were joined together by a variety of junctions, including desmosome-like structures, maculae adherentes, zonulae adherentes and deep infolding of adjacent cells with maculae adherentes. The space of Mall appeared usually as a potential space, filled with collagen fibres and delineated by various cell processes into "spaces mimicking lymphatic vessels". These characteristics of hepatic lymphatics described by Shatzki conform with those of lymphatics elsewhere.

It is commonly said that, in general, the lymphatic system begins "blindly", either in the form of a network or

as a saccular outpocketing (McCallum, 1903). This suggestion has been supported for liver lymphatics by Yamamoto and Phillips (1986). They studied the livers of five New Zealand male rabbits. The liver was first perfused with Ringer's solution via the hepatic artery at a pressure of 150cm of water. Then the resin was injected into the bile duct at a pressure less than 16mm Hg which was sufficient to cause resin to leak into the interstitium, then to be picked up by lymphatics. Casts produced were studied by scanning electron microscopy. Some of the "blind ends" found were connected with structures of the surrounding interstitial tissue; these connections suggested that there might be open junctions in the vascular wall, although they might equally be artifacts, in view of the methods used to demonstrate them.

Castenholz (1984) supported the view of "blind ends" of lymphatics in the rat's tongue, by SEM of resin casts.

The crucial question is "where are these "blind" endings or beginnings of the lymphatics situated?"

Most people who have studied this problem confined their attention to the classical view of lymphatic channels as those lined by endothelium. At the site of origin of the hepatic lymph, i.e. Disse's space, none were found. Disse (1890) when describing the pericapillary (sinusoid) cavities within the liver acini thought that they were the beginning of the hepatic lymphatics, but he expressed his reservations because of lack of lymphatic endothelium. The current belief is that in the liver, the lymphatic

capillaries do not penetrate the lobules as do the blood capillaries, but are arranged around the periphery, i.e. in the portal tracts.

Comparini (1969) studied a group of 20 human livers from different ages, with special emphasis on young subjects (2 and 3 year old children). Samples were fixed by immersion in buffered formaldehyde, embedded in paraffin and serial sections, 8 μm thick, were studied by light microscopy. He found that by following backwards the lymphatics in the serial sections right down to their origin, he was able to ascertain that they started in the areolar tissue of the portal tracts, close to the space of Mall and that there were no direct channels connecting the intralobular tissue spaces and the lymphatics proper.

The next question is "at which level of portal tracts do lymphatics start to appear?"

Most reports and textbook accounts give the impression that lymphatics can be found in any portal tract, regardless of its size and therefore of its situation along the branching tree of connective tissue which accompanies the portal tracts. A recent report by Yamamato and Phillips (1986) on lymphatic casts in rabbits' liver showed that lymphatics extend as far as the terminal portal tracts. Schatzki (1978) denied their existence in "smaller" portal tracts (no dimensions were given) in the rat's liver.

What is the vascular pattern of intrahepatic lymphatics?

Comparini and Bastianini (1965) studied the vascular pattern by Halpern's graphic method of reconstruction. These reconstructions were made on the liver of 2 and 3 year old human children because the parenchymal lymphatic pattern, unlike that in the grown up and aged subjects, was clearly and constantly visible and easily traceable. The material was fixed by immersion, embedded in wax and strictly serial histological sections were prepared and then stained. This method, they claimed gave an accurate picture of the lymphatic pattern.

The first reconstruction showed very small interlobular vessels. In the first section of the series the diameter of the portal vein was 132 μm. The lymphatics had a plexiform pattern, sinuous and laminar near the artery. This arrangement round the artery was characteristic. Reconstructions of more peripheral tracts also showed a plexiform pattern of lymphatics.

The second and the third reconstructions made from tissue nearer the porta hepatis showed the interlobular vessels of the liver just slightly larger than in the first reconstruction but this disposition of the simple formations and their relations were more complex. In the first section of the series the portal vein diameter was 155 μm. At this level, the complexity of the disposition of the single vascular formations and their relations were far more remarkable than further peripherally. The

lymphatic pattern was no longer strictly limited to spaces near the artery but it crept in between the arterial, venous and biliary branches, ensheathing all the structures in a delicate network with large irregular meshes. However, it still appeared denser round the larger arterial branch, which came to be almost entirely hidden. Thus even here the disposition formerly noticed at the more peripheral site was maintained, and the lymphatic network seemed to cover all the components of the interlobular space, matching the complexity of the vascular pattern. A slender perivenous lymphatic arrangement began to be noticeable, whilst no specific relationship could yet be detected between the lymphatics and the biliary ducts. Nevertheless, up to this moment it still seemed advisable to speak of a plexiform lymphatic pattern rather than of real lymphatic vessels, even though well defined vessels sometimes appeared. A peculiar feature of the lymphatics was the irregularity of their diameter, very slender tracts often alternating with wide dilatations. This irregularity of the contours was remarkable everywhere and was frequently interspersed with diverticular formations and blind appendices. All this contributed to the formation of a vasal pattern whose morphological features were unmistakable.

In reconstruction in which the first sectional profile of the portal vein was about 820 μm in diameter, Comparini and Bastianini (1965) also found a remarkable

complexity of the vasal pattern. Venous, arterial and biliary branches crossed each other in many ways, interlaced with the nervous branches and the lymphatic pattern to form an extremely intricate pattern round the larger gauged vessels. The lymphatics still selectively covered a large part of the arterial pattern, even where the vasal pattern was more complex. At this level, a peribiliary lymph pattern also began to be visible, although at first rather modest. Sometimes lymphatics near the small bile ducts were found directly beneath the epithelium, suggesting a simple transfer of biliary material to the lymph vessels.

In the midst of this complexity, Comparini and Bastianini found that, here and there, well defined lymph vessels might show up, only to disappear again into an intricate plexiform arrangement after a short run.

The importance of the lymphatic plexus was not the same all over the areas of the interlobular space, as there were tracts where the lymphatics were extremely dense and tracts where they were poorer.

Comparini (1969) studied the lymphatics that follow the hepatic vein and called them "central lymphatics". These lymphatics began in the same way, i.e. outside the lobule, but they were never so numerous as the deep lymphatics in the portal tracts. The arrangement of the lymphatics around the hepatic vein was always relatively simple. The disproportion between the capacity of the two different sections of the deep lymphatics of the liver

(portal and central lymphatics) was remarkable. Whilst the lymphatics following the portal veins were numerous and grew richer as they proceeded towards the porta hepatis, the lymphatic "satellites" of the hepatic vein were instead only few, even around the larger venous branches.

Comparini (1969) also found that the lymphatics in the portal tracts were mainly found in proximity to the arteries. This relation became gradually more evident following the branches of the hepatic artery right into the larger portal tracts. This relationship was so constantly repeated, it might well be defined as "characteristic". The relationships between the lymphatics and the biliary ducts and veins were no doubt of minor importance. In the porta hepatis, the gradual prevalence of the venous pattern caused the larger lymphatics to adhere also to the walls of the veins.

Yamamato and Phillips (1986) studied the three dimensional model of intrahepatic lymphatics in the rabbit by injecting resin into the bile duct under enough pressure to make it leak into the interstitium and then drained by the lymphatics. They found that the lymphatics formed a rich network around the portal vessels and bile ducts. This plexus was mainly composed of straight and anastomosing short side branches which formed frequent communications between the straight channels. The straight channels ran parallel to the portal tracts and the side channels communicated with them. The number of the

straight channels varied from 2-3, to 6-10 in a big portal tract. Yamamato and Phillip's description seemed much less complex than that of Comparini and Bastianini and there was less emphasis on describing the deep lymphatics as plexiform.

Fujikawa and Magaris (1975) studied the lymphatic capillaries in Glisson's capsule of the rabbit's liver by electron microscopy and found that large lymphatics showed a thin endothelium and a wide lumen, while lymphatic capillaries had a thick endothelium and a very narrow lumen.

The "thin" endothelial layer was 2700Å in thickness. The cells contained numerous pinocytotic vesicles and caveolae, the largest one measured 1300Å in diameter. The endothelial junctions were demonstrated as a simple interdigitation, in which usually two small desmosome-like structures were detectable. The intercellular gaps were irregularly enlarged except for the site of junction. The basal lamina was completely absent. In the sub-endothelial area, not only fine filaments measuring about 100Å in diameter but also large bundles of collagen surrounded by the cell processes of the fibroblasts, appeared to be in close association with the abluminal surface.

Between the deepest part of Glisson's capsule and the sub-capsular limiting plate, a large number of lymphatic capillaries ran in all directions, anastomosing to form a network. Comparini (1969) also found that as they traversed the middle layer of the capsule, they retained

their capillary structure but had a wider lumen. In the superficial layer, the morphology and the distribution of the lymphatic varied. At this level, besides the delicate lymph capillaries there were many ample collecting vessels with valves.

Communications between the deep lymphatics of the liver and the capsular lymphatics are controversial, although many have claimed their presence. Comparini (1969) described in the human liver two possible types of anastomosis:

1. At the capillary level, between the lymphatic capillary network in the deeper layer of the capsule and the lymph vessels coming from the sub-capsular liver parenchyma.
2. At the level of the large superficial collecting vessels, between the large capsular lymphatics and the parenchymal lymph vessels in the sheaths of Glisson's capsule.

Szabo et al (1975) cannulated the right lymph trunk in the neck and the thoracic duct and ligated the cystic and the common bile duct of mongrel dogs. They found practically no anastomoses between the lymphatics of liver capsule and the deep liver lymphatics; very few, if any, connections could be demonstrated, even in the presence of a substantial fluid load or/and occlusion of the major part of the lymphatics draining the liver parenchyma at the porta hepatis.

The plexiform arrangement of the deep hepatic lymphatics is not a unique feature of the liver. Many other tissues have a similar pattern. Castenholz (1984) studied the sub-epithelial lymphatics of the rat's tongue by scanning electron microscopy of interstitially fixed tissue and corrosion casts. A plexus-like arrangement was demonstrated. Similar patterns have also been described in the tongues of other species.

A quite different system of peritubular lymphatic spaces, however, has been described in testicular tissue indicating the organ specific pattern of lymphatics in the internal organs (Clark, 1976).

The literature describing testicular lymphatics is contradictory. It is generally agreed that there is a rich plexus of subserosal lymph vessels in the tunica albuginea and that, in large mammals, these extend into the septula. There is no unanimity, however, as to the existence of lymph vessels in the interstitial tissue.

Fawcett et al (1969) described spaces delineated by attenuated endothelial cells with slender elongated nuclei. They suggested that the spaces bounded by such an endothelium were so variable in calibre and irregular in shape that they would be more accurately described as "sinusoids" than as typical lymphatic capillaries. However, vessels described for the testis conform in most respects to the general criteria in other tissues and undoubtedly constitute the primary or initial elements of a lymphatic system that drain the parenchyma of the testis.

I. FROM SPACE OF DISSE TO PORTAL LYMPHATIC: THEORIES

The route followed by lymph from its origin in the space of Disse to the point where it is picked up by lymphatic of the portal tracts has led to considerable controversy.

Disse (1890), who first described the perisinusoidal space, thought that it was the starting point of hepatic lymphatics, but he hesitated to call it a lymph space because it lacked a complete endothelial wall. This hesitation is still felt by many modern workers, most of whom feel that it is not acceptable to call a structure "a lymphatic" unless it has a typical endothelial lining.

Elias (1949) suggested that typical lymph vessels accompanied the portal vein, hepatic artery and bile duct forming networks in the interlobular spaces and also sent occasional, rather straight spurs into the interior of the lobule. He thought that these spurs were located in the delicate connective tissue surrounding the arteries, venules and bile ducts. The spurs then communicated with the space of Disse which he considered as a "virtual lymphatic lacuna".

Schatzki (1978) stressed the fact that there were no direct communications between lymphatics and the spaces of Disse and Mall.

Henrikson et al (1984),when reviewing the blood-lymph barrier in the liver, thought it was probable that the perisinusoidal space communicates more or less freely with

the spaces in the connective tissue of the portal tracts, around the entry of terminal branches of portal vein or hepatic artery into the sinusoid through the limiting plate.

Leon Weiss (1983) presented what he considered as the most generally accepted view, which contended that the perisinusoidal space of Disse is the site of origin of lymph and that, from this, fluid enters the periportal tissue space of Mall, from which it subsequently diffused to the lymphatic capillaries.

Leeson and Leeson (1985) mentioned only that the fluid formed in Disse's space discharged into interstitial spaces of the connective tissue and then passed indirectly into lymphatic capillaries.

Bloom and Fawcett (1986) presented the view that fluid in Disse's space flowed towards the periphery of the lobule and then percolated into the extracellular spaces around the interlobular twigs of the bile ducts and the portal vein. It thus became the tissue fluid of portal tracts which was drained by lymphatic capillaries.

J. THE DEVELOPMENT OF LYMPHATICS

The developmental origin of the lymphatics has been controversial since the earliest studies in the nineteenth century and still remains so. In 1880, Budge thought that there were two lymphatic systems in the chick embryo. The first, or primitive one, was present in the three-day chick. He thought that, the false amnion and coelom being continuous, there were ducts within the body wall connected with the coelom, analogous to those of the area vasculosa which he had injected from the false amnion. The ducts within the body lying along the dorsal line became pinched off from the coelom and united to form a thoracic duct. With the thoracic duct began the second or permanent lymphatic system which he had injected along the arteries in nine-day chicks. Although this idea of relating the lymphatic system to the serous cavities has proved to be incorrect, it led to the discovery of the true origin, for it was by injecting into the side of the neck in early embryos in the hope of reaching what was called "Budge's space" behind the aorta that the cervical lymph heart was injected and lymph hearts give the key to all the superficial lymphatics.

Ranvier published a long series of articles between 1895 and 1897 on the development of the lymphatic system in the frog and in pig embryos. From his injections of lymphatic capillaries in the skin and intestinal villi of pig embryos, he made the important discovery that the

lymphatics within the capillary plexus grew by budding. He suggested that from the side of a duct appeared a bud which was at first solid, but soon developed a lumen which became larger and advanced until it reached a second one into which it opened, by a process of absorption of the endothelium. At the point of junction a valve developed.

Gulland (1894) stated that the fluid of the blood filtered into the tissue spaces which gradually dilated and flowed together to form the first lymph duct, the wall of which was made from the connective tissue which became compressed around it.

Sala (1900) thought that the first trace of a lymphatic system was the appearance of lymph hearts or spaces in the mesenchyme just lateral to the caudal myotomes. These spaces flowed together and joined the thoracic duct, which formed as two cords of mesenchymal cells extending from the level of the thyroid gland to the level of coeliac axis.

Clark (1912) tried to obtain a complete history of the growing lymphatic sprout and of the growing mesenchymal cells individually and side by side, to determine whether the lymphatics grew by the addition of mesenchyme cells or by sprouting of pre-exisiting lymphatic endothelial cells. He found, in the transparent expansion of the tail of living frog larvae, that mesenchymal cells sent out processes, some fine, some coarser, from the thick central perinuclear area. These branched mesenchyme cells shifted

their position by ameboid movement. Fine processes were sent out from the main body of the cell, which might be quite temporary and might be withdrawn subsequently. The rate of such movement was variable in individual cells and it was pricipally towards the free margin of the fin. Another feature of these cells was that they were seen to divide.

As for the growing lymphatics, two distinct processes were seen to occur: cell division and cell migration. The sprout of cytoplasm so formed grew into the tissues as a solid mass in which a lumen appeared later on, then the nucleus moved in. So it appeared to Clark (1912) that each of the two tissues had a characteristic and independent life, each maintained its independence throughout and a mesenchymal cell was not transformed into lymphatic endothelium.

Clark and Clark (1933) again supported this view in a subsequent study on the living lymphatic vessels in transparent chambers in the rabbit's ear. By prolonged microscopic studies day by day observations of the growing sprout of the new lymphatic proved to be similar to that in the transparent tails of amphibian larvae.

Although this view has prevailed for some time now perhaps, in part at least, because of its resemblance to the mode of growth of blood capillaries, there are reports supporting the contrary view.

Klika et al (1972) studied the development and growth of lymphatics in the epicardium of the chick embryo

from the 9th to the 14th day of incubation. On these days the first lymph vessels reach the embryonic heart, spread over the surface of the ventricular myocardium in the subepicardial mesenchyme and acquire their first valves. A retrograde injection of yellow latex particles in 1.5% gelatin, into the efferent lymphatic trunk was used to prevent the cardiac lymph bed from collapsing. The coronary vascular bed was injected with black latex particles to avoid any confusion with the lymphatics. After injection the heart was dissected out and prefixed for 10 minutes in glutaraldehyde. Using a fine pair of scissors, narrow strips of tissue in which one would most likely see lymph vessels were taken, and processed for electron microscopy. They found that the walls of larger lymph vessels made numerous contacts with the surrounding mesenchyme. This intimate relationship, in contrast with Clark's views, was manifested in two ways, especially in small lymphatics.

1. it was difficult to distinguish morphologically between the two tissues
2. some lymphatics had wide open communications directly with the intercellular spaces of the mesenchyme, and it was evident that the mesenchymal cells can participate directly in the demarcation of the lumen of a lymphatic.

In the case of smaller lymphatics, Klika et al (1972) found this participation to be more evident. In fact, at

the site of origin of lymphatics, at the base of the heart, mesenchymal cells directly circumscribed the lumen of the lymphatic capillary and formed an integral part of its wall. These thin mesenchymal cells did not exist in places still lacking lymphatics. At the site of origin of lymphatic capillaries, the distribution and density of mesenchymal cells was not in any way influenced by the presence of lymphatics. This was indirectly due to incorporation of the intercellular spaces into the lymphatic system, which grew from the base of the heart towards the apex, without an intermediate change occurring in their size.

K. TOPOGRAPHY OF EXTRAHEPATIC LYMPHATICS

The bulk of the hepatic lymph coming from the interior of the liver drains to the nodes at the porta hepatis.

Ritchie et al (1959), and Szabo et al (1975) found in dogs that this route accounts for about 80% of hepatic lymph and that when this route was ligated the thoracic duct lymph flow decreased and the amount of transported proteins dropped by 70%. Comparini (1969) found that in man this route is the primary path of drainage of hepatic lymph.

Shatzki (1978) found in rats that 8-10 large efferent lymphatics grouped in bundles and containing several valves, descended along the course of the hepatic artery, to drain into nodes at the porta hepatis. These nodes have received different names. Yoffey and Courtice (1970) called them "hepatic" as did Popper and Schaffner (1957), while Tilney (1971) called them "portal" nodes.

Intercommunications with lymphatics from neighbouring organs have been reported. Rotenberg (1949) found intercommunications between the efferent lymphatics of the liver and those of the stomach in the regional nodes of the two organs.

A minority of the intrahepatic lymphatics follow the tributaries of the hepatic veins, then collect around the proximal portion of the inferior vena cava and accompany it through its diaphragmatic hiatus and enter the thoracic

cavity. These lymphatics were called "the ascending" lymphatics by Yoffey and Courtice (1970). Most workers agreed that they drain to lymph nodes around the terminal segment of the inferior vena cava. Rusznyak et al (1960) reported connections with the anterior mediastinal lymph nodes and that these lymph vessels inter-communicate with those of the diaphragm and sub-diaphragmatic pleura. Kultner (1950) reported connections with the supraclavicular lymph nodes, especially on the right side.

MATERIALS AND METHODS

A. METHODS OF INVESTIGATING LYMPHATICS

REVIEW OF LITERATURE

The discovery of lymphatics by Aselli (1622) was a chance observation, based on the fact that the intestinal lymphatics are filled with fat droplets in a recently fed animal, and appear milky white.

Routine histological preparations seldom reveal the lymphatic vessels. As a result, many investigators have looked for ways of making them visible.

Identification of lymphatics is made much easier if they are dilated by prior ligation of the efferent lymphatics or the thoracic duct in the living animal. Rudbeck (1653) isolated the larger lymph trunks, ligated them, thereby causing dilatation of their tributaries and making them more readily visible.

Major advances in the study of lymphatic trunks came from their injection with mercury, a method due principally to William Hunter; the beauty of some of his preparations remains unsurpassed even today (Fig. 1). In the nineteenth century, other injection masses were introduced, including Berlin blue, Gerota's blue mass, and carmine gelatin. More recently, the study of lymphatic capillaries has been extended by the use of fine particulate suspensions such as India ink, and colloidal dyes, including Evan's blue and Pontamine Sky Blue. Most recently, scanning electron microscopy of lymphatics injected with low viscosity

polymers has produced beautiful three dimensional pictures.

The demonstration of lymphatics by injection has been criticised because of the risk of artifactual appearances. When the dye is injected retrogradely into a lymphatic trunk, pressure is required to overcome the resistance of the valves, running the risk of rupture of the vessel. When injections are made, not directly into a lymphatic trunk but blindly into the tissue, (the "stab" injection technique), in the hope of entering lymphatic capillaries and filling the regional vessel by antegrade flow, the dye frequently enters torn lymphatic capillaries, to give extensive filling of the local capillary plexus, but artifacts may again be produced by the spread of the dye in streaks through the tissue, mimicking lymphatic capillaries.

A "double injection" technique of contrasting colours was used by McLean and Scothorne (1970) to distinguish between blood capillaries and lymphatics. In their experiment on lymphatics of the rabbit's uterus they injected india ink retrogradely into lymphatics after filling the blood vessels by yellow lead chromate.

The study of lymphatics in the living animal, particularly by the Clarks (1933), using the rabbit's ear chamber has given much information about their permeability, growth and repair, and contractility, but has not been very helpful in resolving problems of the initial lymphatics.

Our present understanding of lymphatic capillaries depends largely upon the use of electron microscopy, e.g. the work of Palay and Karlin (1959) on the intestinal lymphatics, French et al (1960) on diaphragmatic lymphatics. The structure of normal small lymphatics, in various tissues, was studied in detail by Florey and Weiss (1961) and Casley-Smith and Fraley (1961) by use of the electron microscope. Leak and Burke (1966) described in detail the anchoring filaments.

One of the recent most satisfactory methods of demonstrating lymphatics is that of vascular perfusion by Fawcett et al (1969) already described in detail in the Introduction. Vascular perfusion has the advantages of producing rapid fixation of tissues so that lymphatics are fixed in their natural state and of allowing the use of the same material for light and electron microscopy.

B. METHODS OF STUDY OF THE BASIC MATERIAL

The livers of adult rats, weighing 200-240gms, of an inbred Albino-Swiss strain maintained in the Department, were used in this study.

The animal was killed by an over-dose of anaesthetic ether, pinned to a cork board, the abdomen and chest opened and the heart exposed. Mammalian Ringer's solution containing 0.4% lignocaine chloride - as a vasodilator - was infused through a 19G needle in the left ventricle to wash out the blood from the vascular system. This procedure usually took about one minute. A small opening was made in the right atrium to allow outflow of the blood and the perfusate. This perfusion of the hepatic vasculature was carried out through the left ventricle and the systemic circulation rather than through the portal vein because it was felt that there was less chance of overloading the hepatic circulation and causing oedema (Silberberg, 1972).

When vascular wash-out was complete, as judged by blanching of the liver and kidneys, the fixative was introduced by switching the two-way valve on the infusion set.

After preliminary trials of various concentrations of glutaraldehyde (2.5, 3.0, 3.5, 4.0, 4.5, and 5.0%), a 3% solution in Millonig's phosphate buffer, pH 7.2-7.4, osmolality 550 mosm/l, was chosen as giving the best results. About 800ml of the chilled fixative was perfused

over a period of thirty to forty-five minutes, under a gravitational pressure of 130cm of solution (Fawcett, 1969). This pressure was used in the light of the findings of Frenzel et al (1977) that different perfusion pressures have various adverse effects on the sinusoidal endothelium fenestrations and on the hepatocytes.

Perfusion was judged to be satisfactory when there was prompt blanching of the liver, kidneys and mesenteries, when the perfusate leaving the right atrium was clear, and when there was rapid hardening and tanning of the tissues.

The liver was then removed and immersed intact in fresh fixative for a further 24 hrs, after which it was rinsed in buffer several times. Up to this stage the liver was handled as little as possible to avoid artifact. It was then cut into small pieces with fresh sharp blades and left again in buffer overnight. The pieces were then post fixed in 1% osmium tetroxide in phosphate buffer for two hours, followed by rinsing in several changes of buffer solution. Dehydration in an ascending series of ethanols starting with 70% and reaching 100% (four changes of 100%) was carried out before embedding in Spurr's resin.

Blocks were trimmed and semi-thin sections of 1-1.5μm thickness in interrupted series through each block were taken.

Sections are stained by one of a variety of methods: Azur II, methyl green-pyronin, silver nitrate, light green, P.A.S., and H & E. Stained sections were studied by the

light microscope; selected areas of interest in each block were cut in thin sections of 60-80 nm, mounted on uncoated 200 mesh grid and then double stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a JOEL 100S electron microscope.

In all, the livers of 12 rats were used to study the normal appearances, without any experimental manipulation. This material provided the basis for the initial hypothesis.

The recognition of very small lymphatics under the light microscope was based on the criteria already outlined in the Introduction; doubtful vessels were checked by transmission electron microscopy.

C. SCANNING ELECTRON MICROSCOPY

Liver pieces, fixed by perfusing 3% glutaraldehyde rinsed in buffer, were sliced into 100 μm slices by a vibratome, then osmicated and rinsed in buffer several times. They were then dehydrated through ascending grades of ethanol and three changes of amyl acetate, critical-point dried and coated with gold, and finally screened in the scanning electron microscope (JEOL JSM T300).

Some specimens, after dehydration, were embedded in wax and a few 4 μm thick sections were taken. The last section cut was mounted on a glass slide and stained with H & E while the rest of the block was dewaxed by frequent changes of xylene over a 24 hour period. The block was then processed through the rest of the procedure described above. The cut surface was oriented for viewing under SEM.

The stained section was found to be very useful for orientation of the scanning image of the cut surface of the block.

Livers from six rats were used for this experiment.

D. TRACERS

After studying the basic material a hypothesis was formulated, principally on the basis of the presence of precipitated protein in the interstitial fluid and in the lymph.

The hypothesis was then tested by the use of various tracers which, when introduced either directly or indirectly into the circulating blood, find their way into the interstitial fluid and are taken up by lymphatics where their presence can be detected by electron microscopy.

Two types of tracers were used:

1. Natural tracers

   These are chylomicrons and lipoproteins which are normally present in the body; we simply modified the diet of the animals to enhance them.

2. Foreign tracers

   The second category, foreign tracers, is a group of substances not normally present in the body but which are introduced intravenously. The following were used:

   1 - Ferritin

   2 - Pontamine sky blue

   3 - Monastral blue

Special care was taken to inject the minimal dose of these tracers that can serve our purpose in this study, and close watch was kept for any possible ill effect of the injected material.

A brief account is now given of the background information bearing on the use of tracers introduced into the circulation.

When particulate materials - as tracers - enter the blood stream they trigger certain mechanisms which lead eventually to their sequestration from the blood stream by the macrophage system.

When particles make contact with the blood they are coated by a colourless film. Similarly coated particles adhere loosely to each other, so that they may separate if exposed to some of the shearing forces of the circulation (Bloch & McCuskey, 1977). Not all particles aggregate the moment they make contact with the blood. In the case of carbon particles, Bloch and McCuskey (1977) found that the coating film is made of fibrin. This coating film can be prevented by making the blood uncoagulable by injecting heparin prior to the injection of the tracers, but aggregates reappeared some thirty minutes later.

Biozzi et al (1953), Benacerraf et al (1959), Cotran (1965), Bloch and McCuskey (1977) and De Bruyn et al (1983) have all studied the clearance of particulate materials by macrophages. When coated particles, formed in the extrahepatic parts of the circulation, entered the liver sinusoid and touched its wall they adhered; in contrast when such material was prevented from forming the coat by the introduction of heparin or silicon prior to the injection of the particles, these particles did not adhere. The particles were phagocytized after adherence regardless

of their size. The rate of this process appeared to be related i) to the quantity of the particulate material introduced, ii) to the rate of injection and iii) to the degree of saturation of the cells by the phagocytized particles. As the macrophages adapted themselves rapidly after repeated injections and changed their activity towards the particles according to the new level in the blood, ingested particles started to appear in other locations, particularly the endothelium of renal glomeruli and of other blood vessels. The total mass of these endocytic endothelial cells is large and collectively must be considered to be an important mechanism for the sequestration of blood-borne particulates including proteins, and it appears very difficult to saturate such pool.

Ferritin:

Ferritin has been extensively used as a tracer, particularly in studying the permeability of blood capillaries (see e.g. Farquhar et al, 1961). De Bruyn et al (1983) used ferritin to study the endocytosis by bristle-coated pits of protein tracers and their intracellular transport in the endothelium lining the sinusoids of the liver. In the present study, the ferritin used was of the same type - NaF-4503 type 1 from horse spleen (Sigma) in 0.15 mol/l sodium chloride, sterilized and filtered. It was injected in the same dosage of 0.25mg/100gm animal weight, into the tail vein.

Four rats were injected and killed after two minutes. Ferritin granules measure 80-100Å (Leak & Burke, 1968). They are electron dense and are seen as fine black dots in the electron microscope. These four animals were perfused after death by intracardiac injection, as in the study of normal control animals already described.

Electron microscopy of this material revealed no ferritin in the sinusoidal lumen or in the space of Disse. It seemed possible that the process of perfusion had washed away the ferritin. A fifth animal was therefore injected intravenously with ferritin, as before, killed by decapitation, and the liver fixed by <u>immersion</u> in 3% glutaraldehyde.

<u>Pontamine sky blue (P.S.B.)</u>:

P.S.B. has been extensively used to study lymphatics as well as capillary permeability [see e.g. Gurr (1960), McMaster and Parsons (1938, 1939, 1950), Psychoyos (1971)]. P.S.B. 6BX has a molecular weight of 992,832; its chemical formula is $C_{34}H_{24}N_6O_{16}S_4Na_4$, its approximate percentage solubility at 15°C in water is 4.75. It is an acid dye of the diazo series and binds to albumin when injected intravenously (Psychoyos, 1971).

A 3% solution of the dye in saline was prepared and injected via the tail vein at a dosage of 0.5ml/100gm body weight. Animals were killed at various intervals after injection to determine the most suitable time to find dye particles in the liver and its lymphatics. Two rats were killed at intervals of 2,4,6,8,10 and 12 minutes after

injection, giving a total of 12 rats. The eight minute pair proved to be most suitable.

P.S.B. proved to be safe in the recommended dose and was not taken up by any cell. Particles were uniformly rounded, under E.M., slightly variable in size and uniformly electron dense.

Monastral blue (M.B.):

This is a phthalocyanine colloidal blue pigment supplied by Sigma as a 3% solution in 0.85% sodium chloride. Its pH was 9 to 10.

Majno and Palade (1961) used monastral blue in their series of studies of capillary permeability after histamine injection and after injury. They presented it as a suitable alternative to certain lacquer-free carbon particle suspensions (Pelikan), no longer commercially available.

It is non-toxic in the recommended dose of 0.1 - 0.2 ml/100gms of animal weight. The particles vary in shape; some are rod-shaped, others are amorphous, with variable electron density.

Four rats were injected intravenously with the recommended dose and killed 10 minutes later. The liver was then processed in the same way as that of the control material.

Chylomicrons:

The characteristics of chylomicrons have been extensively studied. They are normally produced by the

intestinal epithelium from absorbed dietary fat. The fatty compounds are coated with a phospholipid material. About 90% is drained by intestinal lacteals into the thoracic duct and thence into the circulation. The remaining 10% is drained by the portal vein to the liver. Chylomicrons therefore reach the intrahepatic circulation directly and indirectly.

They measure from 1000Å to 3500Å, and small ones coalesce to form larger ones. Those seen in the liver circulation are comparable to those in the intestine.

Chylomicrons look different when fixed, embedded and stained in different materials (Casley-Smith, 1962).

Two adult rats were fasted overnight and fed next morning with bread liberally soaked in lard. Four hours later they were sacrificed and processed for electron microscopy in the usual way. In addition, 6 young rats which had recently suckled were used: two were less than 24 hours old, two were one week old and two were two weeks old. In all, 8 rats were used in this experiment.

Lipoproteins:

There are very few references to the use of lipoproteins as tracers [see e.g. Hayes and Hewitt (1957].

Six two weeks old rats with stomachs full of milk were used. This material yielded few chylomicrons, but abundant lipoproteins which varied in size from 150Å to 800Å, and were irregular in shape and density (Silberberg, 1972).

E. DEVELOPMENT OF INTRINSIC HEPATIC LYMPHATICS

The intrinsic lymphatics were studied in the livers of groups of Albino Swiss rats aged 24 hours, 1, 2 and 3 weeks and adult. The material was fixed by the same procedure of arterial perfusion, after vascular wash out, but using a smaller gauged needle (24G) to minimize the risk of overloading the circulation. Two animals were used in each group (total of 10 rats), and five blocks of liver tissue from each animal were embedded in Spurr's resin and studied by optical microscopy and transmission electron microscopy. The total number of semi-thin sections from each group was 10. For the newborn rats 18 sections were studied because there were fewer portal tracts. In each semi-thin section, all portal tracts were examined to determine the presence, or absence, of sectional profiles of lymphatics. The diameter of the associated portal vein branch was measured by use of a camera lucida.

F. EXTRINSIC HEPATIC LYMPHATICS

25 adult Albino-Swiss rats, weighing an average of 230 gms, were used.

A 3% aqueous solution of pontamine sky blue was used, in a dose of 0.5ml/100gm body weight (Psychoyos, 1971), injected in the tail vein after light ether anaesthesia. After about 7 minutes the rat was killed by an overdose of anaesthetic ether. Death occurred in about three minutes, so the time interval between the injection of the dye and the death of the animal was approximately 10 minutes, during which time the dye circulated throughout the body, filtered through the capillaries and was taken up by the lymphatics. The rat was then opened and perfused through a 19G needle introduced into the left ventricle, with mammalian Ringer's solution containing 2% lignocaine as a vasodilator; an outlet for the blood and the perfusate was made in the right atrium. Washout of the blood usually took 2 minutes, at the end of which the blood vessels appeared either whitish or very faintly bluish in contrast to the lymphatics and lymph nodes which were dark blue and could be readily identified under a dissecting microscope.

G. PLASMA CELLS

Six Albino-Swiss rats were used for this study. They were fixed by perfusion of 3% glutaraldehyde after vascular wash out. A piece of the liver was taken and embedded in wax, six sections 5-6 μm thick, 15 sections apart were taken from each animal. The sections were stained with methyl green-pyronin to identify plasma cells. Plasma cells were counted in each of the total of 36 sections. The average number of plasma cells in the 6 sections from each animal was calculated, then the average area of the 6 sections was calculated using the MOP-AM02. The result was expressed as number of plasma cells/mm^2.

Plasma cells were identified by their intensely pyroninophilic cytoplasm. Mature cells showed the characteristic eccentric "cart wheel" nucleus and negative golgi image. Immature plasma cells (plasmablasts) had large pale nuclei with more prominent nucleoli, and abundant "foamy" pyroninophilic cytoplasm.

RESULTS

The strategy adopted in this investigation was determined by the following considerations.

i) that most of the hepatic lymph derives ultimately from protein-rich interstitial fluid in the space of Disse;

ii) that typical, endothelially-lined lymphatics are found in the connective tissue of portal tracts (although, as we shall see, not in all of them), and,

iii) that an exhaustive review of the literature had provided no clear consensus about the route which interstitial fluid follows in its passage from space of Disse to a lymphatic vessel.

It seemed likely that the structure of the perilobular limiting plate might provide the clue to the first part, at least, of this controversial route, since the space of Disse lies, almost by definition, within the lobule and the portal tract, equally by definition, immediately outside it. The first question to which an answer was sought, therefore, was this one:

Are there gaps in the perilobular limiting plate?

It is, of course, well known that the perilobular limiting plate is not a continuous sheet of hepatocytes, but is perforated by structures entering the lobule - branches of portal vein and hepatic artery - and by structures leaving it - tributaries of the biliary tree. The way in which these structures pierce the limiting plate

on entering or leaving the lobule is clearly seen in the familiar 3-dimensional scheme of Elias (Fig. 2) and in the more diagrammatic representation provided by Henrikson et al (Fig. 3).

No difficulty was experienced in identifying gaps in the limiting plate associated with blood vessels; examples identified by optical microscopy of semi-thin sections and by T.E.M. are illustrated in Figs. 4a & b. In Fig. 4a, as the portal vein branch (P.V.) enters the sinusoid (S) there is hardly any space visible between the wall of the vein and the hepatocytes of the limiting plate. However, a T.E.M. picture (Fig. 4b) of the same area, reveals, around the entering portal vein branch, a space of the same order of size as Disse's space.

It soon became evident, however, that there were many examples of gaps in the limiting plate which were not obviously associated with vascular or biliary elements.

For example, Fig. 5 shows a gap (G) between two adjacent hepatocytes belonging to the limiting plate. It is about 17 um long and 2 um wide. At its inner end is a cross-sectional profile of a sinusoid (S) and its associated perisinusoidal space of Disse (D); its outer end is directly continuous with the space of Mall (M) and the connective tissue of the portal canal (P). The gap contains collagen fibres, processes of a fibroblast (F) and hepatocytic microvilli (shown at higher magnification in Fig. 6). It seems to provide a route for the passage of fluid from Disse's space into the portal canal.

Similar appearances are shown in Fig. 7 in which the gap is shorter (4 um) and Fig. 9 in which the sinusoid abuts almost directly on the portal canal.

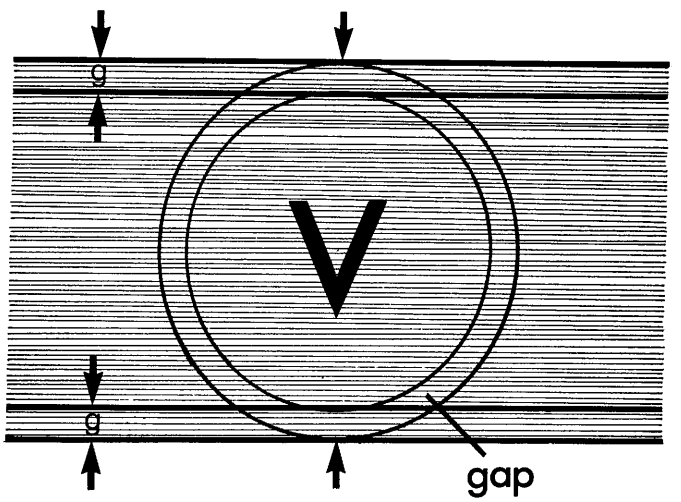
Further examples of gaps in the limiting plate are illustrated in Figs. 8, 9, 10, 11, 12, 13, 14 and 15, which show a progressively closer relation of a sinusoidal cross sectional profile to a portal canal with shorter (17 μm - 0 μm) but wider (1 μm - 32 μm) gaps between limiting plate hepatocytes. An extreme example is shown in Fig. 16, in which a longitudinally sectioned sinusoid (S) is seen to abut directly upon portal canal connective tissue, showing a gap between hepatocytes measuring 32μm in width.

The examples given so far do not reveal the true extent of the further extensions of these gaps within the lobule. Fig. 17 shows a gap which can be traced into continuity with the perisinusoidal spaces associated with two sinusoids (S1 & S2) and the interconnection between them. The inter-hepatocytic ramifications of Disse's space are further emphasized in Fig. 18.

It is, of course, likely that some, at least, of the variations described were dependent upon the plane of section. It is also likely that some of the gaps were in fact associated with a vascular or biliary element which was not included in the sectional plane and which might have been revealed by study of serial thin sections. However, this technique was not followed for two reasons; the first, that it is extremely demanding and that the

returns were thought unlikely to justify the time involved; the second, that examples of gaps not associated with vascular and biliary structures were seen very much more frequently than were gaps associated with such structures.

If all gaps were indeed associated with vascular or biliary elements, random sections would show this association in a majority of cases. That this is so is made clear if one considers the relative width of, say, a portal vein branch piercing the limiting plate and its associated perivascular space ("gap").



If the diameter of the vein V = d, and the width of the gap = g, the section thickness = 1.5 μm, the number of sections showing the gap, without including profiles of the associated vein, would be 2g/1.5; the number of sections showing the vein flanked on either side by a gap, would be V/1.5. The chance of a random section showing the gap and not the vein would be 2g/V. Since V is much larger than g, tangential sections through a "gap" which was associated

with a vein not included in the section would be uncommon. This suggests strongly that, of the large number of gaps seen which were not associated with a vascular (or biliary) element, many were indeed independent entities.

What structures occupy these gaps?

The gap in Fig. 5 contains bundles of collagen fibres some of which are embraced by fibroblastic processes, in addition to hepatocytic microvilli. These three elements constitute the major components occupying the gaps. In other instances, a bundle of collagen occupies the major part of the gap as in Fig. 19 while in other cases the hepatocytic microvilli dominate the scene (Fig. 8). It appears that gaps are mostly occupied by the three previously mentioned elements, with one or other element dominating the picture in any particular section. This variability is probably due to the plane of section. In other gaps, e.g. the wide and shallow ones, Figs. 13, 14 15 and 16, where the sinusoidal endothelium abuts directly on the portal tract, it is hardly justifiable to speak of the contents of such gaps.

How frequent are these gaps?

At the light microscopic level, in a single section through a portal tract there may be recognized 6 gaps (arrows Fig. 20), 7 in Fig. 21 and 4 in Fig. 22. It should be noted that of all these 17 gaps only two show a possible association with the site of entry of a portal vein branch into a sinusoid.

Use of T.E.M. showed much narrower gaps in the limiting plate of similar dimensions to Disse's space, which would not be detected by light microscopy (Figs. 5 and 8). Even the very limited EM fields shown in Figs. 23 and 24 each shows several gaps. It appears then that gaps are frequently found in sectional profiles of the perilobular limiting plate.

If it is accepted that there are many gaps in the perilobular limiting plate through which protein rich interstitial fluid can escape from the space of Disse and its extensions, to enter the perilobular connective tissue (Space of Mall) the next question to be answered is this:

Where are the nearest available lymphatic vessels?

It is commonly assumed that lymphatics extend, in the connective tissue of the portal canals accompanying the structures of the portal triads - artery, vein and bile duct - to their finest terminals. In order to determine if this is indeed the case, a detailed study was made of the presence or absence of lymphatics in a large number of portal canals randomly selected. This was done in the following way: a number of semi-thin sections of liver tissue taken randomly from different blocks and from different adult animals already used for the control study, were used here. In a pilot experiment, 100 portal tracts were examined, and it was noticed that the collection contained many portal tracts whose portal vein branch diameter was small, and very few portal tracts with a portal vein branch diameter of 80 μm and more. In the

light of this, a new series of semi-thin sections, containing large portal tracts was studied: all portal tracts were examined and the distribution of lymphatics was determined in relation to the diameter of the associated portal vein branch.

The criteria used in identifying lymphatics in these semi-thin sections viewed by the optical microscope were: their irregular shape, their thin endothelial lining and their contents of stained precipitated proteins. In case of any doubt, e.g. Fig. 25A, the vessel was checked by electron microscopy (Fig. 25B) which resolved further details of the endothelium such as intercellular junctions, intraendothelial vesicles, the variable thickness of the endothelium and the absence of basement membrane. The results are given in Tables 1 & 2, and particular attention is drawn to the following points:

- although in adult rat liver many of the small portal tracts had lymphatics, these did not extend into tracts in which the portal vein branch was less than 30-40 μm in diameter (Fig. 26).
- lymphatics do not accompany every branch of portal tree; there are portal tracts containing quite large portal vein branches which do not contain lymphatics (Figs. 27A & B).
- all portal vein branches larger than 120 μm were accompanied by lymphatics while only 25% of portal vein branch of less than 50 μm in diameter were accompanied by

lymphatics. In all it is estimated that about 50% of portal tracts in adult rat's liver have lymphatics.

Table 1

Incidence of lymphatics in portal tracts of adult rat liver.

Total No. of portal tracts examined	No. of portal tracts containing lymphatics	% of portal tracts containing lymphatics
133	64	50%

Table 2

Distribution of lymphatics in portal tracts of adult rats in relation to diameter of accompanying portal vein branch. (No. of tracts containing lymphatics/No. of tracts examined)

Portal vein diameter less than 50 μm	Portal vein diameter 50 μm - 80 μm	Portal vein diameter more than 80 μm
15/61	23/39	26/33
24%	58%	78%

In view of these findings, it is clear that fluid escaping through gaps in the limiting plate may have to travel for a substantial distance - in microscopic terms - before reaching an endothelially-lined lymphatic vessel. This leads us to the third question to be answered.

Does the connective tissue of the portal tract into which interstitial fluid escapes, have any special structural characteristics?

In the larger portal canals, the connective tissue is unremarkable. The S.E.M. (Fig. 28) shows rather coarse

collagenous bundles, irregularly arranged, in which are embedded an artery, a vein, a bile duct and a lymphatic; at higher power, a T.E.M. (Fig. 29) shows the closely packed collagen bundles, fibroblasts and as additional features, mast cells, a bundle of nerve fibres and a lymphatic vessel. Because of the density of collagen fibres, there is little interstitial ground substance and the form of fibroblasts is largely obscured. However, careful inspection shows that they have slender cytoplasmic extensions, which partially envelop collagen bundles.

When the collagen is less dense (see, e.g. Fig. 30) these cytoplasmic extensions of fibroblasts are more readily seen; they appear as irregular sinuous strands (or sectional profiles of sheets - see later) of variable thickness, but mostly slender. Where they lie adjacent to a sectioned lymphatic vessel (Fig. 30) it is clear that, in some respects at least, they resemble lymphatic endothelium. In Fig. 30 the distinction is clearly made:

1. by the presence within the lymphatics of a dense precipitate of lymphoprotein and by the characteristic interdigitating junctions (J) between lymphatic endothelial cells, and
2. by the presence of collagen (C) within the loculi enclosed by fibroblastic processes. It should be noted, however, that the loculi also contain a precipitate similar to, but less dense than, that in the lymphatic.

In the looser connective tissue of the more peripheral parts of the portal tract (Fig. 31), this loculation of the interstitium is a uniform characteristic. When studied in thin sections, necessarily random, a wide variety of appearances are seen, as illustrated in the following series of E.M. profiles.

A. In some, e.g. Fig. 32, the fibroblastic nature of the cells and their processes is not in doubt.

B. In others, e.g. Fig. 31, processes apparently from a single cell almost completely enclose two loculi, which still clearly belong to the interstitium. Some loculi (e.g. Fig. 32), are bounded by processes of two fibroblasts with desmosome-like junctions (J).

C. When the processes happen to run lengthwise in the sectional plane, e.g. Fig. 33, and when the enclosed loculus contains a particularly dense protein precipitate, part of the profile (between lines AA' and BB') might well be mistaken for an endothelially-lined lymphatic, although a glance at the collagen bundles within it soon dispels this error (Coll & Coll").

D. In some profiles (Figs. 34 and 35), fibroblast processes form almost completely closed channels, whose true nature can only be determined by electron microscopy (e.g. Figs. 36 and 37 longitudinally sectioned "channels" contain leucocytes, but also tell-tale fine bundles of collagen).

Some transversely sectioned profiles of "channels" also require a second look to distinguish them from

lymphatics, e.g. Figs. 38, 39 and 40).

From this account it is evident, particularly in the looser connective tissue of the more peripheral parts of the portal tract, that -

1. The interstitium is loculated by attenuated cytoplasmic processes of fibroblasts.
2. These are interpreted as sheet-like rather than cylindrical and slender on two pieces of evidence.
   i) that in a large number of random sections, lengthy profiles are most commonly seen; slender processes would hardly be sectioned lengthwise so frequently.
   ii) the appearance in S.E.M. (Fig. 41). These are difficult to interpret with certainty, but they certainly suggest channel-like spaces, bounded by incomplete cytoplasmic sheets. This incompleteness is characteristic; it leaves large gaps communicating with the rest of the interstitium.
3. These loculi are closely associated with collagen bundles.
4. Their contents of precipitated protein and their proximity to the space of Mall (Figs. 42 and 43) suggest that, collectively, they provide an organized, sponge-like system of communicating loculi which receive protein-rich interstitial fluid from the space of Disse through the gaps in the limiting plate already described.

5. They extend into the vicinity of the most peripheral twigs of the lymphatic tree (Fig. 44).

Bundles of collagen in Disse's space (Figs. 45 and 46), in gaps (Fig. 19), and interstitium of portal tracts (Fig. 47) - when viewed by T.E.M. - are found to be bathed in proteinous material and not only as a thin film on the outer coat, so these bundles seem to take some part in the transport of interstitial fluid.

The hypothesis

In the light of the answers given to the three questions posed so far, the following working hypothesis was proposed for testing: that the interstitial fluid formed in the space of Disse passes out of the liver lobule through gaps in the limiting plate, to be carried away by fibroblastic prelymphatic channels to the true lymphatics.

All parts of this proposed pathway were seen to contain precipitated proteinous material. In order to test this hypothesis, it was decided to introduce various tracers in the blood stream and to determine if they could be shown to follow this route from blood, to space of Disse, through gaps to specialized connective tissue, to lymphatics.

Distribution of intravascular tracers in the liver

1. Ferritin

When ferritin was injected through the tail vein the granules were seen in the sinusoidal lumen (Fig. 48), endothelial fenestrations and the space of Disse. Ferritin particles were particularly abundant in the decapitated animal which had been fixed by immersion in order to avoid the washout procedure (Fig. 49). Ferritin was also seen in the gaps of the limiting plate (Fig. 50), in the space of Mall (Fig. 51), in the interstitium of portal tract (Fig. 52), prelymphatics (Fig. 53) and bathing the bundles of collagen (Fig. 54). The lymphatics contained ferritin in a higher concentration than that in any other part of the pathway (Fig. 55).

Ferritin granules were taken up by the sinusoidal endothelium in bristle-coated vesicles (Figs. 56A & B). Fibroblasts of the portal tracts showed such activity very occasionally (Fig. 52).

2. Pontamine sky blue

When the rats injected with Pontamine Sky Blue (P.S.B.) were killed 8 minutes after injection, dye particles were seen within the sinusoidal lumen and Disse's space (Fig. 57). Dye particles were also seen in each part of the suggested pathway for the egress of interstitial fluid from the hepatic parenchyma: the gaps in the periportal limiting plate (Fig. 58), the interstitial connective tissue of the portal tracts (Fig. 59), prelymphatics channels (Fig. 59) and, finally, in the lymphatics, in which the concentrations of dye particles was again higher than in other parts of the pathway (Fig. 60).

No evidence was found of endocytosis of P.S.B. particles by any cell along the route and this made it a particularly useful tracer in testing the hypothesis.

3. Chylomicrons

Chylomicrons are synthesized, as a combination of triacylglyceride, phospholipid and apoprotein, within the intestinal epithelium, which they leave by exocytosis, to enter the lacteals and reach the blood stream by the thoracic duct. They are carried in the blood, as particles 0.5 - 1 um in diameter, to all parts of the body, including the liver.

The rationale for using chylomicrons as a tracer for interstitial fluid movement from Disse's space to lymphatics rested upon the demonstration that chylomicrons are found in Disse's space. If the proposed hypothesis of fluid movement is correct, chylomicrons might be expected to be seen in each part of the pathway in animals killed after a large fatty meal.

The electron microscopic appearance of chylomicrons is known to vary, depending upon the type of diet and upon methods of preparation, especially of fixation, dehydration and embedding [see Casley-Smith (1962)].

In the material used in the present study, chylomicrons appeared as roughly circular profiles, with a thin dark rim and an electron-lucent centre, occurring either singly or in clusters like a collection of soap bubbles of various sizes. There appearances corresponded to those described by Casley-Smith (1962), following the use of osmium tetroxide for fixation and what he referred to as "excessive" alcohol for dehydration.

Chylomicrons were, in fact, identified in each part of the suggested pathway; in the sinusoidal lumen and the space of Disse (Fig. 61); in gaps in the perilobular limiting plate (Fig. 10); in the interstitium of portal tracts (Fig. 63); in fibroblastic prelymphatic channels (Fig. 62) and in lymphatics (Fig. 63).

4. Lipoproteins

The liver is the main source of plasma lipoproteins derived from endogenous sources. The lipoprotein apoprotein is probably synthesized in the rough endoplasmic reticulum and passes into the tubules of the smooth endoplasmic reticulum and Golgi bodies where the lipid is added. Very low density lipoproteins (VLDL) are ultimately secreted into the space of Disse, from which they are all usually assumed to enter the sinusoidal lumen by passing through the fenestrations in the endothelium, i.e. in the reverse direction to that followed by interstitial fluid. A possible additional route for lipoproteins is that suggested for the hypothesis. Lipoprotein "droplets" were in fact seen: in the space of Disse (Fig. 64) the interstitium of the portal tract and lymphatics (Fig. 65).

5. Monastral Blue

Because of their characteristic shape, large size and electron density, Monastral Blue dye particles were readily identified by electron microscopy. They were notably sparse in the sinusoidal lumina, but many were adherent to the sinusoidal surface of Kupffer cells (Fig. 67) which avidly phagocytosed them and sequestered them in large phagosomes (Figs. 66 and 67). Particles were occasionally seen apparently entering Disse's space through an endothelial fenestration or intercellular overlapping junctions (Fig. 68) and within Disse's space (Fig. 69); occasional small clusters of particles were seen in the interstitium of the portal tract and occasional single particles were seen in lymphatics (Fig. 70).

The demonstration of the existence of pre-lymphatic channels in the perilobular connective tissue, and of the failure of lymphatics to accompany all the terminal twigs of the portal venous tree led to the next question studied.

What is the developmental history of the intrinsic lymphatics of the liver?

This was studied in two ways:

i) by determining the incidence of lymphatics in > 100 randomly selected portal tracts in livers from groups of animals aged 24 hours, 1 week, 2 weeks, 3 weeks and adult (TABLE 3).

ii) by determining the distribution of lymphatics in relation to three size classes of portal vein branches: < 50 μm; 50-80 μm; > 80 μm (TABLE 4).

TABLE 3

Incidence of lymphatics in portal tracts of rat liver in relation to age.

AGE	TOTAL NO. OF PORTAL TRACTS EXAMINED	NO. OF PORTAL TRACTS CONTAINING LYMPHATICS	% OF PORTAL TRACTS CONTAINING LYMPHATICS
24 hrs	110	2	2%
1 week	137	9	6%
2 weeks	109	15	15%
3 weeks	134	41	30%
Adult	133	64	50%

TABLE 4

Distribution of lymphatics in portal tracts in relation to diameter of accompanying portal vein branch.

