abnormal protein production, apparently localized plasmacytomas of lymph nodes should be treated by surgical resection if possible, utilizing radiotherapy if surgical removal cannot be accomplished. However, the patient must be suspect for the development of multiple myeloma and continual and prolonged observation is mandatory.

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The Structure of Normal Large Lymphatics: How This Determines their Permeabilities and their Ability to Transport Lymph

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It is well known that the structures of small lymphatics are very similar to those of small blood vessels (reviewed – 3, 4, 12). There are some differences, however, in that the lymphatics are more fragile, with tenous basement membranes, and endothelial intercellular junctions which sometimes lack zonulae adhaerentes and usually lack zonulae occludentes. (The zonulae are regions where the plasma membranes of two

cells come very close together and the cells appear to be more firmly united than elsewhere; the z. occludens is characterised by the outer membranous laminae actually seeming to fuse – 8.)

In the absence of injury or tissue activity the junctions usually stay closed. Activity or even mild injury, however, causes many of them to open allowing many large molecules and particles to enter the vessels. (Similarly, injured blood vessels have many open junctions, which greatly increase their permeabilities to large molecules – 12.) With some obvious exceptions, e. g. fenestrated capillaries and sinusoids, it would seem to be generally true that vessels with low permeabilities to large molecules have predominently closed junctions, while high permeabilities can be attributed to the presence of many open junctions.

Mayerson (13) considered that the two most important properties of the lymphatic system which required explanation were how material enters the vessels and how it is retained in them. The open junctions explain this facile entry; the sealing of the junctions during tissue compression (3, 4) explains why the lymph is not regurgitated into the tissue spaces from the small lymphatics. As the structure of the large lymphatics has not been described, one is unable to explain their lack of permeability to the large molecules. It is well known that they are relatively impermeable to large molecules, (e. g. proteins), but quite permeable to small ones with molecular weights below 2000 (13, 20). From the information we possess about the small lymphatics and the blood vessels, and from the fact that even closed junctions possessing zonulae occludentes are quite permeable to small molecules (5, 6), it would seem very probable that the large lymphatics possess mostly closed junctions. It would also seem likely that the junctions remain closed, in spite of tissue movements etc., because they probably receive support from zonulae, basement membranes, and connective tissue and muscular elements in the vessels' walls. It will be shown here that these suppositions are indeed correct.

Material and Methods

Young adult rats, mice and guinea pigs were used. Various lymph nodes were dissected from the animal and, if larger than 1 mm^3., were divided before being fixed. To isolate the mesenteric lymphatics, pieces of mesentery of about 5 mm^2, were excised, fixed and subsequently trimmed. About half of each thoracic duct was removed, together with some of the mediastinum. The specimen was fixed and then cut into smaller pieces. The mediastinal fragments frequently contained other large lymphatics.

To study the passage of ferritin through the walls of the nodal lymphatics, about 0.25 ml. of a 10% aqueous solution of "Cadmium-free" ferritin (Nutritional Biochemical Coy, U.S.A.) was injected into the hind-foot pads of mice. The inguinal and aortic nodes were removed after 6, 12 and 24 hours. Liproproteins and chylomicra were caused to be present in the lymphatics of Peyer's patches and the mesenteries by, respectively starving the animals and by feeding them Corn oil (1).

Fixation was performed using Caulfield's (7) Osmium tetroxide for 1–2 hours. At times, when the pieces were quite large, glutaraldehyde (4% freshly prepared in Caulfield's medium, without the Osmium) was used for ½–1 hour before using the Osmium

fixative. Any necessary subdivision of the specimen was performed between the two fixations, using trans-illumination. The blocks were dehydrated in ethanol and embedded in prepolymerised methacrylate, araldite, or epon by the usual procedures. Sections were cut on a Huxley ultramicrotome, mounted unsupported or on carbon-collodion films, stained with Lead citrate (9) or Phosphotungstic acid (1/2 % aqueous), and examined with a Siemens Elmiskop I.

Results

Some small lymphatics were seen in the different sites; they had structures similar to those mentioned in the Introduction. Apart from their size, the large lymphatics are very similar to the small ones (Figs. 1–12). There are differences, however, in the intercellular junctions, basement membranes, surrounding connective tissue, and in the smooth muscle of their walls.