No. of tracts containing lymphatics
No. of tracts examined

AGE	Portal vein diameter less than 50 μm	Portal vein diameter 50 μm - 80 μm	Portal vein diameter more than 80 μm
24 hrs	0/44 0%	0/48 0%	2/18 11%
1 week	0/107 0%	3/18 16%	6/12 50%
2 weeks	7/96 7%	6/11 54%	2/2 100%
3 weeks	15/105 14%	14/17 82%	12/12 100%
Adult	15/61 24%	23/39 58%	26/33 78%

1. Rats 24 hours after birth: In the youngest group studied - rats less than 24 hrs old - very small lymphatics were seen only in major portal tracts, which contained a portal vein branch of diameter greater than 180 um (Fig. 71). These few and small lymphatics very soon disappeared as one followed the portal tracts peripherally. The more peripheral parts of the portal tract tree were devoid of lymphatics (Fig. 72A & B). The paucity of lymphatics in the neonatal rat liver is emphasized by the fact that only 2% of all sectional profiles of portal tracts contained lymphatic profiles.
2. In the one week old rats, the lymphatics extended further along the branching tree of portal tracts.

Small lymphatics were seen in portal tracts whose portal vein diameter was about 60 μm, beyond which lymphatics were lacking. Of all the portal tracts studied, only 6% had lymphatics (Table 1).

3. In the next group of two weeks old rats, lymphatics were found extending into smaller portal tracts, reaching those whose portal vein branch was 30-40 μm in diameter. They were now present in 15% of all the portal tracts studied (Table 3).
4. In the three weeks old rats a higher proportion of the portal tracts whose associated portal vein branch was 30-40 μm in diameter possessed lymphatics, and lymphatics were now seen in 30% of all portal tracts.
5. The last group included the adult rats already described, where lymphatics did not extend beyond portal tracts whose associated portal vein diameter was 30 - 40 μm, and did not accompany every branch of the portal tree. Lymphatics were present in about 50% of portal tract sectional profiles.

Therefore, it may be concluded (i) that in the rat's liver, lymphatics grow centrifugally from the hilum towards the periphery but (ii) that they stop short of its end and do not follow every branch of the portal tract tree.

These conclusions are based on statistical and morphometric considerations. While they show the centrifugal extension of lymphatics along the vascular tree, from its larger stems (portal vein branches > 80 μm)

to its smaller peripheral twigs (portal vein branches < 50 μm), they do not, of course, establish the method of this lymphatic growth. As outlined in the Introduction, there are at least two possibilities (i) extension by budding of endothelium from the tips of the growing tree and (ii) extension by the incorporation of new cells differentiating from the mesenchymal bed into which the lymphatics are extending. The next section examines this question.

How do intrahepatic lymphatics grow?

Light microscopy of semi-thin sections showed that young lymphatics had incomplete loculi on both the luminal and abluminal sides of their wall (Fig. 73). The transmission electron microscope showed that some cells which formed part of the wall of a lymphatic showed characteristics of mesenchymal cells such as sending out processes into the neighbouring area to surround loculi which contained protein material (Figs. 74, 75, 76, 77, 78 & 79). They clearly differed from the adjacent well differentiated endothelium. These loculi were also seen very near to lymphatics and sometimes a small distance away (Fig. 80).

These findings, along with the absence of mitotic figures and budding out of lymphatic endothelium, strongly suggest that lymphatics grow by gradual differentiation of mesenchymal cells into lymphatic endothelium.

What is the pattern and morphological characteristics of intrinsic hepatic lymphatics?

A - Their relationship to other structures in the portal canals

In working out the relation of the lymphatics to the arteries, veins or bile ducts, they were placed in one or other four categories:

1. those mainly related to an artery.
2. those mainly related to a portal vein branch
3. those mainly related to an artery and a vein
4. those mainly related to a bile duct

The third category had to be included, as it was often difficult to decide if a particular lymphatic was related to an artery or to a vein.

In 100 portal tracts selected in advance as containing lymphatics, there were present 153 sectional profiles of lymphatics. 35 (i.e. 22.8%) of these were exclusively related to arteries (see e.g. Fig. 81), while 50 (32.6%) were related to veins (Fig. 82). However, 68 (44.4%) were related equally to arteries and veins (Fig. 83). No lymphatic was seen to be mainly associated with a bile duct.

As to the number of lymphatics in portal tracts:

67% had a single lymphatic

19% had two

8% had three

4% had four

1% had five

1% had six

B. Do they exist as trunks or are they plexiform?

The above results suggest that lymphatics in the rat's liver are principally in the form of trunks and not in the form of a plexus.

Extra hepatic lymph drainage routes and regional nodes

At the hilum of the liver there emerged efferent lymphatic trunks which follow one of three possible pathways:

1. In the hilum of the liver a number of lymphatics emerged to follow the portal vein (Fig. 84), running on its wall, usually two on its ventral side and one on its dorsal side. These lymphatics usually united to form a single lymphatic trunk which descended on the wall of the portal vein to join a small lymph node. Since this lymph node was very near the portal vein and received lymphatics only from the liver, it is referred to as the "portal node".
2. Two or three lymph trunks, one usually larger than the others, came out of the liver, descended obliquely to the left, and drained into one or two nodes in the porta hepatis near the gastroduodenal artery and vein. In the literature, these are currently called "portal nodes" but it is suggested that they are better called "hepatic nodes". Finer branches of these trunks joined

the posterior gastric group of nodes.

3. A single lymphatic vessel, usually large, came from the hilum of the liver at a higher level than the previously described trunks, descended obliquely to the left, reached the oesophagus and divided into two branches. One ran dorsally, and the other ventrally to the oesophagus; the two re-united to form a single trunk which ran downwards and then bifurcated. The left limb joined a pale node lying at the right edge of the splenic group (Fig. 85). The right limb joined the posterior gastric lymph nodes.

Lymphatics accompanying the hepatic veins traversed the diaphragm, and united in two vessels that ran on both sides of the inferior vena cava, to ascend and join the mediastinal lymph nodes.

Plasma cells in the liver

Plasma cells at various stages of their development were seen in various areas of the liver. On average there were about 15 plasma cells per square millimeter of adult rats' liver (Table). They were not uniformly distributed. In the sinusoid (Fig. 86) they were scarce and mostly young, rarely found between hepatocytes (Fig. 87) but the main area of concentration was in the interstitium of the portal tracts (Figs. 88A & B, 89A & B and 90) where the majority were mature plasma cells.

No. of animal	Average No. of plasma cells	Average area of sections in mm2
1	1238	53.6
2	664	53.0
3	203	28.8
4	668	38.8
5	753	56.9
6	326	26.0
	3852	257.1mm2

3852 plasma cell/257.1mm^2 = ~ 15 plasma cell/mm^2

DISCUSSION

How far do the results, presented above, go to establish the route followed by the interstitial fluid from its site of formation in Disse's space, to its entry into lymphatics in the portal tracts?

Because the study has been based entirely on morphological methods, the evidence has been largely circumstantial: the normal fine structure of each part of the pathway has been analysed in some detail and several new findings and concepts have emerged. The hypothesis based on study of normal material has been tested by studying the distribution of various tracers - natural and foreign - introduced into the circulation. The results obtained are consistent with the hypothesis advanced, but they fall short of final proof for reasons which will emerge from the following discussion. Each part of the proposed pathway will now be examined in turn.

I. Gaps in the perilobular limiting plate

a) Have they been recognized previously?

A careful search of the literature, both at the start of this study, and again after the gaps were identified, has not found any previous description. A single sentence in Krstic (1984) hints at their existence, but no other reference has been found.

b) Do the gaps occur only in association with blood vessels entering the lobule?

Henrikson et al (1984) suggested that connections

existed between the space of Disse and the portal tract, around the entry of terminal portal vein branches. Such gaps do undoubtedly occur, and although they are very narrow and contain little interstitial connective tissue, collectively they should provide a significant connection between Disse's space and the portal tract interstitium. The independence of at least some of the gaps is indicated by the fact that they are wider than those clearly associated with entering blood vessels, and are much more numerous than could be accounted for by tangential section, through connective tissue spaces around blood vessels.

c) Are gaps related to bile ductules?

Gaps, in this study, were not seen to be related to bile ductule epithelial cells when these formed, with a hepatocyte, a duct of Hering. However, only one example was seen when a gap was related to a bile ductule (Fig.11).

d) Is there any collateral evidence of independent gaps in the perilobular limiting plate?

Scanning electron microscopy of corrosion casts has extended our knowledge and understanding of liver morphology. Among those who have used this method, Kordan and Kessel (1980) were concerned with the internal organization of the liver microvasculature, and particularly the mode of termination of branches of the portal vein and of the hepatic artery and their connection with the sinusoids. Two of their findings are relevant to

the question under discussion.

1. The presence of "shell-like" collections of casting material surrounding divisions of the portal vein and hepatic artery. These may be continuous with the casting material extending into the space of Disse, and probably represent the space of Mall.
2. The second and more interesting finding of Kordan and Kessel (1980) was their demonstration of sinusoids which ended blindly at the periphery of the lobule. They were described as neither constricted nor irregular; their ends were smooth, rounded and often expanded and they were remote from the point of entry of any vessel. They gave many possible explanations of these appearances but did not seem to consider that they might really exist, as blind-ending sinusoids. Taken with the findings of the present study, it seems likely that blind-ending sinusoids do indeed exist and that they come to lie tangential to the portal tract through gaps in the limiting plate.

II. Considerations of the second part of the pathway involved a detailed study of the interstitial tissue of the portal tract. This led to the recognition - it is thought for the first time - of channels, incompletely lined by fibroblasts and/or mesenchymal cells, to which the name of prelymphatics has been assigned.

The term and the concept raise for discussion several questions.

a) Are prelymphatics artifacts?

The writer believes that they are not. The technique used, (involving vascular washout with mammalian Ringer's solution, followed by perfusion fixation through the arterial system at physiological pressures) was comparable with standard techniques in other organs. The liver was not handled in any way until after it had hardened in fresh fixative overnight, when it was cut into small pieces by a sharp razor blade without any undue pressure. Meticulous dehydration and embedding followed that. Semi-thin sections were cut and the quality of fixation was checked by looking at cellular details, especially the mitochondria and glycogen. In case of any doubt, transmission electron microscopy was used. Poorly fixed material was discarded.

The tracers used fell into two categories.

i) those, such as Pontamine Sky Blue, Monastral Blue and Ferritin, which have been widely used for many years and which are not known to have toxic effects.

ii) those, such as chylomicrons and lipoproteins, which are normal endogenous constituents and which may be presumed to be entirely harmless.

In view of all these considerations, it is felt that the prelymphatic channels described exist as such in the living animal.

b) How may the "prelymphatic channels" be interpreted; are they truly "prelymphatics"?

Comparini and Bastianini (1965) spoke of the difficulty of identifying lymphatics, and found, in

reconstructions of human liver, that the lymphatics existed in a "plexiform" pattern rather than one of more typical lymph vessels, even though typical lymph vessels were sometimes seen. Most of the plexus was made of very irregular slender "tracts" of varying diameter which alternated with ample dilatations of irregular contours, frequently interspersed with diverticular formations and blind appendices.

Schatzki (1978), in rats, saw in the portal tracts and in the space of Mall numerous dilated "channels" lined by a very thin layer of what he called endothelium; he also recognized true lymphatics. The "channel" contrasted with the very much smaller blood capillaries which were lined by a much thicker endothelium.

In the light of the present study, it seems that when Comparini and Bastianini (1965) and Schatzki (1978) spoke of "lymphatics" they really spoke of two different types of structures. The first was "true" endothelially-lined lymphatics. The second was what Comparini and Bastianini (1965) described as "tracts" while Shatzki (1978) called them "channels". Others (McMaster & Parsons, 1939, 1950) have simply called them "gaps" or "clefts" in the interstitium. It seems that there has been some hesitation in interpreting the peripheral elements of lymph drainage system, on the part of those who think of the lymph drainage system in terms only of classical endothelially-lined lymphatic vessels.

The findings of the present study strongly suggest that, at the periphery of the classic lymphatics there is an open system of prelymphatics.

c) Are prelymphatics part of the interstitium or of lymphatics?

The writer thinks that prelymphatics are best considered as specialized or organized interstitial tissue spaces which perform the function of conducting lymph, and that the cells which provide an incomplete lining for these prelymphatics are essentially related developmentally to mesenchymal cells and fibroblasts which could in their turn differentiate into lymphatic endothelium. Fibroblasts and lymphatic endothelium are two different lines of differentiation of mesenchymal cells which later on complement each other functionally. It appears that when Casley-Smith (1982) suggested using the term "prelymphatics" it was the functional aspect that dictated the term, and the present study provided structural support for his view.

d) Is the pattern of distribution of prelymphatics in the liver consistent with their proposed function?

All the evidence from the present study supports an affirmative answer.

Prelymphatics exist mainly in the more peripheral parts of the portal tracts, where true lymphatics are either absent altogether or where they exist only as small initial lymphatics. In all such areas the connective tissue is loose.

In the major portal tracts and at the porta hepatis the lymphatics are larger, endothelially-lined collecting vessels, the connective tissue is well packed with coarser collagen fibres and prelymphatics hardly exist. There is a gradual transition between the conditions at the "twigs" of the connective tissue tree and those around its main "trunks", at the porta hepatis.

This pattern of distribution is consistent with the suggested function of the prelymphatics, of transporting lymph to the point when it can enter the initial lymphatics.

e) Is the idea of the transport of interstitial fluid/lymph by a non-endothelial system a new one?

The idea of the prelymphatics is not in fact new; in the last thirty years or so many workers have been interested in the lymph drainage of tissues in which "true" lymphatics were lacking. The concept began with the description of a continuous series of spaces - later given various names - of which prelymphatics is only one. Then the reality of their continuity with, and drainage by, lymphatics was demonstrated, mainly by the use of tracers such as carbon or ferrocyanide or by the ligation of efferent lymphatics.

There follows a concise review of the development of the concept of prelymphatics and its relation to the present findings in the liver.

The modern view of the interstitial tissue is that it

exists as a two-phase system. The more solid gel-phase contains effectively no free water, although a large amount of water is present, bound to the matrix of glucosaminoglycans (GAGS). The fluid sol-phase is far less in volume and contains free water with a minimum amount of GAGS. No doubt there are areas of gradation from one state to the other, where the two phases meet.

In normal tissues one often sees gaps between the formed elements of the connective tissue, especially near vessels (Casley-Smith, 1976a; Casley-Smith et al, 1976; Simionescu et al, 1972). These spaces contain protein-rich fluid, but neither cells, fibres, GAGS, nor proteoglycans. Since the latter two are invisible in ordinary preparations, their absence can be demonstrated only by the use of special staining techniques (Merker and Gunther, 1972; Wight and Ross, 1975). These spaces are likely to be the water-rich phase, i.e. the sol-phase. For many years it was thought that the sol-phase was in the form of many isolated small (50 nm or so) vacuoles lying in the gel-phase (Chase, 1959). However, high voltage electron microscopy of thick sections has shown that in fact the sol-phase exists as irregular randomly arranged and interconnected channels (Casley-Smith and Vincent, 1978). The size and number of these can be estimated by filling them with a precipitated tracer which has high charge density so that it will be retained in the sol-phase rather than entering the gel-phase (Browning, 1979, Browning and Casley-Smith, 1981; Casley-Smith and Vincent, 1978, 1980;

Casley-Smith et al, 1979).

The "walls" of these channels were regarded by Casley-Smith (1982) as formed by a gradual transition from the gel-phase to the sol-phase, over a distance of 1nm - 100nm. The effective radii of the channels were described as about 60nm (Chase, 1959; Dennis, 1959; Casley-Smith, 1975a) but certainly as varying greatly with tissue site, activity or injury. Bondareff (1957) found that similar structures became smaller as the animals became older.

In most regions of the body, such channels are relatively short, some tens to hundreds of micrometers, in other regions such as the brain, the retina and medullary bone, some of them measure tens of centimeters.

The difficulty is that the system of tissue channels is so randomly arranged and interconnected, tortuous and overlapping. They were demonstrated by Casley-Smith and Vincent (1978) who injected rabbits with semi-polymerized methylmethacrylate and sodium ferriferrocyanide and produced casts for SEM and sections for TEM. They found regions in which the plastic had penetrated the tissues as thin (~ 100nm) long filaments which, they considered, probably represented true tissue channels. Such channels could be seen in small numbers near the arterial capillaries of the ilium and kidney. Quite often the channels seemed to join together to form semi-continuous sheets, although these were frequently perforated in many places. The transmission electron microscopic findings

were similar to those of Chase (1959) and of Dennis (1959) who found fine diffusely scattered deposits of the ferriferrocyanide precipitate throughout the tissues; however, the heaviest deposits were in a few sharply localized regions. These channels sometimes ended at a lymphatic junction, which was either open or appeared openable. Such connections have also been traced by serial sectioning (Collan and Kalima, 1974; Kalima and Collan, 1976) and it was evident that such combinations of paths and open junctions will not be seen so frequently, simply because of the random nature of the sections and the tortuosity of the paths.

Hauck (1982) found similar tissue channels with similar properties in the mesenteries of rabbits and cats by using incomplete dark field transillumination.

These tissue channels seem to form a fine circulatory system transmitting the bulk flow of fluid through the tissues and providing paths from the arterial to the venous side of the capillary system. No doubt some of them also provide paths which terminate at the walls of initial lymphatics (Casley-Smith, 1982). This system of tissue channels and the initial lymphatic system are continuously connected, forming a common converging drainage system to the larger lymphatic transport vessels. All these results suggest the interpretation that the most peripheral part of the lymph vessel system is a completely open one (Hauck, 1982).

Some tissue channels, however, especially in the

brain and retina (Casley-Smith, 1976d; Casley-Smith et al, 1976a; Foldi et al, 1968a,b; Ottaviani and Azzali, 1965; Varkonyi et al, 1969, 1970) and perhaps in cortical bone (Deysine, 1976), are particularly large and follow quite definite, well defined courses in the adventitia of blood vessels. They become dilated and full of proteins when the central collecting lymphatics are ligated, just as do the true lymphatics in artificial lymphoedema. If carbon is injected into the cerebral cortex, the tissue channels carry it into the cervical lymphatics and lymph nodes (Casley-Smith, 1976a). It must therefore be concluded that these non-endothelialized tissue spaces do in fact carry protein and fluid from the depth of the cerebral cortex to discharge their contents into the cervical lymphatics near the great vessels in the neck; in doing so they travel for long distances outside the skull, largely in the adventitia of the internal carotid artery.

These tissue channels are responsive to various stimuli. As might be expected, if there is increased flow in the tissue, their numbers and dimensions seem to have a negative feed back (Casley-Smith, 1982), i.e. are self-regulating by virtue of the conflicting effects of high flow rates in the large channels eroding their walls, but larger channels also tending to have slower flow rates because they allow quicker equilibrium. Other calculations, based on these data, also agree well with macrophysiological results (Casley-Smith, 1976a).

Another example is their response to injury. Casley-Smith (1980), studied the response of tissue channels after making wounds in different regions of the abdominal wall of white mice. Normally there were only a few small channels. A few days after injury, there were many more small channels and some new larger ones, but after one week the number of both small and large channels was maximal, and then decreased after two weeks. After one month the small and medium sized channels had diminished still further but in the two largest groups showed significant increase.

Tissue channels displaying all these properties responding to stimuli and performing such a special role seemed to deserve a special name. For them Casley-Smith (1982) thought that "prelymphatic system" was justified, at least for the larger ones and for those which terminated opposite a lymphatic junction. Hauck (1973, 1982) after finding similar results thought that it would be better to call them "low resistance channels" or "preferential fluid pathways".

This concept of the prelymphatics has aroused some controversy. They have been described in many tissues in mammals, e.g. the intestine (Kalima and Collan, 1976), cortical bone (Deysine, 1976), the tongue (Casley-Smith, 1976) and many other regions (Rodbard & Taller, 1969) and also in the octopus (Browning and Casley-Smith, 1981). 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. Fiedler (1975)

had a more serious objection; he considered that many of these spaces are art facts caused by immersion fixation or some perfusion fixation defects. Others like Silberberg (1982) found that a system of low resistance channels is a most intriguing idea, however, he expressed his reservations by asking two main questions. Are the channels really seen under physiological conditions? Are the contents of the channels of a higher protein content than the surrounding gel?

f) What is the difference between the two types of prelymphatics?

The basic differences are of size and of the nature of the lining. The prelymphatic fibroblastic channels described in the present study are large enough to accomodate lymph and macrophages and have a diameter of the order of 15-20 μm or more. The prelymphatic channels of Casley Smith seem to have a diameter of the order of only about 100nm.

The lining of the two types also differs: the wall of the "sol-phase prelymphatics" is the gradation from the gel-phase to the sol-phase, which cannot be distinguished either by optical or by electron microscopy, while the walls of "fibroblastic prelymphatics" as described in the present study are well-defined and recognizable morphologically. It is the absence of a definite wall in the sol-phase prelymphatics which attracted much criticism of the concept and indeed raised doubt about their

existence.

Sol-phase prelymphatics have been described mainly in organs lacking lymphatics, such as the brain and the retina, but they have also been found in organs, like the intestines, which have typical lymphatics. In the brain and retina prelymphatics were thought to be the sol draining system, while fibroblastic prelymphatics serve as a complementary system, existing at the periphery of the true lymphatics.

g) Is there any other sort of non-endothelialized part of the micro-circulation in any organ?

It might be expected that a pattern of non-endothelial channels might be present in other viscera. Search of the literature has produced some examples, of which two are selected, one in the spleen, the other in the kidney.

The spleen:

Leon Weiss (1985) described one variety of non-endothelialized vascular spaces in the spleen. He studied the link between terminal arterioles and splenic sinuses, in an attempt to solve the old controversy about whether the intermediary circulation of the spleen is "open" or "closed". He described reticular cells, with "sail-like" processes, which provided an incomplete lining for channels which conducted blood flow from arterioles to sinusoids. He concluded that the splenic circulation was "open" in the sense that the channels linking arterioles and sinusoids were not lined by endothelium; but it behaved largely as a

"closed" circulation in the sense that preferred channels were defined by the reticular cells. Resemblances between these channels in the spleen, which carry blood, and those described in the present study, which carry interstitial fluid, are seen to be closer when it is recalled that the fibroblastic nature of the reticular cells of the spleen, and of other lymphoid organs, is now well established.

The kidney:

Niiro et al (1986) studied the renal cortical lymphatics in rats, rabbits and hamsters and noticed, in the hamster only, what he called "a unique structural feature". At points of junctions between two adjacent endothelial cells, one of the cells was sometimes seen to bifurcate. One process made a junction with the adjacent endothelial cell. But the most interesting thing in connection with the present study, is that the other process extended into the interstitium and partly or even completely enclosed a space. The contents of such spaces were shown to be precipitated proteins. Niiro et al (1986) called them "channel-like" structures and found that they occurred commonly. It seems that in the kidney, as in the liver, there are connective tissue cells of hybrid nature, having properties of both endothelial and connective tissue cells.

h) What is the developmental history of the prelymphatic channels and of lymphatics in the liver?

This part of the study was undertaken to throw more

light on the relationship of these two parts of the proposed pathway.

The statistical evidence presented in the Results points clearly to a centrifugal spread of lymphatics, starting at the porta hepatis and extending into the portal tract connective tissue. In brief, at 24 hours after birth, lymphatics were found in only about 2% of sectional profiles of portal tracts, those which contained the largest branches of the portal vein. At 2 weeks, lymphatics were found in 15% of all portal tracts examined, at 3 weeks in 30% and in adults, in about 50% of tracts. Lymphatics were not found at all, even in adults, in the more peripheral parts of the portal tracts with portal vein branches of less than 30 μm in diameter. Beyond this point the place of endothelially-lined lymphatics was taken by "fibroblastic channels".

These findings prompted the next question: How do the lymphatics grow and extend into the liver? By a process of budding and migration of endothelial cells from pre-existing lymphatics or by differentiation, in situ, of mesenchymal cells and their progressive assimilation by the tips of the lymphatic tree?

The evidence points to the second possibility; although extension of the lymphatic tree occurred rather rapidly between weeks 1 and 2, no signs of endothelial mitosis and sprouting from pre-existing lymphatics were seen. On the contrary, frequent examples were found of small lymphatics, lined by typical endothelium and ending

open mouthed in continuity with mesenchymal cells whose processes bounded irregular fluid filled tissue spaces. These appearances were indeed similar to those already described for the smaller portal canals in the adult. The developmental evidence therefore suggests that the "prelymphatic channels" at the periphery of the adult liver represent a persistence in the adult of a pattern of organized loose mesenchyme which in the new born extended almost to the porta hepatis.

This concept of the centrifugal growth of a lymphatic tree by the progressive differentiation of mesenchymal cells into endothelium and their assimilation at the tips of the growing tree, is not a new one although it has still to gain general acceptance. It was described most elegantly by Klika et al (1972) as the mechanissm by which lymphatics grow in the epicardium from the base of the heart of the chick embryo. Their conclusions are quite explicit: "..... mesenchymal cells form part of the wall of the primitive lymph vessels ....." "The vascular wall is connected to the surrounding mesenchyme by numerous processes and in places it communicates freely with the mesenchymal intercellular space". "The development of the lymph capillary in the periphery is promoted by ..... incorporation of mesenchymal cells into the lining of the lymph bed".

III. In the present study, attention has been drawn to the possible role of collagen fibres in the transport of

interstitial fluids.

a) fine reticular fibres ran lengthwise in the longer gaps in the limiting plate, where they suggest the function of a wick, draining fluid from Disse's space.

b) in the connective tissue of portal tracts, collagen bundles appear to be permeated by coagulated interstitial fluid protein, in which injected tracer particles can also be identified (see Figs. 47 and 54).

c) deficiences in the wall of fibroblastic prelymphatics are commonly seen to be filled, in part, by collagen.

The role of collagen in the movement of interstitial fluid was first demonstrated in the classic study of McMaster and Parsons (1939) who injected the lymphatics in the skin of the ears of mice with Pontamine Sky Blue. They observed fine wavy lines of dye radiating out of the lymphatics; these lines could be bent and twisted by pressure with a microprobe and they sprang back to their original position when pressure was removed, as if the dye were fixed upon or between some tissue elements. They suggested that the lines of colour were formed by the dye moving between the connective tissue fibres or along them and came to the conclusion that, indirectly, connective tissue fibres might play an important part in the transport of certain substances through resting tissue.

Aterman (1963) overloaded the circulation with an intravenous injection of dextran followed by a second injection of Evans blue. He found that parts of the connective tissue framework were prominently outlined by

their intense blue staining. This suggested not so much a staining of the fibres themselves as of a thin sheet surrounding them.

Hauck (1982) injected a fluorescent dye into the microcirculation in the mesentery of rabbits and cats and showed a typical fluorescent pattern of linear structures in the extravascular space, confirmed later by using incomplete dark field illumination. He suggested that this network was formed mainly by the elastic fibres of the interstitial connective tissue. If this is the case, the elastic fibres besides fulfilling their mechanical function also have a passive transport or "guide rail" function for fluid and dissolved molecules between micro blood vessels and the drainage system, making the low resistance pathway.

The results of the present study similarly suggest that bundles of collagen may take part in the transfer of fluids and particulate material through the interstitium. It is interesting to note that the larger particulate tracers, such as Pontamine Sky Blue, were seen only at the edges of collagen bundles, while smaller particles - ferritin and lipoproteins - were seen <u>within</u> the bundles.

IV. <u>The pattern of intrinsic lymphatics and their relation to the structures in the portal canals</u>

The finding that 67% of portal tracts have only a single lymphatic makes it clear that, in the rat, most of the hepatic lymphatics are <u>not</u> plexiform. The 33% of tracts which had two or more lymphatics were mainly those

near the porta hepatis, especially around the arteries to which they were intimately related. Further peripherally, lymphatics were not preferentially related to the arterial tree, but were related equally to arterial and venous branches. These relationships do not have any obvious functional significance.

V. Extrahepatic lymphatics

The extrahepatic lymph trunks and the nodes draining them were mapped out by intravenous injection of Pontamine Sky Blue, a method introduced by Psychoyos (1971).

Within eight minutes the lymph trunks were easily distinguished from veins and arteries by their dark blue colour, as were the lymph nodes. Lymphatics were traced to the main nodes:

1) The lymph trunks which were found accompanying the portal vein, to join a lymph node very near to the same vein, have not been reported previously. The node receives lymph only from the liver; it is designated as the "portal node" because of its intimate relation with the portal vein.
2) The lymph nodes in the porta hepatis which receive several lymphatics from the liver have been described previously. Yoffey and Courtice (1970) called them "hepatic", a term which we prefer to Tilney's (1971) "portal nodes".
3) Lymphatics from the liver were also traced to the posterior gastric nodes, in agreement with the findings

of Hass (1936).

4) A lymph trunk, not previously recognised, ran round the oesophagus to join the pale node of the splenic group.

5) Concerning the ascending group of hepatic lymphatics our findings support the view that these lymphatics travel along the hepatic veins, inferior vena cava and join the mediastinal nodes.

These findings were constant in the 25 rats used in this experiment.

VI. Plasma Cells

Brown et al (1982) found that the human bile contains abundant secretory IgA, and it was suspected that the human liver, like that of the rat, transfers polymeric IgA from plasma to bile. They examined the human thoracic duct lymph, portal vein blood and aortic blood for content and molecular size of IgA. None was found to have a higher total concentration of IgA or higher proportion of polymeric IgA than that in peripheral venous blood, and concluded that a relatively large production of human biliary IgA might originate from synthesis in hepatobiliary tissues.

Mononuclear cells containing IgG, IgA, and IgM, collectively termed "Ig cells" have been seen in all human liver examined (Hadziyannis et al, 1969). These major contributors to antibody formation are a family of cells usually referred to a "plasma cells in different stages of development".

Immunoglobulin-containing cells were found in the portal tracts and fibrous septa (Hadziyannis et al, 1969) but they were also observed in connective tissue elsewhere. Only a minority were in the sinusoids, but the major location in the liver was in proximity to bile ducts. Nagura et al (1983) agreed on that and estimated that there were about 1.3 cells per $0.0025mm^2$. It is agreed that the few IgA containing plasma cells present in the periportal connective tissue are inadequate to account for all the IgA present in human hepatic bile (Nagura et al, 1983).

An incidental finding in the course of the present study was the presence of significant numbers of plasma cells within the liver. This was unexpected and led to a systematic search. Plasma cells at various stages of development were counted, not only in the periportal areas and bile ducts - as in previous studies - but throughout the liver: in 4-6 μm wax sections stained with methyl green-pyronin. The figure arrived at (i.e. ~ 15 cells/mm^2) is much less than that of Nagura et al (1983), i.e. 1.3 cells per $0.0025mm^2$ because we did not exclude the large area of lobular tissue which contained much fewer cells, but the presence of plasma cells was a constant finding.

SUMMARY

1. It is generally agreed that the liver produces a substantial amount of lymph. In fact it is the largest single source of lymph in the body, contributing 15-20% of the total. This hepatic lymph is largely originating from the interstitial fluid in the space of Disse. However, it is still not clear how the fluid in the space of Disse may end up in the lymphatics of the portal tracts, as lymphatic vessels were not seen to penetrate into the hepatic lobules.

2. This study aimed to investigate the route followed by interstitial fluid from Disse's space to the lymphatic vessels of the portal tracts.

   The livers of adult Albino swiss rats, fixed by vascular perfusion, were studied by light and electron microscopy.

3. Frequent gaps were found between hepatocytes of the perilobular limiting plate. They were often independent of vessels entering the lobule. These were usually wider than the space of Disse, and contained hepatocytic microvilli, bundles of collagen fibres, and occasional slender fibroblastic processes extending into the lobule from portal tract interstitium. Large gaps (32 μm wide or more) allowed the sinusoidal endothelium to abut directly on portal tract interstitium. These gaps allowed free communication between the space of Disse and portal tract

interstitium.

4. In the periportal connective tissue, long flattened processes of mesenchymal cells or fibroblasts formed incomplete linings of well defined spaces, which contained precipitated protein and occasional leukocytes. The lining cells were often associated with collagen fibres. The discontinuities in the wall of these spaces were confirmed by S.E.M. These mesenchymal/fibroblastic "channels" were seen in the perilobular space of Mall, in the interstitium of portal tracts and in proximity to lymphatics. They were not true lymphatics, but appeared to function as prelymphatic channels, leading the protein-rich interstitial fluid to adjacent terminal lymphatics, from which they could be distinguished, but only by T.E.M.

5. In the light of all these findings, it is suggested that interstitial fluid in Disse's space had found its way through gaps in the perilobular limiting plate to reach the perilobular space of Mall, from which it drained by mesenchymal/fibroblastic prelymphatics to reach the initial lymphatics.

6. This hypothesis was tested by studying the distribution, in the liver, of several artificial and natural tracers introduced into the circulation of the artificial tracers, ferritin, Pontamine Sky Blue and Monastral Blue. Ferritin and Pontamine Sky Blue were identified by T.E.M. at all points along the proposed

pathway. Most of the Monastral Blue particles were phagocytised. Natural tracers - chylomicrons and lipoproteins - introduced by feeding the animals with a high fat diet, were also found to be distributed along the proposed pathway.

7. Lymphatic profiles were found in only about 50% of portal tracts of adult rats livers. They did not accompany every branch of the portal tree, and when they did, they stopped short of the terminal twigs; lymphatic profiles were not seen in portal tracts whose associated portal vein branch diameter was 30-40 um or less.

   In 100 portal tract profiles it was found that in 44.4% lymphatics were related equally to both arteries and veins, 32% mainly to veins and 22.8% to arteries. it was also found that 67% of portal tracts' profiles (mainly in small, peripheral, portal tracts) had a single lymphatic, 19% had 2, 8% had 3, 4% had 4, 1% had 5 and 1% had 6. Multiple lymphatic profiles were mainly found in large portal tracts. This suggests that lymphatics in rat liver are principally in the form of trunks and not in the form of a plexus.

8. The histogenesis and topographic distribution of lymphatics in the livers of groups of Albino-swiss rats aged 24 hours, 1,2,3 weeks and adults were studied by optical and T.E.M.

   At 24 hours, lymphatics were found in only about 2% of

sectional profiles of portal tracts, only in association with the largest branches of the portal vein. At 1 week, lymphatics were found in portal tracts further from the porta hepatis, associated with portal vein branches down to 60 um in diameter. At 2 weeks, lymphatics were found in 15% of tracts, at 3 weeks in 30% and in adults in 50% of portal tracts. This statistical evidence suggests that lymphatics develop by centrifugal extension from the porta hepatis accompanying many branches of the portal vein. The E.M. evidence indicated that this extension occured mainly by the differentiation, in situ, of mesenchymal cells, and their progressive assimilation by the tips of the lymphatic tree. Frequent examples were found of small lymphatics lined by typical endothelium ending open-mouthed in continuity with mesenchymal cells bounding irregular fluid filled tissue spaces.

9. The extensive lymphatics of the liver were studied by intravenous injection of Pontamine Sky Blue. Lymphatic trunks emerged from the porta hepatis in three groups:

   i) two trunks ran on the portal vein and joined a small node (portal node) near the portal vein.

   ii) a few trunks joined the hepatic node at the porta hepatis.

   iii) one trunk ran obliquely, curved around the oesophagus and bifurcated to join the posterior gastric nodes and a pale node of the splenic group.

Lymphatics around the hepatic vein ran along the inferior vena cava to join the mediastinal lymph nodes.

10. Plasma cells at various stages of development were frequently found in the interstitium of portal tracts in apparently normal rat livers. Young ones were mainly seen in the sinusoids, and mature ones in the portal tracts. There were approximately 15 cells/mm^2 of liver tissue.

REFERENCES

ABBAS, B. (1984): Studies on the splenic haemolymph nodes and the intrinsic lymphatics of the spleen. B.Sc. thesis, University of Glasgow.

ALLEN, L. (1967): Lymphatics and lymphoid tissues. Annual Review of Physiology, 29, 197-224.

ASELLI, G. (1627): Quoted by Rusznyak, I., Foldi, M., Szabo, G. (Eds.). Lymphatics and lymph circulation. Oxford, Pergamon Press Ltd. 1960, 20-28.

ATERMAN, K. (1963): The structure of the liver sinusoids and the sinusoidal cells. In: The Liver. Rouiler, C. (Ed.). Vol. 1, 61-136. New York, Academic Press.

ATERMAN, K. (1981): Connective tissue: an eclectic historical review with particular reference to the liver. Histochemical Journal 13, 341-396.

BARONE, P., INFERRERA, C., CARROZZA, G. (1967): Quoted by: Motta & Porter (1974).

BATTING, C.G., LOW, F.N. (1961): The ultrastructure of human cardiac muscle and its associated tissue spaces. American Journal of Anatomy 108, 199-230.

BENACERRAF, B., McCLUSKY, R.T., PATRAS, D. (1959): Localization of colloid substances in vascular endothelium, a mechanism of tissue damage. I. Factors causing the pathological deposition of colloid carbon. American Journal of Pathology 35 (1), 75-82.

BIOZZi, G., BENACERRAF, B, HALPERN, B. (1953): Quantitative study of the granulopectic activity of the reticuloendothelial system. II. A study of the kinetic of the granulopectic activity of the reticuloendothelial system in relation to the dose of carbon injected, relationship between the weight of the organs and their activity. British Journal of Experimental Pathology 34, 441-457.

BLOCH, E.H., McCUSKEY, R.S. (1977): Biodynamics of phagocytosis: an analysis of the dynamics of phagocytosis in the liver by in vivo microscopy. In: Kupffer cells and other liver sinusoidal cells. Eds. Wisee, E., Knook, D.L. pp21-32. Elsevier. North Holland Biochemical Press, Amsterdam.

BLOOM, W., FAWCETT, D.W. (1986): A Textbook of Histology. Eleventh edition. pp. 704. W.B. Saunders Co., Philadelphia and London.

BONDAREFF, W. (1957): Submicroscopic morphology of the connective tissue ground substance with particular regard to fibrillogenesis and ageing. Gerontologia 1, 222-233.

BOUSSAUW, L., LAUWERYNS, J.M. (1969): Quoted by Casley-Smith, (1977).

BRACE, R., TAYLOR, A., GUYTON, A. (1976): Time course of hindlimb lymph protein concentration. Federal Proceedings 35, 851.

BRINKHOUS, K.M., WALKER, S.A. (1941): Prothrombin and fibrinogen in lymph. American Journal of Physiology 132, 666.

BROWN, W.R., SMITH, P.D., LEE, E., McCALMON, R.T., NAGURA, H. (1982): A search for an enriched source of polymeric IgA in human thoracic duct, portal vein, blood and aortic blood. Clinical Experimental Immunology 48, 85-90.

BROWNING, J. (1979): Demarcation of tissue channels by ferrocyanide deposits: use of an alternative precipitant. Microvascular Research 19, 380-384.

BROWNING, J., CASLEY-SMITH, J.R. (1981): Tissue channel morphology in Octopus. Cell and Tissue Research 215, 153-170.

BRUNS, R.R., PALADE, G.E. (1968b): Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. Journal of Cell Biology 37, 277-299.

BUDGE, (1880; 1887): Quoted by Sabin, F.R. (1902).

BURKEL, W.E. (1970): The fine structure of the terminal branches of the hepatic arterial system of the rat. Anatomical Record 167, 329-350.

BURKEL, W.E., LOW, F.N. (1966): The fine structure of rat liver sinusoids, space of Disse and associated tissue space. Americal Journal of Anatomy 118, 769-784.

CASLEY-SMITH, J.R., FLOREY, H.W. (1961): The structure of normal small lymphatics. Quarterly Journal of Experimental Physiology 46, 101-106.

CASLEY-SMITH, J.R. (1962): The identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals. Journal of Cell Biology 15, 259-277.

CASLEY-SMITH, J.R. (1963): Pinocytic vesicles, an explanation of some of the problems associated with the passage of particles into and through cells via these bodies. Proceedings of the Australian Society for Medical Research 1, 58.

CASLEY-SMITH, J.R. (1964): Endothelial permeability - The passage of particles into and out of diaphragmatic lymphatics. Quarterly Journal of Experimental Physiology 49, 365-383.

CASLEY-SMITH, J.R. (1964a): An electron microscopic study of injured and abnormally permeable lymphatics. Annals of New York Academy of Science 116, 803-830.

CASLEY-SMITH, J.R. (1965): Endothelial permeability. II. The passage of particles through the lymphatic endothelium of normal and injured eras. British Journal of Experimental Pathology 46, 35-49.

CASLEY-SMITH, J.R. (1967a): The fine structures, properties and permeabilities of the endothelium. And: The fine structures and permeabilities of lymphatics under some pathological conditions. In: Collette, J.M., Schoffeniels, E. (Eds). New trends in basic lymphology. Karger, Basil, pp. 19-39 and 124-137.

CASLEY-SMITH, J.R. (1969a): The structure of normal large lymphatics: How this determines their permeability and their ability to transport lymph. Lymphology 2, 15-25.

CASLEY-SMITH, J.R. (1969b): The dimensions and numbers of small vesicles and the significance of these for endothelial permeability. Journal of Microscopy 90, 251-268.

CASLEY-SMITH, J.R. (1970a): How the lymphatic system overcomes the inadequacies of the blood system. And: The dilatation of lymphatics by oedema and their collapse following hyaluronidase. And: The transportation of large molecules, by small vesicles, through the endothelium. In: Viamonte, M. et al. (eds). Progress in lymphology II. Thieme, Stillgart. pp. 51-54, 122-124, 255-260.

CASLEY-SMITH, J.R. (1972): The role of endothelial intercellular junctions in the functioning of the initial lymphatics. Angiologica 9, 106-131.

CASLEY-SMITH, J.R. (1973): The lymphatic system in inflammation. In: Zweifach, B.W., Grant, L., McCluskey, R.C. (Eds). The inflammatory process. 2nd Edition. Vol. 2. Academic Press, New York. pp. 161-204.

CASLEY-SMITH, J.R. (1975a): A theoretical support for the transport of macromolecules by osmotic flow across leaky membrane against a concentration gradient. Microvascular Research 9, 43-48.

CASLEY-SMITH, J.R. (1975b): The entrance of material into initial lymphatics and their relationship with fenestrated blood capillaries. In: Witte, M., Witte, C. (Eds.). Proceedings of the Fourth International Congress on Lymphology. Tucson, University of Arizona Press, Tucson.

CASLEY-SMITH, J.R., O"DONOGHUE, P.J., CROCKER, K.W.J. (1975c): The quantitative relationship between fenestrae in jejunal capillaries and connective tissue channels: proof of "Tunnel capillaries". Microvascular Research 9, 78-100.

CASLEY-SMITH, J.R., FOLDI-BORCSOK, E., FOLDI, M. (1976): The prelymphatic pathways of the brain as revealed by cervical lymphatic obstruction and the passage of particles. British Journal of Experimental Pathology 57, 179.

CASLEY-SMITH, J.R. (1976a): The structural basis for the conservative therapy of lymphoedema. In: Clodius, L. (Ed.). The pathophysiology and treatment of lymphoedema. Thieme, Stuttgart.

CASLEY-SMITH, J.R. (1976d): The prelymphatic pathways of the brain; lymphostasis, and the benzo-pyrones. In: Foldi, M. (Ed.). III. Ringelheim Symposium. Springer, Berlin.

CASLEY-SMITH, J.R. (1977): Lymph and lymphatics. Chapter 19. In: Microcirculation. Vol.1. Part P. 423-502. Haley, G., Burton, M.K. (Eds.). University Park Press, Baltimore, London, Tokyo.

CASLEY-SMITH, J.R., CLODIUS, L. BORCSOK, E., GRUNTZIG, J., FOLDI, M. (1978): The effects of chronic cervical lymphostasis on regions drained by lymphatics and by prelymphatics. Journal of Pathology 124, 13-17.

CASLEY-SMITH, J.R., VINCENT, A.H. (1978): The quantitative morphology of interstitial tissue channels in some tissues of the rat and rabbit. Tissue and Cell 10, 571-584.

CASLEY-SMITH, J.R., FOLDI-BORCSOK, E., FOLDI, M. (1979): A fine structural study of the tissue channels' numbers and dimensions in normal and lymphoedematous tissues. Journal of Lymphology Band III, 49-58.

CASLEY-SMITH, J.R. (1980): The fine structure and functioning of tissue channels and lymphatics. Lymphology 12, 177-183.

CASLEY-SMITH, J.R. (1980a): The structure and functioning of blood vessels, interstitial tissues and lymphatics. In: Foldi, M. (ed.). Lymphology, Stuttgart.

CASLEY-SMITH, J.R., VINCENT, A.V. (1980): Variations in the numbers and dimensions of tissue channels after injury. Tissue and Cell 12(4), 761-771.

CASLEY-SMITH, J.R. (1982): Prelymphatics, a question of terminology? Experientia 38, 1123.

CASLEY-SMITH, J.R. (1982): Mechanisms in the formation of lymph. International review of physiology 26, 147-187.

CASTENHOLZ, A, (1984): Morphological characteristics of initial lymphatics in the tongue as shown by scanning electron microscopy. Scanning Electron Microscopy 11, 1343-1352.

CHASE, W.H. (1959): Extacellular distribution of ferrocyanide in muscle. A.M.A. Archives of Pathology 67, 525-532.

CLARK, E.R. (1909): Observations on living growing lymphatics in the tail of the frog larva. Anatomical Record 3, 183-198.

CLARK, E.R. (1912): Further observations on living growing lymphatics. Their relation to the mesenchyme cells. American Journal of Anatomy 13, 351-361.

CLARK, E.L., CLARK, E.R. (1921): The character of lymphatics in experimental oedema. Anatomical Record 21, 21-127.

CLARK, E.R., CLARK, E.L. (1933): Further observations on living lymphatic vessels in the transparent chamber in the rabbit ear; their relation to tissue spaces. American Journal of Anatomy 52 (2), 273-305.

CLARK, R.V. (1976): Three dimensional organization of testicular interstitial tissue and lymphatic space in rats. Anatomical Record 184, 203-226.

COLLAN, Y., KALIMA, T.V. (1974): Topographical relations of lymphatic endothelial cells in the initial lymphatics of the intestine. Lymphology 7, 175-184.

COMPARINI, L. (1969): Lymph vessels of the liver in man: microscopic morphology and histotopography. Angiologica 6, 262-274.

COMPARINI, L., BASTIANINI, A. (1965): Graphic reconstructions in the morphological study of the hepatic lymph vessels. Angiologica 2, 81-95.

COTRAN, R.S. (1965): The delayed and prolonged vascular leakage in inflammation. II. An electron microscopic study of the vascular response after thermal injury. American Journal of Pathology 46, 589-620.

COTRAN, R.S., GUTTUTA, M., MAJNO, G. (1965): Study on inflammation. Fate of intramural vascular deposits induced by histamine. American Journal of Pathology 47, 1045-1077.

COURTICE, F.C. (1960): The flow and composition of hepatic lymph in the normal and hypercholesterolaemic rabbit. Australian Journal of Experimental Biology 38, 403-412.

COURTICE, F.C. (1971): Lymph and plasma proteins. Barriers to their movement throughout the extracellular fluid. Lymphology 4, 9-17.

COURTICE, F.C. (1981): The lymphatic circulation. In: Structure and Function of the Circulation. Schwartz, N.T. (Ed.). Plenum, New York. pp. 487-602.

CREMER, H., MUELLER, N., BECHTELSHEJMER, H. (1974): Morphological studies on the rat liver and a biochemical analysis of the serum following experimental ligature of the thoracic duct. Lymphology 7, 69-80.

CURRY, F.E., MASON, A., MICHEL, C.C. (1976): Osmotic reflection coefficients of capillary walls to low molecular weight hydrophilic solutes measured in single perfused capillaries of the frog mesentery. Journal of Physiology 261, 319-336.

DAY, T.D. (1950): Connective tissue permeability and the mode of action of hyaluronidase. Nature. London 166, 785-786.

DE BRUYN, P.H., CHO, Y., MICHELSON, S. (1983): In vivo endocytosis by bristle coated pits of protein tracers and their intracellular transport in the endothelial cells lining the sinuses of the liver. I. Endosomal disposition. Journal of Ultrastructure Research 85, 272-289. II. Endosomal-lysosomal transformation. Journal of Ultrastructure Research 85, 290-299.

DENNIS, J.B. (1959): Effects of various factors on the distribution of ferrocyanide in ground substance. Archives of Pathology 67, 533-549.

DEYSINE, M. (1976): Lymphatic spaces in cortical bone, anatomy and physilogy. In: Proceedings of the Fourth International Congress on Lymphology. Witte, M., Witte, C. (Eds.). University of Arizona Press, Tucson.

DISSE, J. (1890): Ueber die lymphbahnen der saugethierleber. Archives Mikroscopik Anatomie 1, 203-223.

DOBBINS, W.O., ROLLINS, E.L. (1970): Intestinal mucosal lymphatic permeability. An electron microscopic study of endothelial vesicles and cell junctions. Journal of Ultrastructure Research 33, 29-59.

DRINKER, C.K. (1931): The permeability of the capillaries of the dog to proteins. American Journal of Physiology 97, 40.

DRINKER, C.K. (1942): The lymphatic system: its part in regulating nd volume of tissue fluid. Lane Medical Lectures, California: Stanford University.

DRINKER, C.K., FIELD, M. (1931): The protein content of mammalian lymph and the relation of lymph to tissue fluid. American Journal of Physiology 97, 32.

ELHAY, S., CASLEY-SMITH, J.R. (1976): Mathematical model of the initial lymphatic. Microvascular Research 12, 121-140.

ELIAS, H. (1949): A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary system. American Journal of Anatomy 87, 379 -427

FARQUHAR, M.G., WISSIG, S.L., PALADE, G.E. (1961): Glomerular permeability. I. Ferritin transfer across the normal capillary wall. Journal of Experimental Medicine 113, 47-65.

FAWCETT, D.W. (1955): Observations on the cytology and electron microscopy of hepatic cells. Journal of National Cancer Institute, U.S.A., 15, 1475-1502.

FAWCETT, D.W., PAUL, W., HEIDGER, M., LEAK, L.V. (1969): Lymph vascular system of the interstitial tissue of the testis as revealed by electron microscopy. Journal of Reproduction and Fertility 19, 109-119.

FICHTELIUS, K.E., DIDERHOLM, H. (1959): On the recirculation of lymphocytes from the lymph to the blood. Acta Haematologica 22, 322-328.

FICHTELIUS, K.E., DIDERHOLM, H., STILLSTORM, J. (1961): The importance of different lymph nodes in lymphocyte recirculation. Acta Anatomica 44, 177-187.

FICHTELIUS, K.E., GROTH, O. (1963): Recirculation of lymphocytes via the liver and its lymph. Acta Haematologica 29, 233-241.

FIEDLER, H.H. (1975): Discussion in basic lymphology. Folia Angiologica Supplementary 3, 95.

FIELD, M.E., DRINKER, C.K. (1931): Conditions governing the removal of protein deposited in the subcutaneous tissue of the dog. American Journal of Physiology 98, 66-69.

FIELD, M.E., LEIGH, O.C., HEIM, J.W., DRINKER, C.K. (1934): The protein content and osmotic pressure of blood serum and lymph from various sources in the dog. American Journal of Physiology 110, 174-181.

FOLDI, M. (1969): Diseases of lymphatics and lymph circulation. Charles C. Thomas, Springfield, III.

FOLDI, M. (1977): The lymphatic system. Lymphology 1, 44-56.

FOLDI, M., CSILLIK, B., VARKONYI, T., ZOLTAN, O.T. (1968b): Lymphatic cerebral haemangiopathy. Ultrastructural alterations in blood capillaries of the brain after blockage of cervical lymph drainage. Vox Sang 2, 214-222.

FOLDI, M., CSILLIK, B., ZOLTAN, O.T. (1968a): Lymphatic drainage of the brain. Experientia 24, 1283-1287.

FRALEY, E.E., WEISS, L. (1961): An electron microscopic study of the lymphatic vessels in the penile skin of the rat. American Journal of Anatomy 109, 85-101.

FRENCH, J.E., FLOREY, H.W., MORRIS, B. (1960): The absorption of particles by the lymphatics of the diaphragm. Quarterly Journal of Experimental Physiology 45, 88-103.

FRENZEL, H., KREMER, B., HUCKER, H. (1977): The liver sinusoids under various pathological conditions. A TEM and SEM of rat liver after respiratory hypoxia, telecobalt-irradiation and endotoxen application. In: Kupffer cells and other liver sinusoidal cells. Wisse, E., Knook, D.L. (Eds.). Elsevier: North Holland Biomedical Press, Amsterdam.

FRIEDMAN, M., DYRES, S.O., OMOTO, C. (1956): Some characteristics of hepatic lymph in intact rat. American Journal of Physiology 184, 11-17.

FUJIKAWA, K., MAGARI, S. (1975): An electron microscopic study of the lymphatic capillaries in the interlobular connective tissue of the rabbit liver. Acta Anatomica Nippon 50, 129-137.

GASKELL, W.H. (1876): Quoted by Starling (1898).

GROTTE, G. (1956): Passage of dextran molecules across the blood lymph barrier. Acta Chirurgica Scandinavica Supplementum 211, 1-84.

GULLAND (1894): Quoted by Sabin (1902).

GURR, E.B. (1960): Encyclopaedia of microscopic stain's. Pontamine Sky Blue. Leonard Hill Books Ltd. pp. 337-338.

GUYTON, A.C. (1963): A concept of negative interstitial pressure based on pressures in implanted perforated capsules. Circulation Research 12, 399-414.

GUYTON, A.C. (1965): Interstitial fluid pressure. II. Pressure-volume curves of interstitial space. Circulation Research 16, 452-460.

GUYTON, A.C. (1966a): Interstitial fluid pressure. III. Its effect on resistance to tissue mobility. Circulation Research 19, 412-419.

GUYTON, A.C. (1966b): Interstitial fluid pressure. IV. Its effect on fluid movement through the capillary wall. Circulation Research 19, 1022-1030.

HADDY, F., SCOTT, J., GREGA, G. (1972): Effects of histamine on lymph protein concentration and flow in the dog fore limb. American Journal of Physiology 223, 1172-1180.

HADZIYANNIS, T., FEIZI, T., SCHEVER, P.J. SHERLOCK, S. (1969): Immunoglobulin-containing cells in the liver. Clinical Experimental Immunology 5, 499-514.

HARGENS, A.R., ZWEIFACH, B.W. (1976): Transport between blood and peripheral lymph in intestine. Microvascular Research 11, 89-101.

HASS, H. (1936): Die architektur der lymphgefabe der lebereapsel in ihren bezichungen zur indegewebsstruetur und flussigkeitsstromung. Virchows Archives 297, 384.

HAUCK, G. (1972): Pathways between capillaries and lymphatics. European Journal of Physiology Suppl. 336, S55-S57.

HAUCK, G. (1982): The connective tissue space in view of the lymphology. Experientia 38, 1121.

HAYES, T.L., HEWITT, J.E. (1957): Visualization of individual lipoprotein molecules in the electron microscope. Journal of Applied Physiology 11, 425-428.

HEATH, T.J., LASCELLES, A.K., MORRIS, B. (1962): The cells of sheep lymph. Journal of Anatomy 96, 397-408.

HEDBYS, B.O., MISHIMA, S. (1962): Flow of water in corneal stroma. Experimental Eye Research 1, 262-275.

HEIDENHAIN, R. (1891): Quoted by Courtice (1981).

HEINE, H. (1986): Basalmembranen als Regulations systeme Zwischen Epithelialen Zellverbanden und Bindegewebe. Gegenbaurs Morphol. Johrb (1986), 132 (3), 325-332. (English Abstract).

HENRIKSON, J.H., HORN, T., CHRISTOFFERSON, P. (1984): The blood-lymph barrier in the liver: A review based on morphological and functional concepts of normal and cirrhotic liver. Liver 4, 221-232.

HOGG, C.M. (1980): The renal haemolymph node in the rat. An investigation into the origins of erythrocytes within its sinuses. B.Sc. Thesis. University of Glasgow.

HOGG, C.M., REID, O., SCOTHORNE, R.J. (1982): Studies on haemolymph nodes. III. Renal lymph as a major source of erythrocytes in the renal haemolymph node of the rat. Journal of Anatomy 135 (2), 291-299.

HORII, I., ITO, I., SUGIMOTO, K. (1950): Management of lymphocytes in liver and lymphocyte infiltration in hepatic interstitium. Acta Chirurgica Medica, University of Kioto 28, 39-46.

HUANG , T.W. (1978): Composite epithelial and endothelial basal laminas in human lung. American Journal of Pathology 93, 681-685.

HUDACK, S., McMASTER, P. (1932): The permeability of the wall of the lymphatic capillary. Journal of Experimental Medicine 56, 223-238.

ITO, I. (1951): Cytological studies on stellate cells, cells of Kupffer and fat-storing cells in the capillary wall of the human liver. Acta Anatomica Nippon 26 (2), 42-49.

ITO, I., SHIBASAKI, S. (1968): Electron microscopic study on the hepatic sinusoidal wall and the fat-storing cells in the normal human liver. Archives Histologica Japonicum 29, 137-192.

JACOBSSON, S., KJELLMER, I. (1964): Flow and protein content of lymph in resting and exercising skeletal muscle. Acta physiologica Scandinavica 60, 278-281.

KALIMA, T.V. (1971): The structure and function of intestinal lymphatics and the influence of impaired lymph flow on the ileum of rats. Scandinavian Journal of Gastroenterology 6 (10). 11-18

KALIMA, T.V., COLLAN, Y. (1976): A serial section study of lymphatic "inlet valves" in intestinal villi. In: Proceedings of IV International Congress of Lymphology. Witte, M., Witte, C. (Eds.). University of Arizona Press.

KAMPMEIER, O.F. (1969): Evolution and comparative morphology of the lymphatic system. Charles C. Thomas, Springfield, III.

KARNOVSKY, M.J. (1970): Morphology of capillaries with special reference of muscle capillaries. In: Alfred Benzon Symposium II. Capillary permeability. The transfer of molecules and ions between capillary, blood and tissue (1970). CRONE, C. & LASSEN, N. (Eds.). pp. 341-350.

KANO (1952): Quoted by WAKE (1980).

KEFALIDES, N.A. (Ed.): Biology and chemistry of basement membranes. New York, Academic Press, Inc.

KENT, G., GAY, S., INOUYE, T., BAHU, R., MINICK, O.T., POPPER, H. (1976): Vitamin A-containing lipocytes and formation of type III collagen in liver injury. Proceedings of National Academy of Sciences 73, 3719-3722.

KLIKA, E., ANTALIKOVA, L., RYCHTER, Z., JELINEK, R. (1972): Inception and manner of development of the lymph vessels in the chick embryo heart. Lymphology 5, 137-148.

KORDAN, R.H., KESSEL, R.J. (1980): Three-dimensional organisation of the hepatic microcirculation in the rodent as observed by scanning electron microscopy of corrosion casts. Gastroenterology 79, 72-81.

KRSTIC, R.V. (1984): Illustrated encyclopaedia of human histology. pp. 326.

KUHN, N., OLIVIER, M.L. (1965): Ultrastructure of the hepatic sinusoid of the goat (Capra hircus). Journal of Cell Biology 26, 977-979.

KULTNER (1950): Quoted by YOFFEY & COURTICE (1970).

LANDIS, E.M. (1925-1926): The capillary pressure in frog mesentery as determined by micro-injection method. American Journal of Physiology 75, 548-570.

LANDIS, E.M. (1930a): The capillary blood pressure in mammalian mesentery as determined by the micro-injection method. American Journal of Physiology 93, 353-362.

LANDIS, E.M. (1930b): Micro-injection studies of capillary blood pressure in human skin. Heart 15, 209-228.

LANDIS, E.M., PAPPENHEIMER, J.R. (1963): Exchange of substances through the capillary wall. In: Handbook of Physiology, Section 12. Circulation Vol. II, pp. 961-1034. Hamilton, W.F., Dow, P. (Eds.). American Physiological Society. Washington D.C.

LASCELLES, A.K., MORRIS, B. (1961): Surgical techniques for the collection of lymph from unanaesthetized sheep. Quarterly Journal of Experimental Physiology 46, 199-205.

LAURENT, T.C. (1966a): Physicochemical characteristics of acid glycosamino-glycans. Federal Proceedings 25, 1037-1038.

LAURENT, T.C. (1970): The structure of hyaluronic acid. In: The chemistry and molecular biology of the intercellular matrix. Balazs, E.A. (Ed.). Vol. II, pp. 703. Academic Press, New York.

LAUWERYNS, J.M., BOUSSOUW, L. (1969): The ultrastructure of pulmonary lymphatic capillaries of newborn rabbits and of human infant. Lymphology 2, 108-129.

LAUWERYNS, J.M., BAERT, J.B. (1974): The role of the pulmonary lymphatics in the defences of the distal lung. Morphological and experimental studies of the transport mechanisms of intratracheally instillated particles. Annals of New York Academy of Sciences 221, 244-275.

LEAK, L.V. (1970): Electron microscopic observations of lymphatic capillaries and the structural components of the connective tissue-lymph interface. Microvascular Research 2, 361-391.

LEAK, L.V. (1976): The structure of lymphatic capillary in lymph formation. Federal Proceedings of American Society of Experimental Biology 35, 1863-1871.

LEAK, L.V. (1984): Physiology of lymphatic system. In: Blood Vessels and Lymphatics in Organ System. Abramson, D.I., Dobin, P.B. (Eds.). pp. 134-164. Harcourt Brace Jovanovich, Academic Press Inc.

LEAK, L.V., BURKE, J.F. (1966): Fine structure of the lymphatic capillary and the adjoining connective tissue area. American Journal of Anatomy 118, 785-810.

LEAK, L.V., BURKE, J.F. (1968): Electron microscopic study of lymphatic capillaries in the removal of connective tissue fluids and particulate substances. lymphology 1, 39-52.

LEESON, LEESON, PAPARO (1985): "Textbook of Histology" Fifth edition. Saunders W.B. pp. 377.

LILLIE, R.D. (1952a): Staining of connective tissue. Archives of Pathology 54, 220-233.

LOW, F.N. (1961): The extracellular portion of the human blood-air barrier and its relation to the tissue spaces. Anatomical Record 139, 105-124.

LOW, F.N., BURKEL (1965): A boundary membranes concept of ultrastructural morphology. Anatomical Record 151, 489-490.

LUDWIG, C. (1858): Quoted by Rusznyak et al. (1960).

MAJNO, G. (1965): Ultrastructure of the vascular membrane. In: Handbook of Physiology. Section 2, Circulation 3, pp. 2293-2375. Hamilton, W.F., Dow, P. (Eds.). Waverly Press, Baltimore.

MAJNO, G., PALADE, G.E. (1961): Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability, and electron microscopic study. Journal of Biophysical Biochemical Cytology 11, 571-605.

MAJNO, G., PALADE, G.E., SCHOEFL, G.I. (1961): Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree, a topographic study. Journal of Biophysical Biochemical Cytology 11, 607-625.

MALL, F.B. (1888): Reticulated and yellow elastic tissue. Anatomi. Anz. 3, 397-401.

MALL, F.B., (1891): Quoted by ATERMAN (1981).

MALL, F.B. (1906): A study of the structural unit of the liver. American Journal of Anatomy 5, 227-308.

MARESCH, R. (1905): Quoted by ATERMAN (1981).

MAYERSON, H.S. (1963): On lymph and lymphatics. Circulation 28, 839-842.

MENEFEE, M.G., MUELLER, C.B., BELL, A.L., MYERS, J.K. (1964): Transport of globin by the renal glomerulus. Journal of Experimental Medicine 120, 1129-1138.

MERKER, H.J., GUNTHER, T. (1972): Ultrastructure of interstitial tissue. European Journal of Physiology supplementary 336, 533-535.

MORRIS, B. (1956b): Absorption of lipids from the peritoneal cavity. Australian Journal of Experimental Biology and Medical Sciences 41, 318-325.

MORRIS, B., SASS, M.B. (1966): The formation of lymph in the ovary. Proceedings of the Royal Society of Biology 164, 577-591.

MOTTA, P., PORTER, K.R. (1974): Structure of rat liver sinusoids and associated tissue spaces as revealed by scanning electron microscope. Cell and Tissue Research 148, 111-125.

McCALLUM, W.G. (1903): On the mechanism of absorption of granular materials from the peritoneum. Bulletin Johns Hopkins Hospital 15, 105-115.

McCARREL, J.D., THAYER, S., DRINKER, C.K. (1941): The lymph drainage of the gall bladdder together with observations on the composition of liver lymph. American Journal of Physiology 133, 79-85.

McLEAN, J.M., SCOTHORNE, R.J. (1970): The lymphatics of the endometrium in the rabbit. Journal of Anatomy 107, 39-48.

McMASTER, P.D., HUDACK, S.S. (1934): The participation of skin lymphatics in repair of the lesions due to incisions and burns. Journal of Experimental Medicine 60, 479-501.

McMASTER, P.D., PARSON, R.J. (1938): Path of escape of bital dyes from the lymphatics into the tissues. Proceedings of Society of Experimental Biology 37, 707-709.

McMASTER, P.D., PARSON, R.J. (1939): I. The method of interstitial spread of vital dyes. Physiological conditions existing in connective tissue. Journal of Experimental Medicine 69, 247-263.

McMASTER, P.D., PARSONS, R.J. (1939): II. Physiological conditions existing in connective tissue. The state of the fluid in the intradermal tissue. Journal of Experimental Medicine 69, 265-282.

McMASTER, P.D., PARSONS, R.J. (1950): The movement of substances and the state of the fluid in the intradermal tissue. Annals of New York Academy of Sciences 52, 992-1003.

NAGURA, N., TSUTSUMI, Y., HASEGAWA, H., WATANABE, K., NAKANE, P.K., BROWN, W.R. (1983): IgA plasma cells in biliary mucosa, a likely source of locally synthesized IgA in human hepatic bile. Clinical Experimental Immunology 54, 671-680.

NIIRO, G.K., JAROSZ, H.M., O'MORCHOE, P.J., O'MORCHOE, C.C.C. (1986): The renal cortical lymphatic system in the rat, hamster and rabbit. American Journal of Anatomy 177, 21-34.

OPPEL, A. (1891): Quoted by Aterman (1981).

OTTAVIANI, G., AZZALI, G. (1965): Quoted by Casley-Smith (1982).

PALADE, G.E. (1961): Blood capillaries of the heart and other organs. Circulation 24, 368-384.

PALADE, G.E., BRUNS, R.R. (1968): Structural modulations of plasmalemmal vesicles. Journal of Cell Biology 37, 633-649.

PALADE, G.E., SIMIONESCU, M., SIMIONESCU, N. (1979): Structural aspects of the permeability of the microvascular endothelium. Acta Physiologica Scandinavica Suppl. 463, 11-32.

PALAY, S.L., KARLIN, L.J. (1959a): An electron microscopic study of the intestinal villus. I. The fasting animal. Journal of Biophysical Biochemical Cytology 5, 363-370.

PAPP, M.E., ROHLICH, P., RUSZNYAK, I., TORO, I. (1962): An electron microcopic study of the central lacteal in the intestinal villus of the cat. Z. Mikroskopique Anatomie 57, 475-486.

POPPER & SCHAFFNER (1957): Lymphatic vessels, tissue spaces, innervation and stroma of the liver. In: Liver: Structure and Function. Popper & Schaffner (Eds.). McGraw-Hill Book Company. pp. 149-156.

PSYCHOYOS, A. (1971): Methods for studying changes in capillary permeability of the rat endometrium. In: Methods in Mammalian Embryology. Joseph, C., Daniel Jr. (Eds.). pp 334-338. W.H. Freeman and Company, San Francisco.

RANVIER: Quoted by Sabin (1902).

RENKIN, E.M. (1977): Multiple pathways of capillary permeability. Circulation Research 41, 735-743.

RENKIN, E.M., CURRY, F.E. (1978): Transport of water and solutes across capillary endothelium. In: Handbook of Biological Membranes. Giebisch, G., Tosteson (Eds.). Berlin: Springer Verlag.

REYNOLDS, E.S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17, 208-212.

RHODIN, J.A.G. (1964): Ultrastructure and function of liver sinusoid. In: Proceedings of IV International Symposium of Reticuloendothelial system. Kyoto, Japan, pp. 108-124.

RITCHIE, H., GRINDLY, J., BOLLMAN, J. (1959): Flow of lymph from the canine liver. American Journal of Physiology 196, 105-109.

RODBARD, S., TALLER, S. (1969): Plastic casts of extracapsular spaces and lymphatic vessels. Medical Experiments 19, 65-69.

ROTENBERG (1949): Quoted by RUSZNYAK et al. (1960).

RUDBECK, O. (1653): In: Lymphatics and lymph circulation physiology and pathology. Rusznyak, R., Foldi, M., Szabo, G. (Eds.). Oxford: Pergamon Press Ltd. (1960), pp. 20-28.

RUSZNYAK, I., FOLDI, M., SZABO, G. (1960): Lymphatics and lymph circulation, physiology and lathology. Oxford: Pergamon Press Ltd.

RUSZNYAK, I., FOLDI, M., SZABO, G. (1967): Lymphatics and lymph circulation. Youlten (Ed.). London Medical College. Pergamon Press.

RUTILI, G., ARFOS, K.E. (1977): Protein concentration in interstitial and lymphatic fluids from subcutaneous tissue. Acta Physiologica Scandinavica 99, 1-8.

RUTILI, G., HAGANDER, P. (1982): Transport of macromolecules in subcutaneous tissue. Acta Physiologica Scandinavia.

SABIN, F.R. (1902): On the origin of the lymphatic system from the veins and development of the lymphatic hearts and thoracic duct in the pig. American Journal of Anatomy 1, 367.

SABIN, F.R. (1916): The method of growth of the lymphatic system. Science 44, 145.

SABIN, F.R. (1916): The origin and development of the lymphatic system. John Hopkins Hospital Reports 17, 347-440.

SABIN, F.R. (1904): On the development of the superficial lymphatics in the skin of the pig. American Journal of Anatomy 3, 183-195.

SALA, (1900): Quored by Sabin (1916).

SAMANTA, A., SAINI, V., CHHUTANI, P., PATRA, B., VASHISTA, S., DATTA, D. (1974): Thoracic duct and hepatic lymph in idiopathic portal hypertension. Gut 15, 903-906.

SATSUKI, S., TSUNODA, K., SHINDO, K. (1956): Quoted by WAKE (1980).

SCHATZKI, P.F. (1978): An electron microscopy of lymphatics of the porta hepatis. Acta Anatomica 102 (1), 54-59.

SCHIPP, R. (1967): Structure and ultrastructure of mesenteric lymphatic vessels. In: Collete, J.M., Jantel, G., Schoffeniels (Eds). New Trends in Basic Lymphology. pp. 50-55. Birkhauser, Stuttgart.

SCHMIDT, F.C. (1960): Quoted by ATERMAN (1981).

SCHNACK, H., STOCKINGER, L., WEWALKA, F. (1967): Adventitious connective tissue cells in the space of Disse and their relation to fibre formation. Review of International Hepatology 17, 855.

SCHOLANDER, P.F., HARGENS, A.R., MILLER, S.L. (1968): Negative pressure in the interstitial fluid of animals. Science 161, 321-328.

SHEA, S.M., KARNOVSKY, M.J. (1966): Brownian motion: a theoretical explanation for the movement of vesicles across the endothelium. Nature 212, 353-355.

SIEGFRIED, M. (1892): Quoted by ATERMAN (1981).

SILBERBERG, A. (1972): Acessibility of interstitial space and structure of connective tissue. European Journal of Physiology Suppl. 336, 537-539.

SIMIONESCU, N., SIMIONESCU, M., PALADE, G.E. (1972): Permeability of intestinal capillaries. Journal of Cell Biology 53, 365-392.

SIMPSON, L.O. (1980a): Basement membrane and bilogical thixotropy. A new hypothesis. Pathology 12, 377-389.

SIMPSON, L.O. (1981): Biological thixotropy of basement membranes: The key to the understanding of capillary permeability. In: Progress in Microcirculatory Research. Garlick, D. (Ed.). The University of South Wales. Committee in Postgraduate Medical Education.

SMITH, J.B., McINTOSH, G.H., MORRIS, B. (1970): The traffic of cells through tissues: a study of peripheral lymph in sheep. Journal of Anatomy 107, 87-100.

STARLING, E.H. (1894): The influence of mechanical factors on lymph production. Journal of Physiology (London) 16, 140-155.

STARLING, E.H. (1896): On the absorption of fluid from the connective tissue spaces. Journal of Physiology (London) 19, 312-326.

STARLING, E.H. (1898): Production and absorption of lymph. In: Textbook of Physiology. Schafer, E.A. (Ed.). Vol. 1, pp. 285-311. Caxton, London.

STARLING, E.H. (1909): In: The Mercer's Company Lectures on the fluids of the body. Constable, London.

STEINER, J.W., (1961): Investigations of allergic liver injury. I. Light fluorescent and electron microscopic study of the effects of soluble immune aggregate. American Journal of Pathology 38, 411-436.

SUNAGA, Y. (1954a): Quoted by WAKE (1980).

SZABO, G., MAGYAR, Z. (1978): The relationship between tissue fluid and lymph. II. Enzymes in tissue fluid and lymph. Lymphology 11, 101-105.

SZABO, G., MAGYAR, Z., JAKAB, F. (1975): The lymphatic drainage of the liver capsule and hepatic parenchyma. Research of Experimental Medicine 166, 193-200.

SZABO, G., MAGYAR, Z., MOLNAR, G. (1973): Lymphatic and venous transport of colloids from the tissue. Lymphology 6, 69-79.

TAYLOR, A., GIBSON, W., GRANGER, H., GUYTON, A. (1973): The interaction between intracapillary and tissue forces in the overall regulation of interstitial fluid volume. Lymphology 6, 192-208.

TILNEY, N. (1971): Patterns of lymphatic drainage in the adult rat. Journal of Anatomy 109, 369-383.

VARKONYI, T., POLGAR, J., ZOLTAN, J.T., CSILLIK, B., FOLDI, M. (1970): Lymphostatic retinal haemangiopathy. Experientia 26, 67-68.

VIRAGH, S.Z., PAPP, M., TORO, E., RUSZNYAK, I. (1966): Cutaneous lymphatic capillaries in dextran-induced oedema of the rat. British Journal of Experimental Pathology 47, 563-567.

WAKE, K. (1980): Perisinusoidal stellate cells (fat storing cells, interstitial cell, lipocytes), their related structure in and around the liver sinusoids, and vitamin A-storing cells in extrahepatic organs. International Review of Cytology 66, 303-353.

WEISS, L. (1961): An electron microscopy of vascular sinuses of the bone in the rabbit. Bulletin of John Hopkin's Hospital 171, 199-???

WEISS, L. (1983): Histology, Cell and Tissue Biology. Fifth edition. MacMillan Press.

WEISS, L. (1985): The blood supply of the spleen, the blood spleen barrier. In: Proceedings of VII Internation Anatomical Congress. pp. A773.

WIGHT, T.N., ROSS, R. (1975): Proteoglycans in primate arteries. Journal of Cell Biology 67, 660-674.

WISSE, E. (1970): An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoid. Journal of Ultrastructure Research 31, 125-150.

WITTE, S. (1965): Studies in extracellular circulation. Bibliography Anatomy 5, 76.

WITTE, S. (1975): Microscopic technique for the in situ characterisation of concentration of tissue components and penetrating molecules. Biotheology 12, 173.

WOLF-HEIDIGGER, G. (1941): Quoted by Burkel & Low (1966).

WOLF, M. (1905): Quoted by ATERMAN (1981).

WOLFF, J.R. (1977): Ultrastructure of the terminal vascular bed as related to function. In: Microcirculation. Kaley, G., Altura, B.M. (Eds.). Vol. 1, pp. 95-130. Baltimore University Park.

WOOD, R.L. (1963): Evidence of species differences in the ultrastructure of the hepatic sinusoid. Zeit. Zellforsch 58, 679-692.

YAMAGESHI, M. (1959): Electron microscope studies on the fine structure of the sinusoidal wall and fat storing cells of rabbit livers. Archives Histologica Japonicum 18, 223-261.

YAMAMOTO, K., PHILIP, M.J. (1986): Three dimensional observations of the intrahepatic lymphatics by scanning electron microscopy of corrosion casts. Anatomical Record 214, 67-70.

YOFFEY, J.M., COURTICE, F.C. (1970): Lymphatics, lymph and lymphomyeloid complex. London: Academic Press.

STUDIES OF THE LYMPHATICS OF THE LIVER AND THE UPTAKE OF INTERSTITIAL FLUID FROM THE SPACE OF DISSE

Vol. 2

FIGURES

By

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Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine

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Fig. 1

An example from the Hunterian collection of lymphatics injected with mercury showing the superficial lymphatics of the liver forming a close arborescent plexus beneath the peritoneal covering of the organ. The larger trunks are seen running off into one of the ligaments on their way to the draining node.

Fig. 2

Elias's 3-dimensional scheme showing the way branches of the hepatic artery, the portal vein and the bile duct pierce the perilobular limiting plate on entering or leaving the lobule.

Note: the narrow space between each structure and the adjacent hepatocyte.

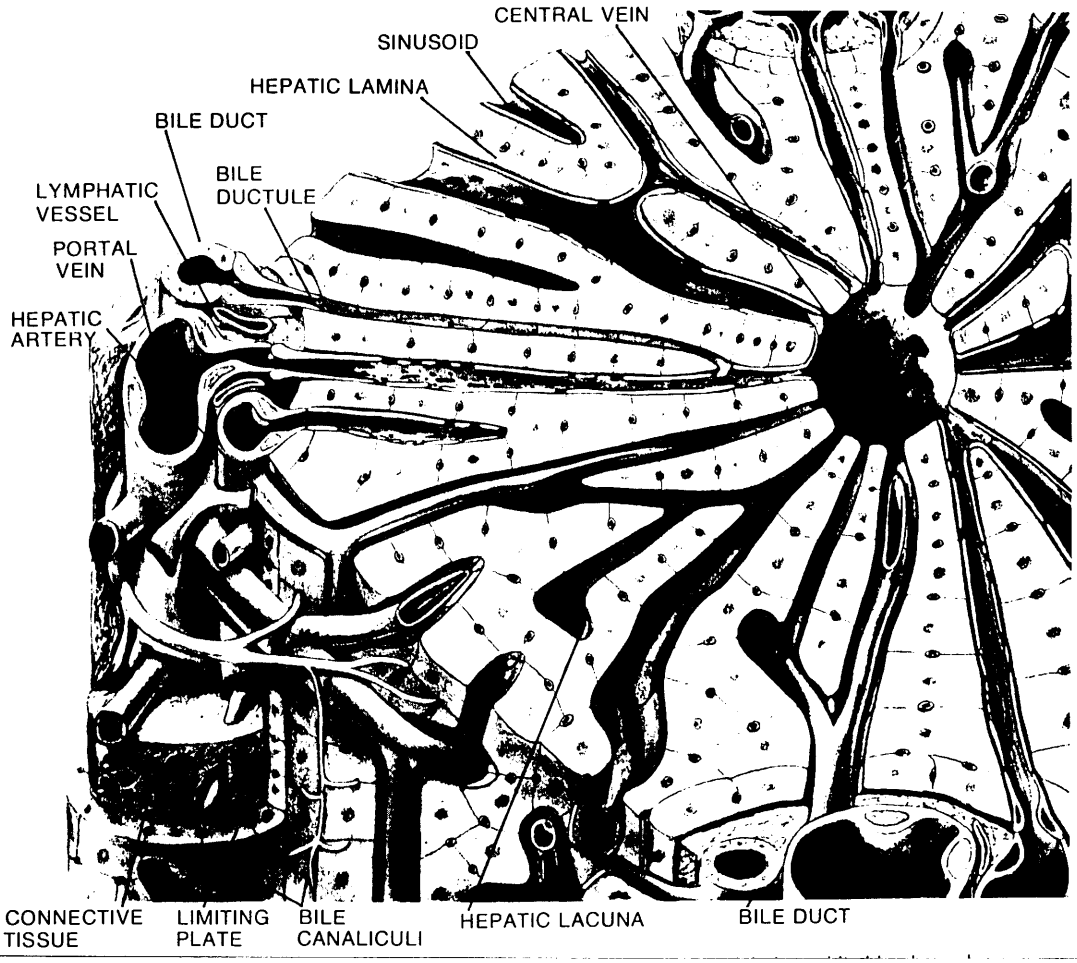

CENTRAL VEIN
SINUSOID
HEPATIC LAMINA
BILE DUCT
BILE DUCTULE
LYMPHATIC VESSEL
PORTAL VEIN
HEPATIC ARTERY
CONNECTIVE TISSUE
LIMITING PLATE
BILE CANALICULI
HEPATIC LACUNA
BILE DUCT

Fig. 3

Schematic drawing of blood-lymph barrier in the normal liver presented by Henrikson et al (1984) showing the way in which the portal vein (PV) and the hepatic artery (HA) pierce the limiting plate and entering a sinusoid (S). The flow along the blood-lymph barrier is shown in arrows. The flow at the point of entry of these vessels [described by Henrikson et al (1984) as less defined] is shown by dotted arrows.

Note: the proximity of the lymphatics (L) to the point of entry of the vessels (i.e. gaps) suggested by Henrikson et al.

AF - anchoring filaments
HV - hepatic vein
SP - sieve plates of the sinusoidal endothelium

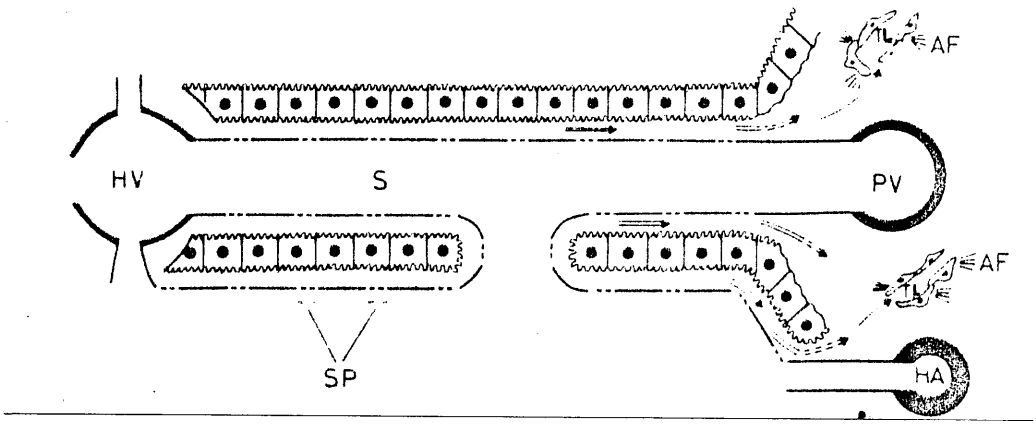

AF
HV
S
PV
AF
SP
HA

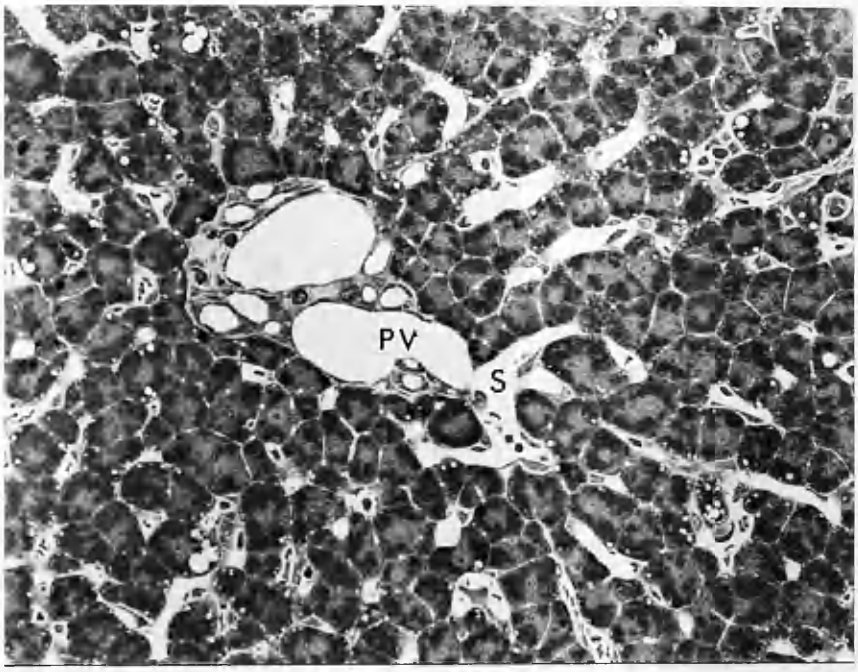
Fig. 4

A. As the portal vein branch (PV) enters the sinusoid (S) it is difficult to recognize a space between the wall of the vein and the hepatocytes of the limiting plate.
x 312

B. A T.E.M. of the same area which reveals that the space between the wall of the vein and the hepatocytes of the limiting plate is of the same order of size as Disse's space.

PV - portal vein
S - sinusoid

x 5880


PV
S

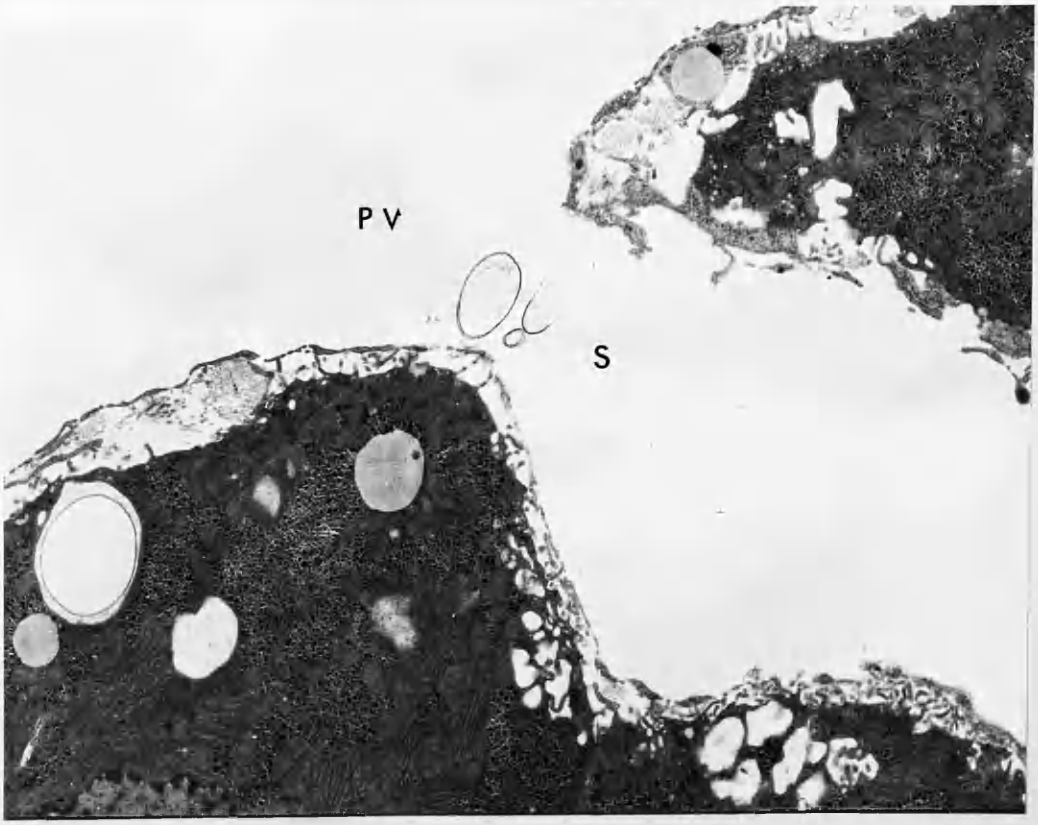

PV
S

Fig. 5

A gap (G) between 2 hepatocytes of perilobular limiting plate. It is 17 µm long, 2 µm wide, and extends between Disse's space (D) of a sinusoid (S) and portal tract (P) and space of Mall (M). It contains a fibroblastic process embracing a bundle of collagen.
x 7840


P
M
G
D
S

Fig. 6

A higher magnification of the contents of the previous gap in Fig. 5 showing the three main contents.

1. C - collagen
2. F.P. - fibroblastic process
3. M.V. - microvilli

x 28000

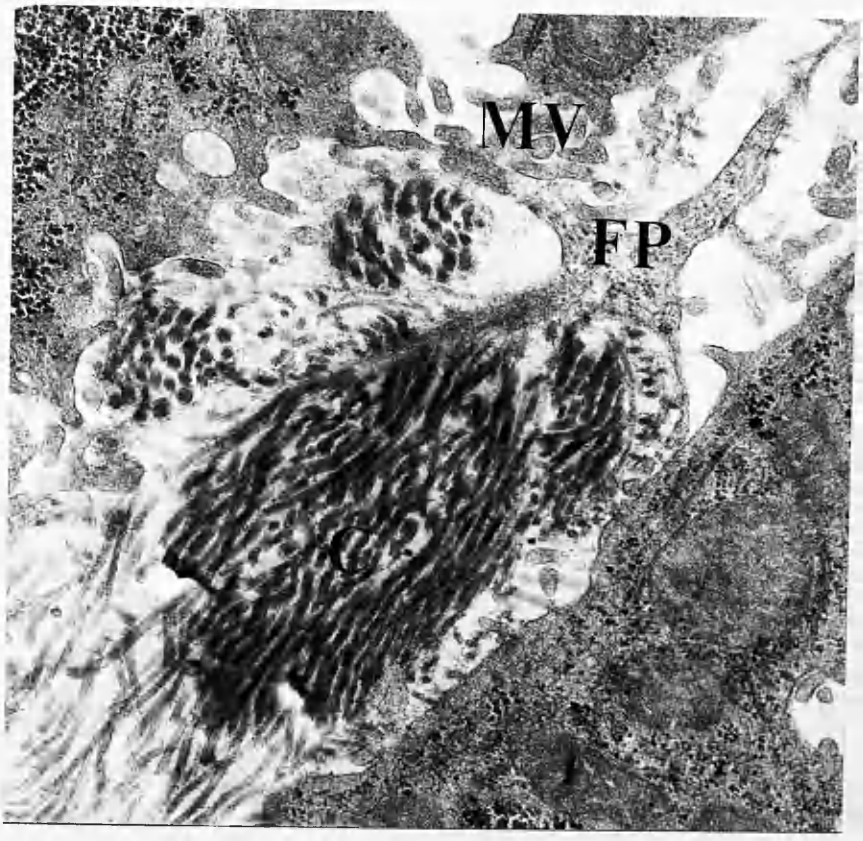

MV
FP

Fig. 7

A gap (G), 2 µm long, 4 µm wide between two hepatocytes of the limiting plate. It extends between Disse's space (D) of a sinusoid (S) and the space of Mall (M) and portal tract (P).
x 5880


P
M
G
D
S

Fig. 8

A gap (G), about 8 µm long and 1 µm wide, between two hepatocytes of a limiting plate.
x 7840

Fig. 9

A gap (G), about 5 μm long and 5 μm wide, between two hepatocytes of a limiting plate.

Note: A fibroblast (F) whose processes extend into the space of Disse (D).

x 7840

G
F
D

Fig. 10

A gap (G), about 3 µm long and 5 µm wide, between two hepatocytes of a limiting plate.

CM - chylomicrons

x 7840

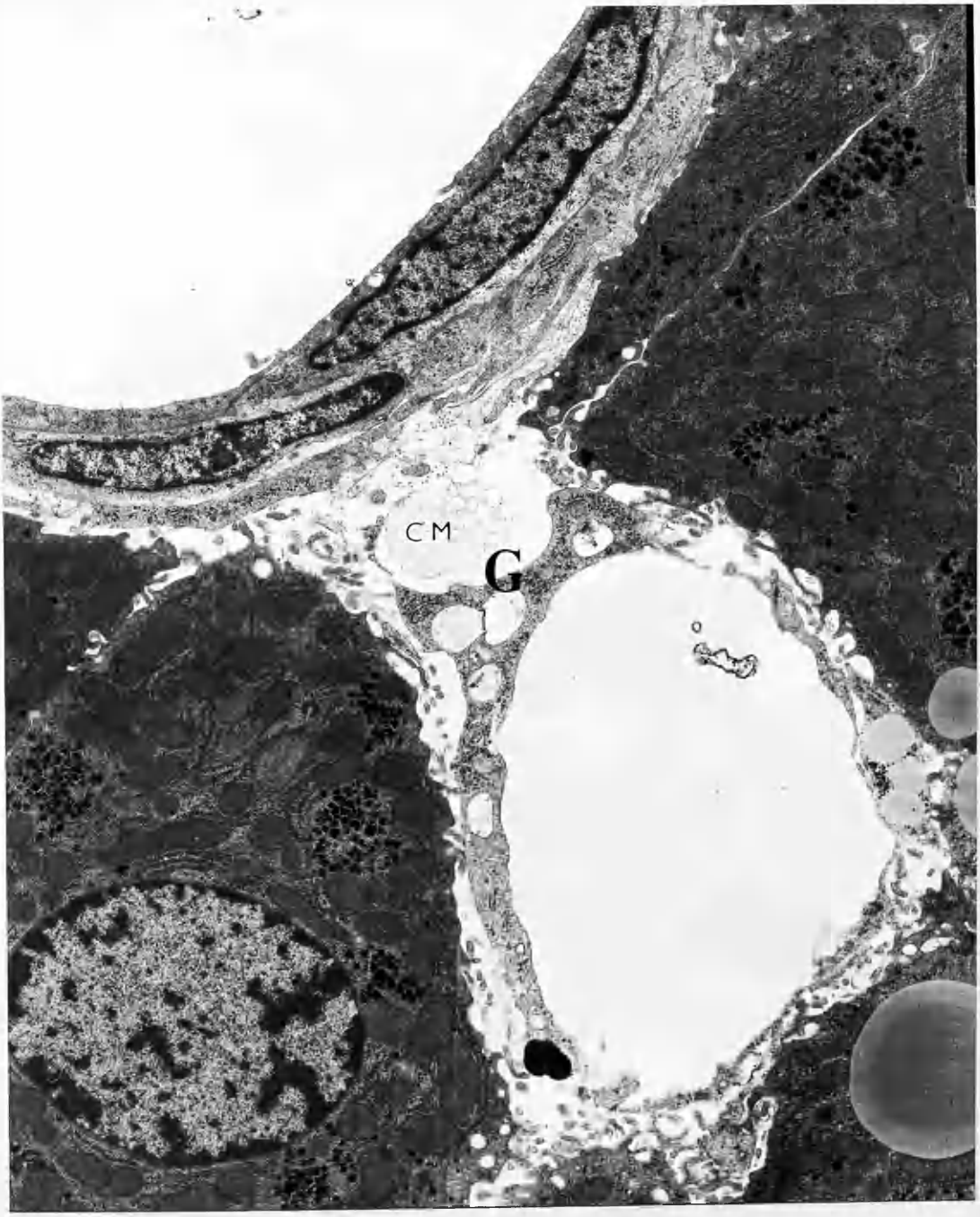

CM
G

Fig. 11

A gap (G), about 3 µm long and 2 µm wide, between two hepatocytes of a limiting plate.

Note: a bile ductule (BD) faces the gap.

x 7840

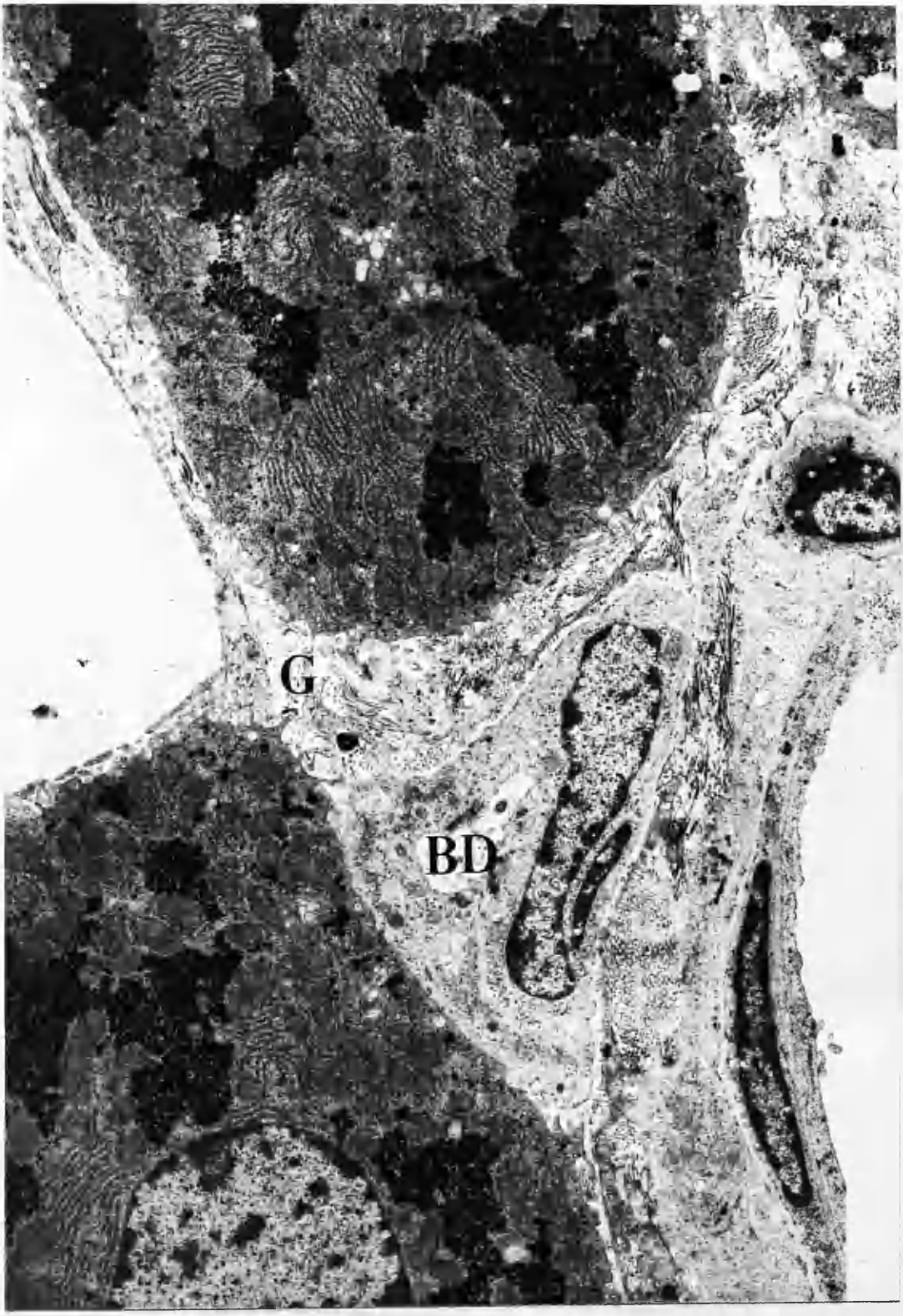

G
BD

Fig. 12

A gap (G), about 1.5 µm long and 3 µm wide, between two hepatocytes of a limiting plate.
x 9800

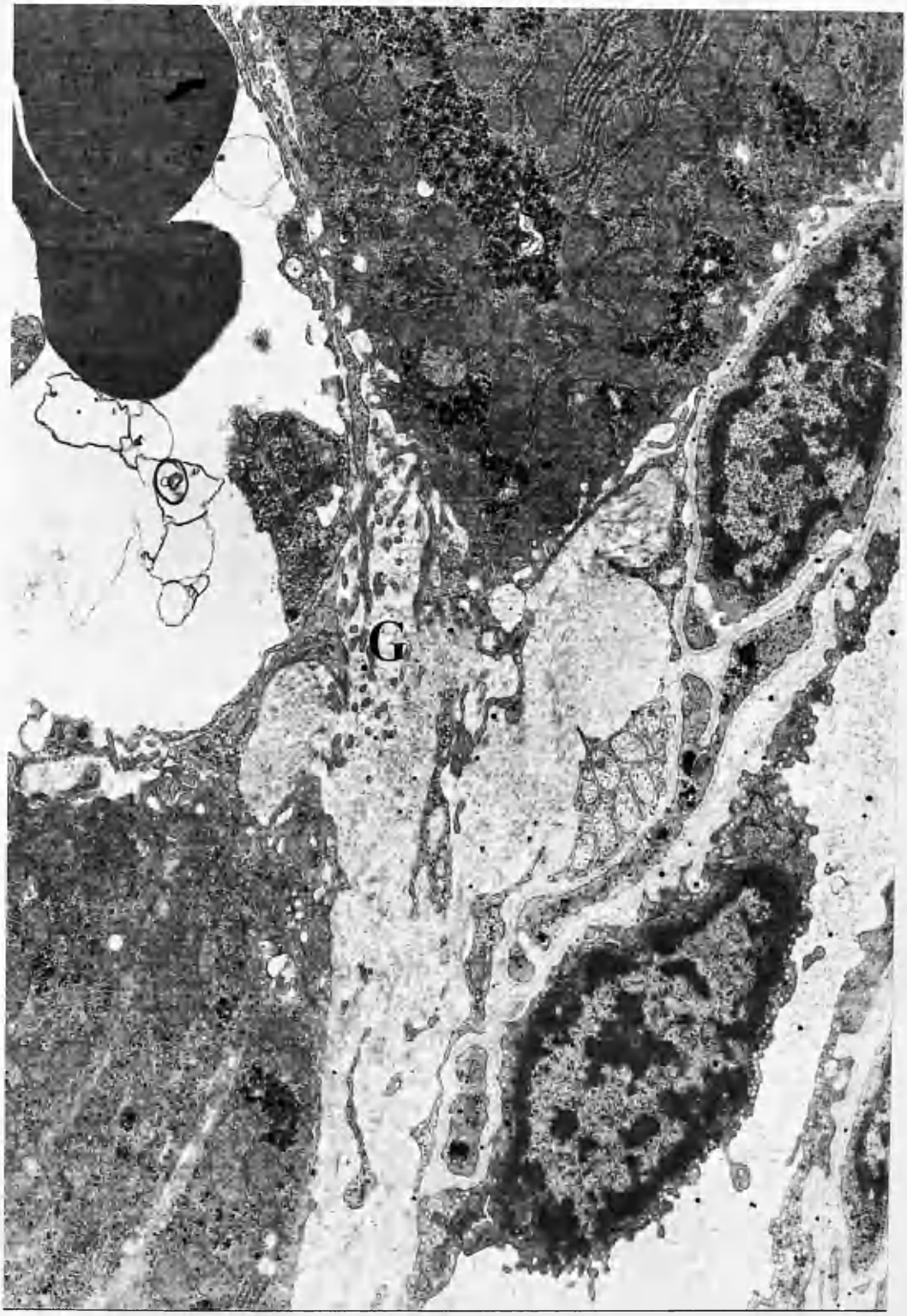

G

Fig. 13

A gap (G), about 3.5 μm wide, between two hepatocytes of a limiting plate.

Note: the sinusoidal endothelium (S.E.) nearly abuts on the portal tract interstitium (PT).

x 5880

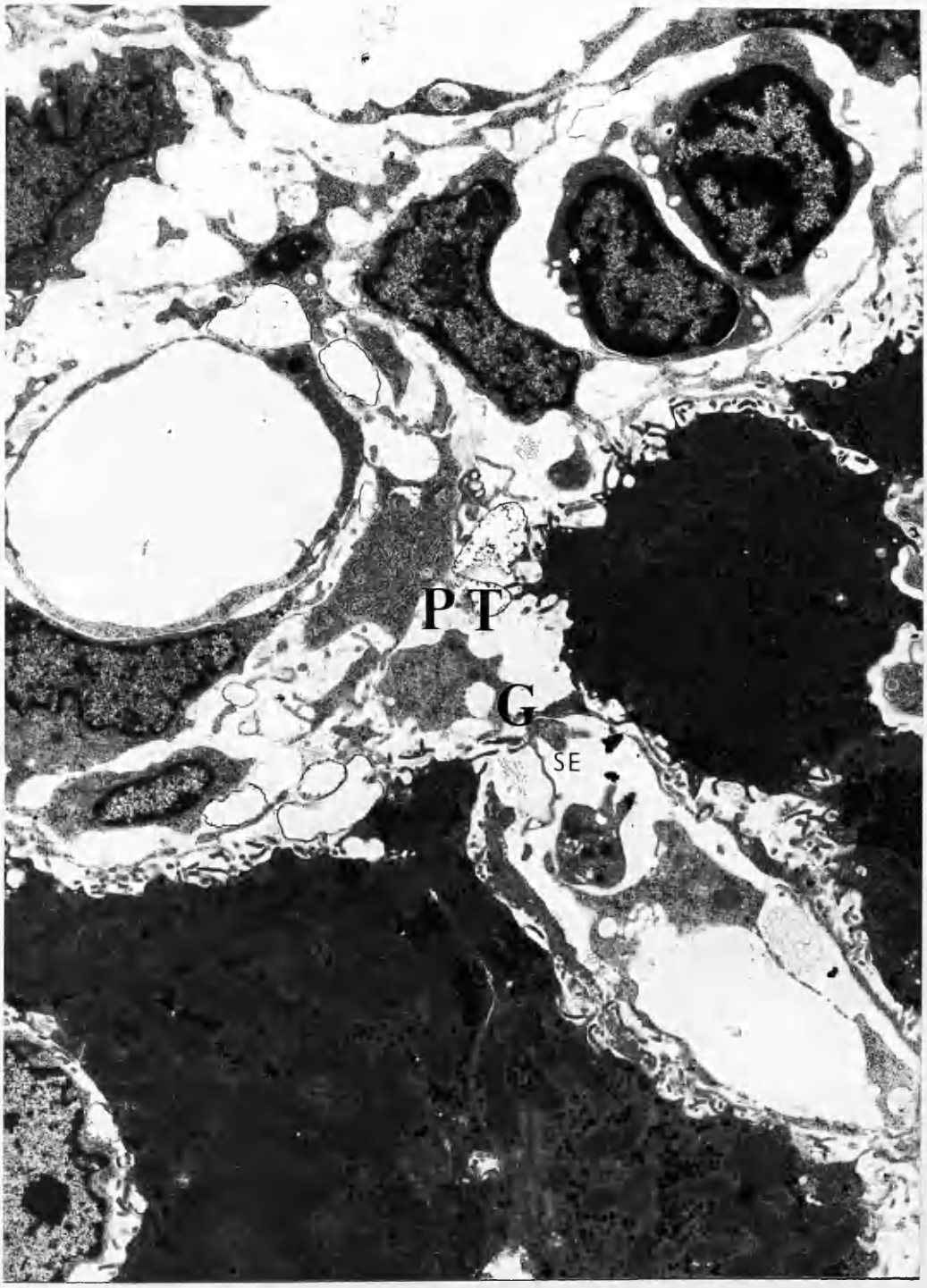

PT
G
SE

Fig. 14

A gap (G) about 3.5 μm wide between two hepatocytes of a limiting plate where the sinusoidal endothelium (SE) abuts directly on the portal tract interstitium (PT).
x 15680


PT
G
SE

Fig. 15

A gap (G) about 12 μm wide between two hepatocytes of a limiting plate where the sinusoidal endothelium (SE) abuts directly on the interstitium of the portal tract (PT).
x 5886

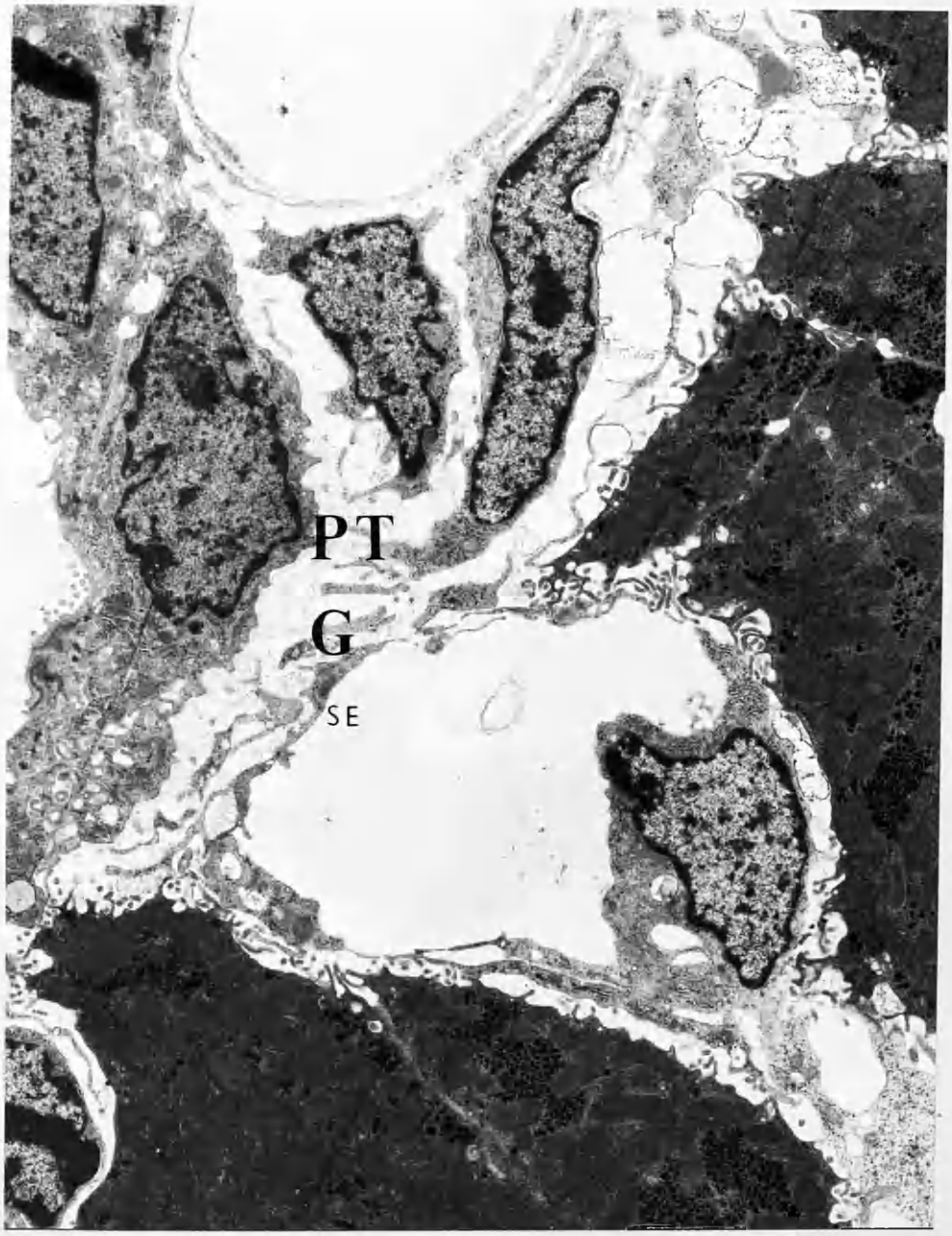
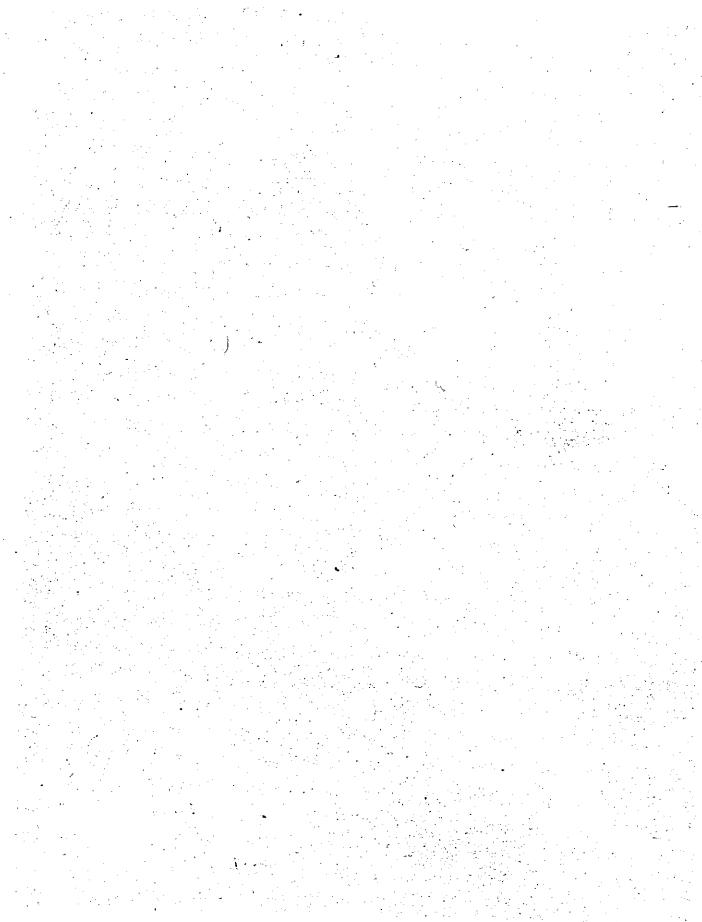

PT
G
SE

Fig. 16

The endothelium (E) of a longitudinal sectional profile of a sinusoid (S) abuts directly on the interstitium of the portal tract (PT) through a gap (G), which is about 32 µm wide, between two hepatocytes of a limiting plate.
x 3920




S E
G PT

Fig. 17

The gap (G) can be traced into continuity with the perisinusoidal spaces of Disse (D) associated with the two sinusoids S1 and S2 and their interconnections.
x 5880

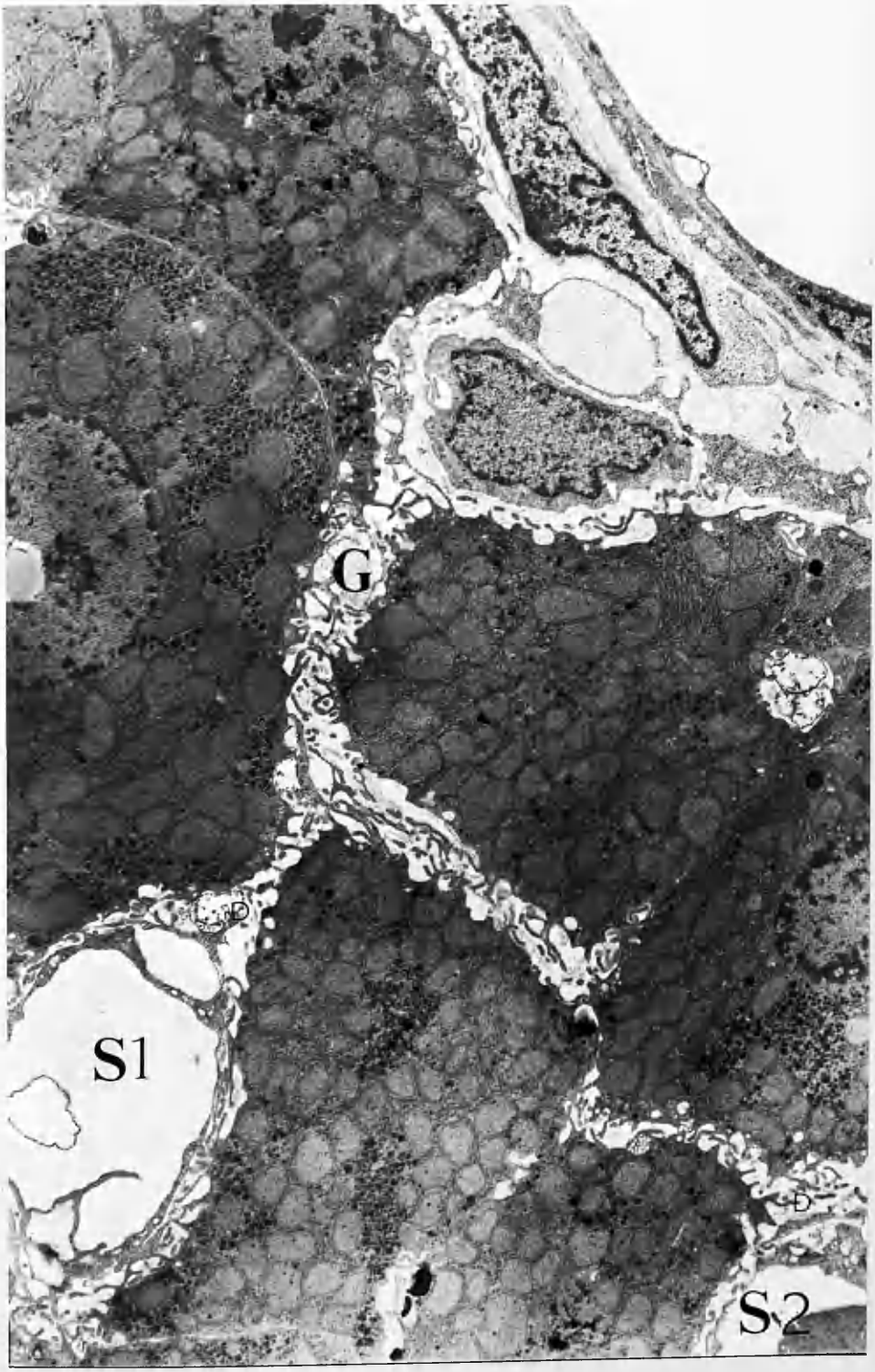

G
S1
S2

Fig. 18

This is one example of the ramifications of the space of Disse (D) between hepatocytes.

Note: the contrast has been deliberately increased to emphasize the intercellular expansions of Disse's space.

x 5880

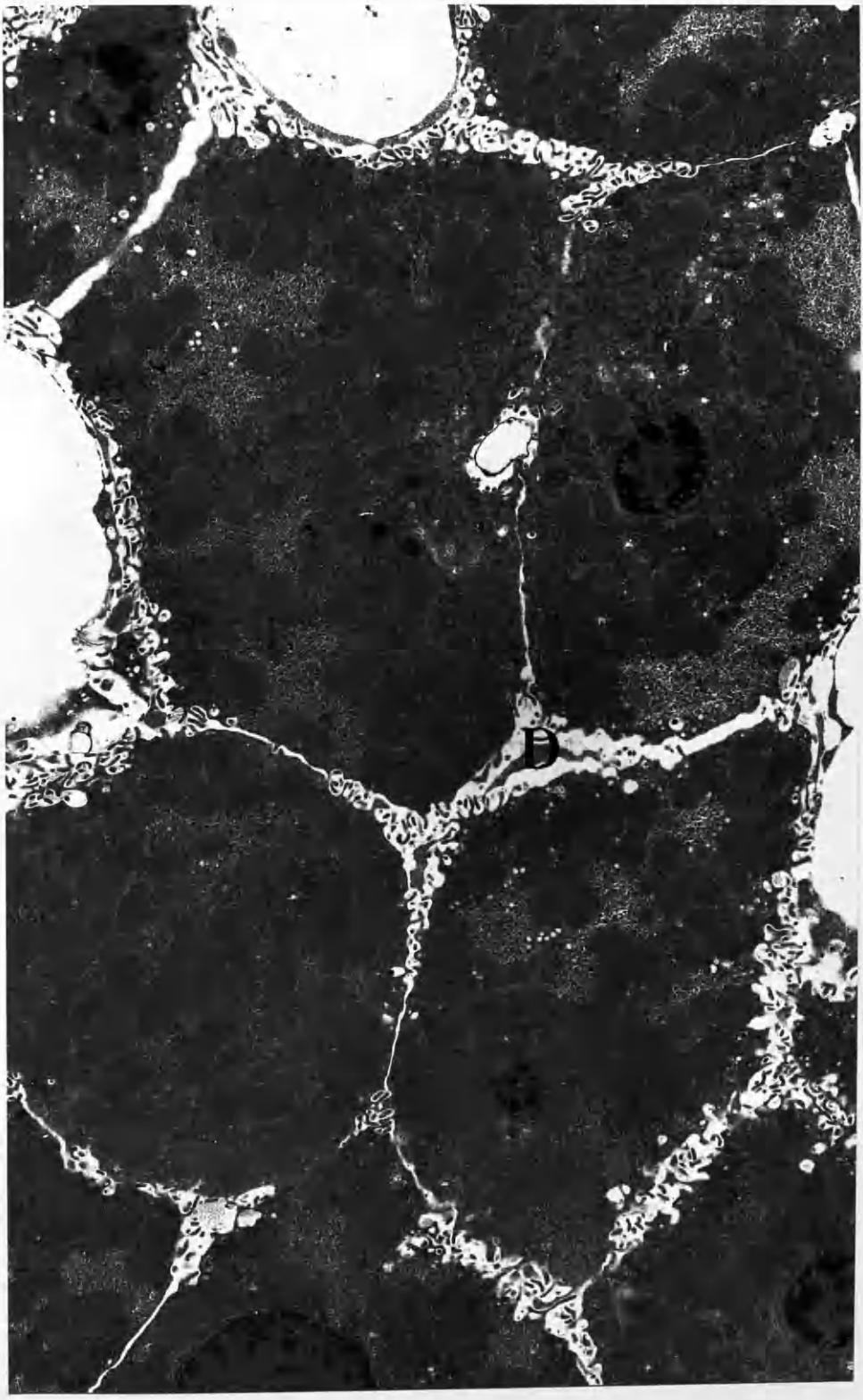

D

Fig. 19

This gap (G) between two hepatocytes of a limiting plate is mainly occupied by a bundle of collagen fibres which extend into spaces of Disse of several sinusoids.
x 3920

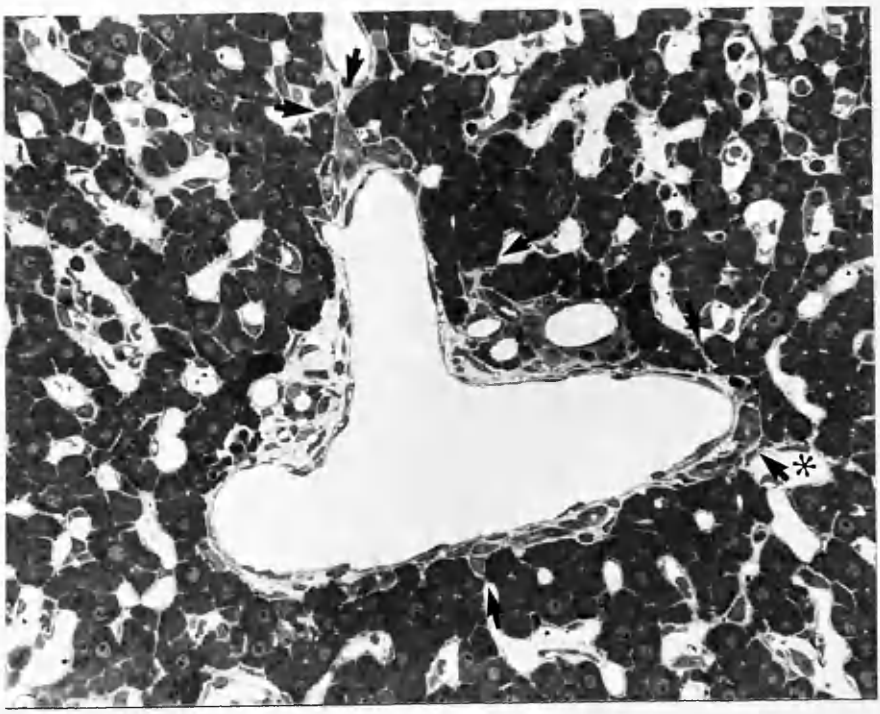
G

Fig. 20

In the sectional profile of this portal tract one can recognize 6 gaps (arrows) in the perilobular limiting plate, onle one of which (*) may possibly be associated with an entry of a portal vein branch.
x 360

Fig. 21

In the profile of this portal tract it is possible to recognize 7 gaps (arrows) in the perilobular limiting plate of which only one (*) can be related to an entry of a portal vein branch.
x 312



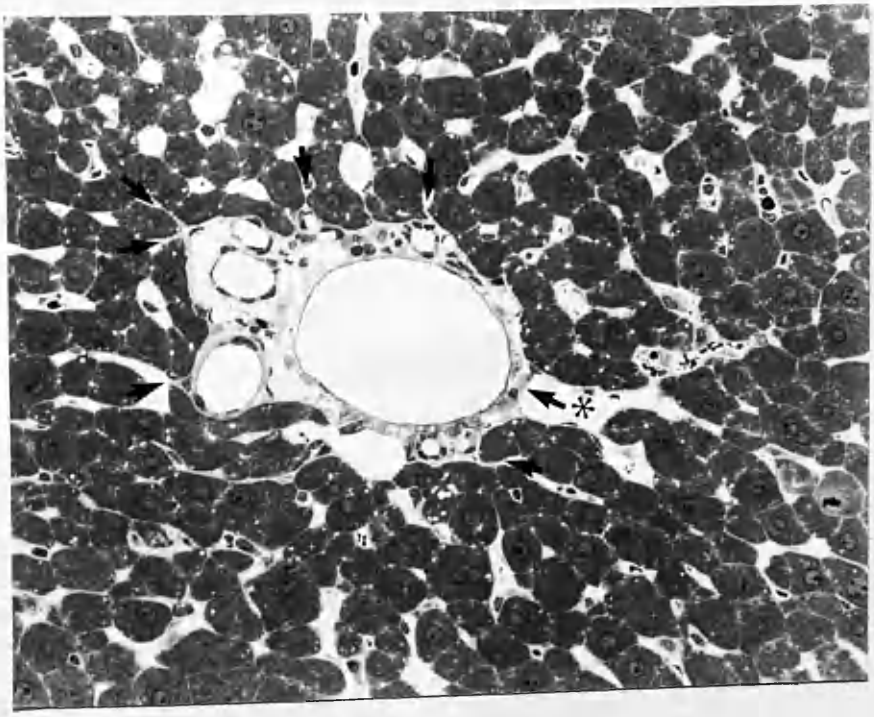


Fig. 22

There are 4 gaps (arrows) in the perilobular limiting plate of the sectional profile of this portal tract. x 312

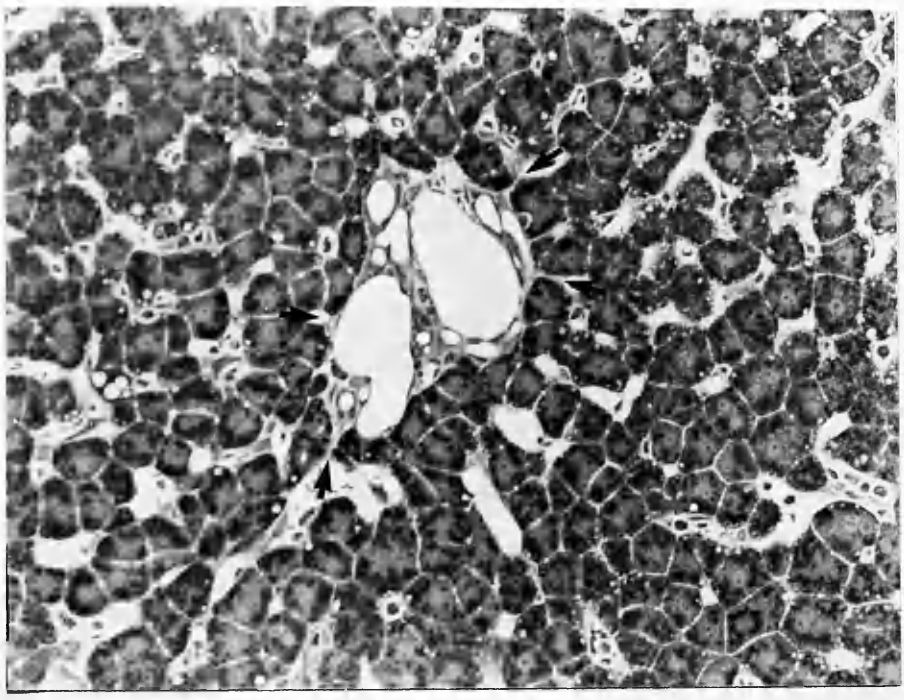


Fig. 23

It is possible to identify 2 gaps (G1, G2) in a TEM field at a relatively low power of 1400.
x 3920

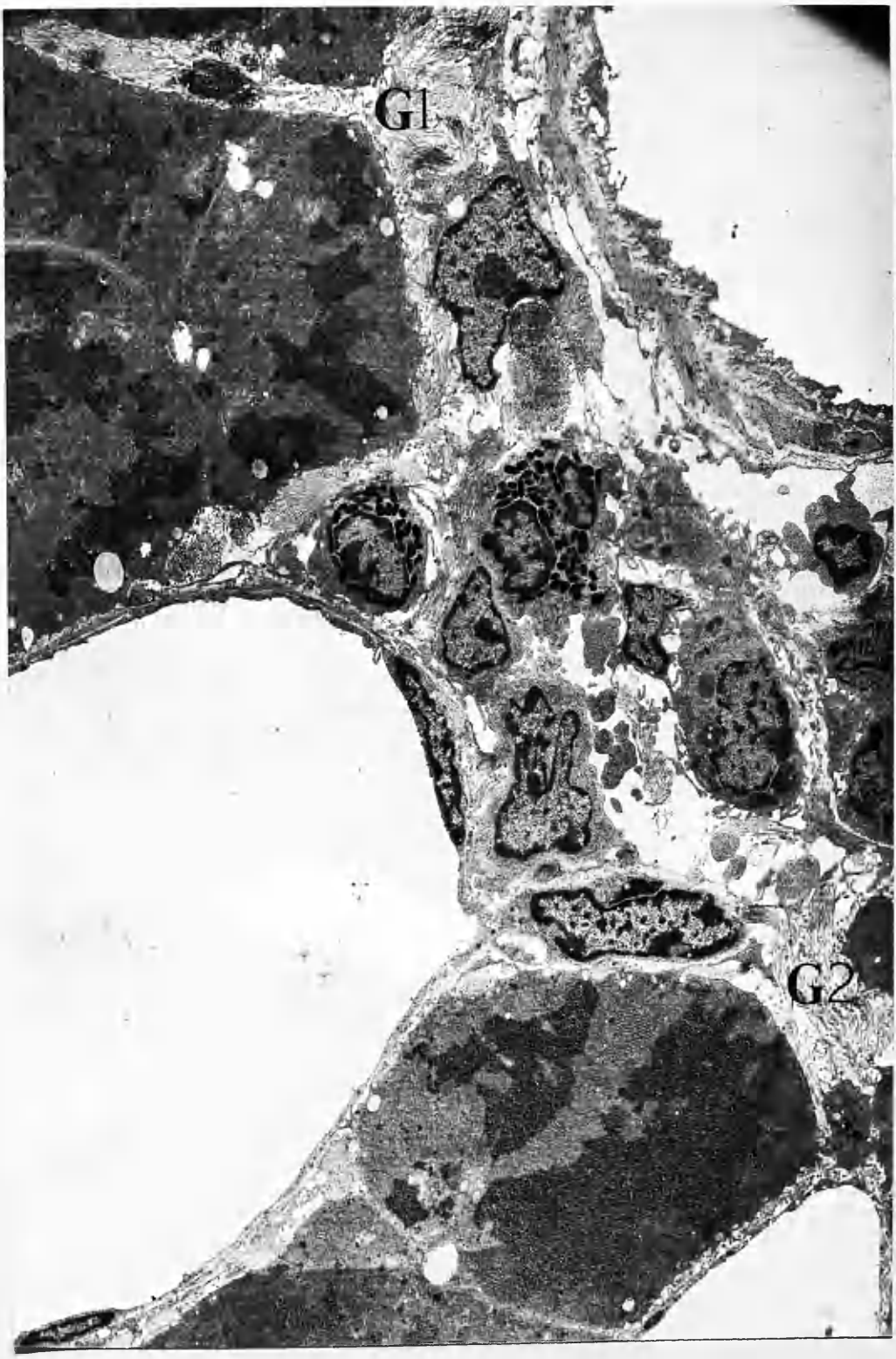

G1
G2

Fig. 24

Shows 3 adjacent gaps (G1, G2, G3) between 4 hepatocytes of a limiting plate.
x 5880

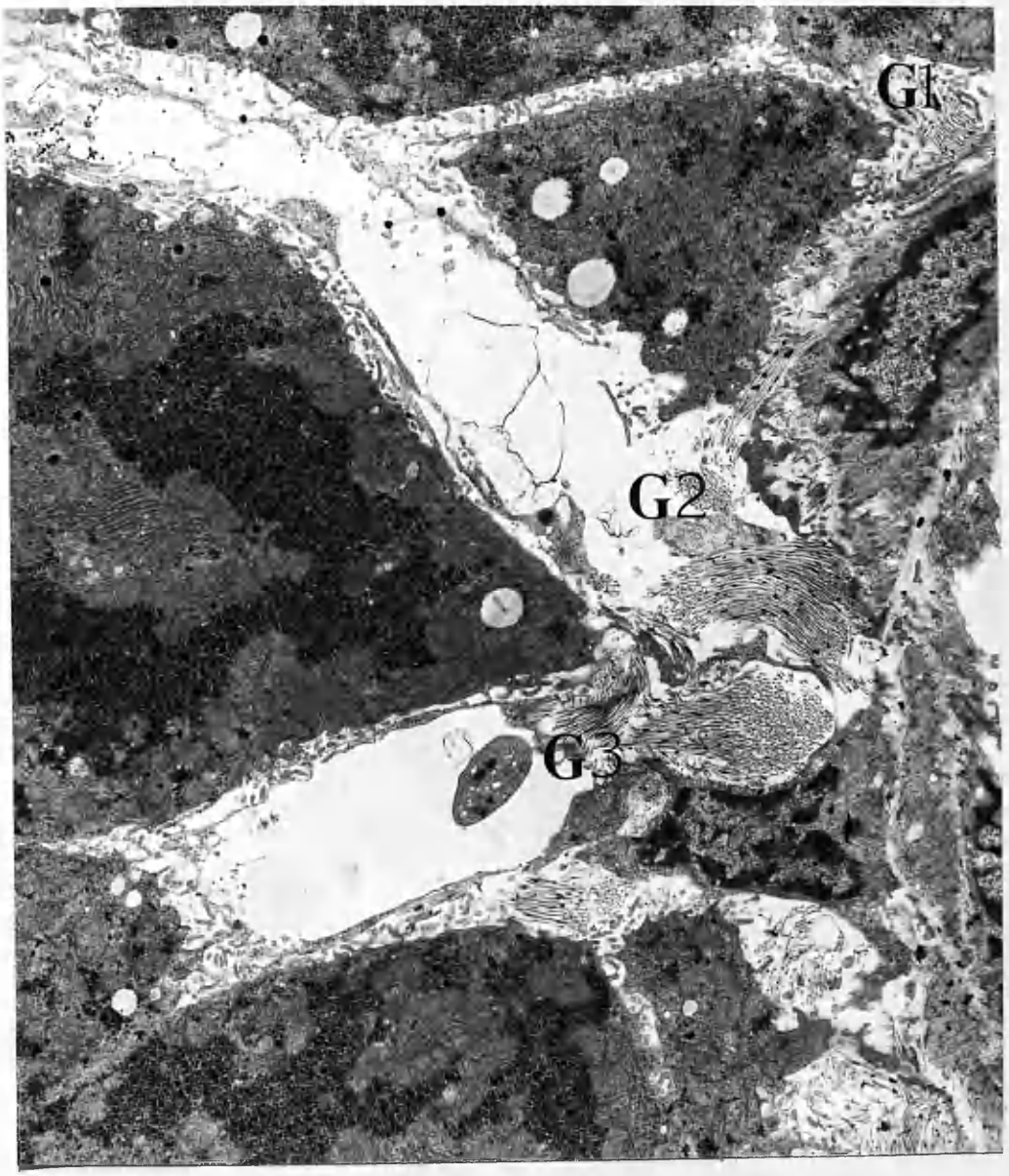
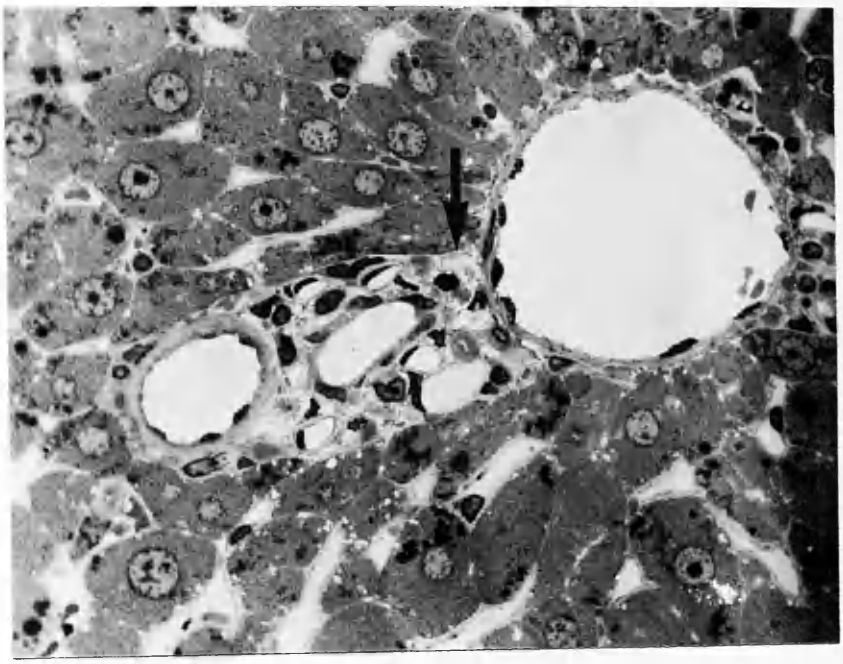

G1
G2
G3

Fig. 25

A. Photomicrograph of the smallest lymphatic profile (arrow) identified by optical microscopy.
x 320

B. An electronmicrograph of the adjacent section of the same area confirming that the vessel is in fact a lymphatic capillary (about 3 μm in diameter).
x 5880



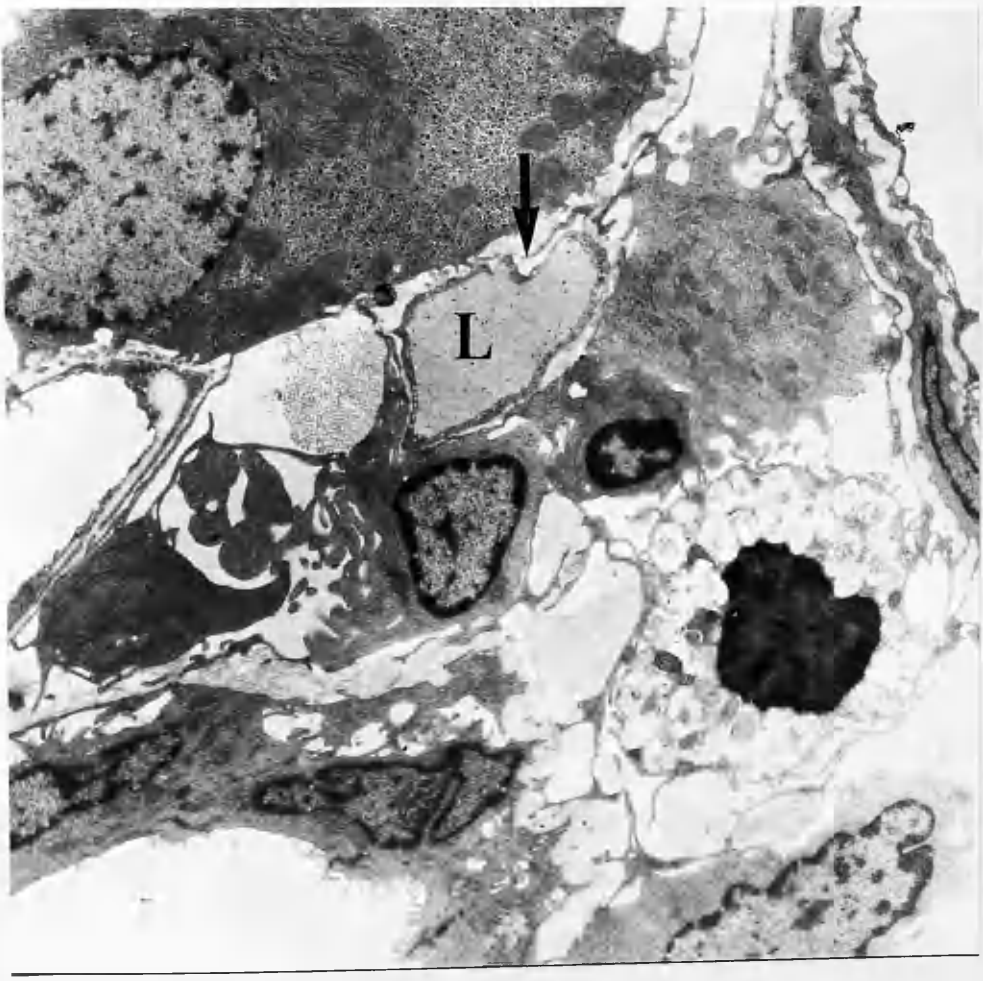

L

Fig. 26

Shows that in this portal tract profile, whose portal vein branch diameter is about 40 μm, lymphatics are absent.
x 520

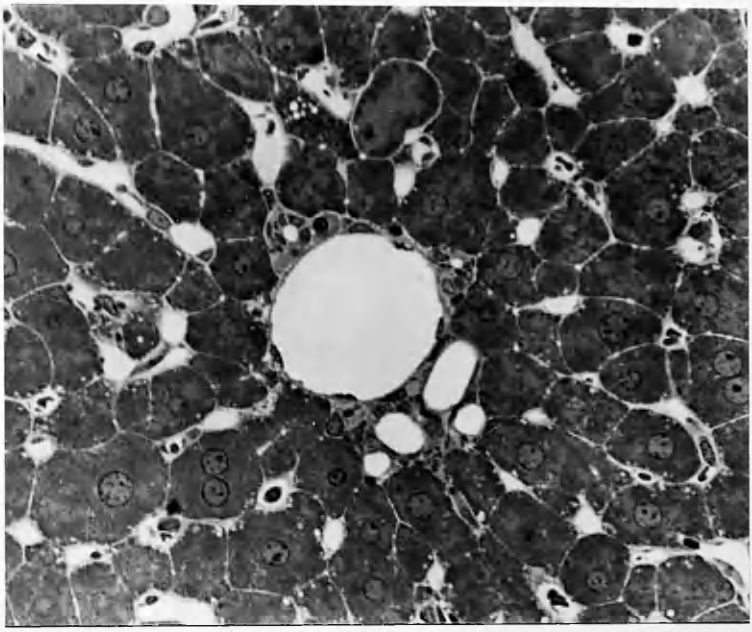
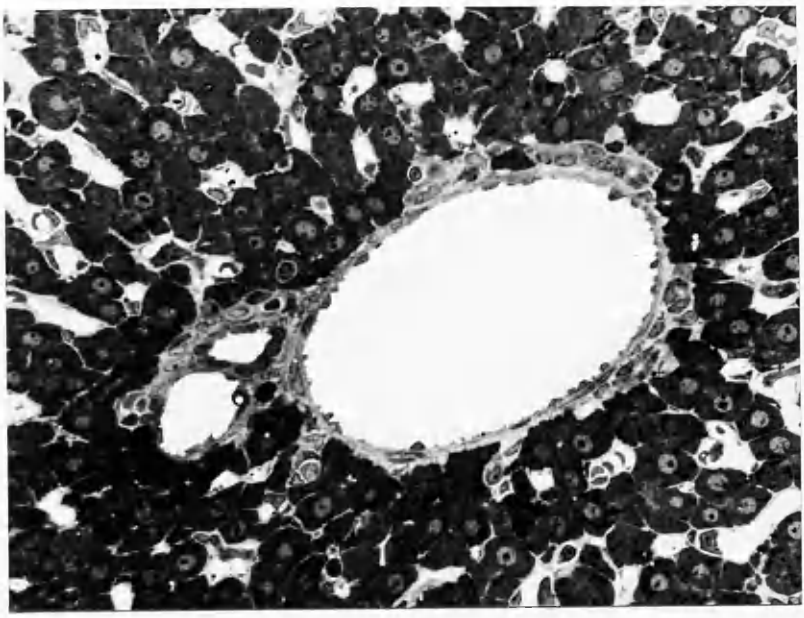


Fig. 27

A. Shows that there are no lymphatics in a portal tract sectional profile whose accompanying portal vein branch diameter is about 73 μm.
x 430

B. No lymphatic vessel could be seen even in this portal tract sectional profile, whose accompanying portal vein branch is about 125 μm in diameter.
x 430



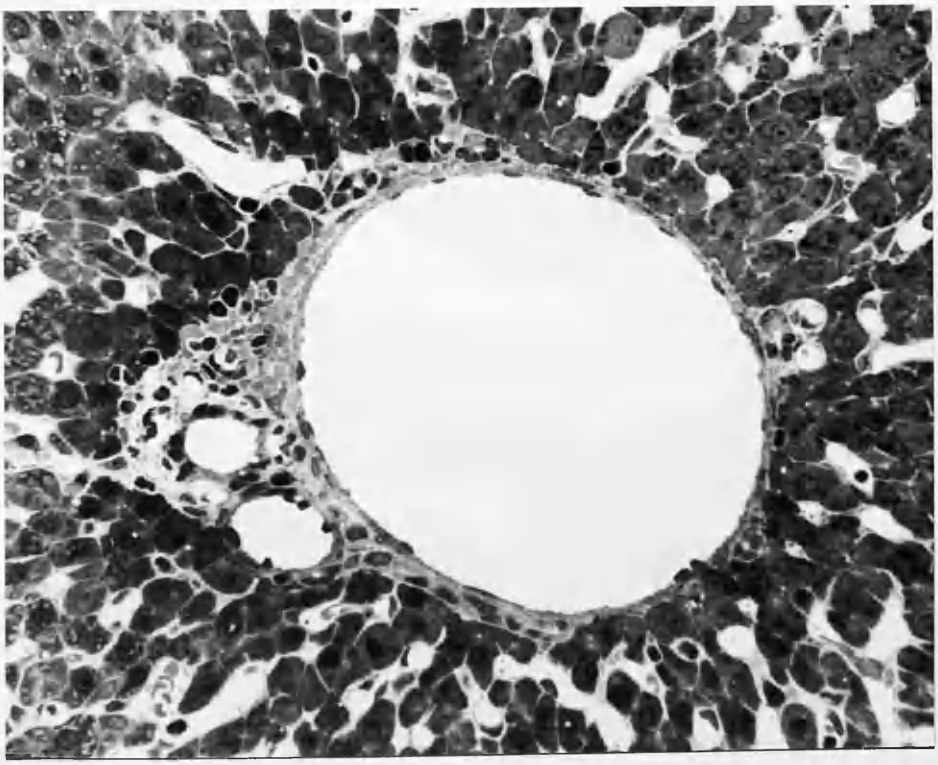


Fig. 28

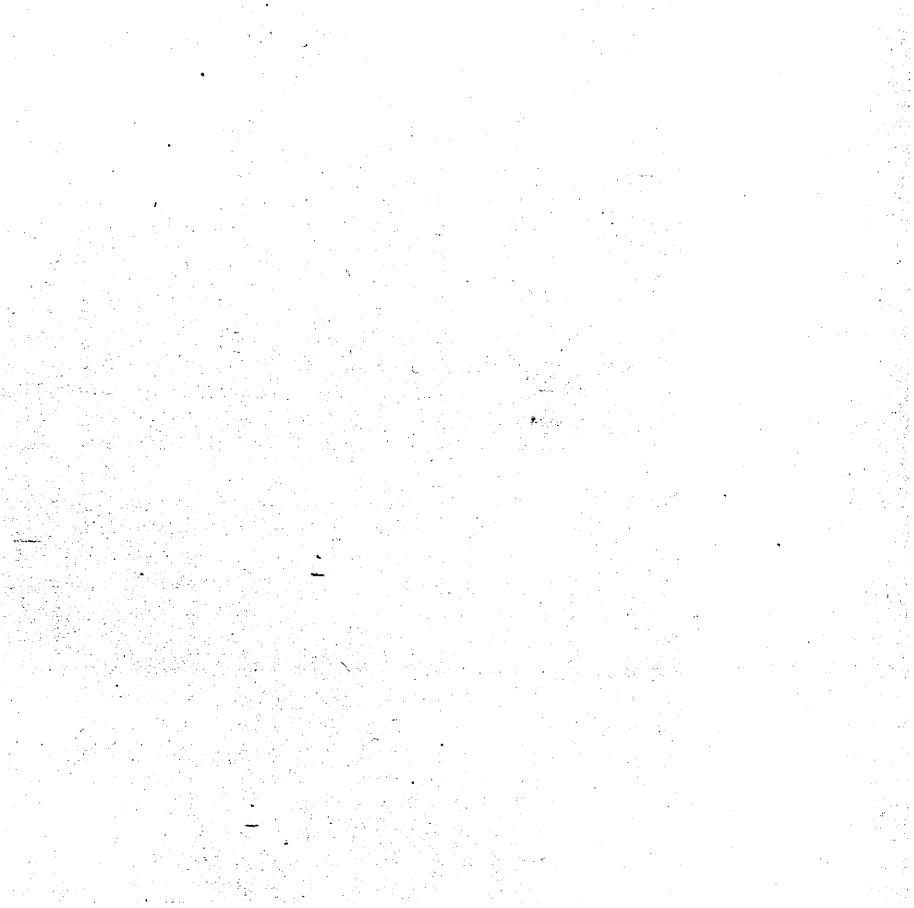
A SEM of part of a large portal tract.

Note: - the coarse, irregulary arranged collagen bundles (C).

- a typical scalloped lymphatic vessel (L) with a thin wall and proteinous contents.

BD - bile duct
C - capillary
V - vein

x 1030



C
D
V
L
C
01 L8!0 52 001

Fig. 29

A high magnification of a small area in the interstitium of a large portal tract.

Note: closely packed collagen bundles (C).
- a fibroblast (F)
- mast cell (M)
- bundle of nerve fibres (NF)
- a lymphatic vessel (L)
- a selnder fibroblastic process

x 15680

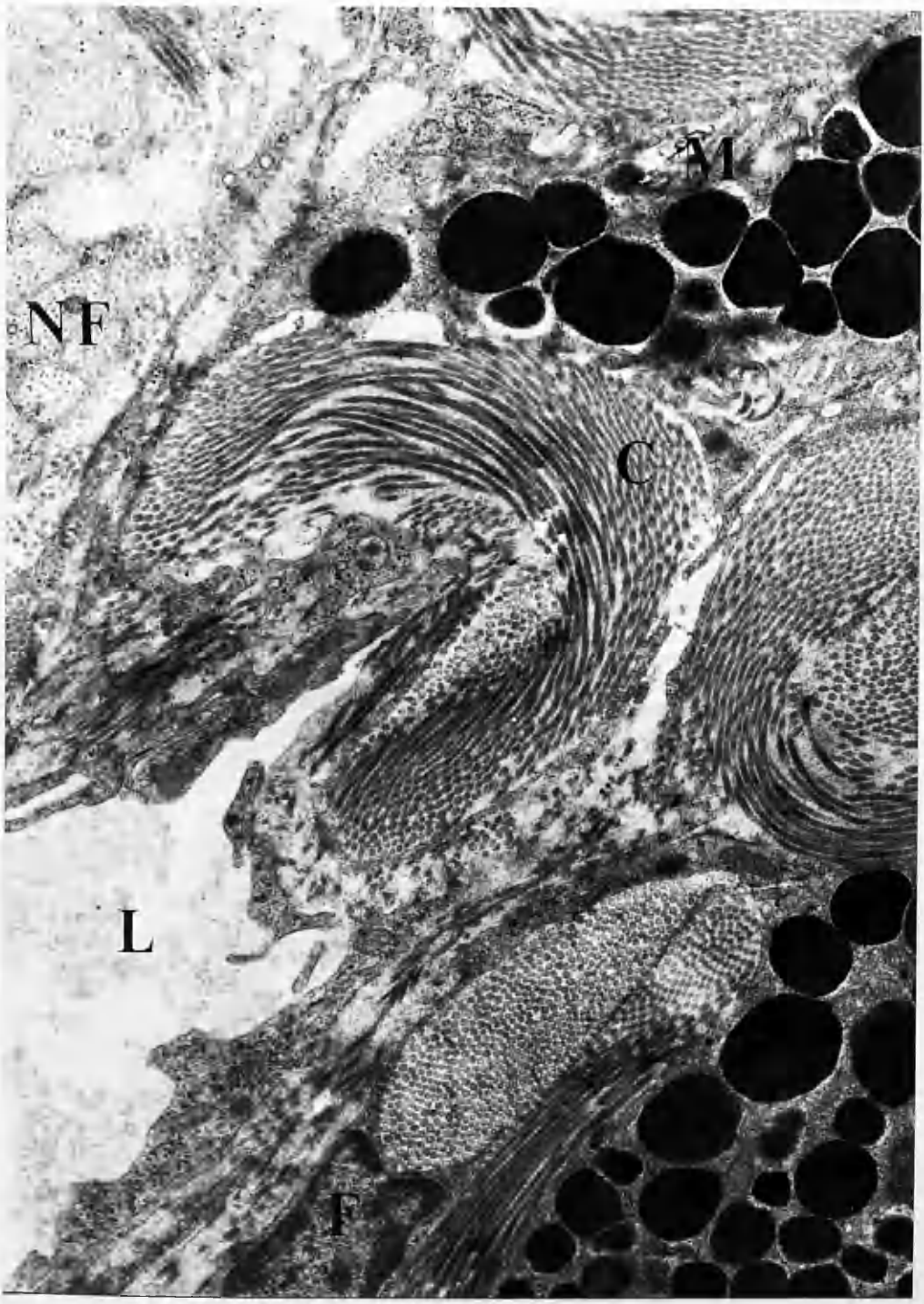

M
NF
C
L
F

Fig. 30

Showing an area in the interstitium of portal tract smaller than the previous one in Fig. 29.

Note:
- looser connective tissue
- compare the cytoplasmic processes of the fibroblast with the lymphatic endothelium of the lymph vessel (L)
- the intercellular junctions (J) between lymphatic endothelial cells.
- compare the density of the proteinous material in lymphatics with that in the loculi bounded by fibroblastic processes.
- the varying density of the collagen bundles.

x 15680

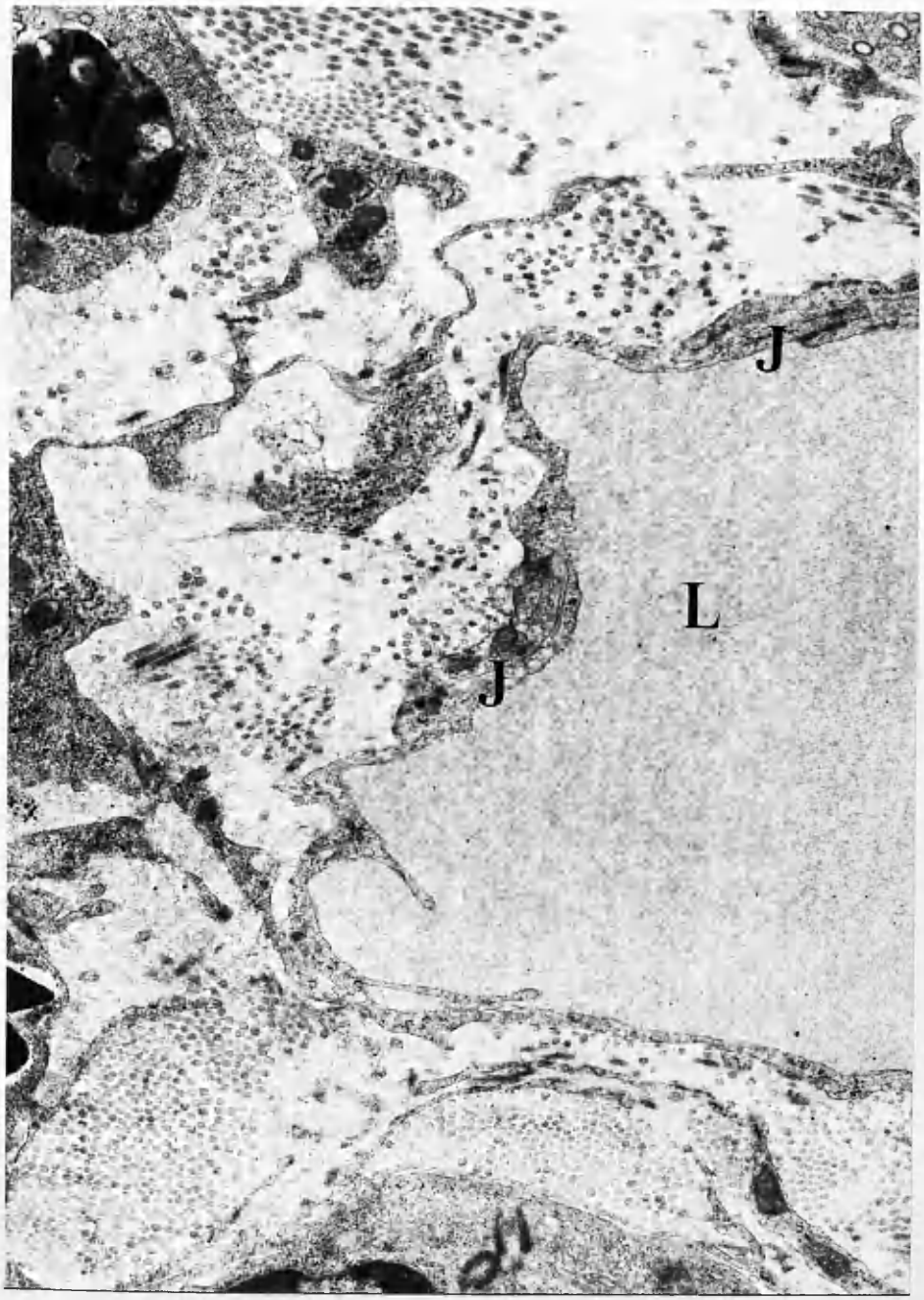

J
L
J

Fig. 31

Shows interstitial area of an even smaller and more peripheral portal tract.

Note: - much looser connective tissue.
- fibroblasts (F1, F2) sending out processes into the interstitium, thus creating loculi.

x 7840

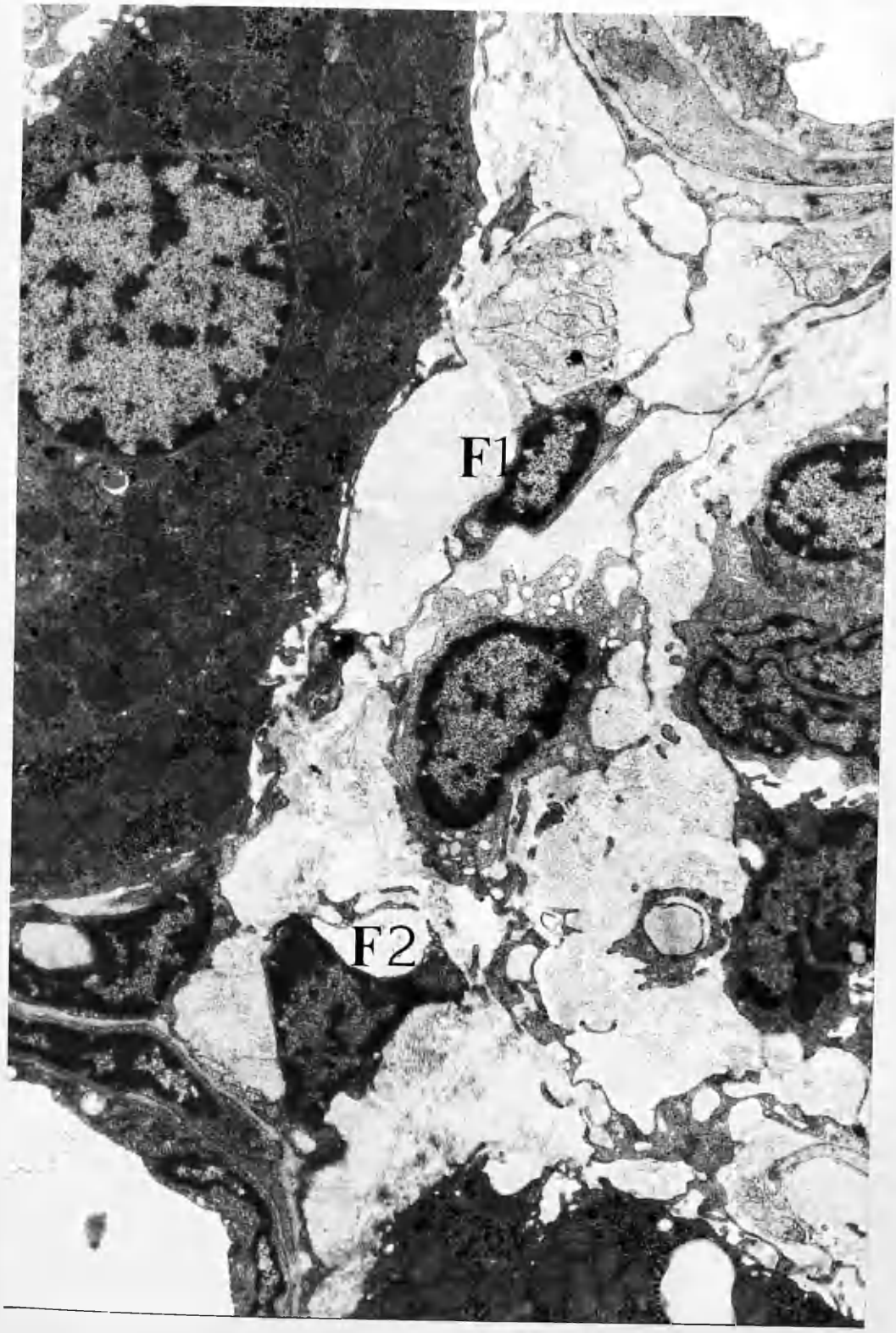
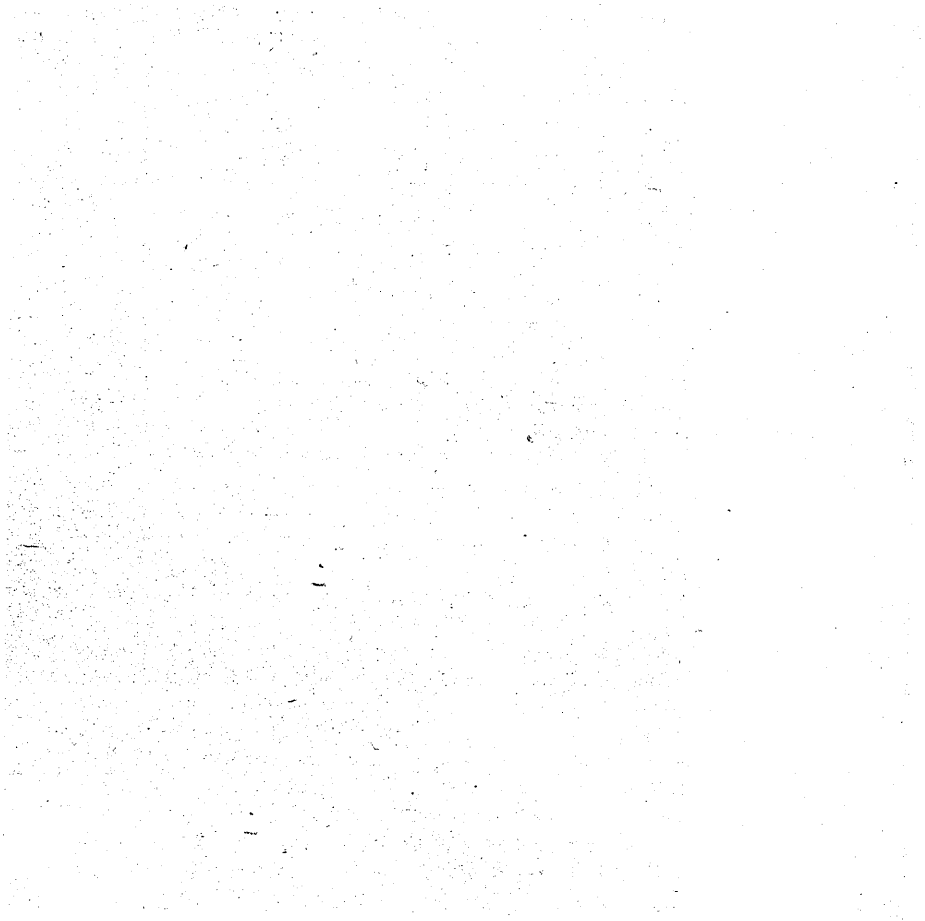

F1
F2

Fig. 32

Loculi (LOC) created by multiple processes of different fibroblasts with desmosome-like junctions (J).
x 19600



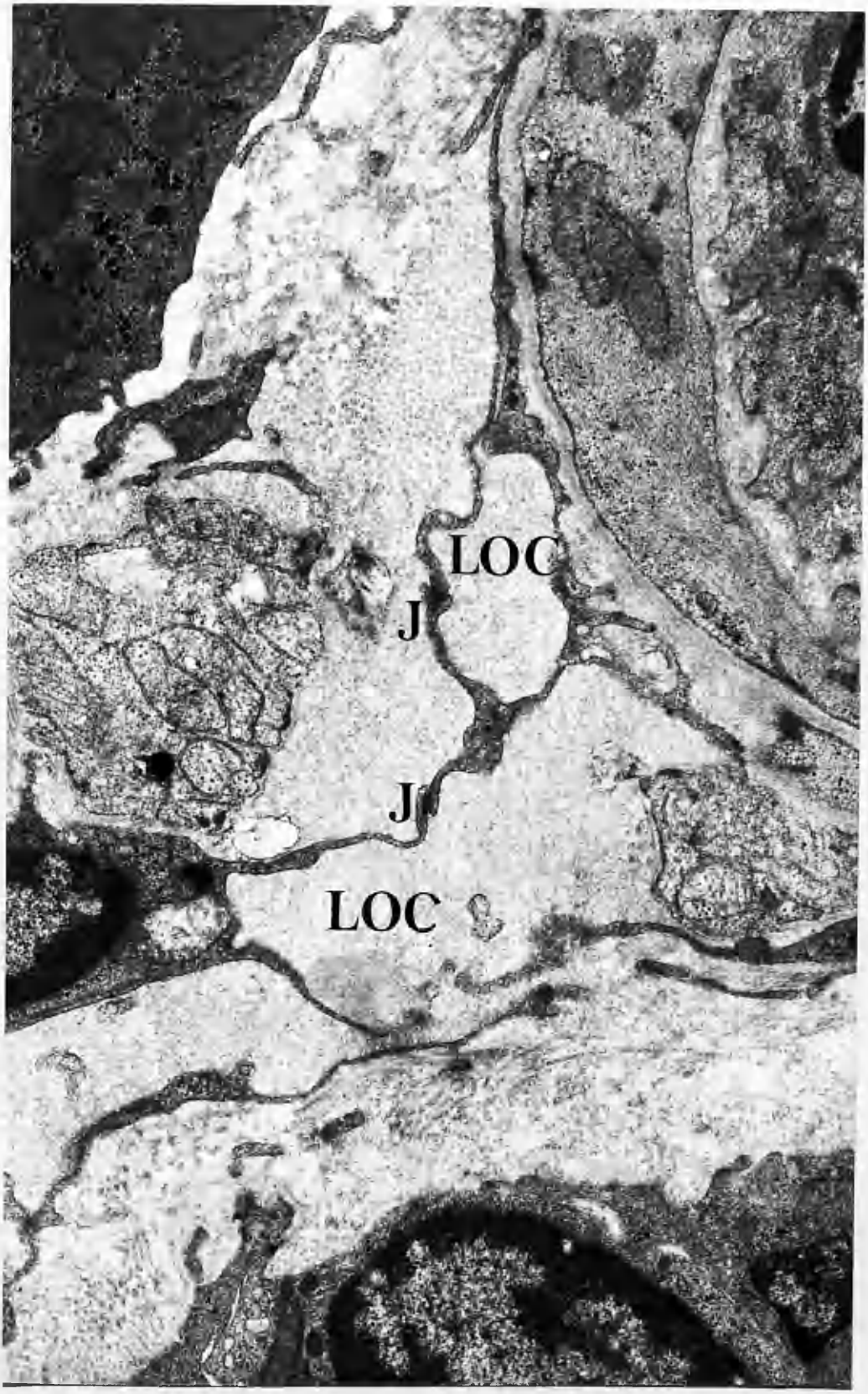
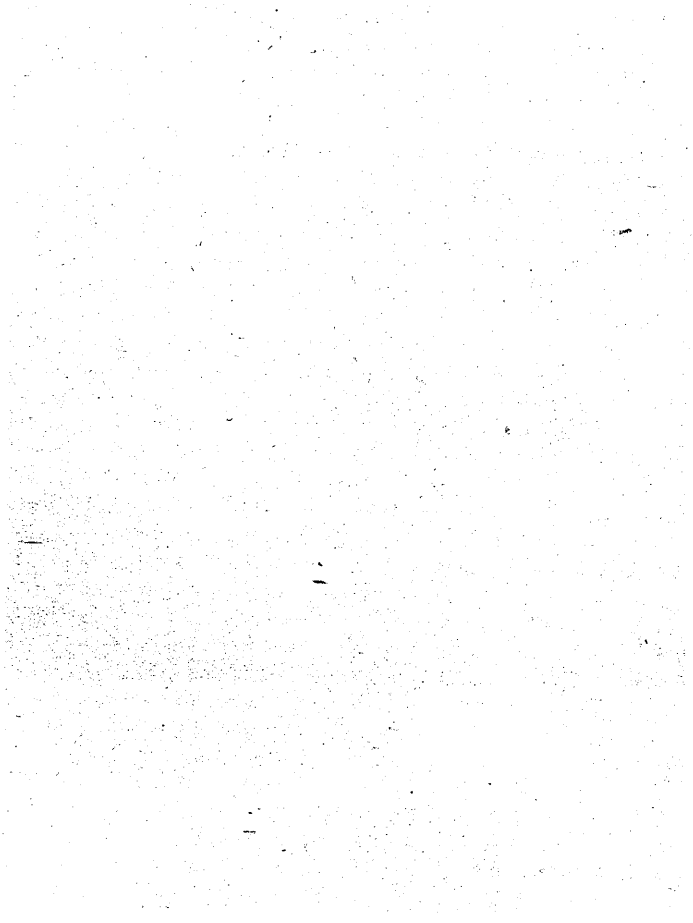

LOC
J
J
LOC

Fig. 33

Showing multiple fibroblastic processes (FP) enclosing a loculus that contains dense proteinous precipitate.

Note: - the area between the two lines AA', BB' might well be mistaken for endothelially lined lymphatics.
- collagen bundles CC' also make small part of the wall of the loculus.

x 15680



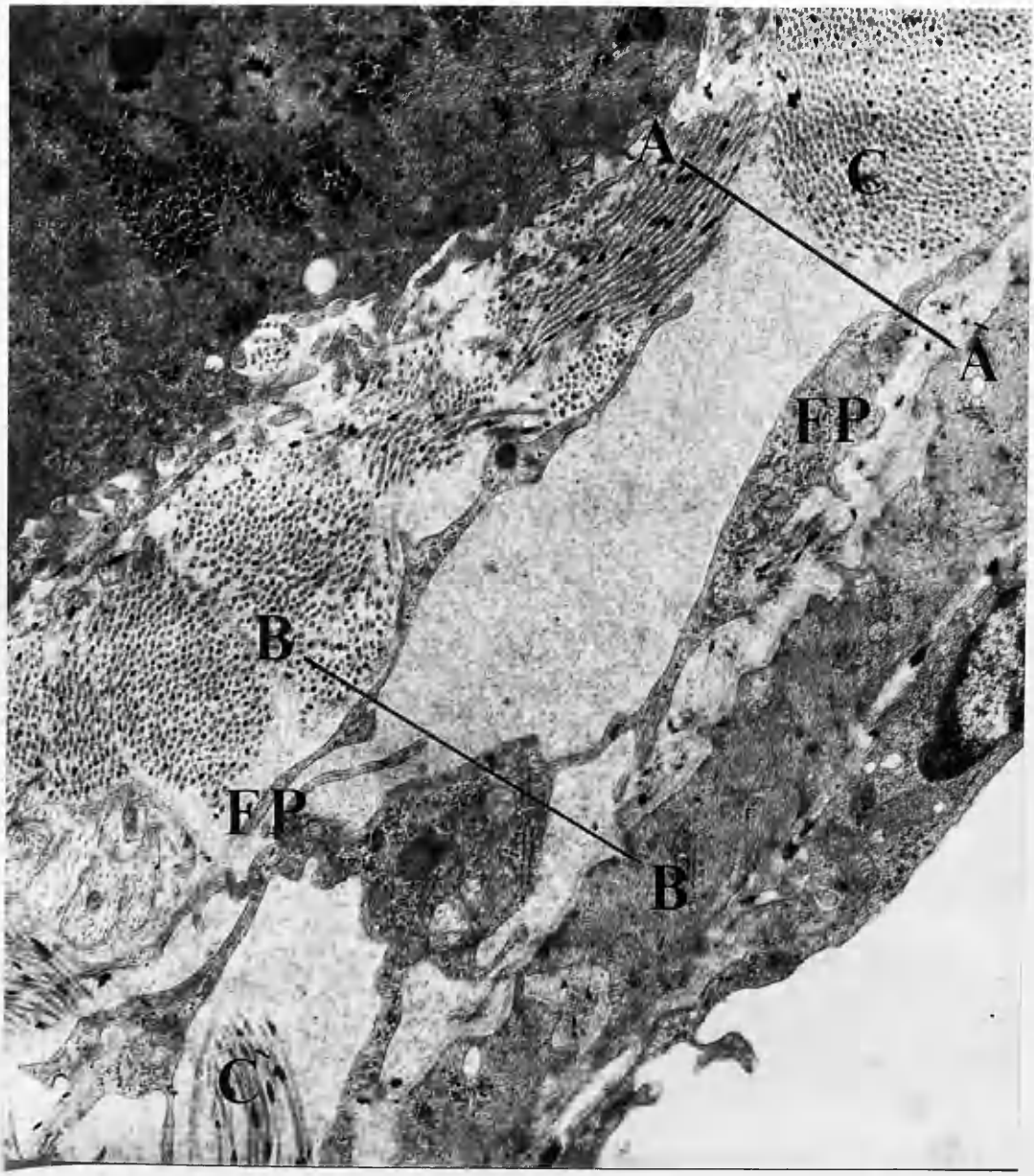
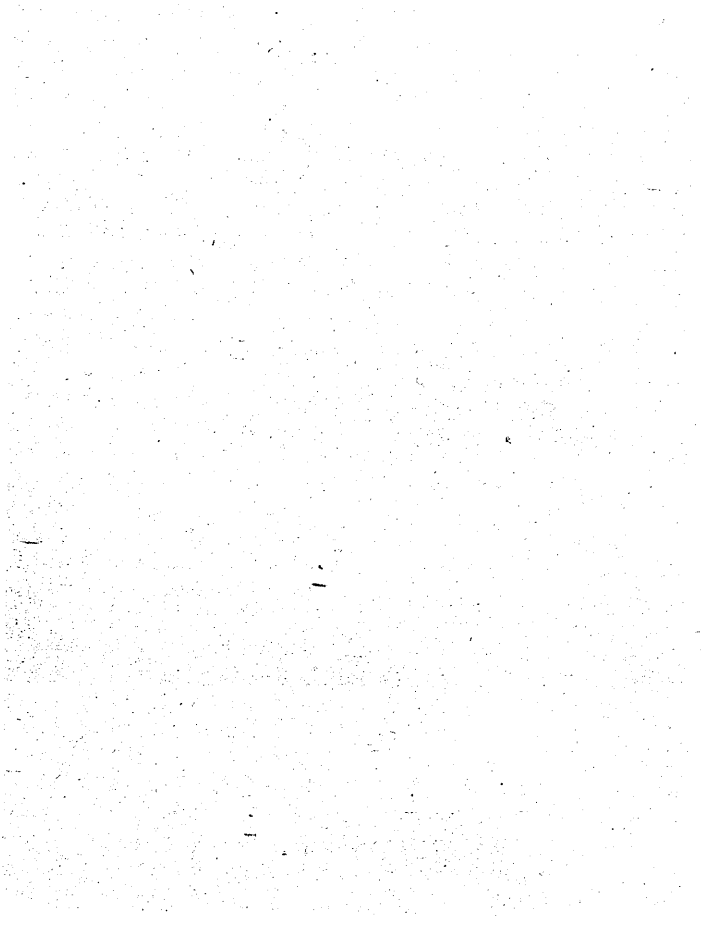

A
C
A
FP
B
FP
B
C

Fig. 34

Fibroblastic processes completely enclose a channel which might easily be mistaken for a lymphatic, although clearly it is not.

Note: - the nature and density of the contents
- the gaps (arrows) between fibroblastic processes that make the channel.

x 11760



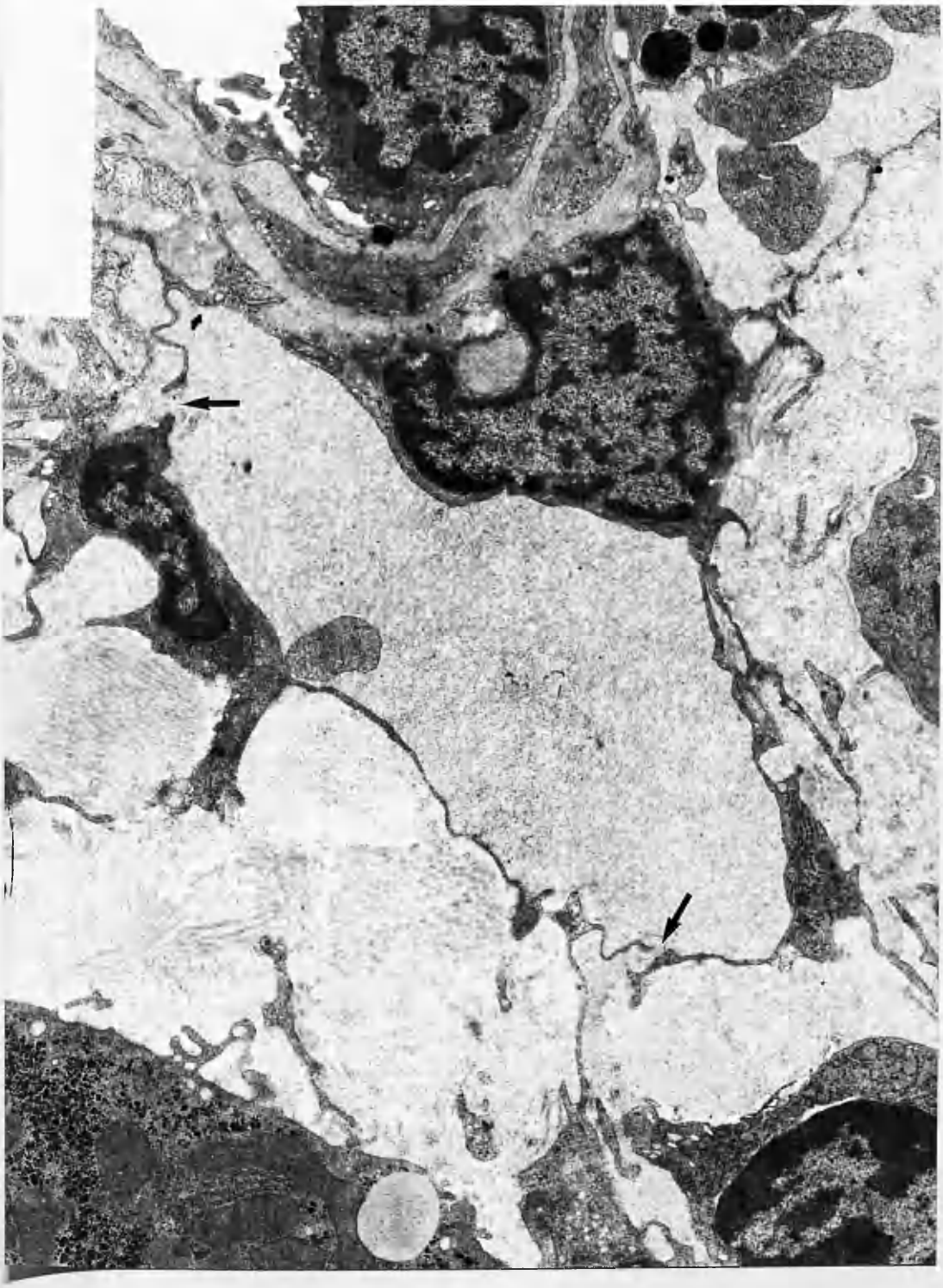


Fig. 35

A cross sectional profile of a fibroblastic channel (FC) which appeared to be a lymphatic by light microscopy. TEM revealed the fibroblastic nature of the lining, the collagen within the lumen (C) and the multiple openings.

- compare the contents with that of the neighbouring true lymphatics (L).

x 15680

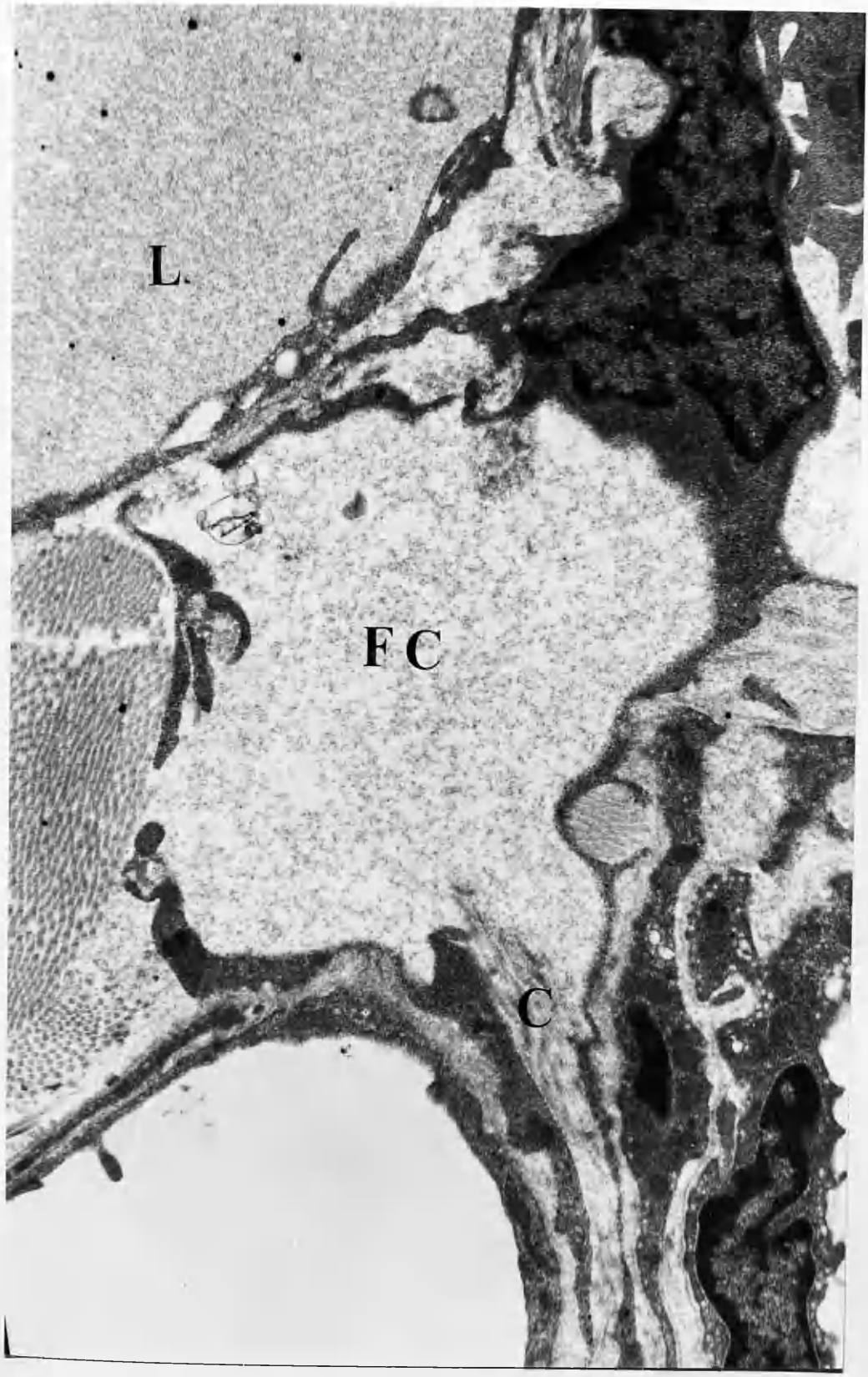

L.
F C
C

Fig. 36

Shows a longitudinal sectional profile of a fibroblastic channel which contains many cells (macrophages and neutrophils).

Note: the thin, interrupted and branching lining of fibroblastic processes.

x 7840

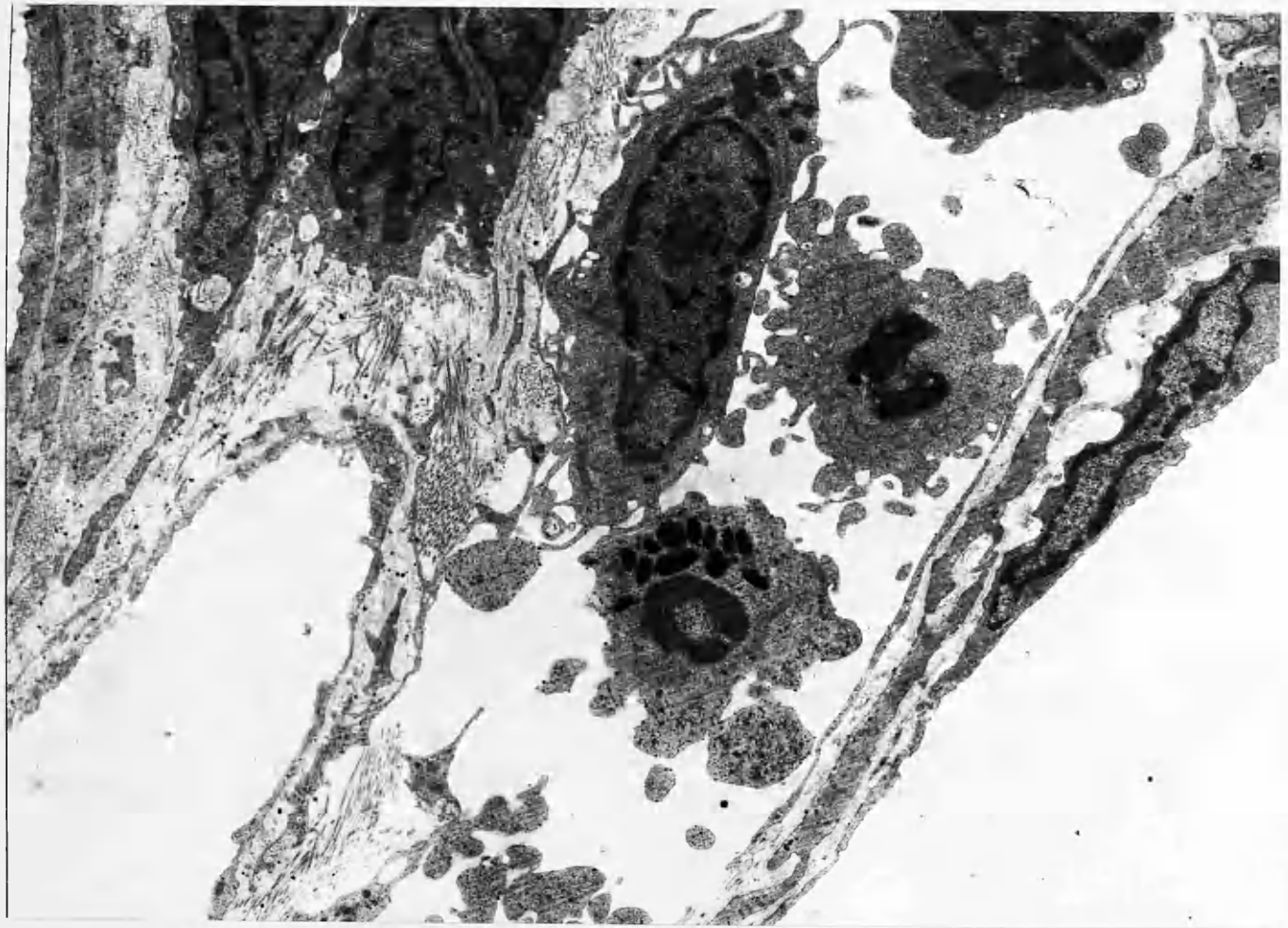
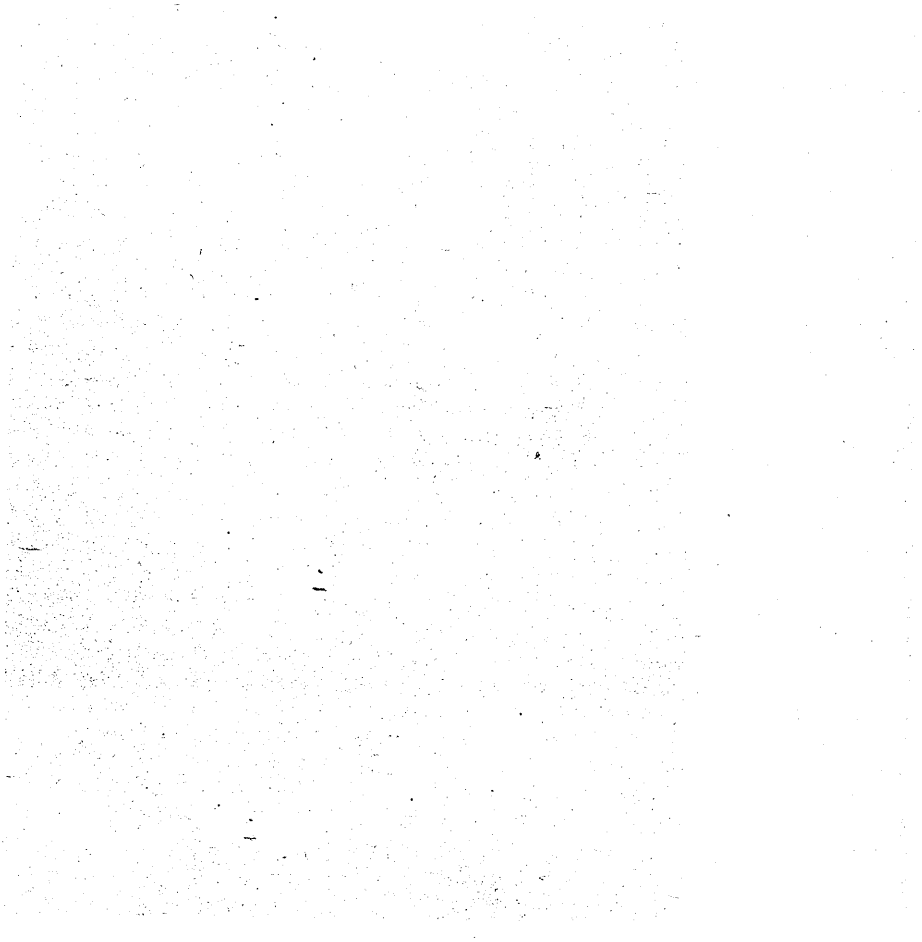


Fig. 37

Shows a longitudinal sectional profile of a fibroblastic channel.

Note:
- contents of precipitated protein and a mononuclear cell
- thin interrupted lining
- collagen (C) on the luminal and abluminal sides of the wall

x 15680



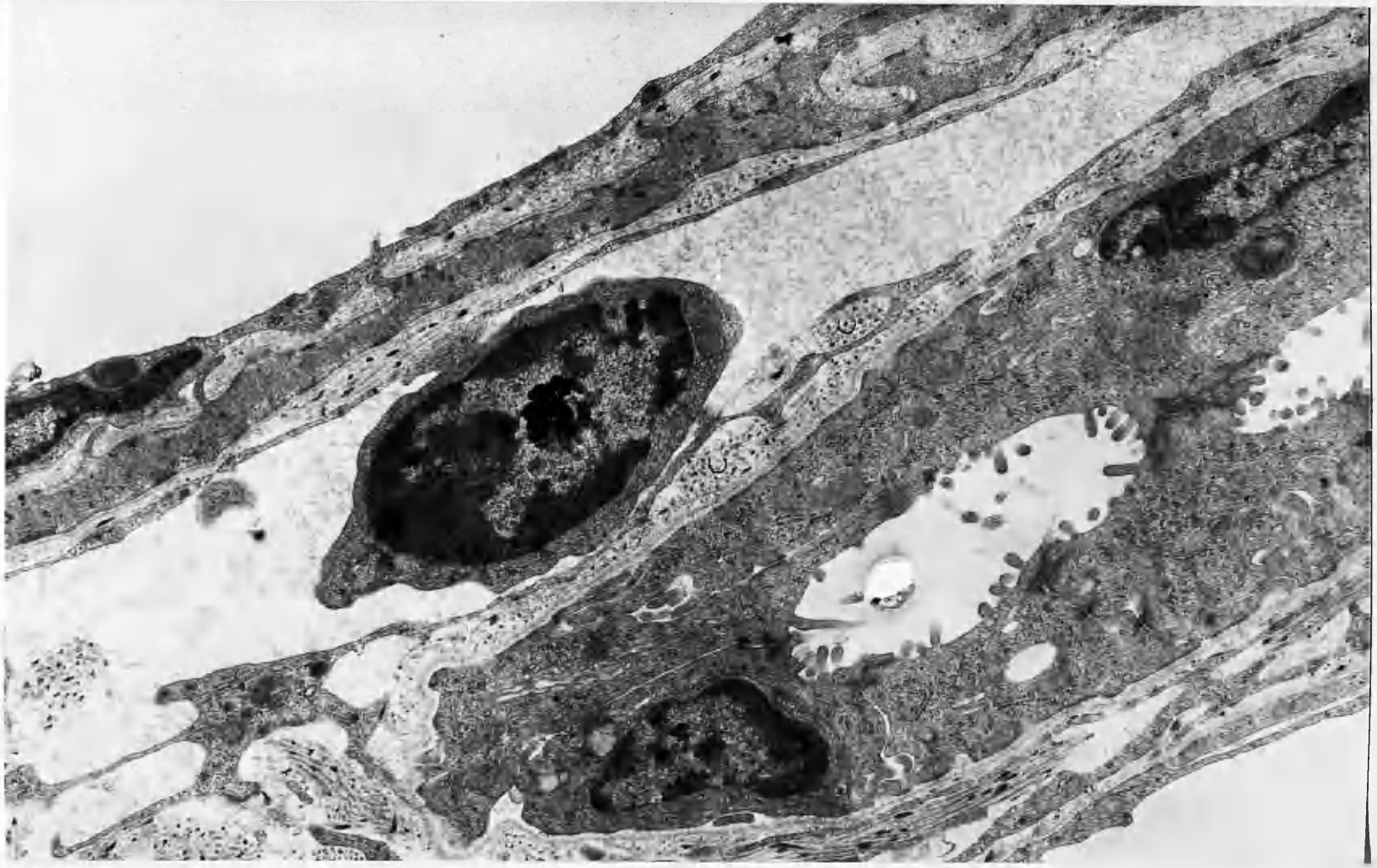
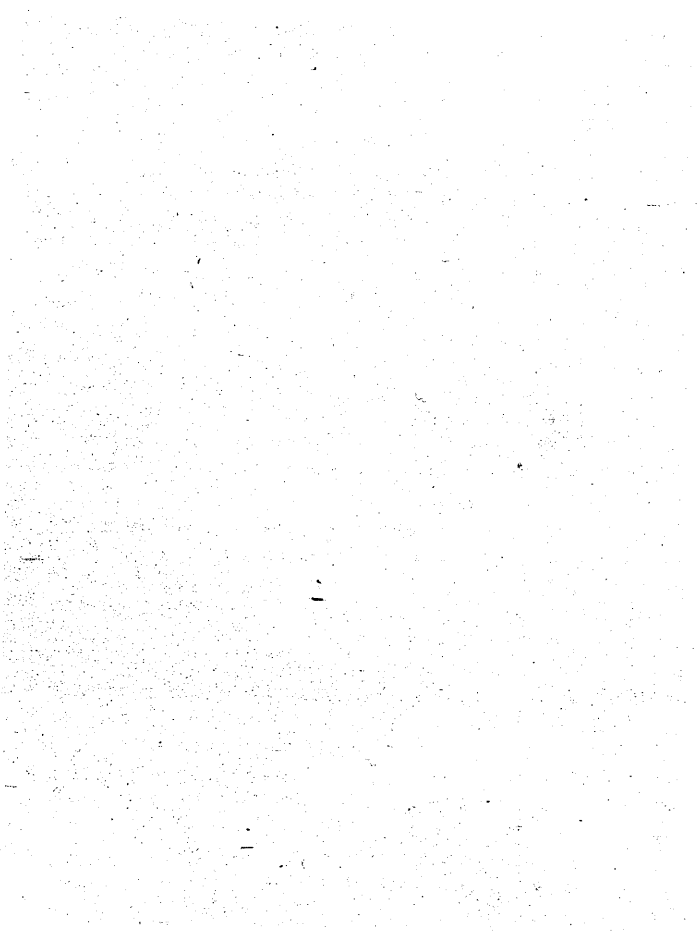


Fig. 38

Shows a cross sectional profile of a fibroblastic channel, almost completely enclosed by the processes of a single fibroblast. The gap left is completed by a bundle of collagen.
x 15680



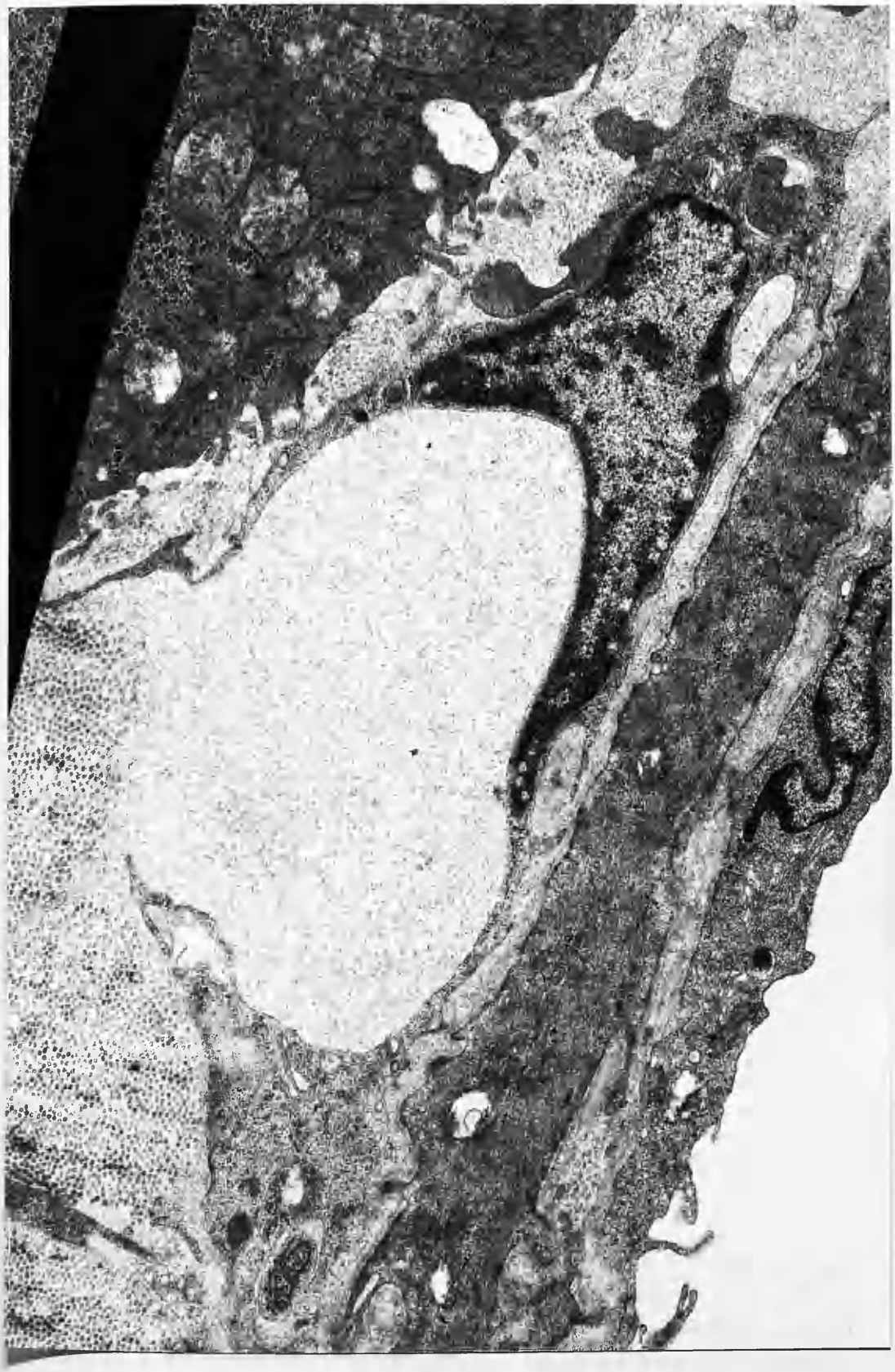


Fig. 39

Shows a sectional profile of a channel completely enclosed by fibroblastic processes.
x 7840

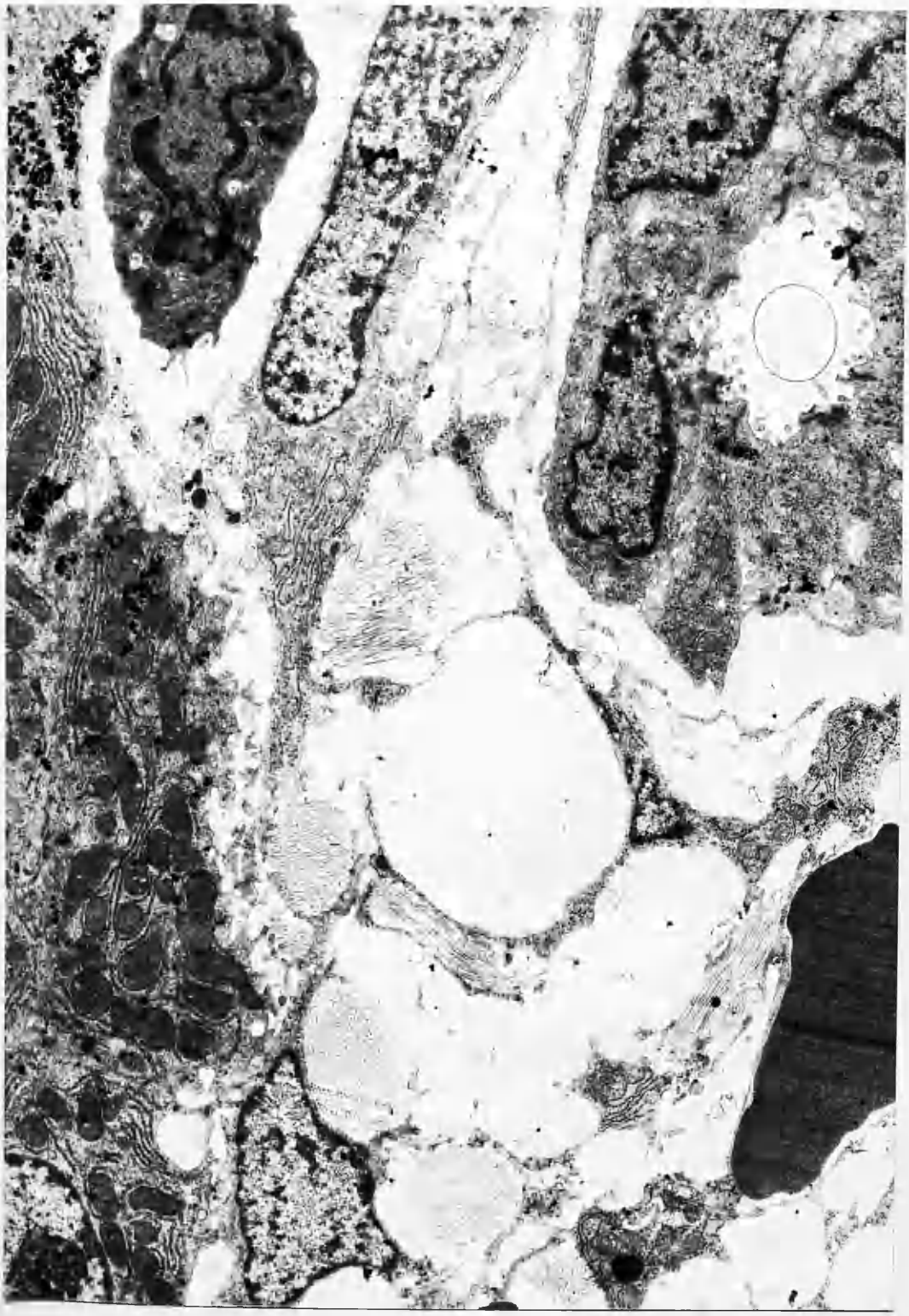


Fig. 40

Shows a fibroblastic channel (FC) adjacent to a blood capillary in a portal tract. Compare the size and proteinous content with that of the adjacent blood capillary.

Note: - the thin branching processes of fibroblastic cytoplasm which bound the channel
- the deficiency in the lining.

x 3920

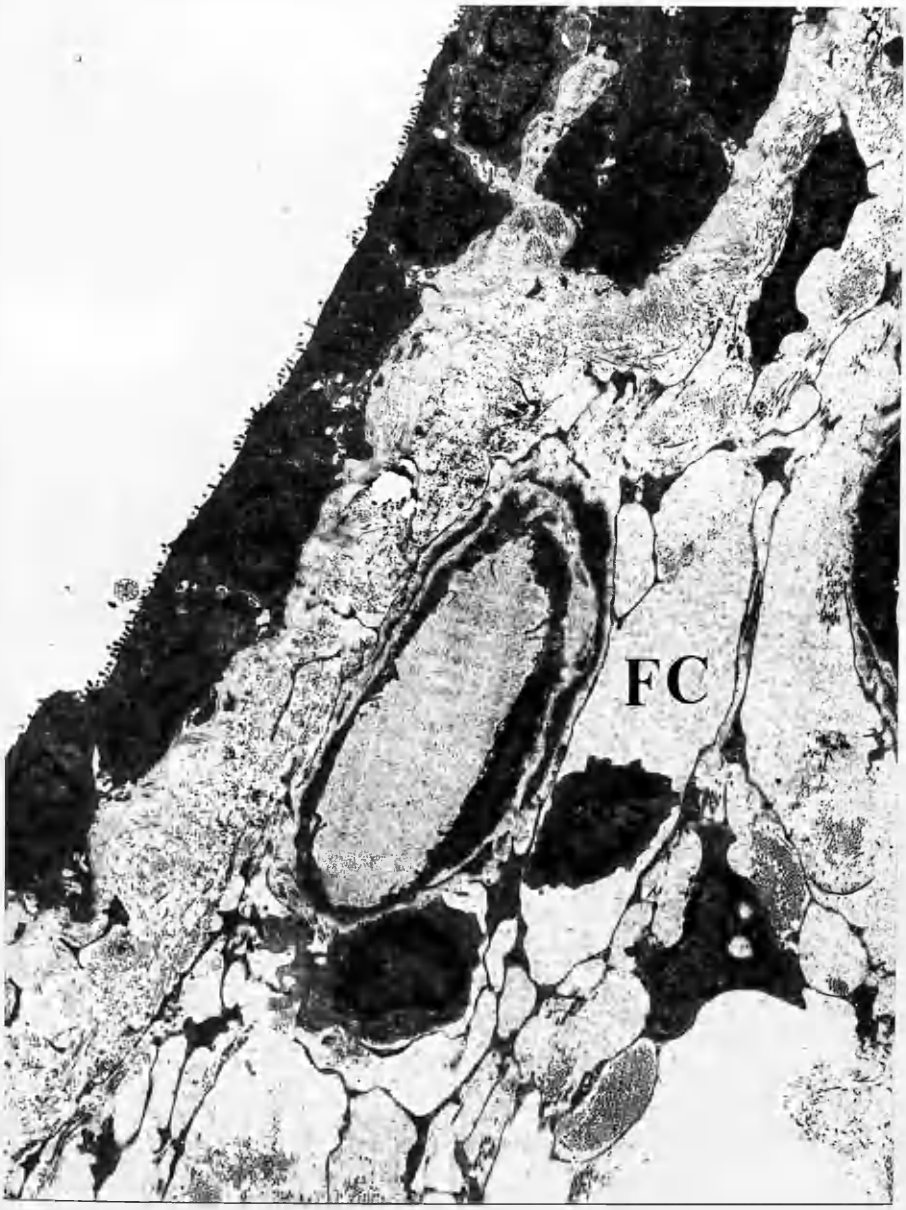

FC

Fig. 41

Shows the SEM appearance of fibroblastic channels which suggests that the wall is most likely made of sheets of fibroblastic processes (SH) with multiple gaps (G) and that the wall may be completed by collagen (C).
x 19000

C
SH
02 h81 0 52 1

Fig. 42

Shows a nearly complete fibroblastic channel (FC); the gap facing the hepatocyte of the limiting plate is completed by collagen (C).

Note: the relation of the gap in the fibroblastic channel to the space of Mall (M).

x 28000

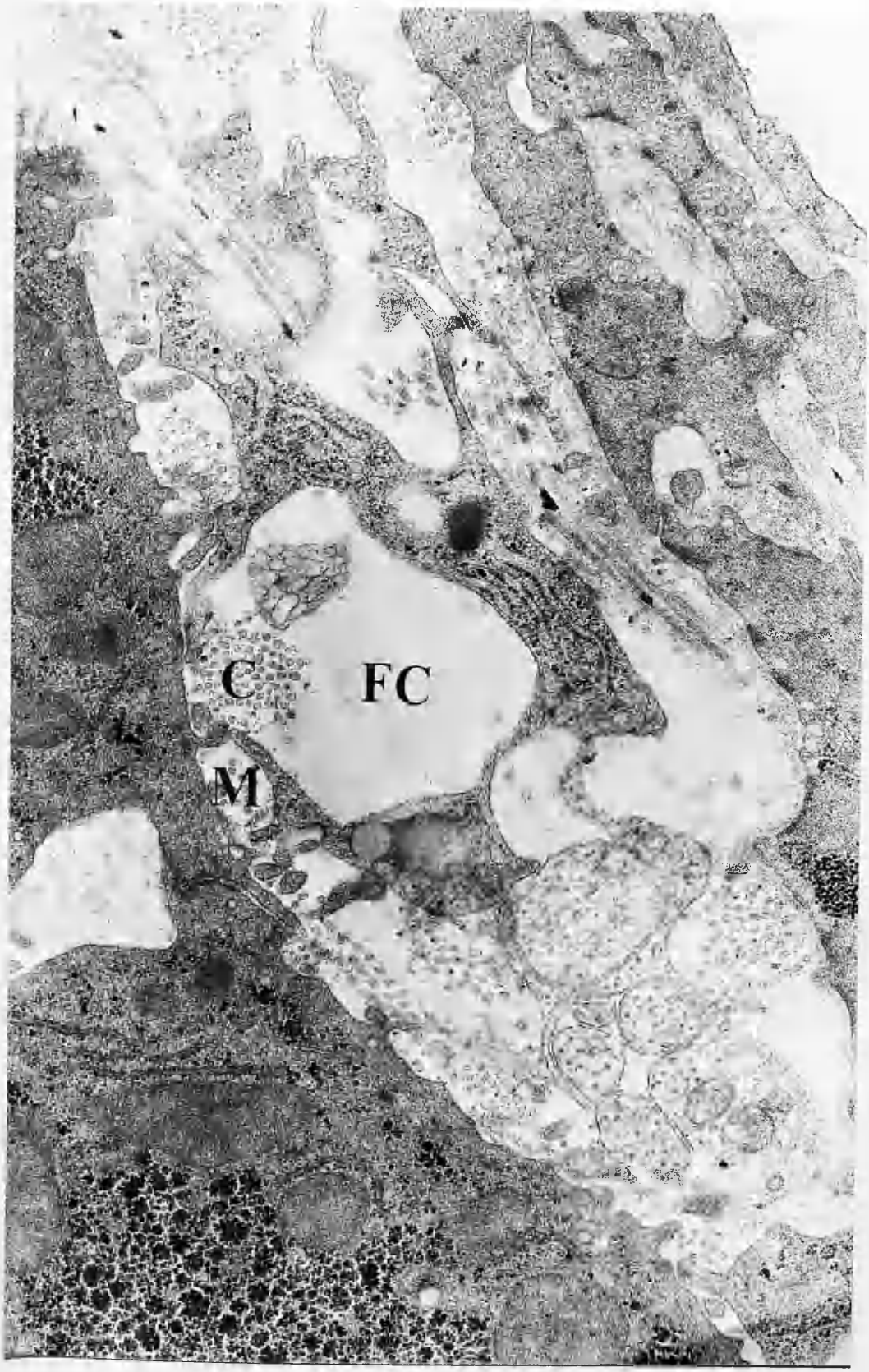

C
FC
M

Fig. 43

Illustrates the proximity of the fibroblastic channel (FC) to the space of Mall (M).
x 11760

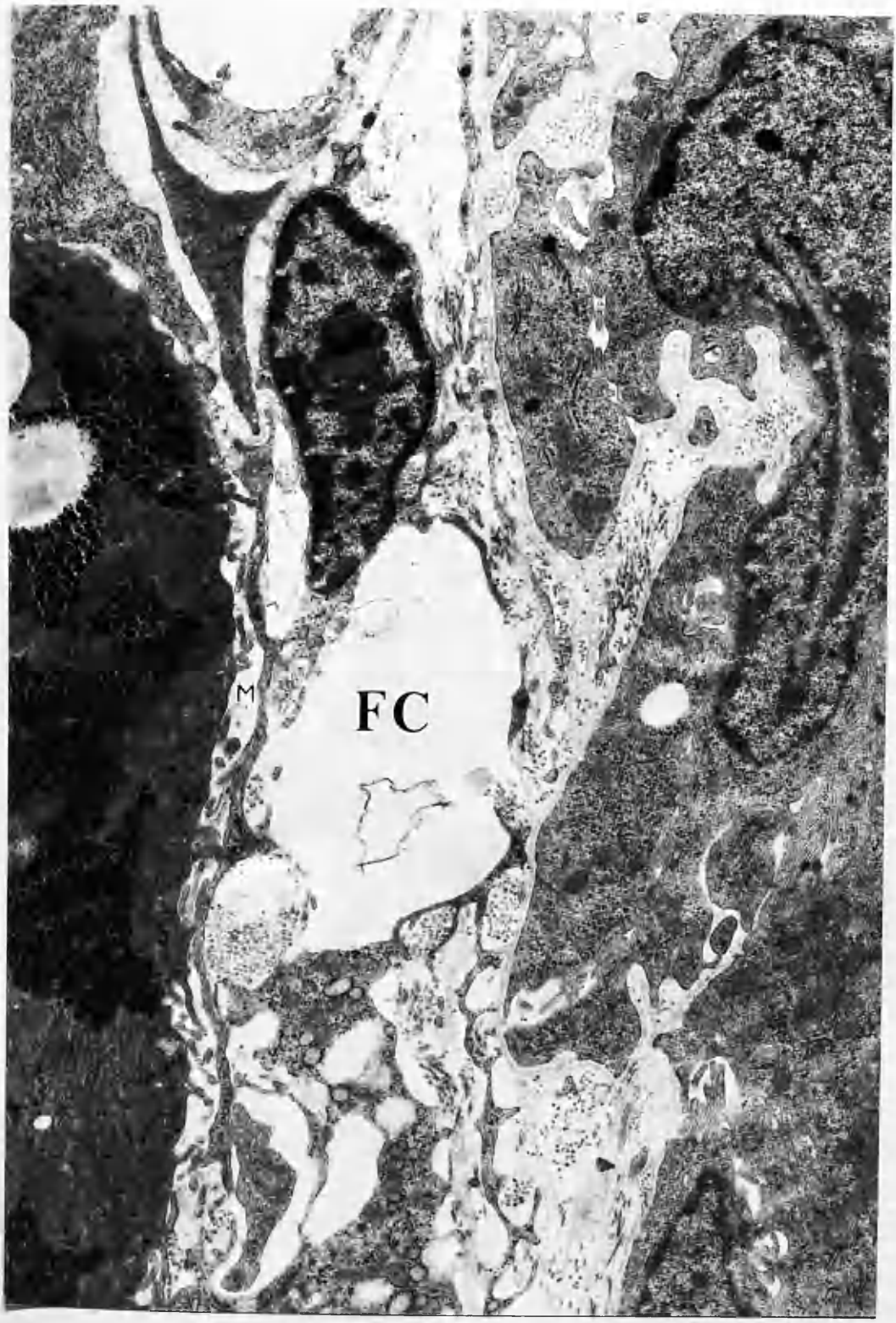

FC
M

Fig. 44

Demonstrates the proximity of the fibroblastic channel (FC) to the true lymphatic (L).

Note: the position of the gap (arrow) in the wall of the fibroblastic channel, which faces the true lymphatic.

x 5880

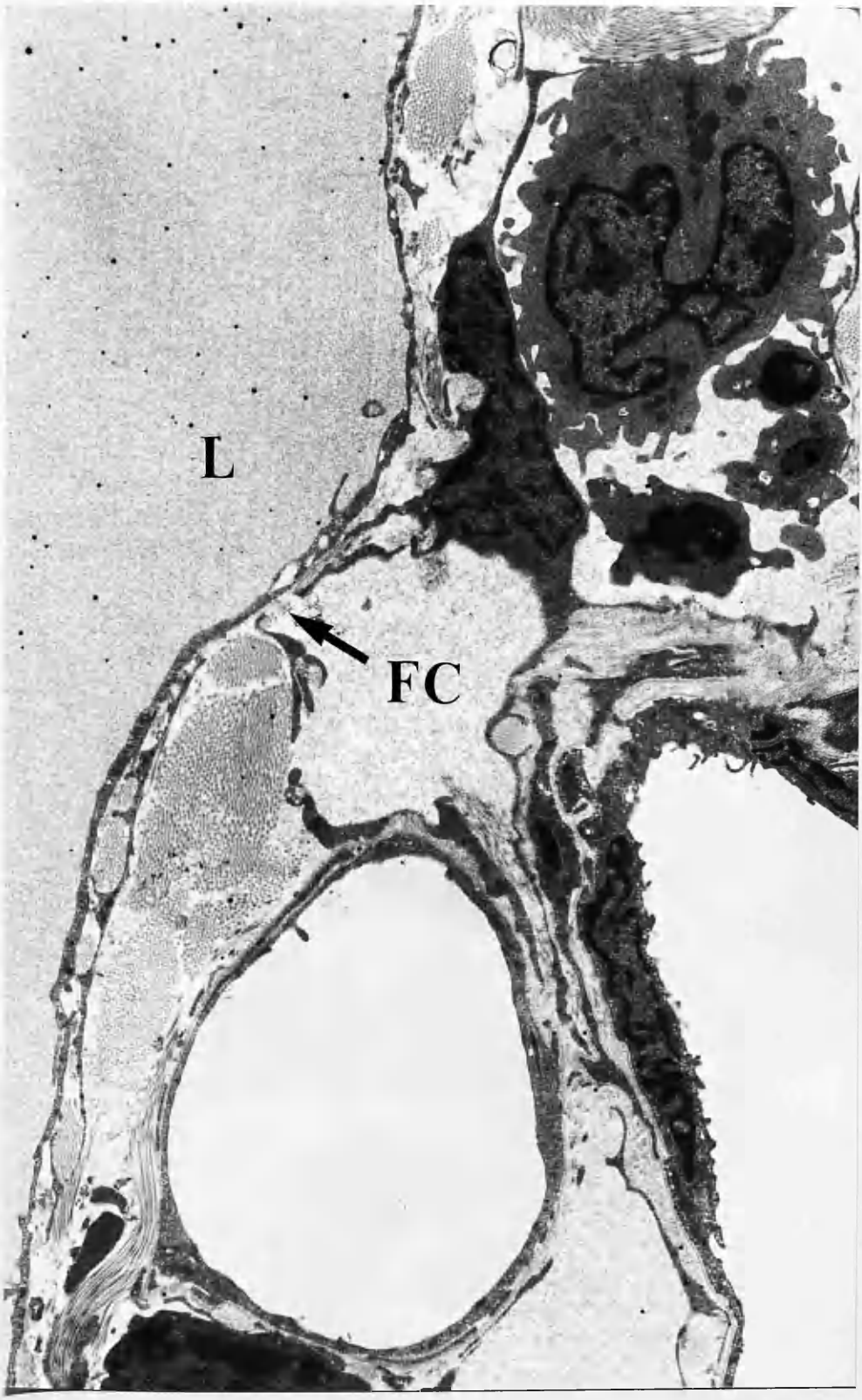

L
FC

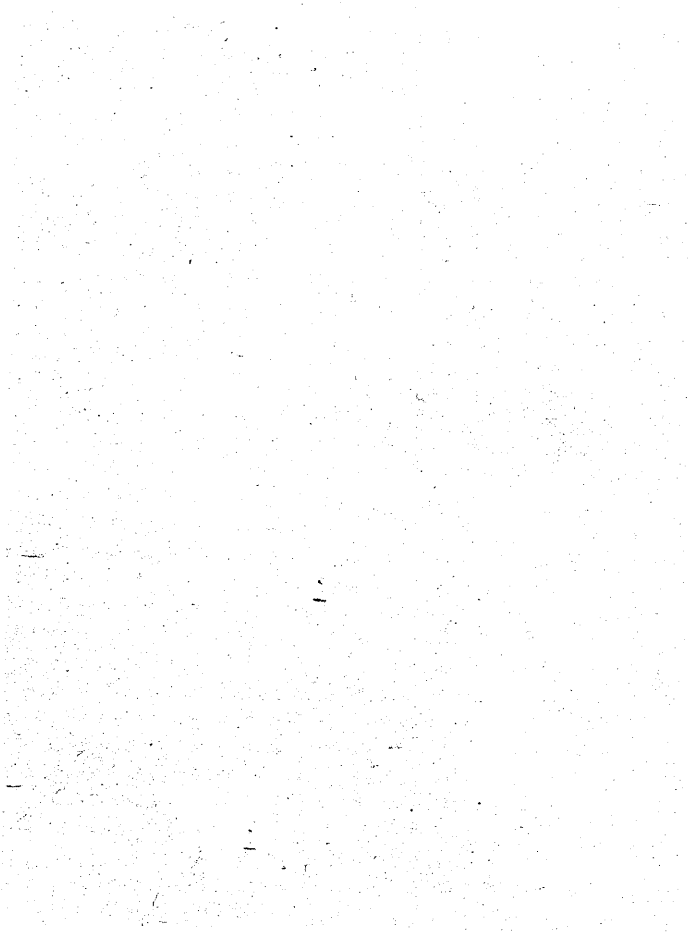
Fig. 45

Shows a bundle of collagen (C) in the space of Disse (D) bathed in proteinous material.
x 58800

C
D

Fig. 46

Shows two bundles of collagen (C) in the space of Disse, bathed in proteinous material.
x 19600



C
C

Fig. 47

Shows a collagen bundle, in a portal tract, bathed in proteinous material.
x 50400

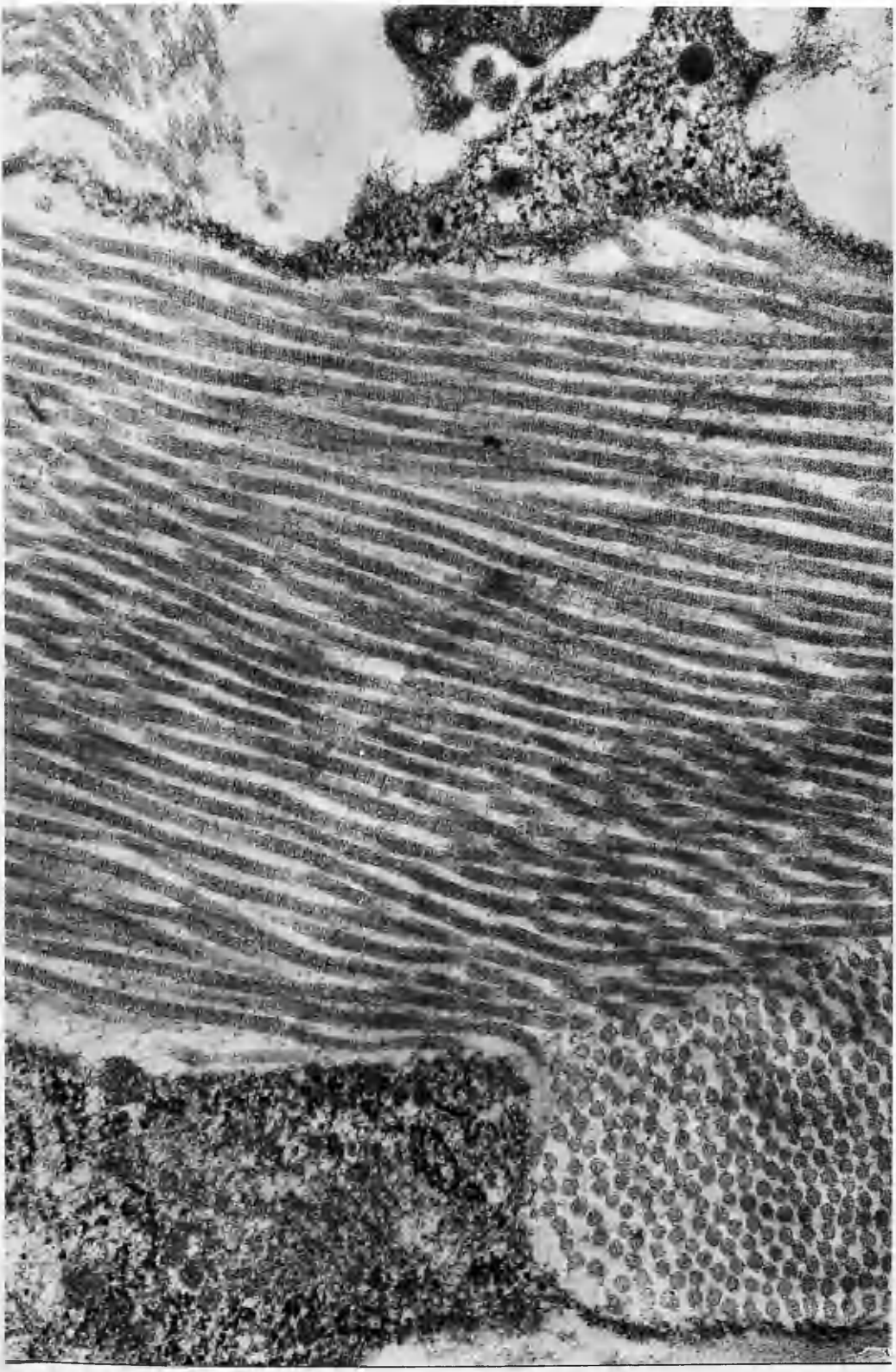


Fig. 48

Ferritin granules can be seen in the sinusoid (S) lumen and the space of Disse (D). These granules are scanty presumably because fixation was by perfusion. x 50400

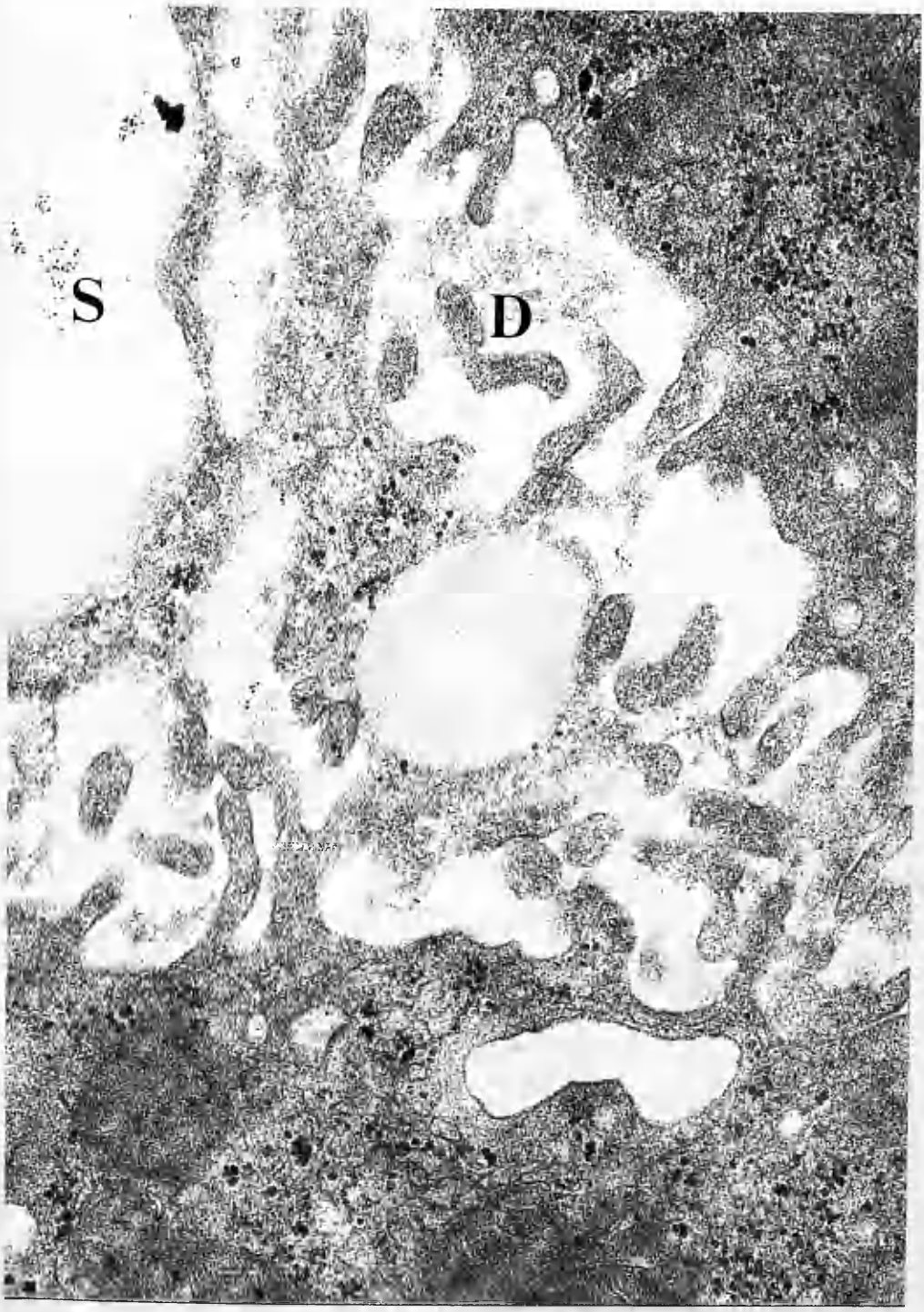
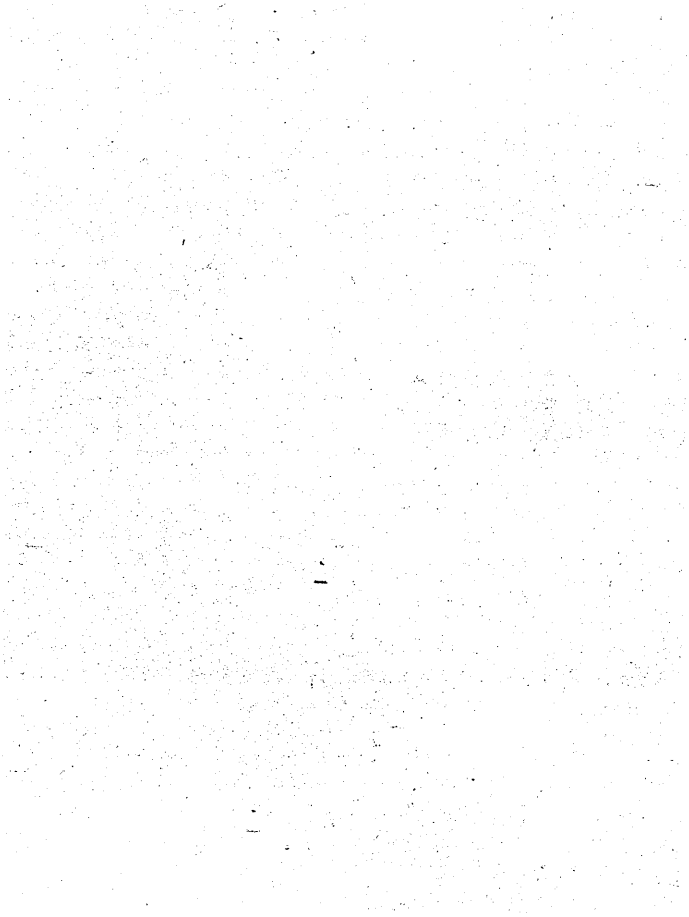

S
D

Fig. 49

Ferritin granules are seen in abundance in the sinusoidal lumen (S), in endothelial fenestrations (arrows) and in Disse's space (D). This specimen was taken from a decapitated animal and fixed by immersion.
x 39200



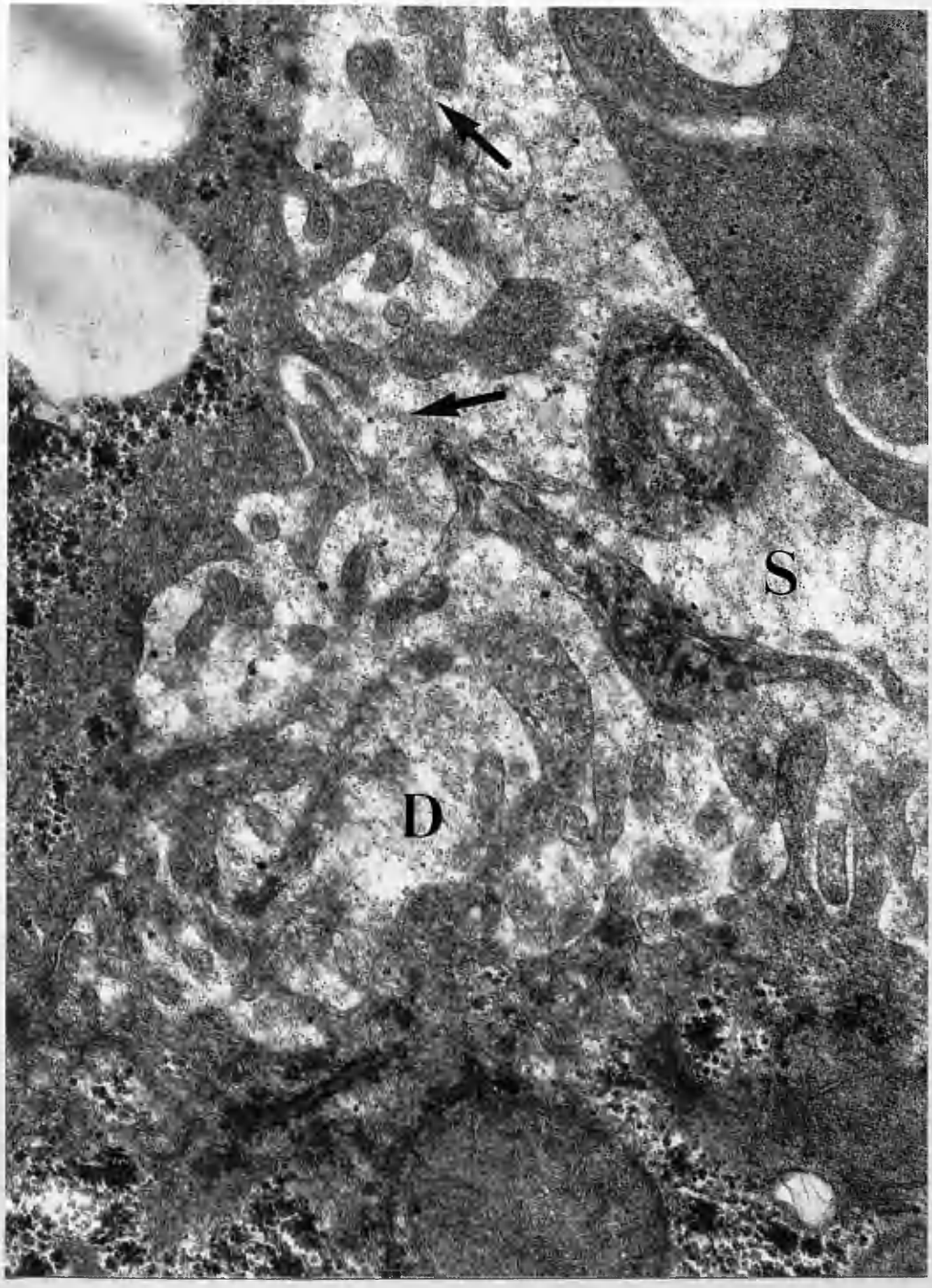
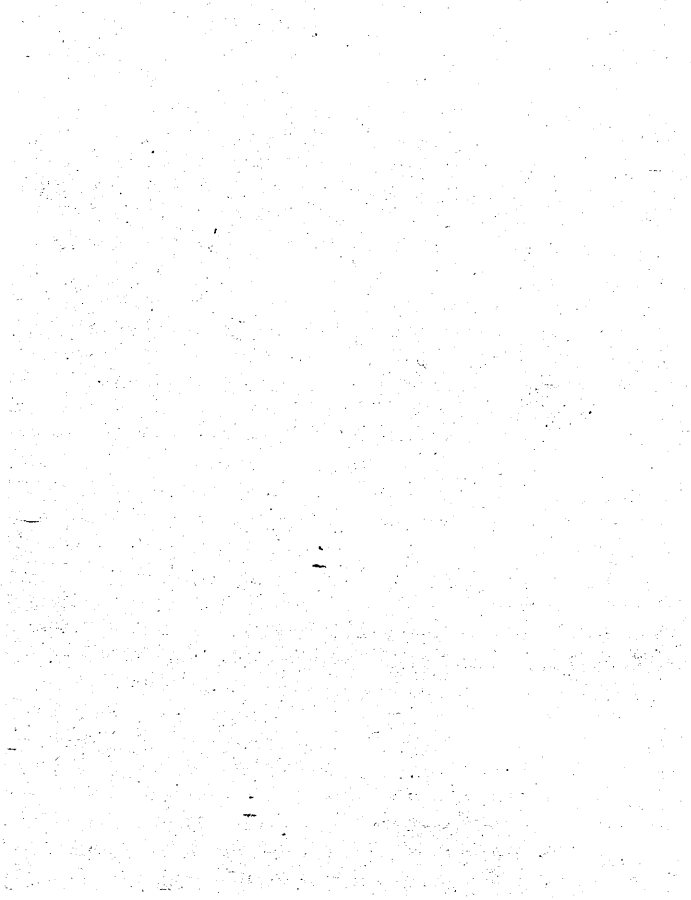

S
D

Fig. 50

A gap (G) in a limiting plate with ferritin granules between its various components.
x 50400



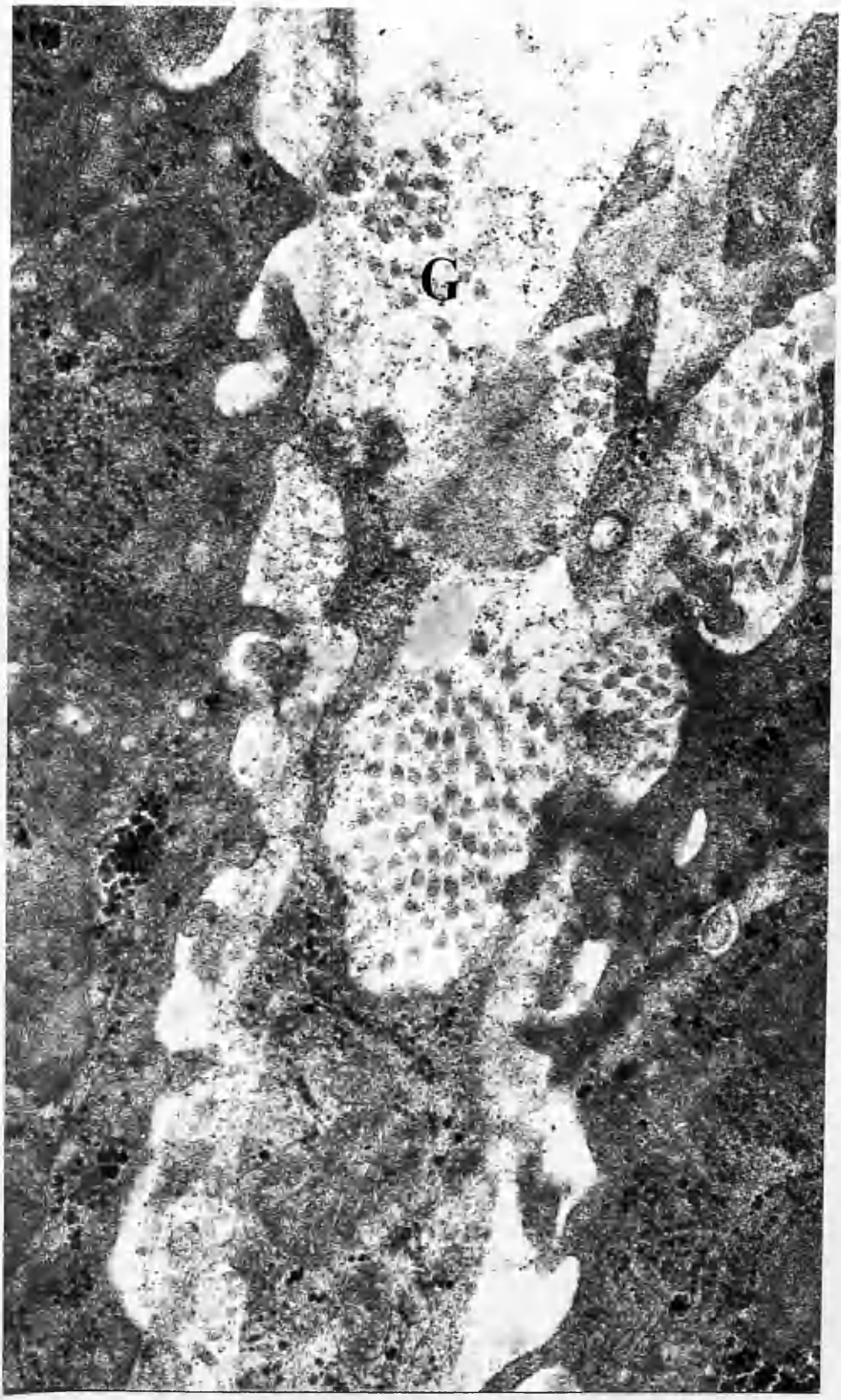

G

Fig. 51

Ferritin granules in the space of Mall (M).
x 15680

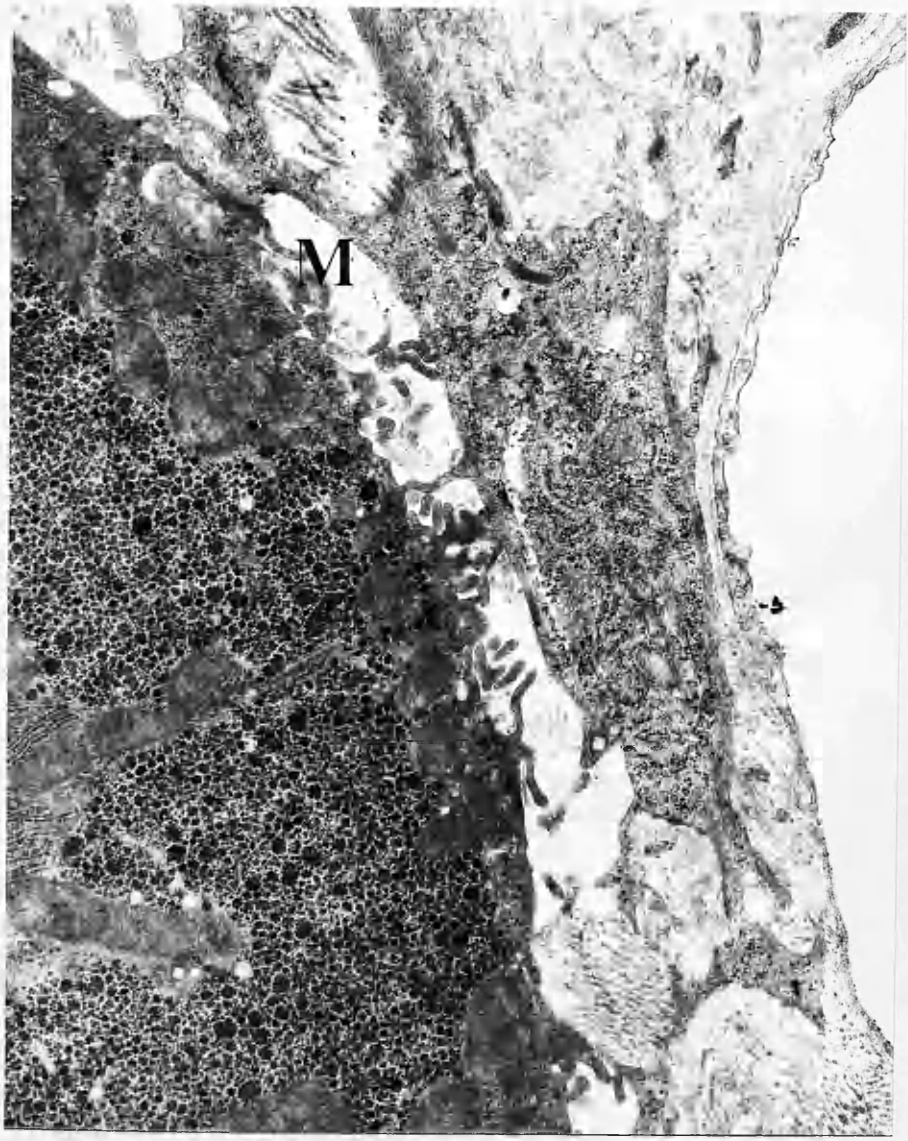

M

Fig. 52

Ferritin granules in the interstitium of a portal tract.

Note: (arrow) indicates site of early formation of a bristle coated vesicle taking up ferritin granules.

x 50400

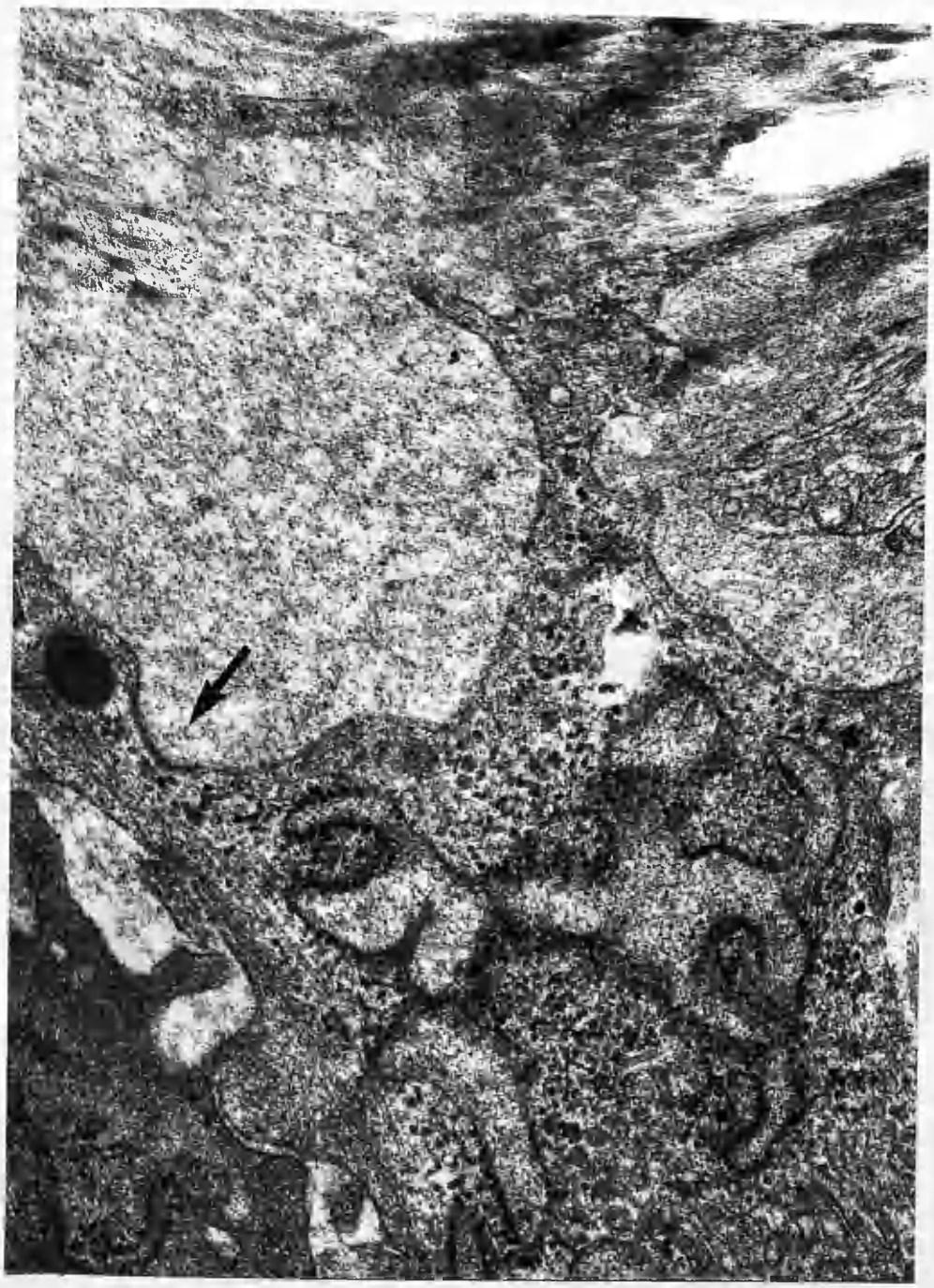
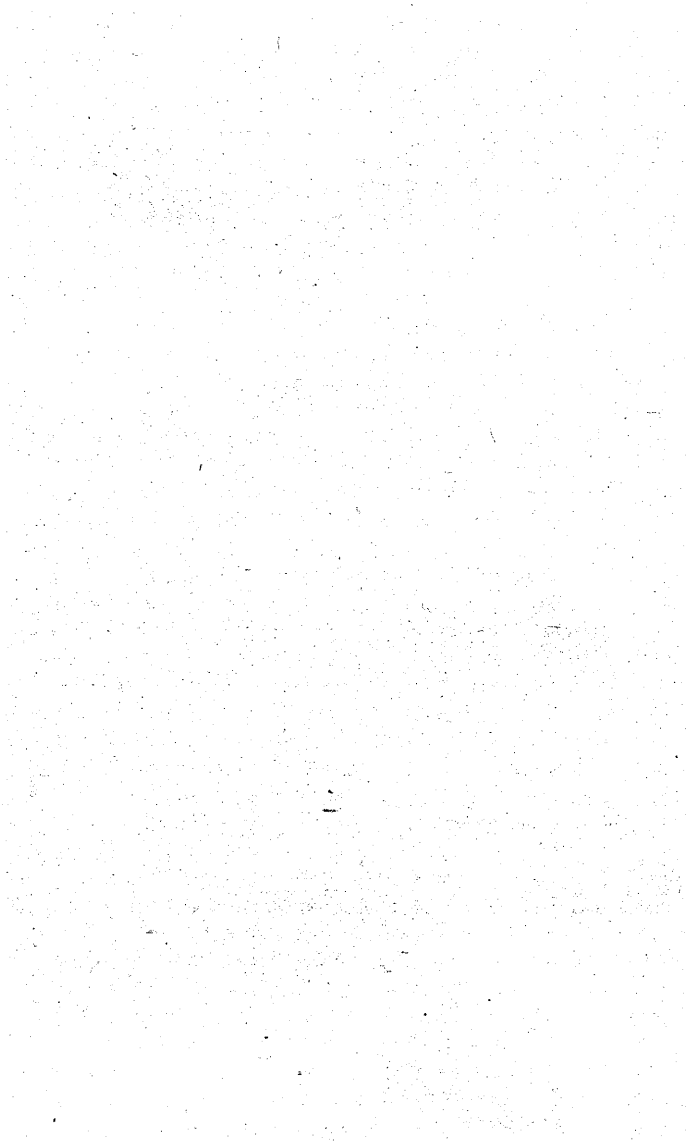


Fig. 53

Ferritin granules in a fibroblastic channel.
x 15680



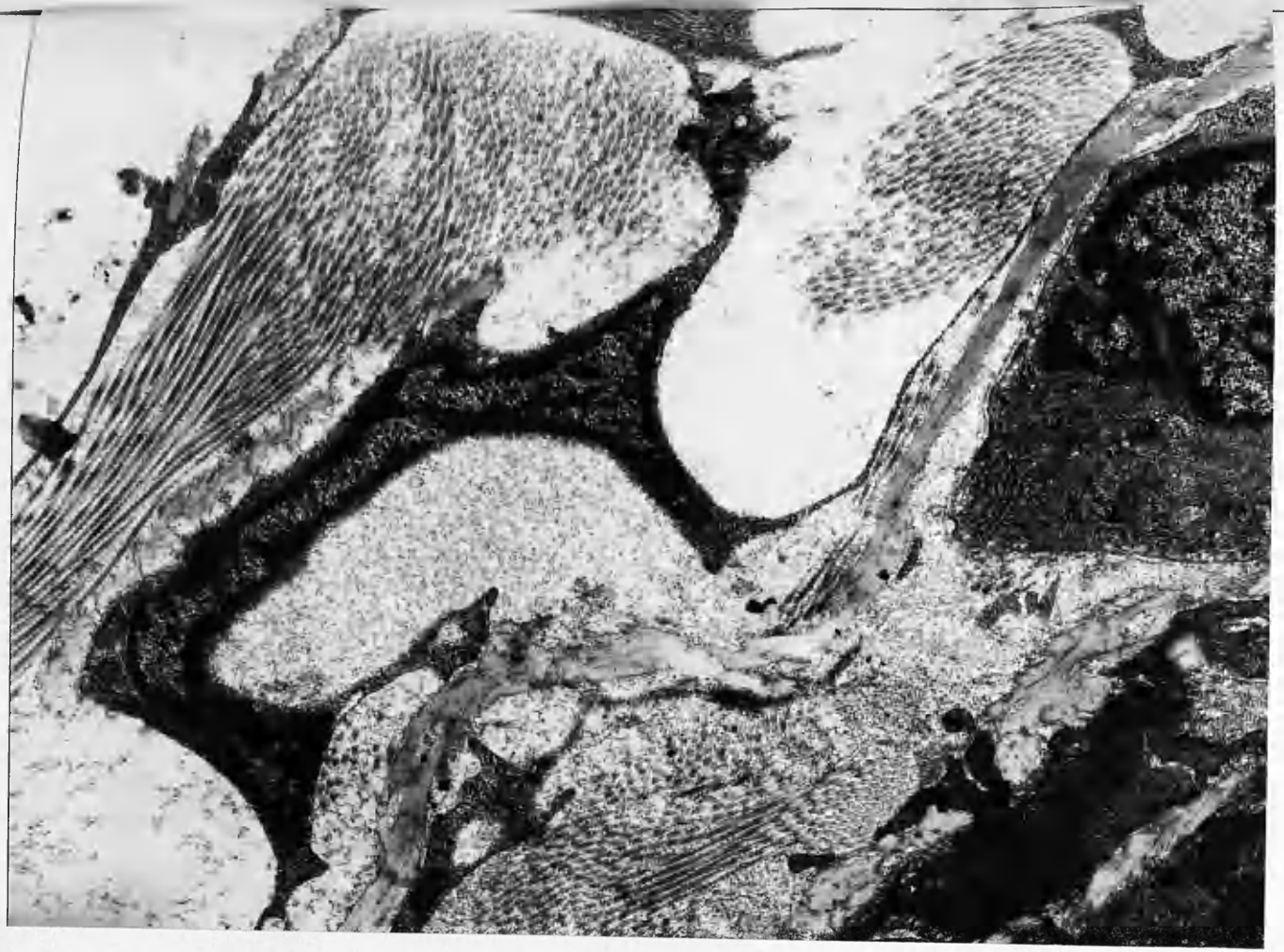


Fig. 54

Shows ferritin granules bathing a bundle of collagen fibres in the interstitium of a portal tract.

Note: compare with Fig. 47.

x 50400

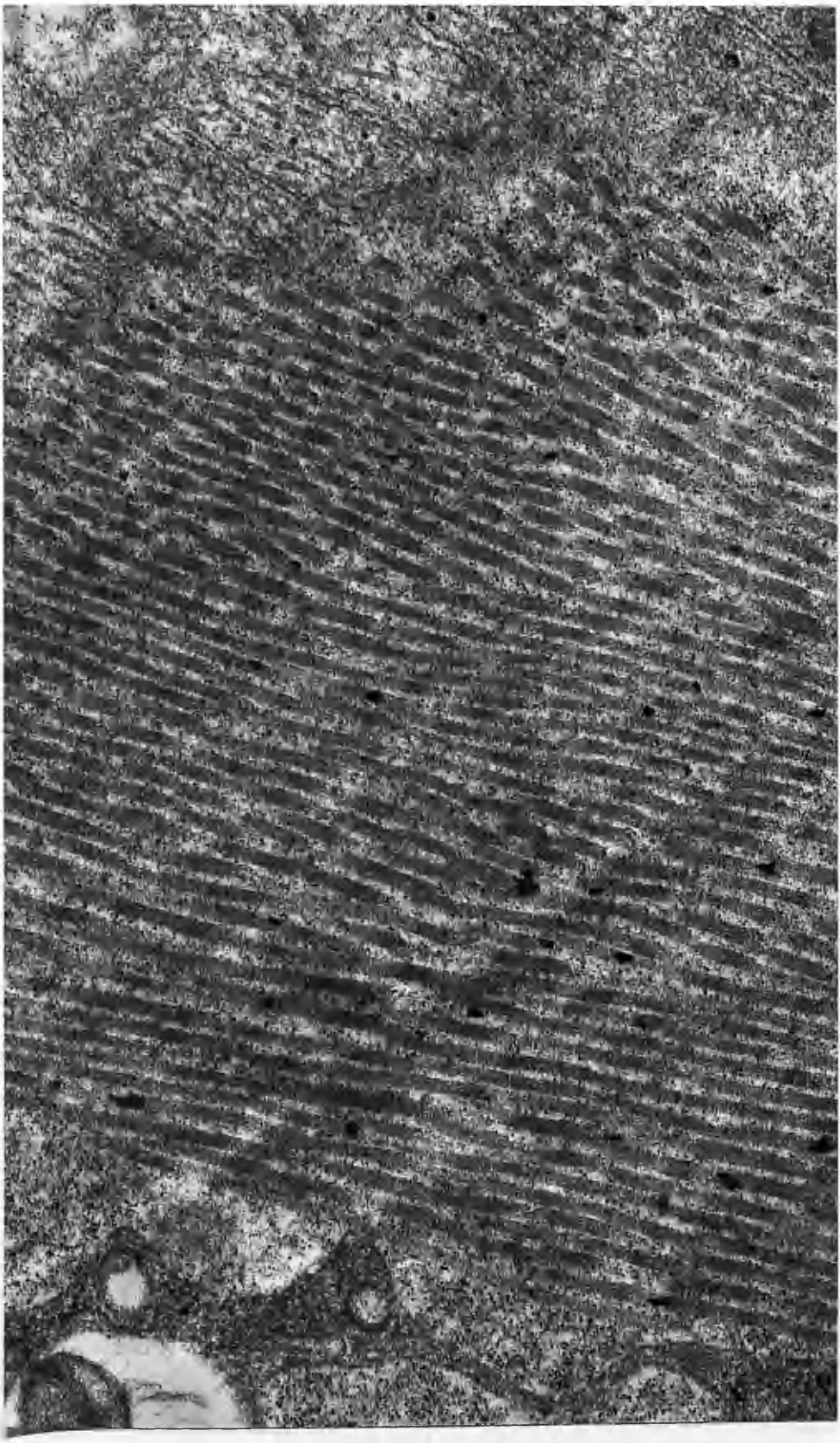


Fig. 55

Shows that lymphatics contained a higher concentration of ferritin granules than did other parts of the suggested pathway.
x 50400

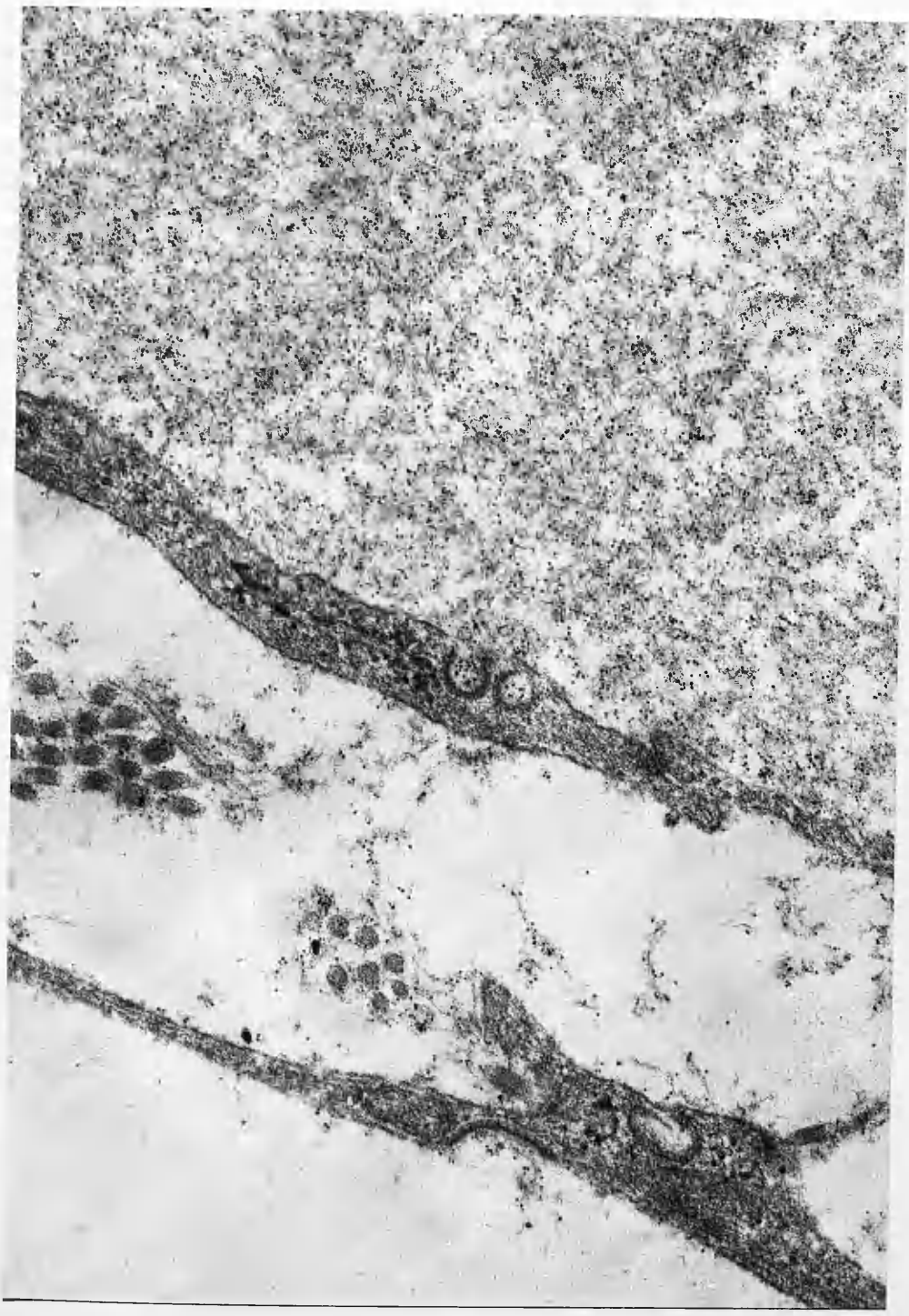
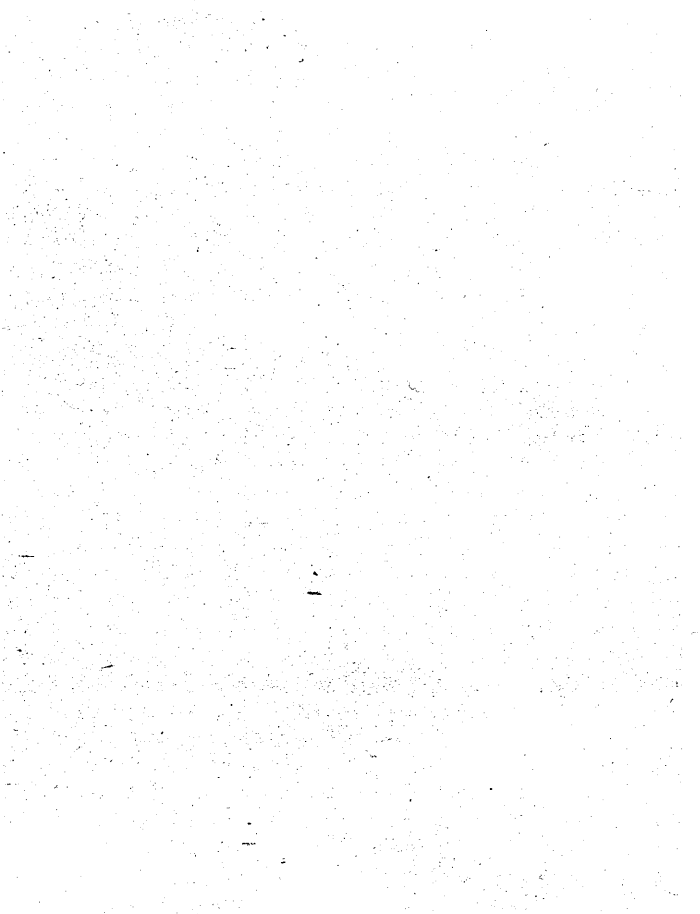


Fig. 56

A. Shows that ferritin granules were taken up by the sinusoidal endothelium (SE) in bristle coated vesicles (arrows).
x 11760



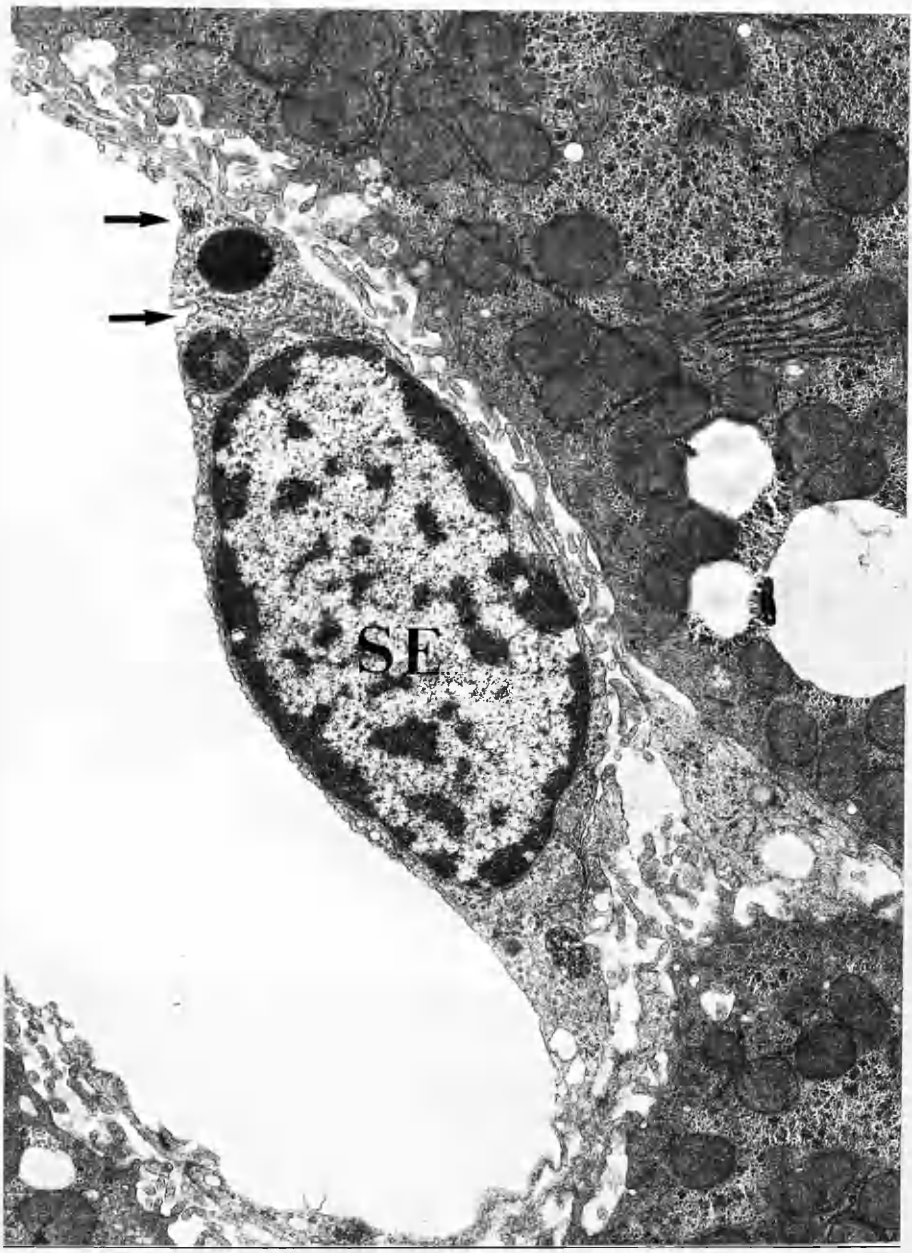

SE

Fig. 56

B. A higher power of the part of the sinusoidal endothelium taking up the ferritin granules in bristle coated vesicles (arrows).
x 39200

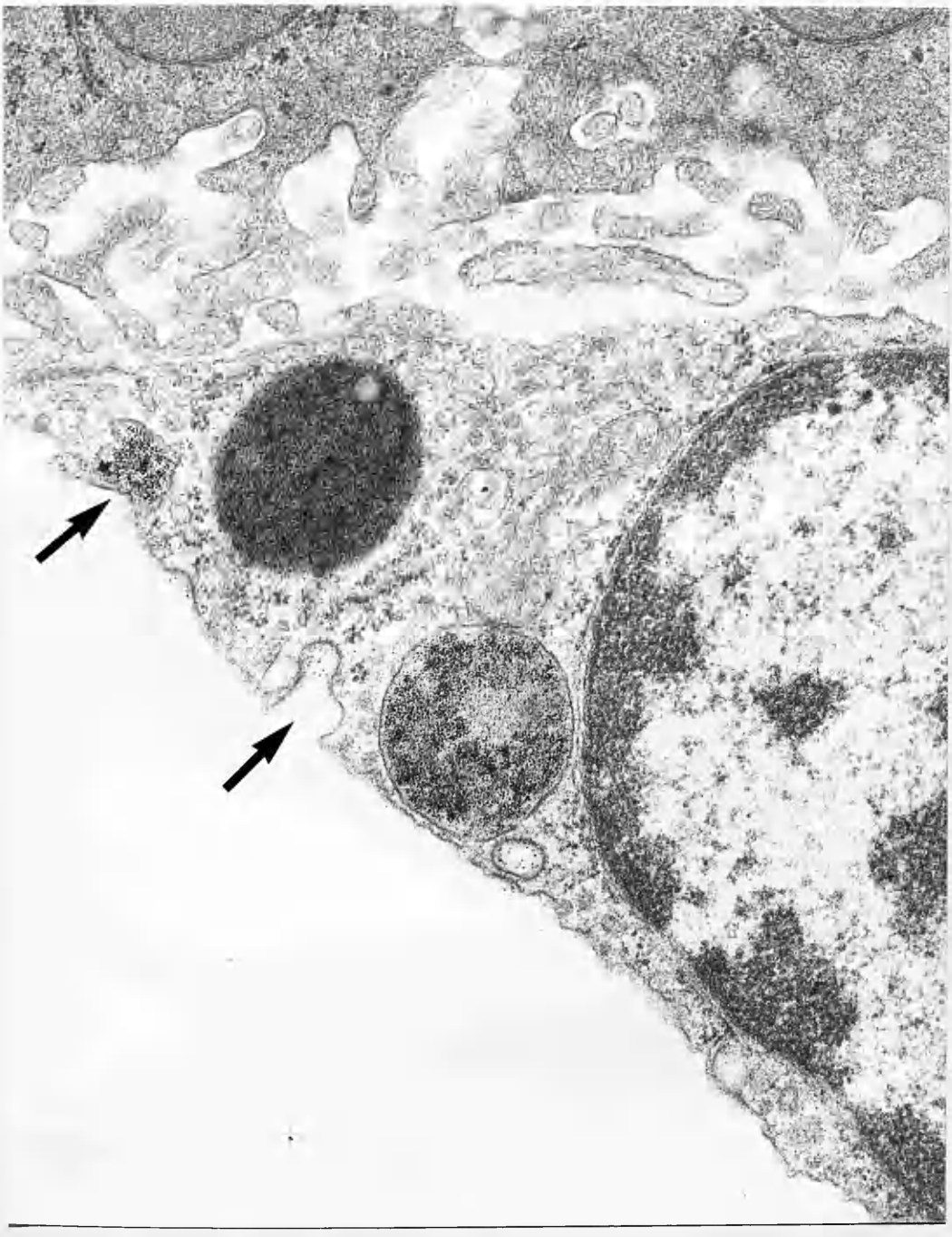
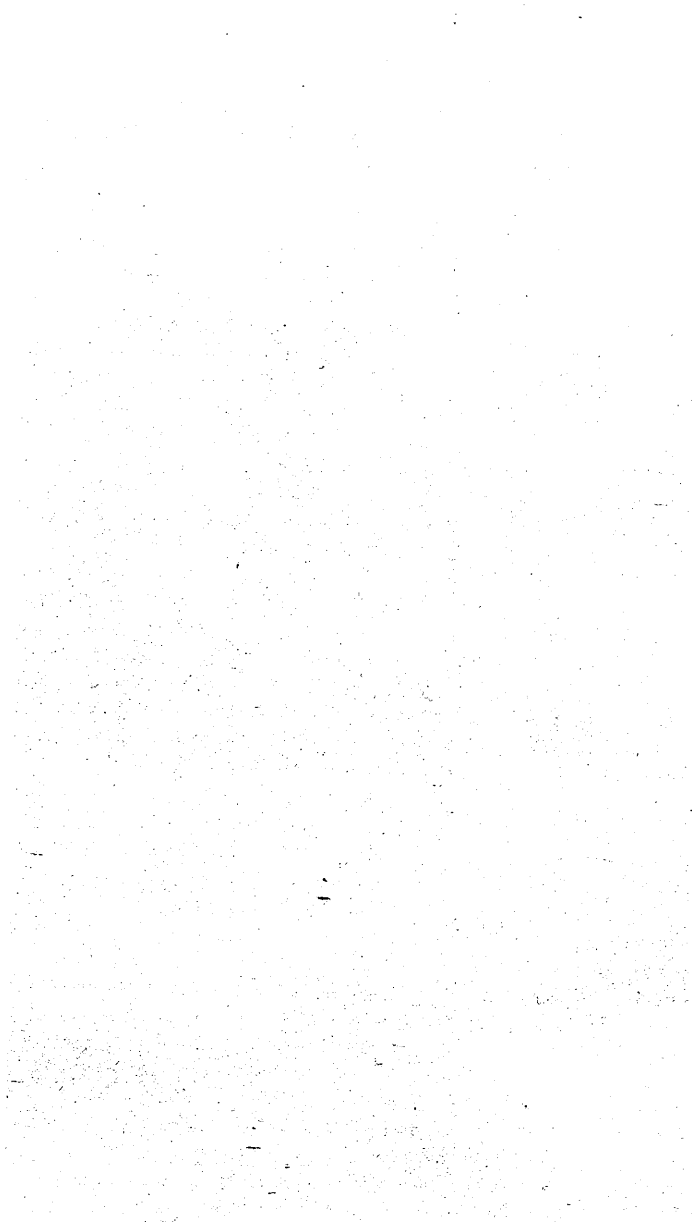


Fig. 57

Shows Pontamine Sky Blue particles in the sinusoidal lumen, fenestrations (arrows) and Disse's space. x 11760



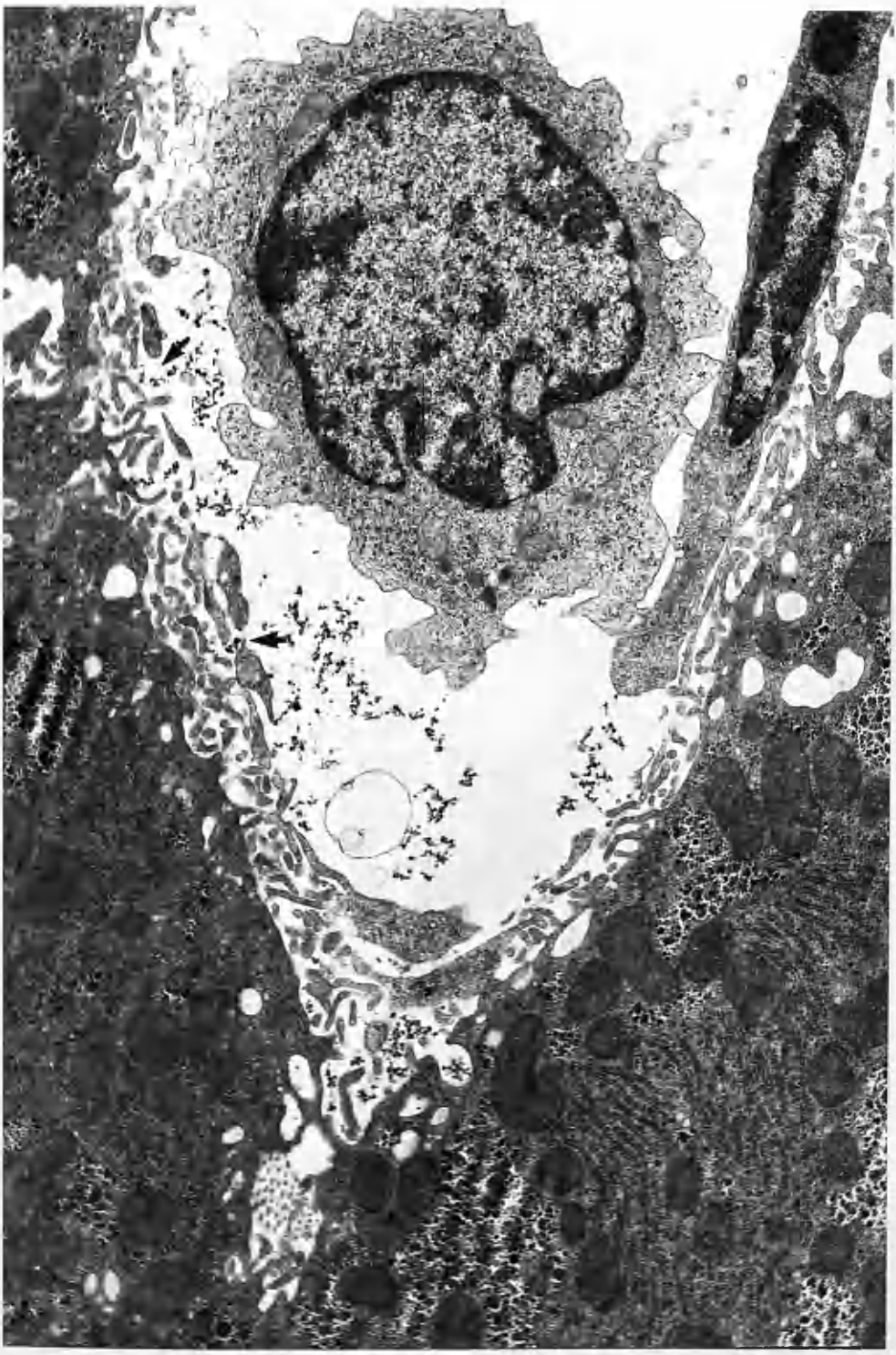


Fig. 58

Shows Pontamine Sky Blue particles in a gap (G) in the limiting plate.
x 11760

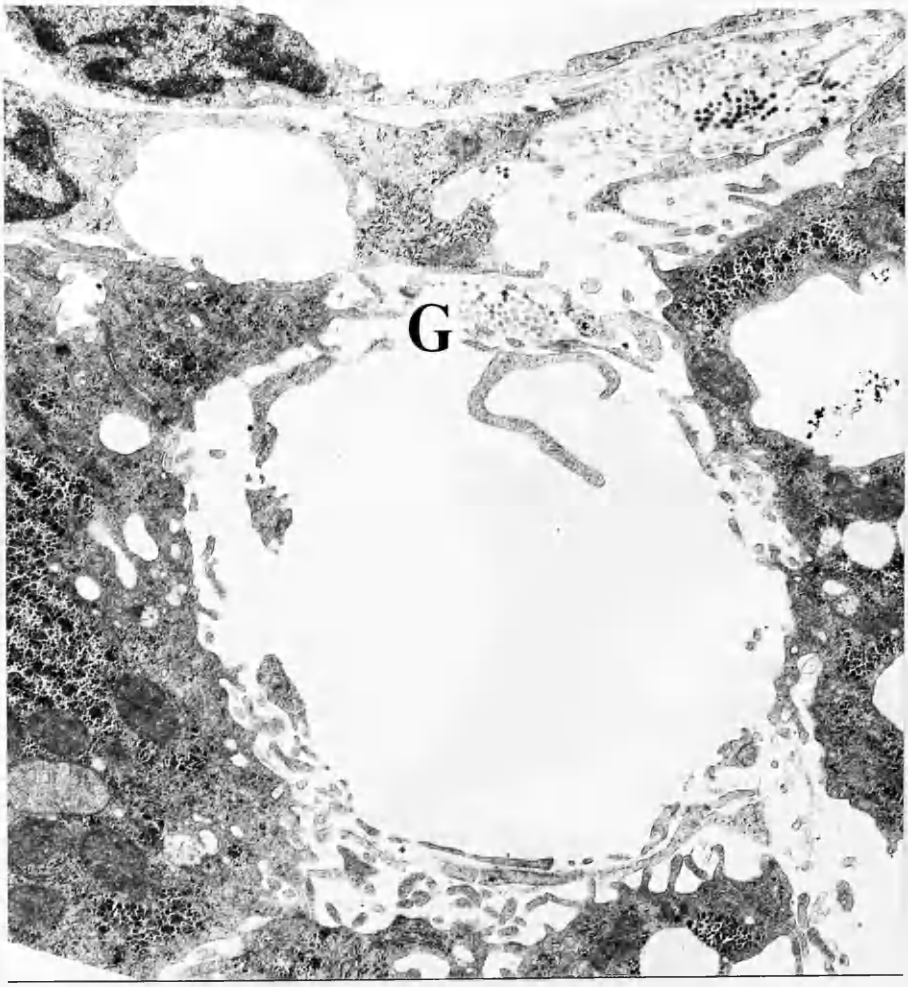

G

Fig. 59

Shows Pontamine Sky Blue particles in various parts of the interstitium of a portal tract.

Note: PSB particles in fibroblastic channels FC.

x 15680

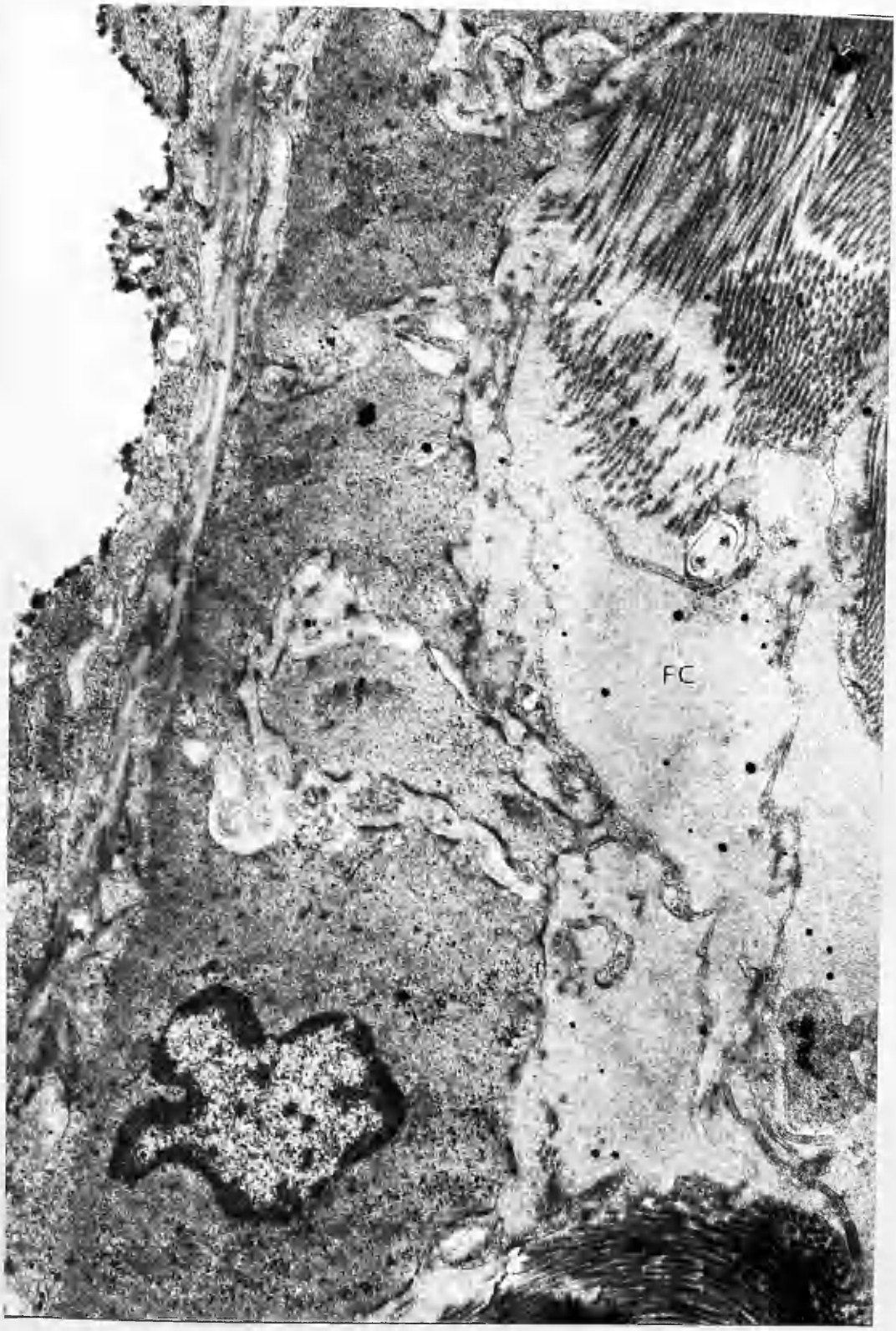

FC

Fig. 60

Shows Pontamine Sky Blue particles in the space of Mall (M), in portal tract interstitium, and in the lymphatic.

Note: the high concentration of the particles in the lymphatics.

x 9800

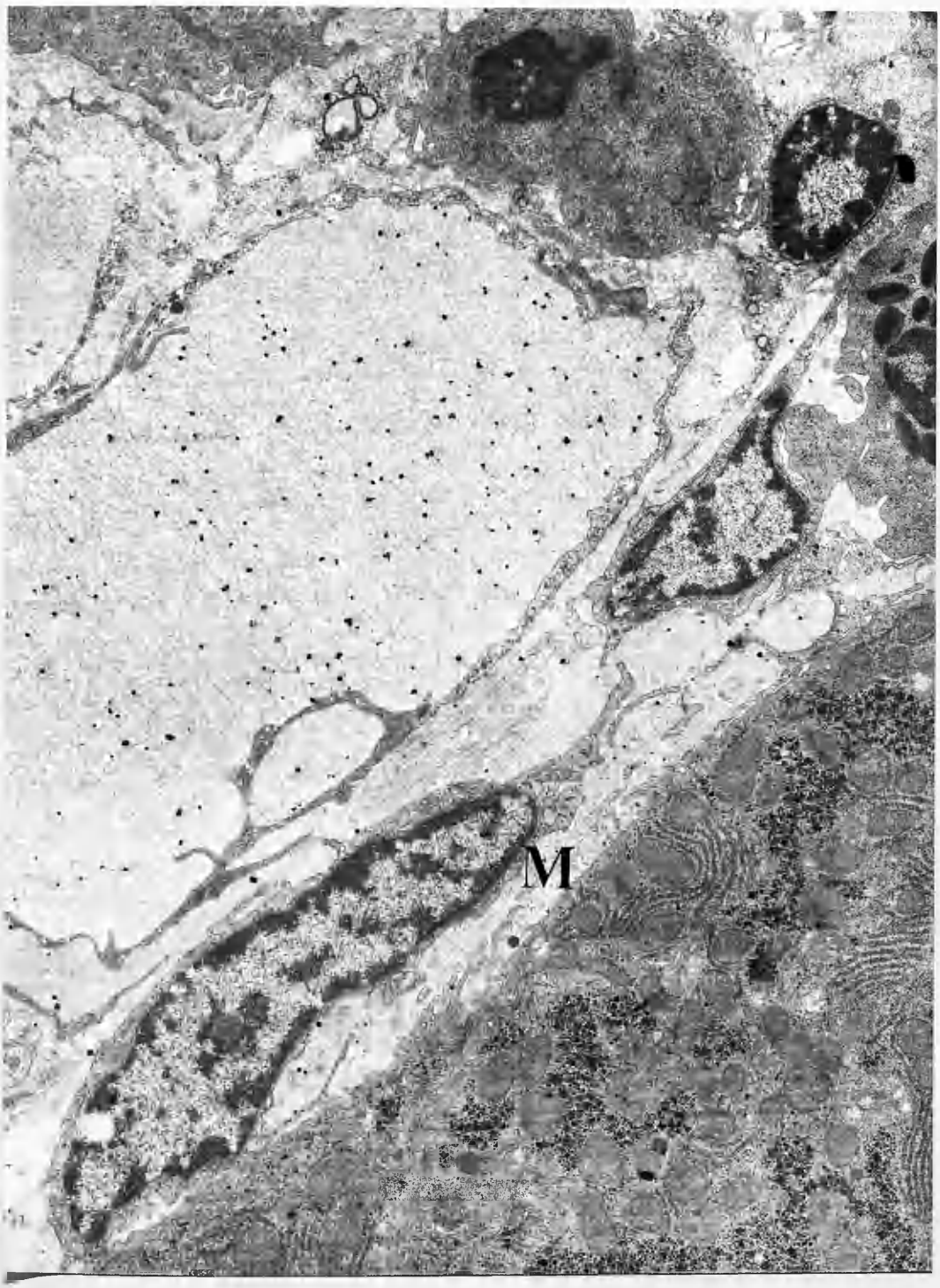

M

Fig. 61

Shows Pontamine Sky Blue particles and chylomicrons in the sinusoidal lumen.

Note: A - a cluster of various sizes of chylomicrons
B - a large chylomicron
C - a cluster of small chylomicrons in the space of Disse

x 11760

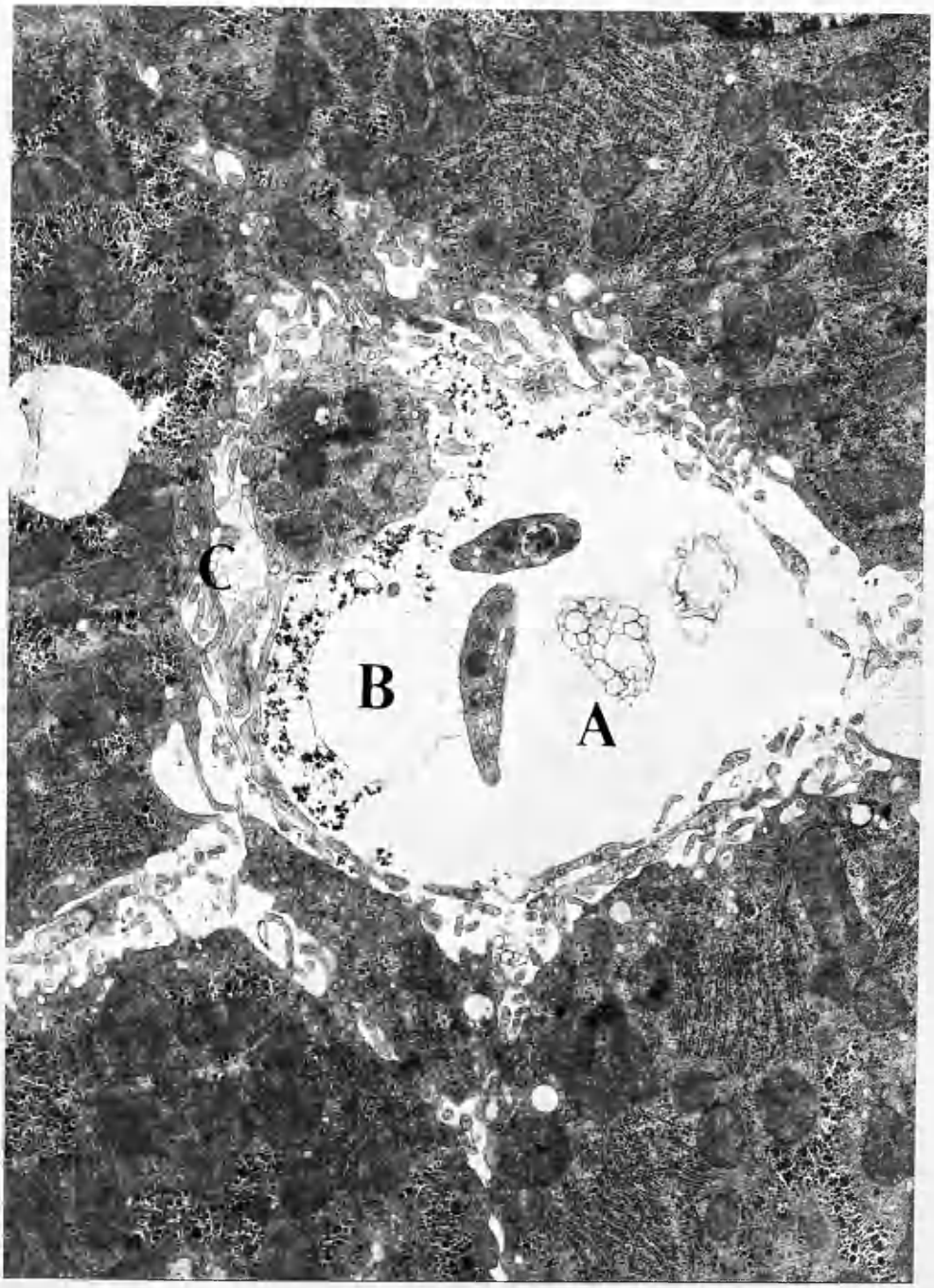

C
B
A

Fig. 62

Shows chylomicrons (arrows) in a longitudinal sectional profile of a fibroblastic channel.
x 28000

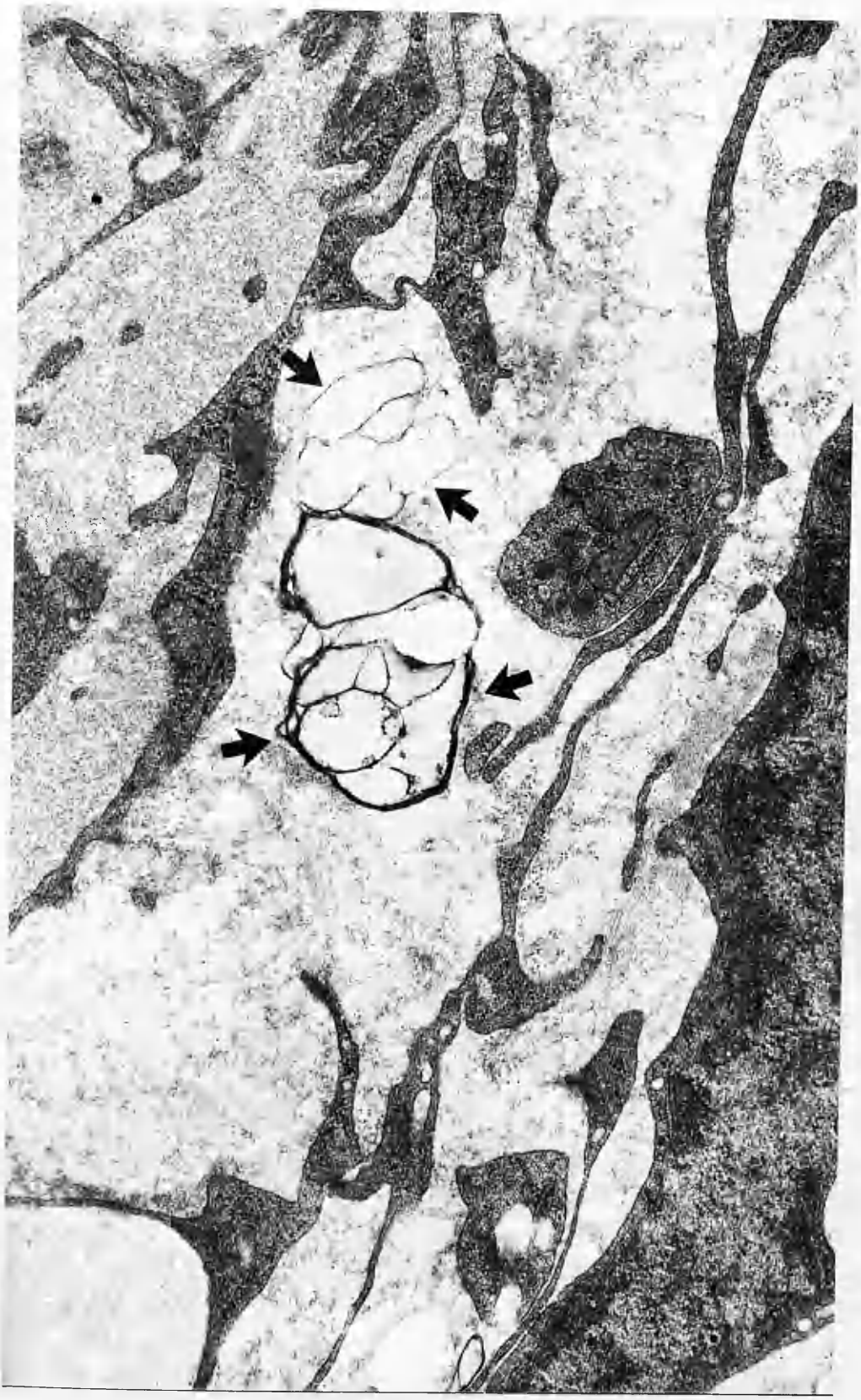
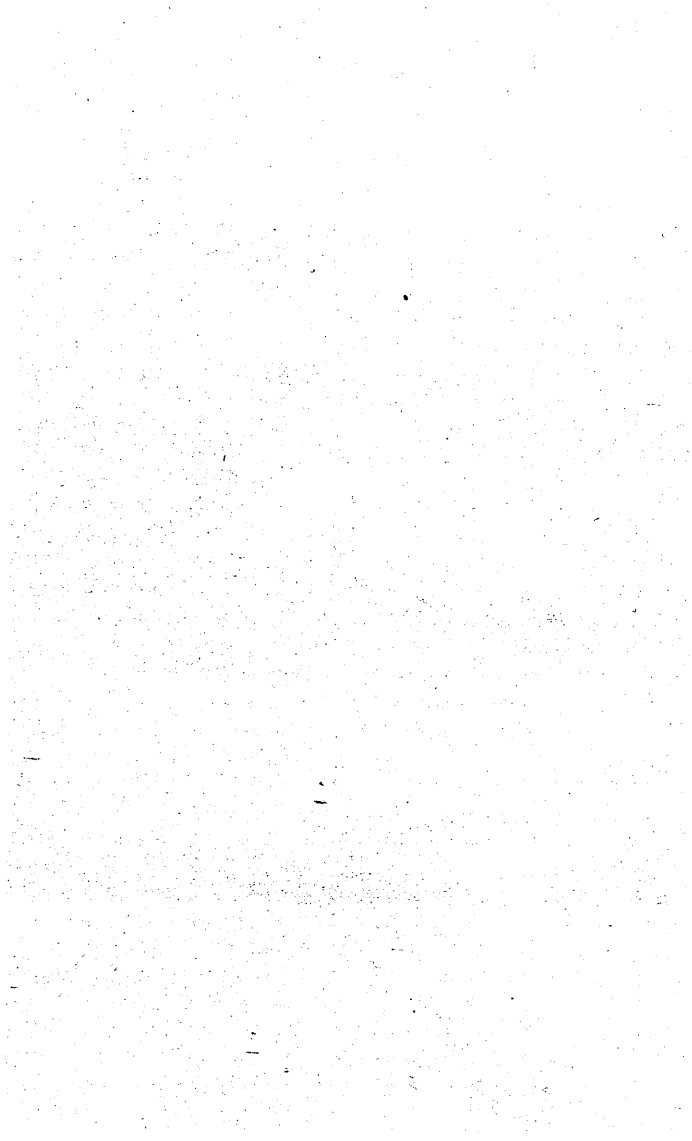


Fig. 63

Shows chylomicrons (arrowed) in the interstitium of a portal tract and in a lymphatic vessel.
x 7840



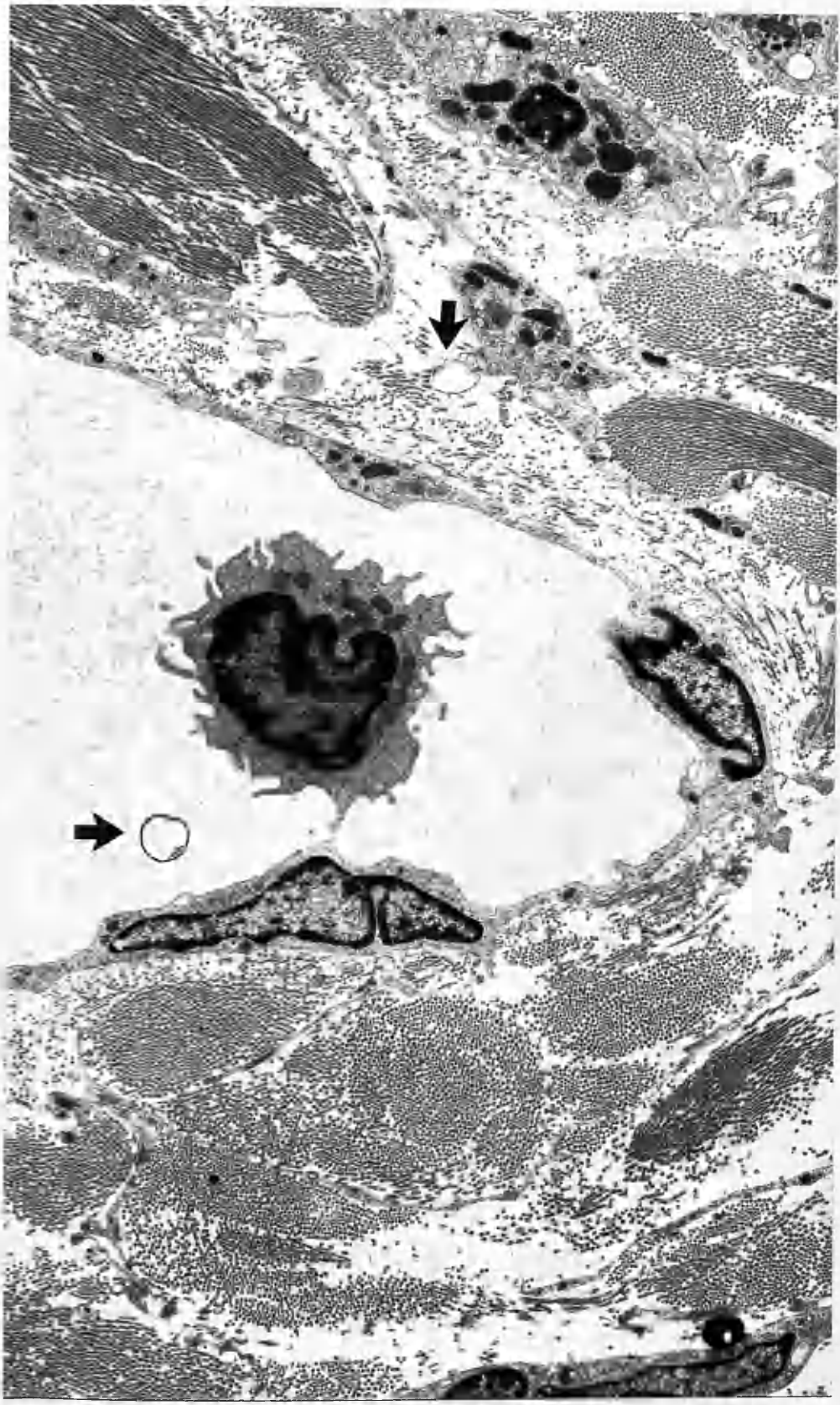


Fig. 64

Shows lipoprotein particles in the sinusoidal lumen (S) and in space of Disse (D).
x 11760

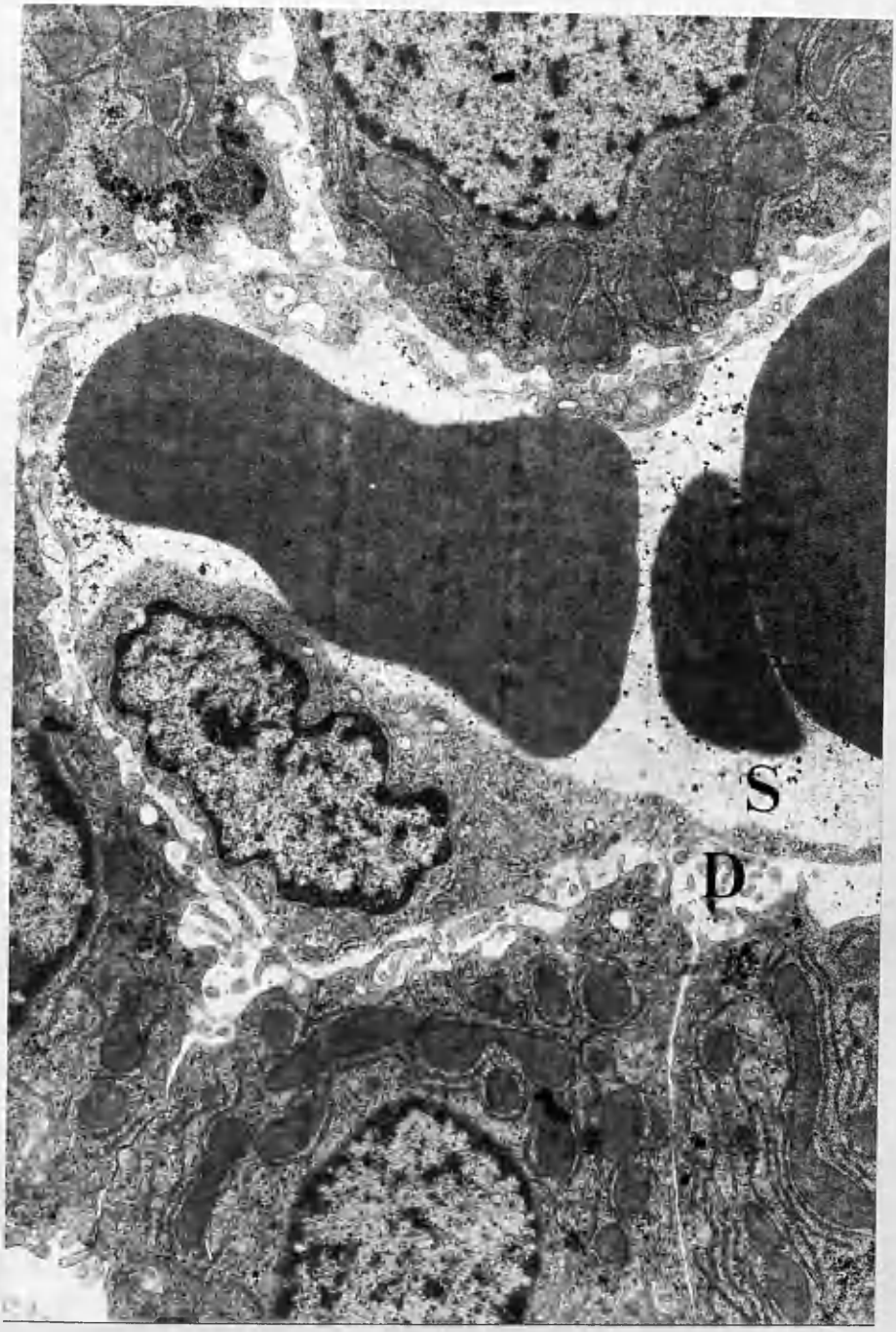

S
p

Fig. 65

Shows lipprotein particles in various spaces of the portal tract interstitium and in a lymphatic (L). x 7840

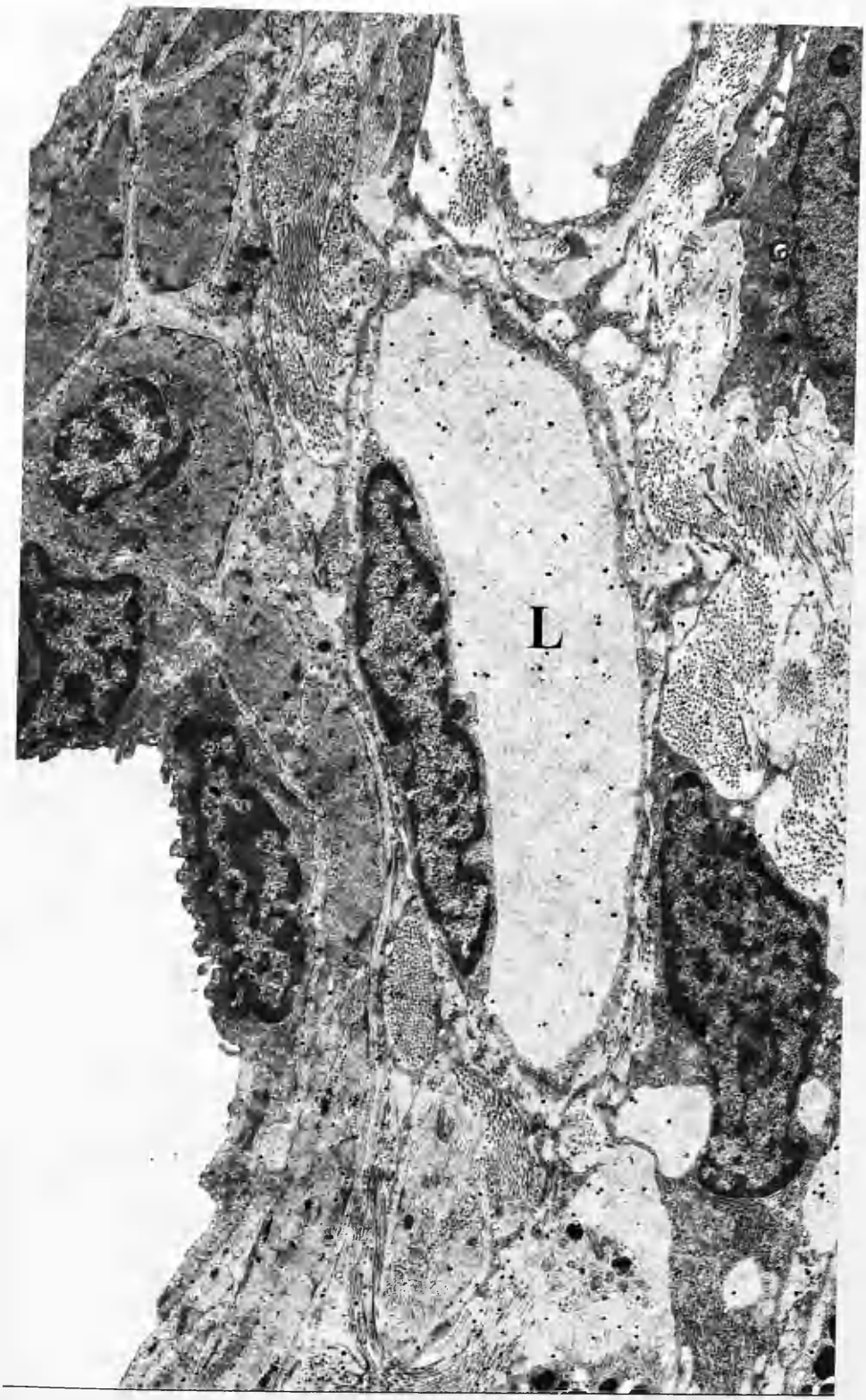

L

Fig. 66

Shows Kupffer cells full of Monstral blue particles.
x 320

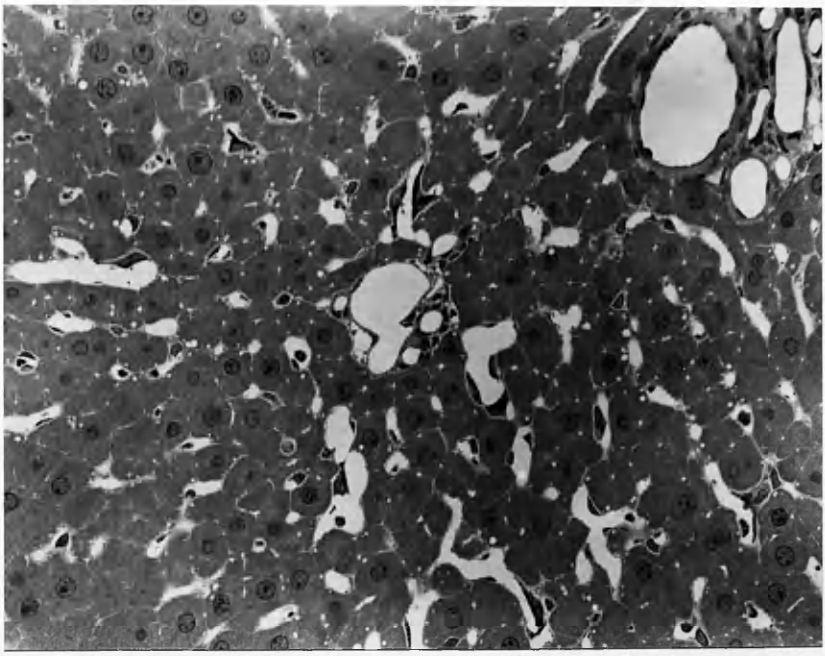
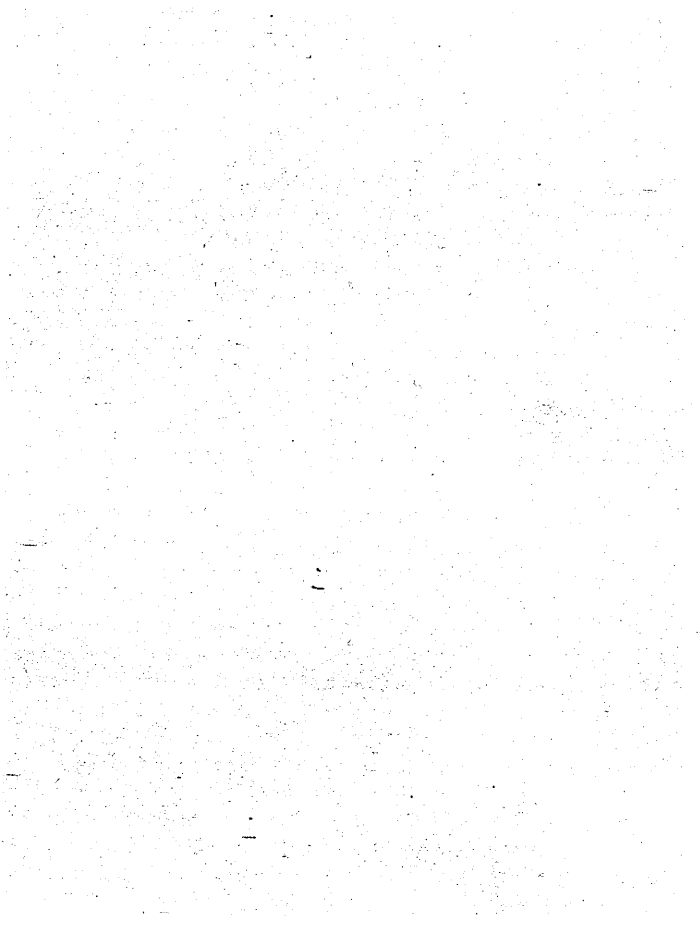


Fig. 67

Shows many Monstral blue particles adherent to the sinusoidal surface of the Kupffer cell which avidly phagocytosed the particles.
x 19600



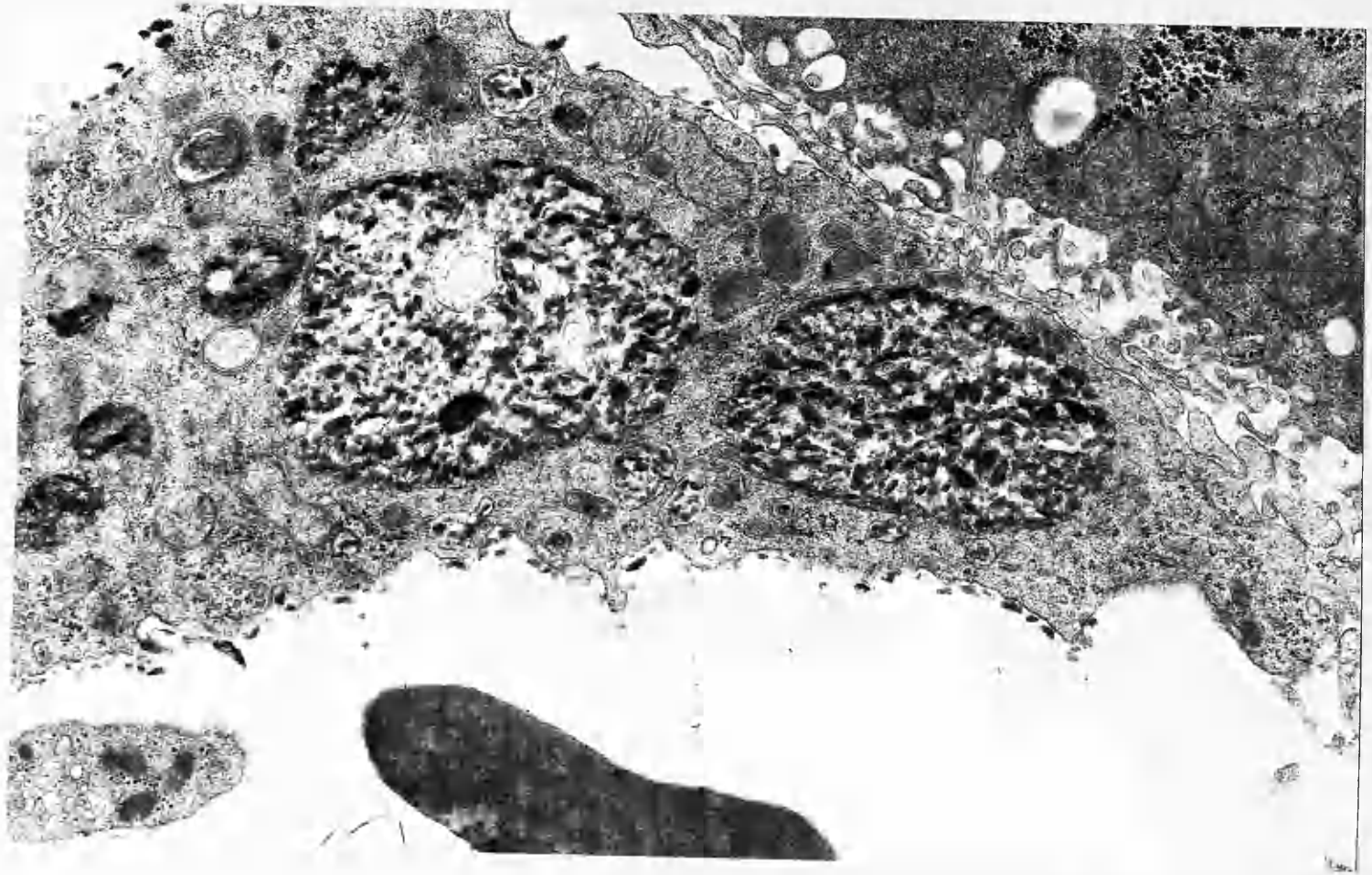


Fig. 68

Shows a few Monastral blue particles passing through interendothelial overlapping junctions.
x 39200

Fig. 69

Shows that only a few Monastral blue particles reached the space of Disse (D).
x 50400

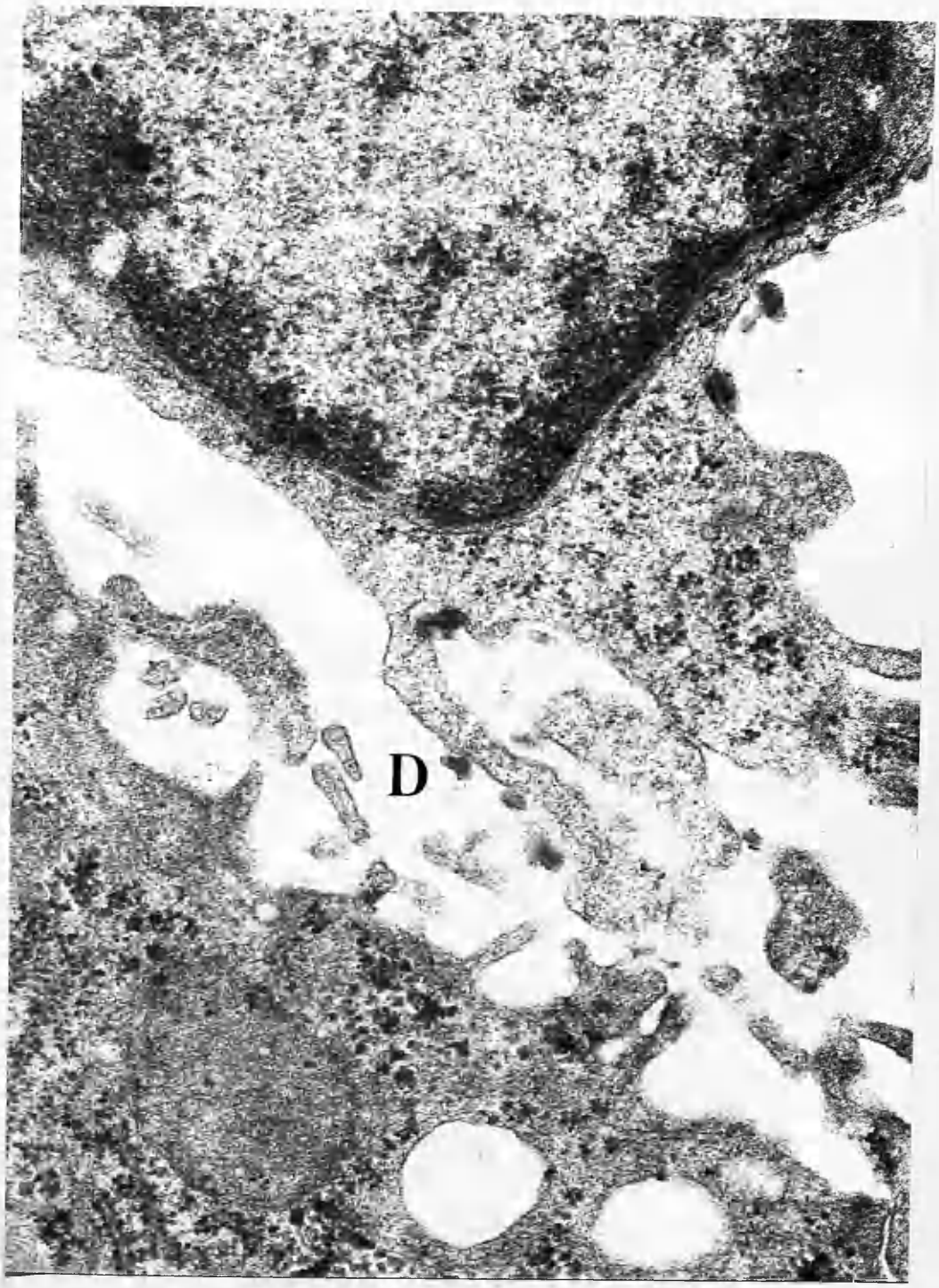

D

Fig. 70

Shows a rare sight of a small cluster of Monastral blue particles in the interstitium of a portal tract and even within a lymphatic.
x 28000

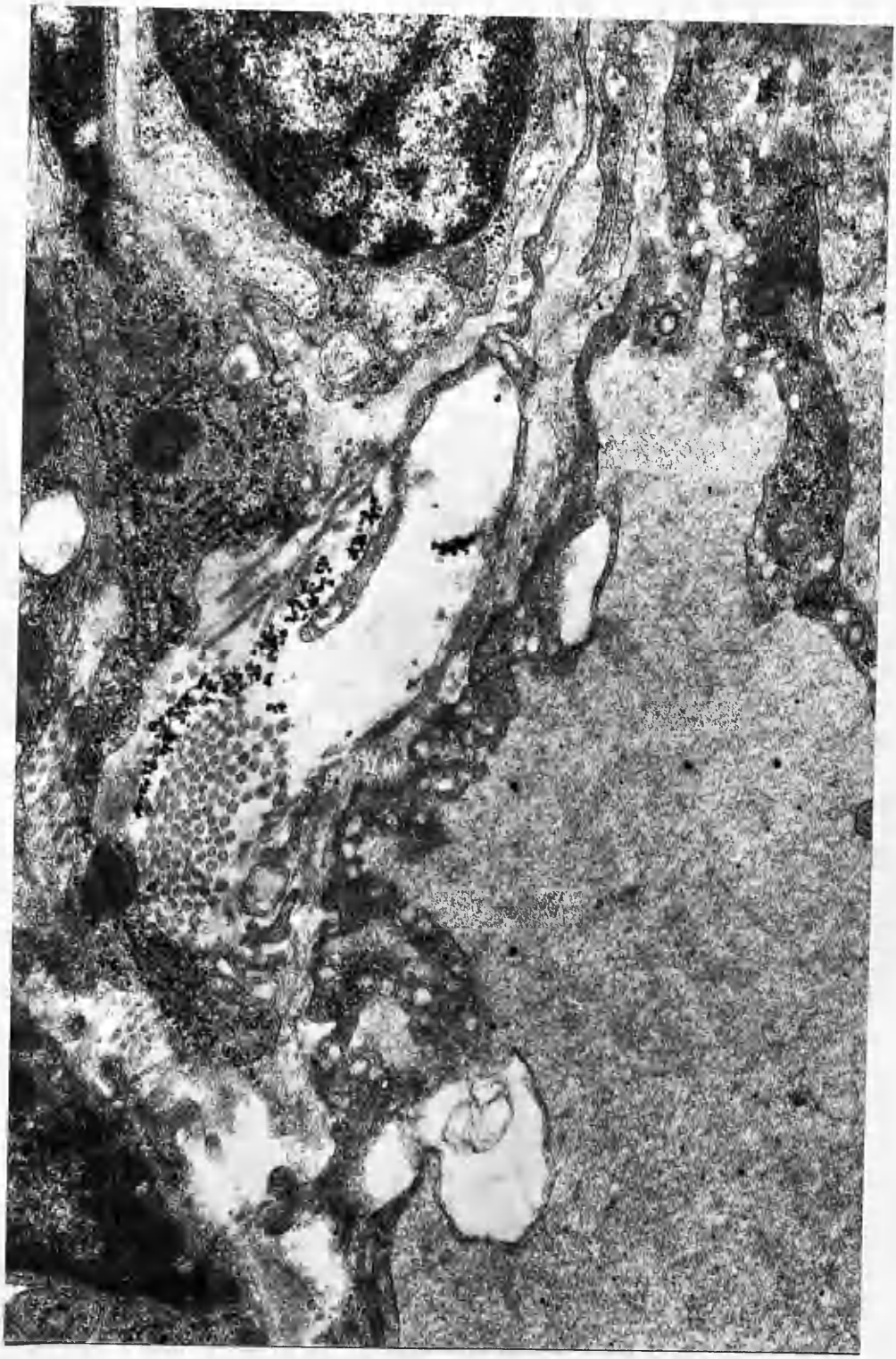


Fig. 71

Shows sectional profile of a large portal tract whose accompanying portal vein branch diameter is greater than 180 μm.

Note: a single lymphatic (arrowed) in the portal tract

24 hr old animal.

x 225

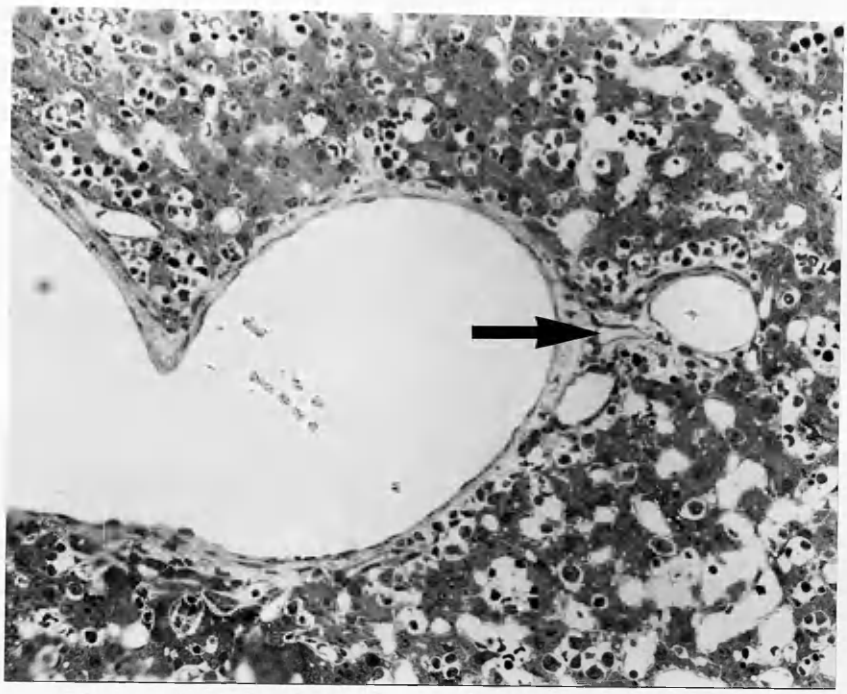


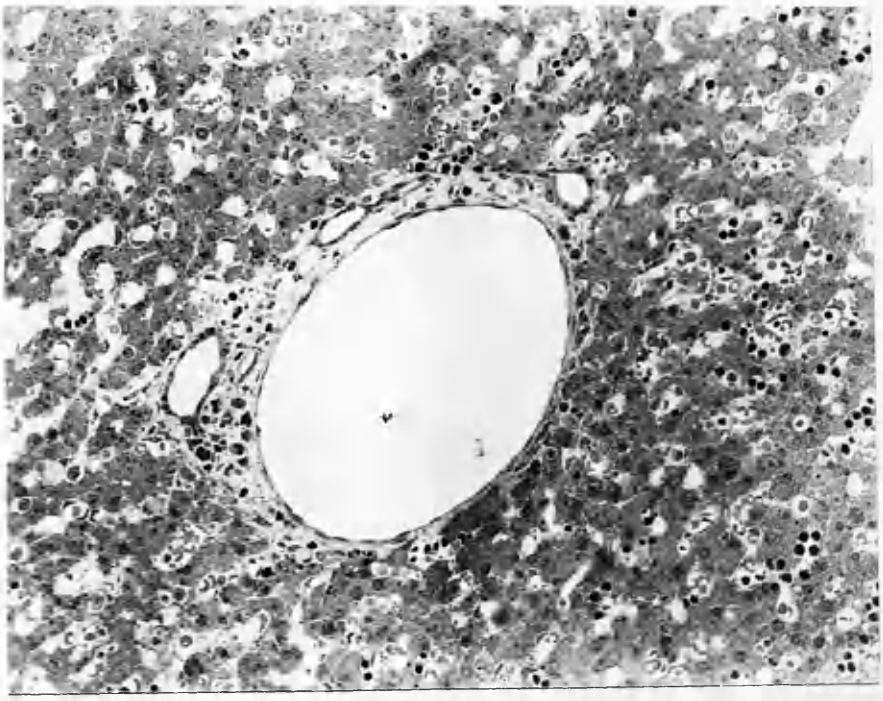
Fig. 72

A & B

Shows sectional profiles of more peripheral portal tracts, with portal vein branch diameters of less than 180 μm, lacking lymphatics.

24 hr old animal.

x 360



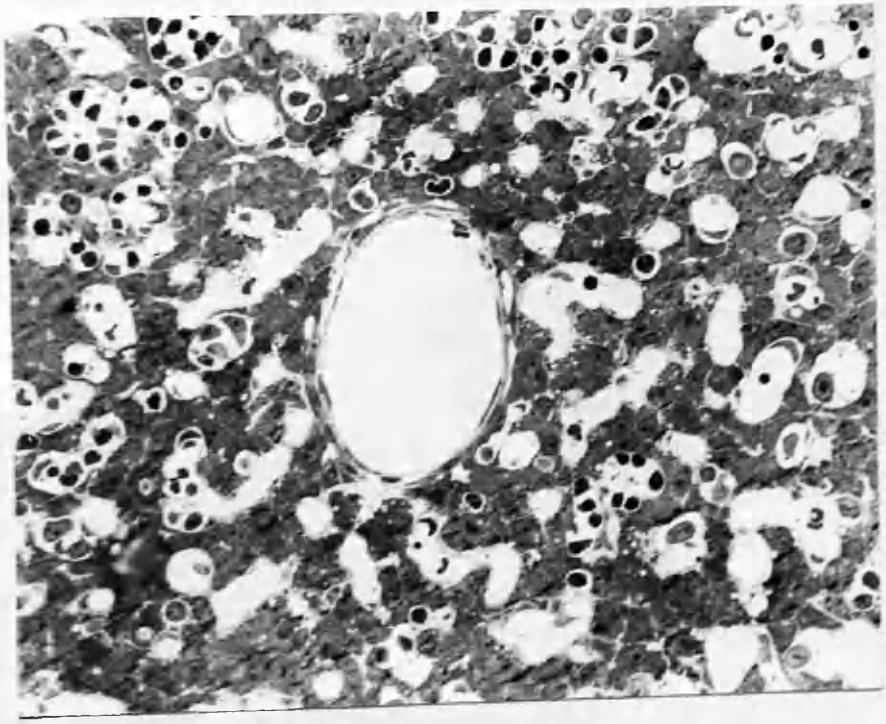
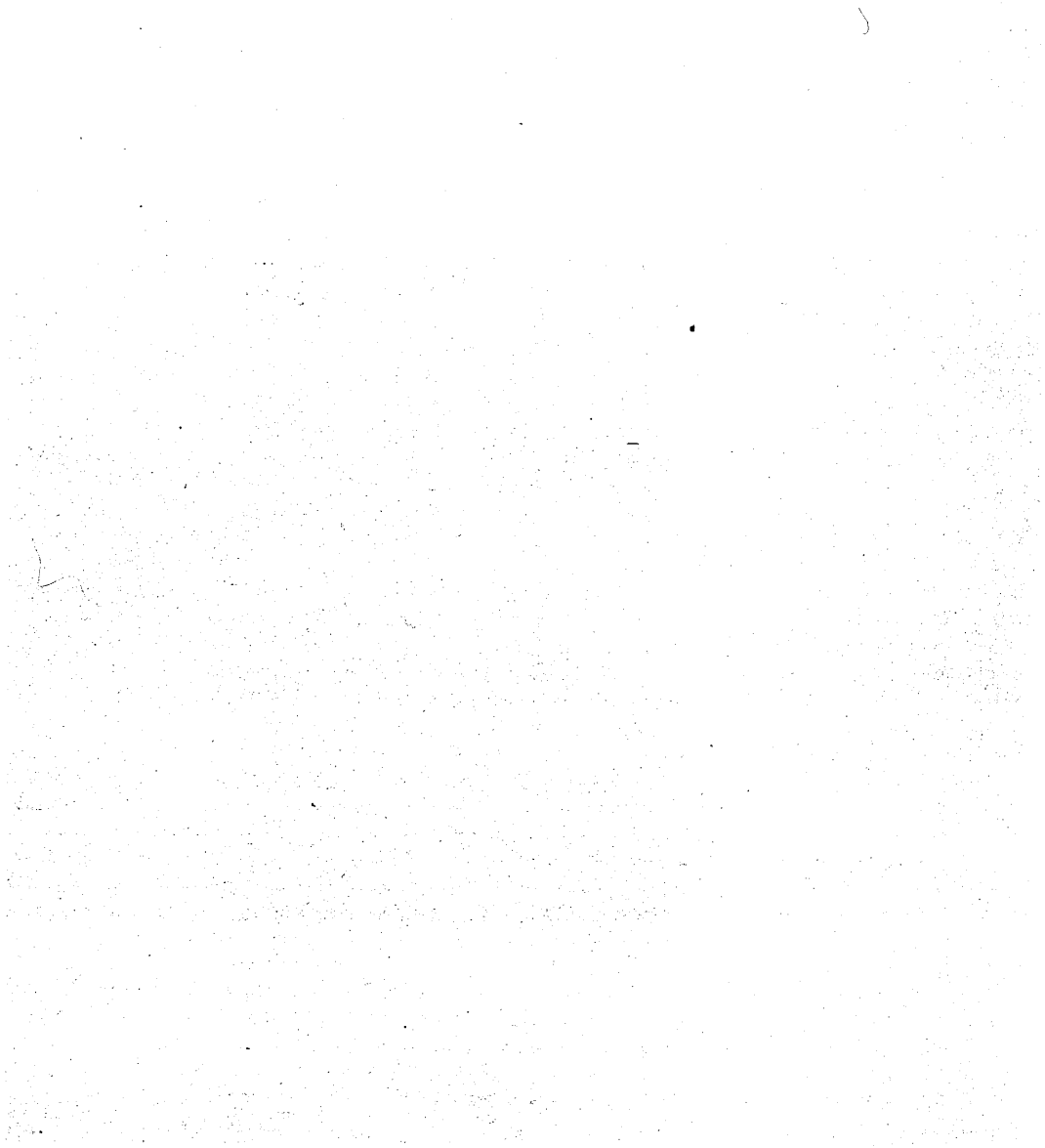


Fig. 73

In this semi-thin sectional profile of a developing lymphatic in a 2 weeks old animal, the lymphatic (L) shows two loculi on the luminal and abluminal sides of the wall.
x 320



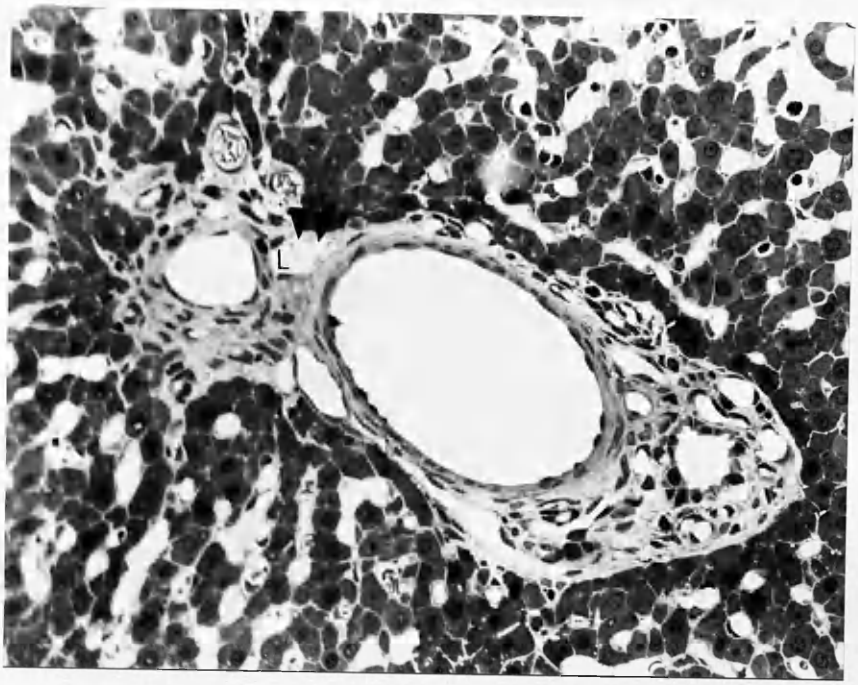

L

Fig. 74

The electron microscopic appearance of lymphatic capillaries shows considerable developmental variations from its "typical" patterns depending upon the age of the animal and the position of the lymphatic within the liver.

In a 2 weeks old rat:

Note: The lining is continuous, but at (A) shows typical endothelial characteristics, and at (B) shows characteristics intermediate between mesenchymal cells and endothelial cells.

x 5940

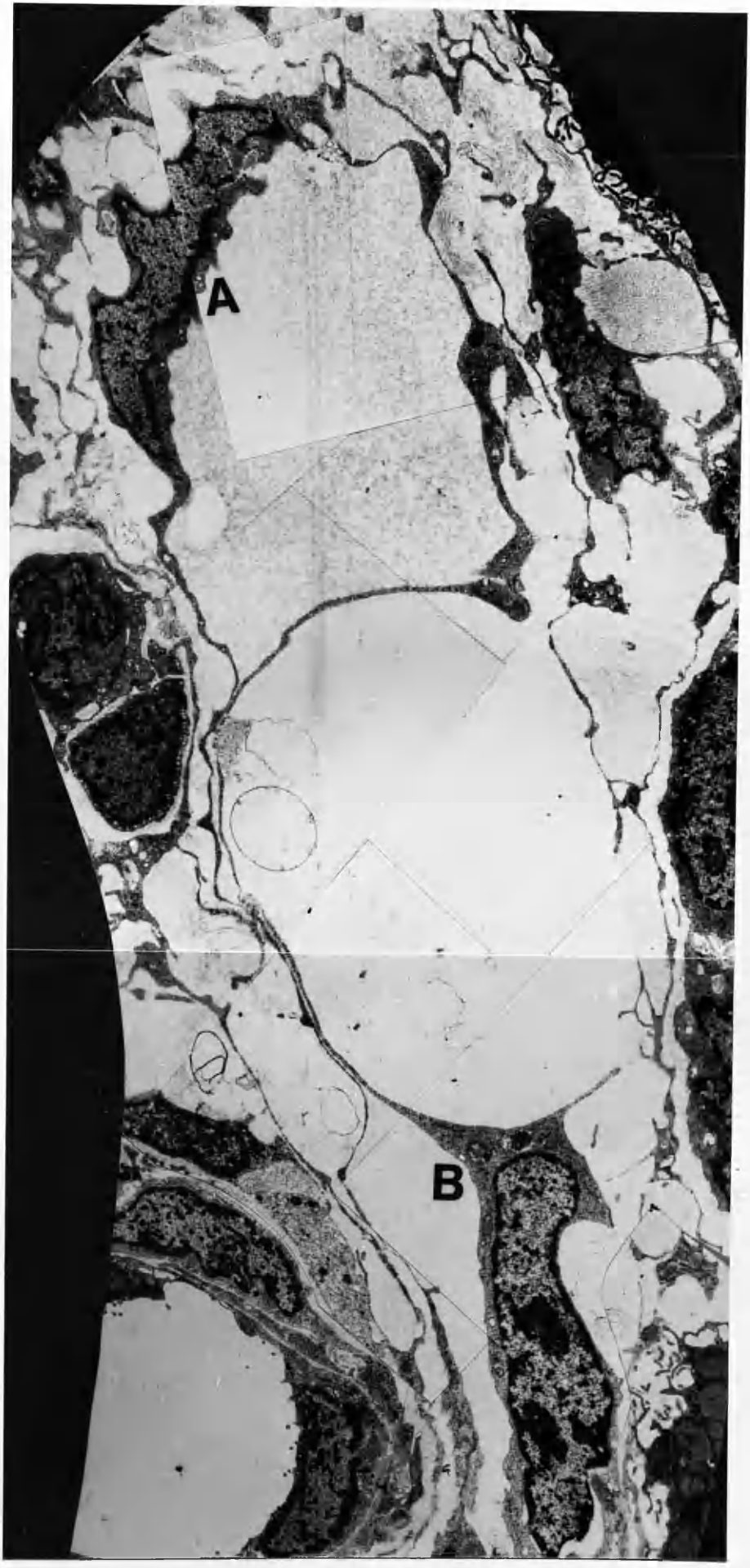

A
B

Fig. 75

Shows two cells A & B which contribute to the wall of an irregular loculated channel in a 24 hour old animal. Both cells retain some mesenchymal characteristics.
x 3920


B
A

Fig. 76

Shows a lymphatic capillary profile in a 2 week old animal.

Note: both cells A & B have "mesenchymal" characteristics.

x 5880

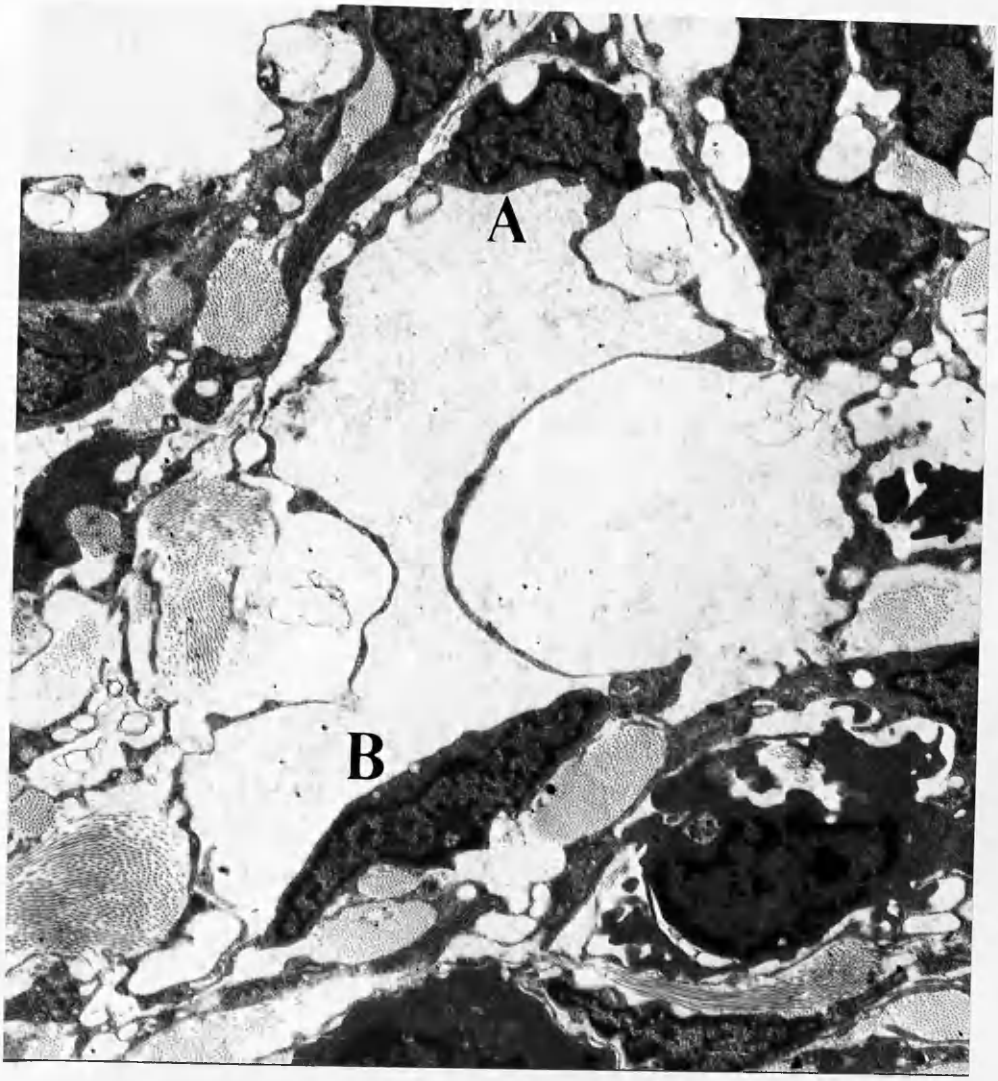
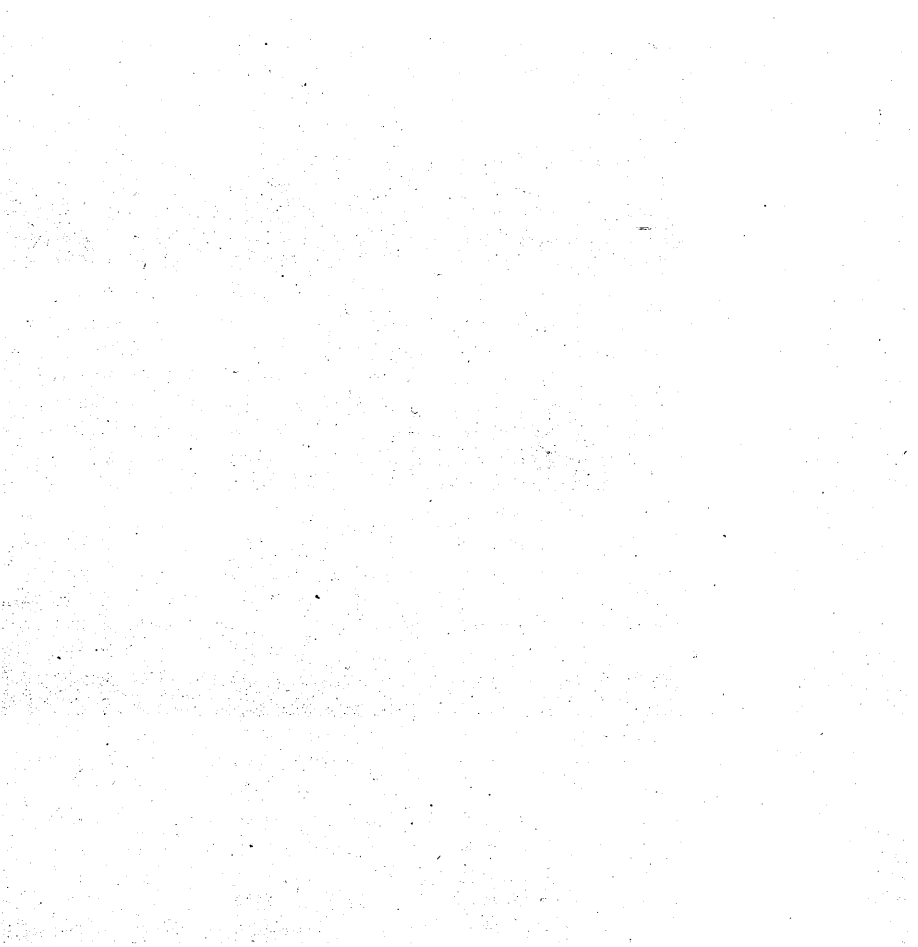

A
B

Fig. 77

Shows a lymphatic sectional profile where the lining cell A has more mesenchymal characteristic compared to cell (B) which looks more like a "characteristic" endothelial cell.
x 3920



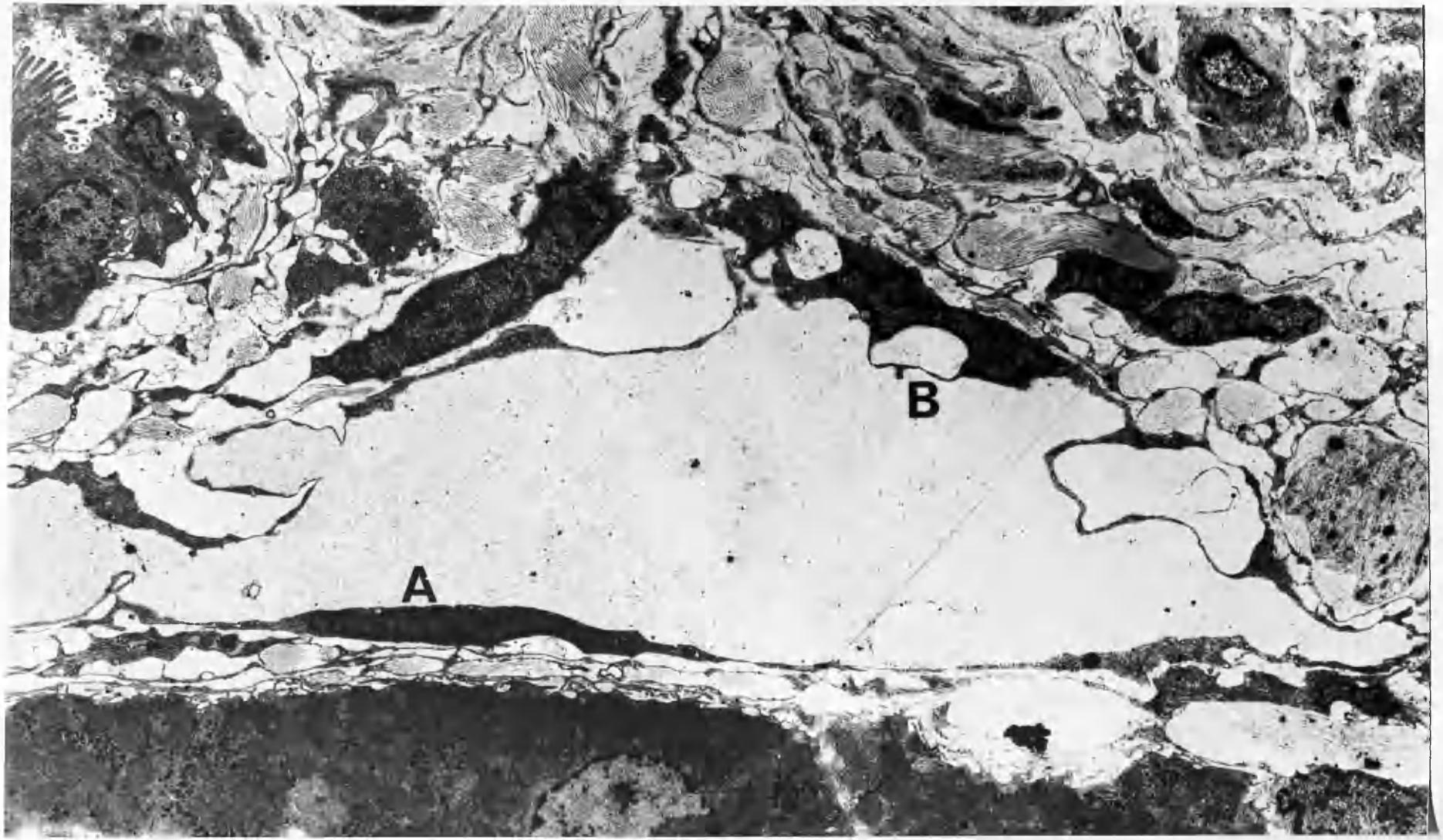

B
A

Fig. 78

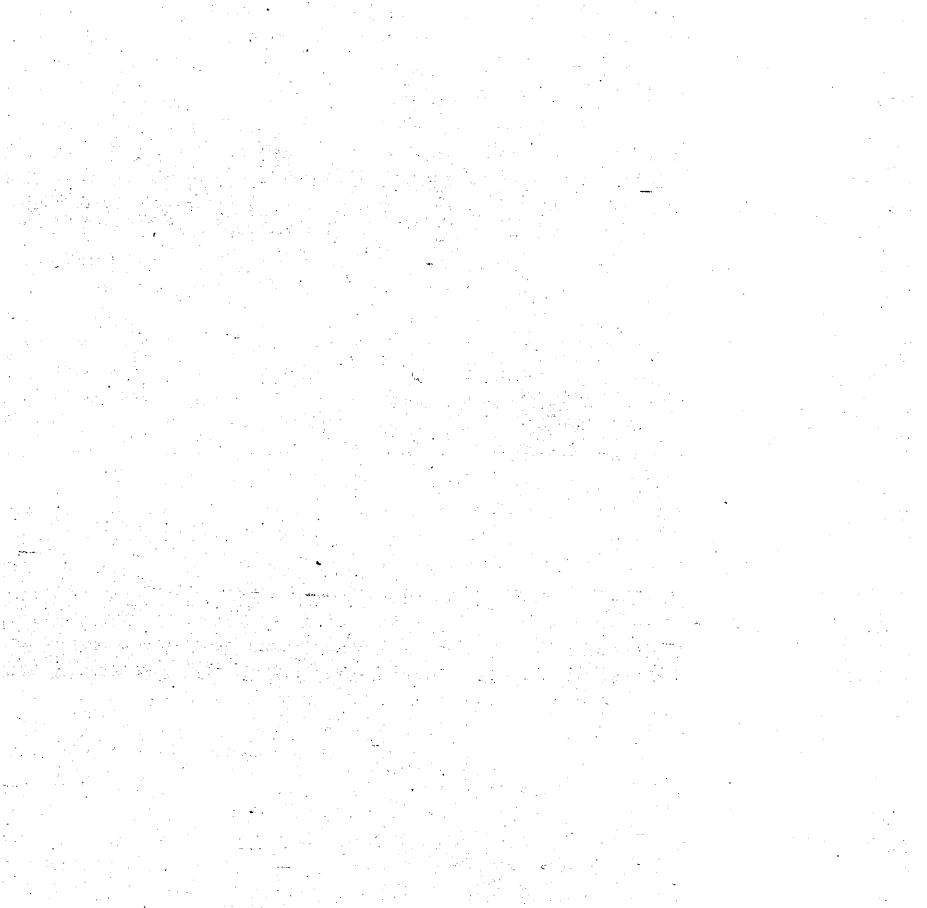
A, B & C

A series of 3 serial sectional profiles of lymphatic capillaries lined by cells which differ in appearance fom typical endothelium. From their abluminal surfaces, cytoplasmic processes extend into continuity with typical mesenchymal cells, with which they help to enclose spaces in the interstitium.

A x 3920
B x 3920
C x 3920

Fig. 79

The lining cell (A) of the lymphatic vessel, in a 2 week old rat, bounds a vacuole which opens into the interstitium.
x 7056



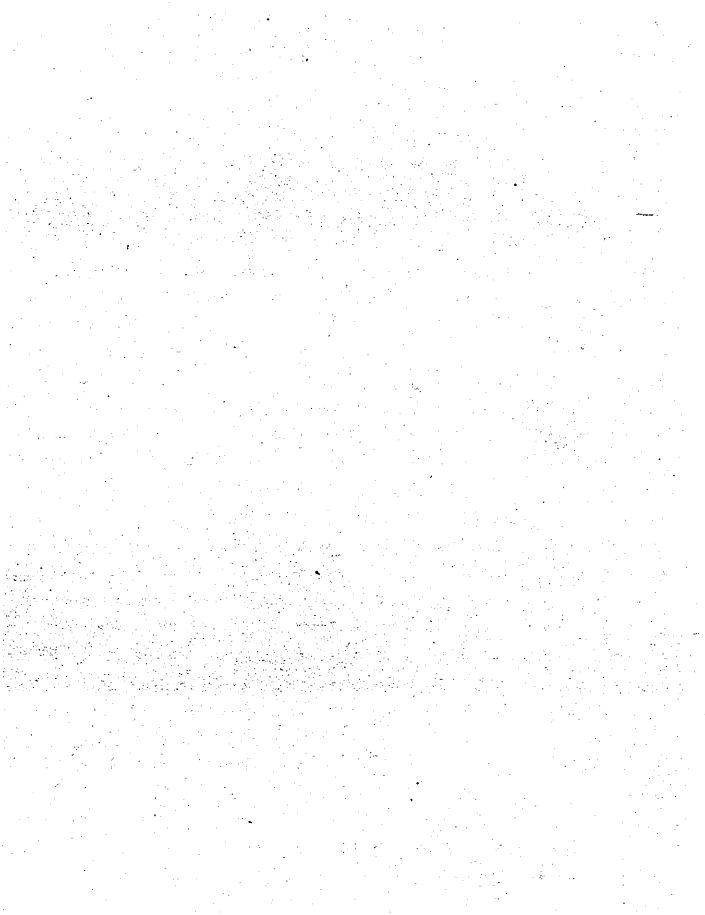

A

Fig. 80

In this sectional profile of a lymphatic of a 3 week old animal, note:

A - numerous vacuoles in the wall of the lymphatic
B - adjacent mesenchymal cell with multiple vacuoles
C - a channel containing precipitated protein

x 3920



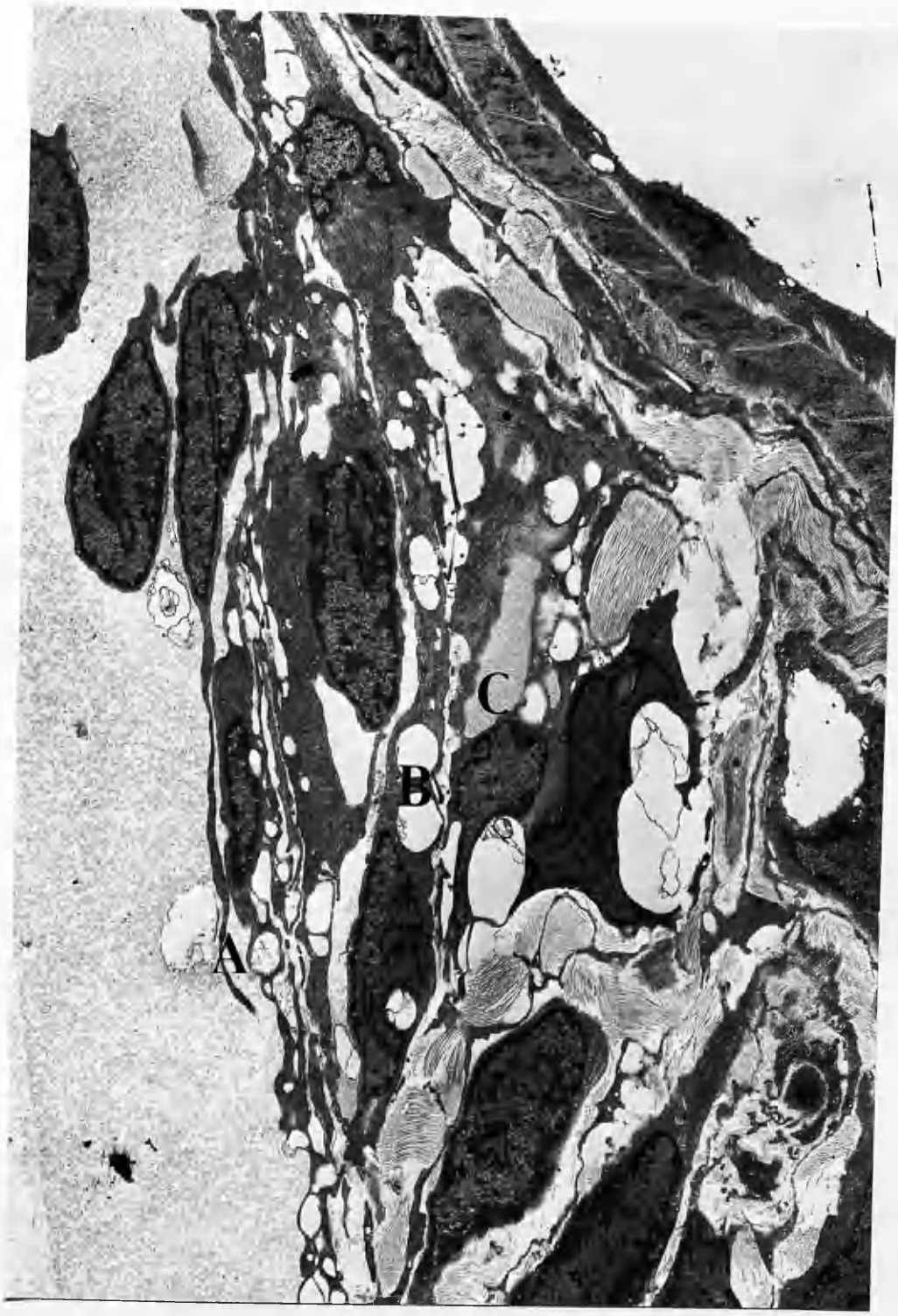

A
B
C

Fig. 81

Shows that, in some sectional profiles, lymphatics (L) can be exclusively related to arteries.
x 312

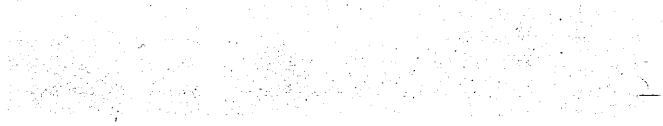
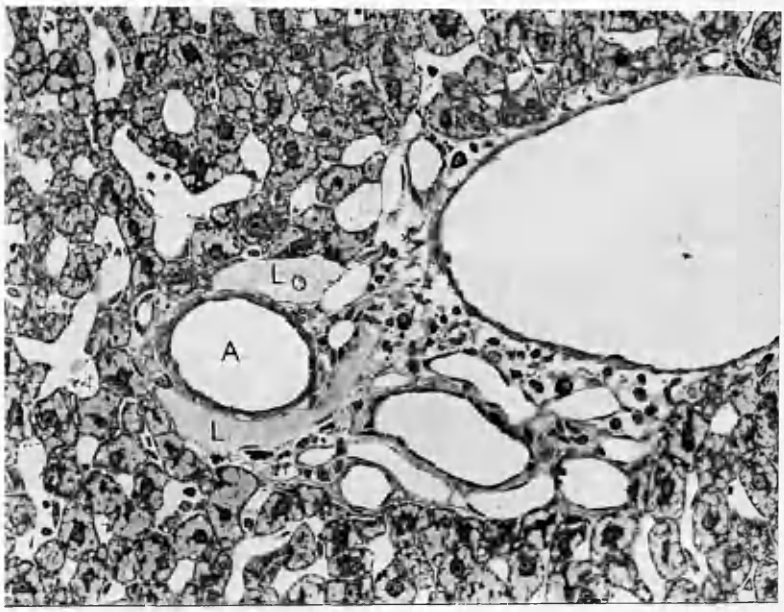


Fig 82

Shows that lymphatics (L), in other instances, are mainly related to veins.
x 100


Lo
A
L

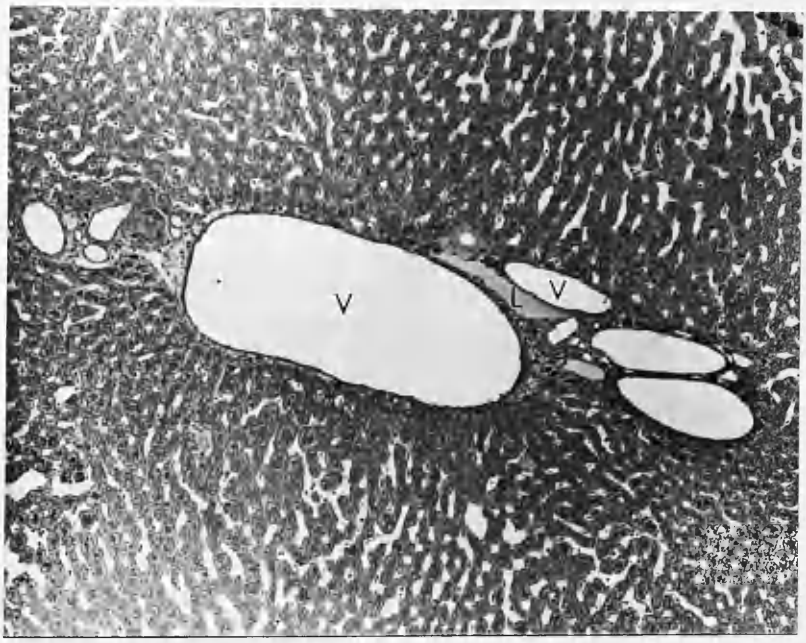

V
L
V

Fig. 83

Shows that sometimes it may be difficult to decide if a lymphatic (L) is associated with an artery (A) or a vein (V).
x 312

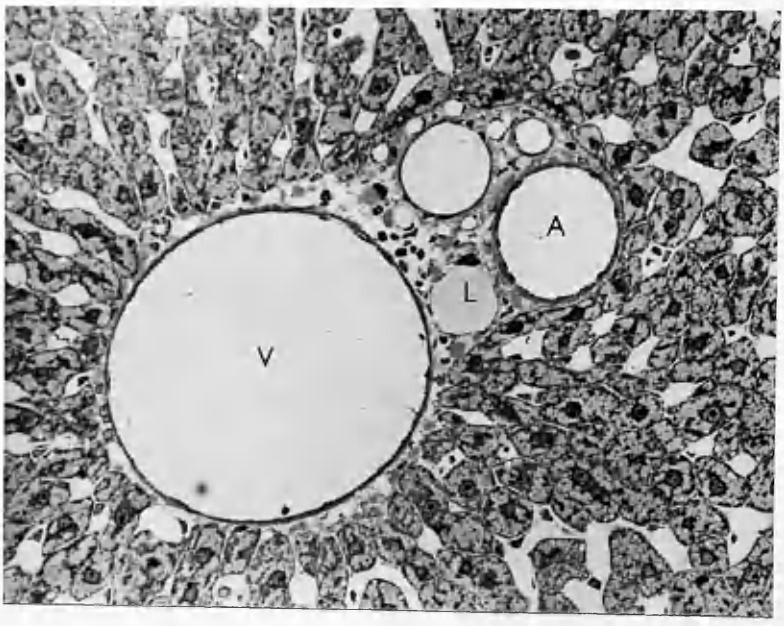

A
L
V

Fig. 84

3 lymphatic trunks (arrows) run over the portal vein (PV), join together to form a single trunk which joins a lymph node (portal node - PN) very near the portal vein.
x 6

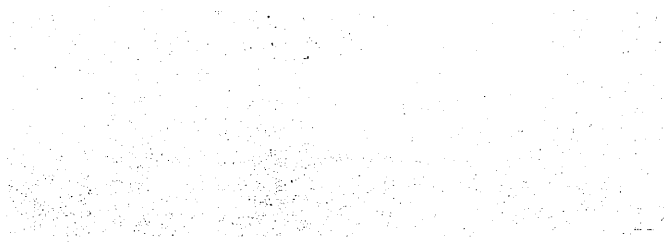
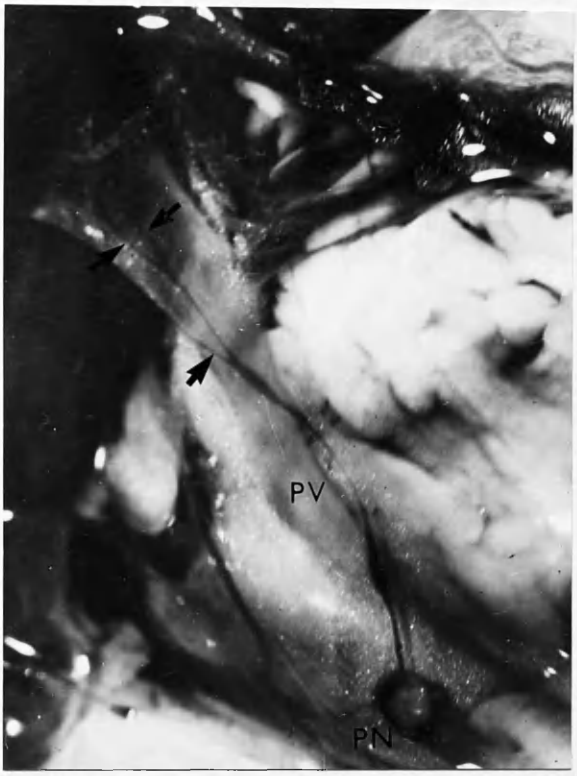


Fig. 85

A lymph trunk coming from the hilum, reaches the oesophagus (OE), bifercates and rejoins again to descend down and joins a posterior gastric node (GN) and a pale node of the splenic node (SN).
x 6


PV
PN

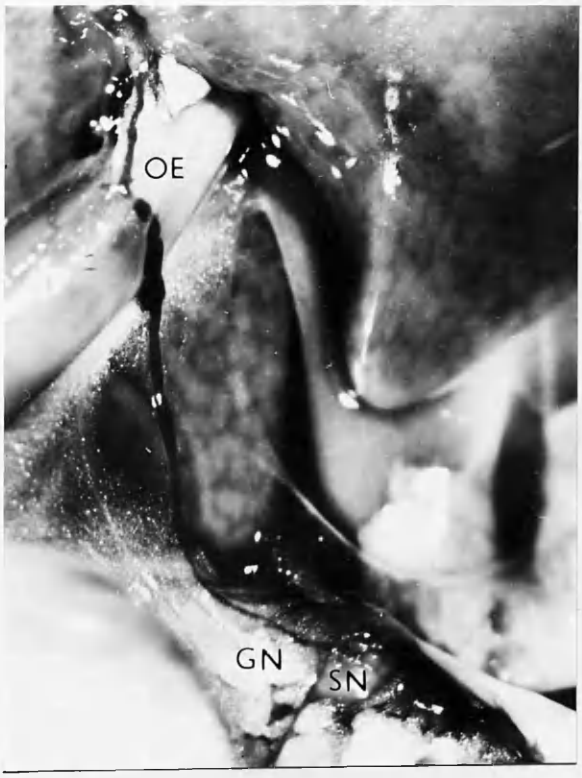
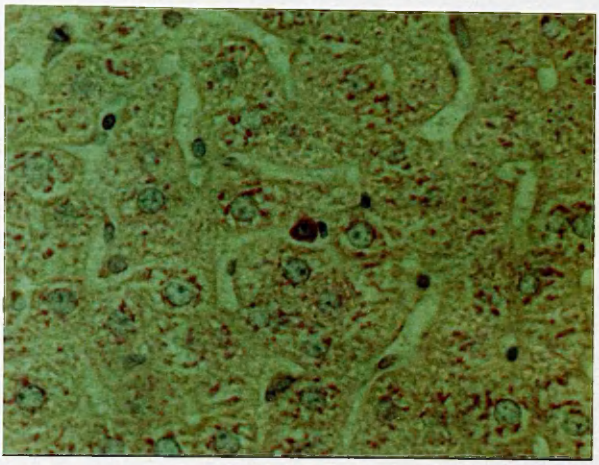

OE
GN
SN

Fig. 86

Shows a plasma cell in sinusoid.
x 500

Fig. 87

Shows two plasma cells between hepatocytes.
x 500



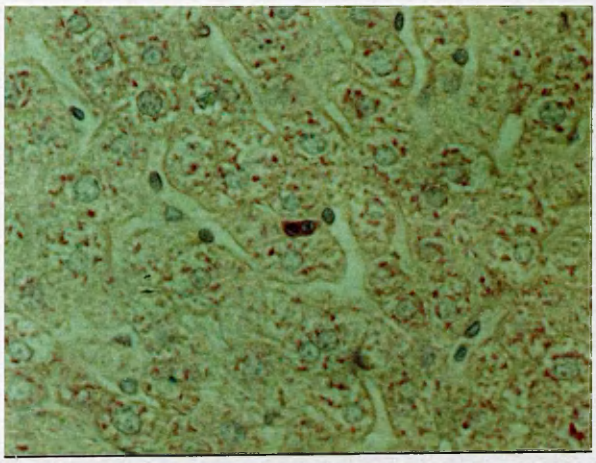
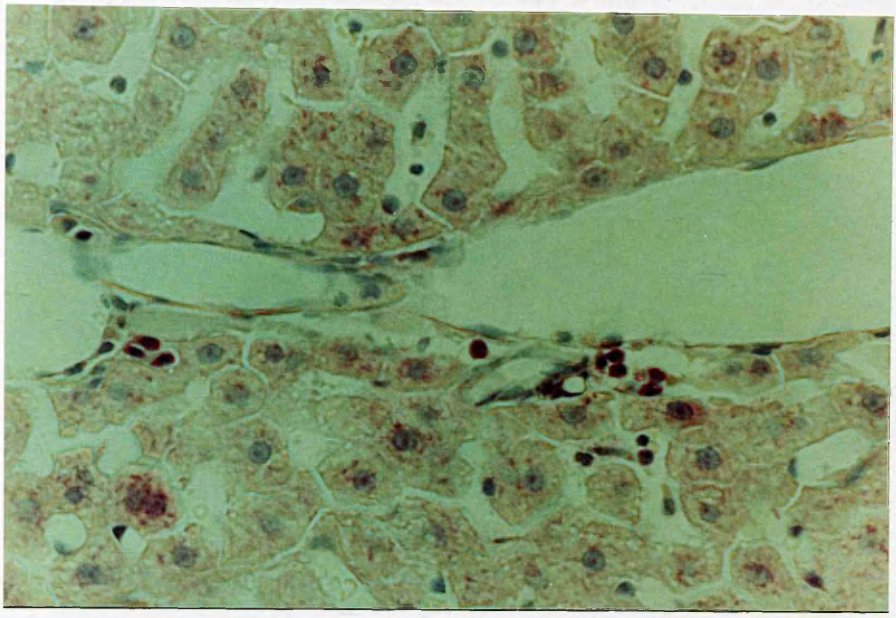


Fig. 88

A. Shows many plasma cells in a large portal tract.
x 500

B. In a smaller portal tract plasma cells can also be found.
x 500



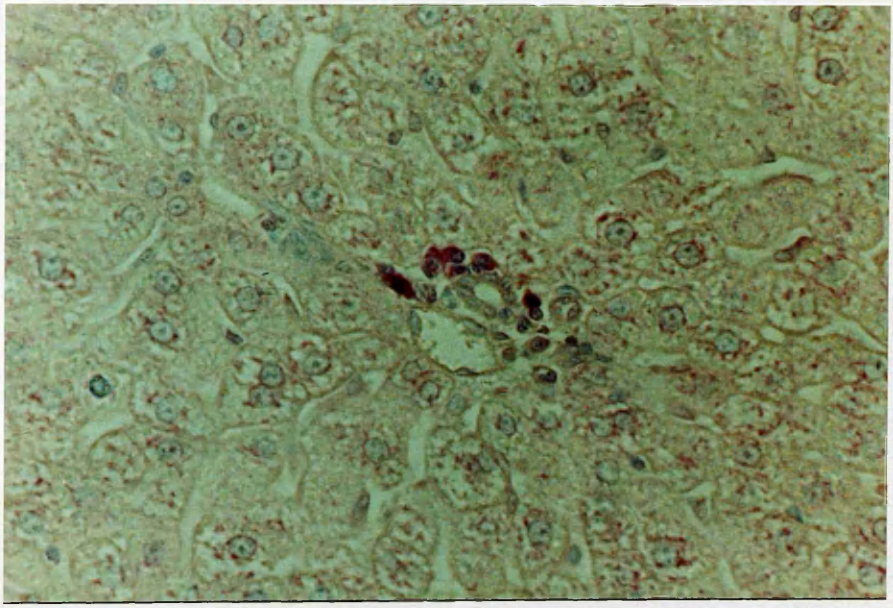


Fig. 89

A. Shows mature plasma cells in the interstitium of a portal tract.

Note: - cog-wheel arrangement of the chromatin in the nucleus
- cytoplasm is full of rough endoplasmic reticulum

x 3920

B. Two mature plasma calle in the interstitium of a portal tract.
x 5880