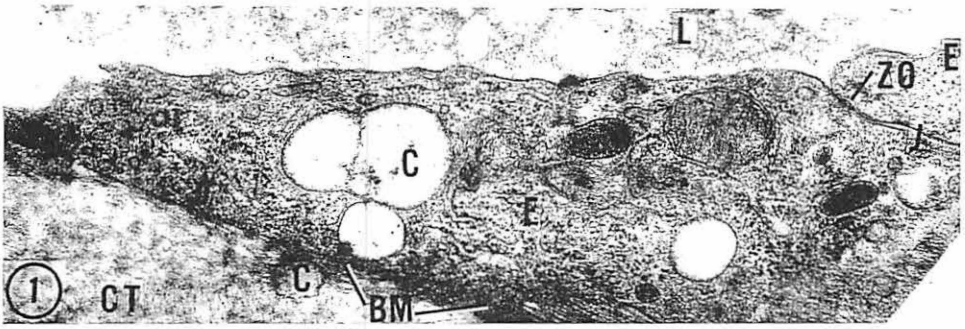
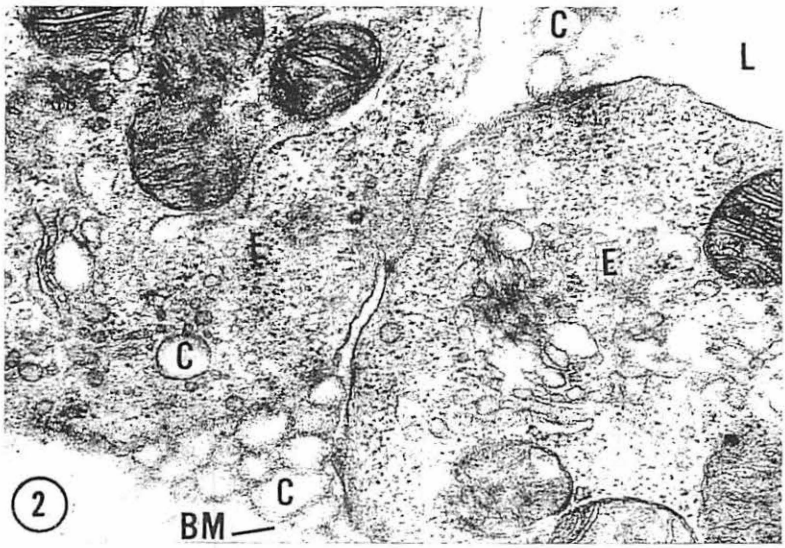


Fig. 1 *Peyer's* patch in rat jejunum. The animal had been fed Corn oil; chylomicra are present in the lymph. They (C) have pale centres because the Osmium has been removed by the epon, but their rims of phopholipid are still visible. They may be seen in the lumen of a lymphatic (L), in large vesicles in the endothelium (E) and in the connective tissue surrounding the vessel (CT). There is an intercellular junction (J) which has a zonula occludens (ZO). A basement membrane (BM) is moderately developed. Epon, lead stained, 30,000x.

Both the peripheral and the central lymphatics in Peyer's patches have a few open junctions (~ 1 in 5–10 junctions) - Fig. 2. The closed junctions have relatively frequent zonulae adhaerentes and occludentes (Fig. 1). They have moderately developed basement membranes and some surrounding connective tissue elements (Fig. 1, 2). The lymphatics of the inguinal and aortic nodes have almost no open junctions and very frequent zonulae (Figs. 4–6). Their basement membranes and the connective tissues of their walls are well developed, especially in the peripheral sinuses (Fig. 4). (It is interesting, that the walls of the peripheral lymphatics seem better developed than those of the central ones; even in Peyer's patches the open junctions were less frequent in the peripheral vessels.)

The deep, collecting lymphatics of the gut have few open junctions, frequent zonulae of both types, and moderate basement membranes. They have some connective tissue which at times includes elastic elements. The large lymphatics of the mesentery and of the mediastinum (Fig. 7–9) have no open junctions. There are zonulae adhaerentes and occludentes in nearly every junction. Their basement membranes are well develo-

ped and the connective tissue elements are very prominent, frequently including elastic tissue and smooth muscle cells.



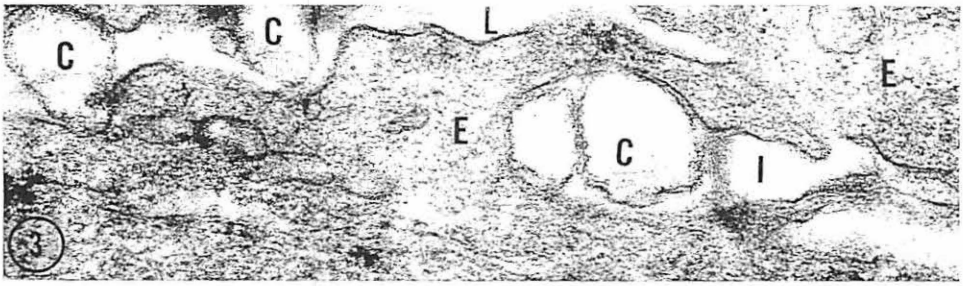


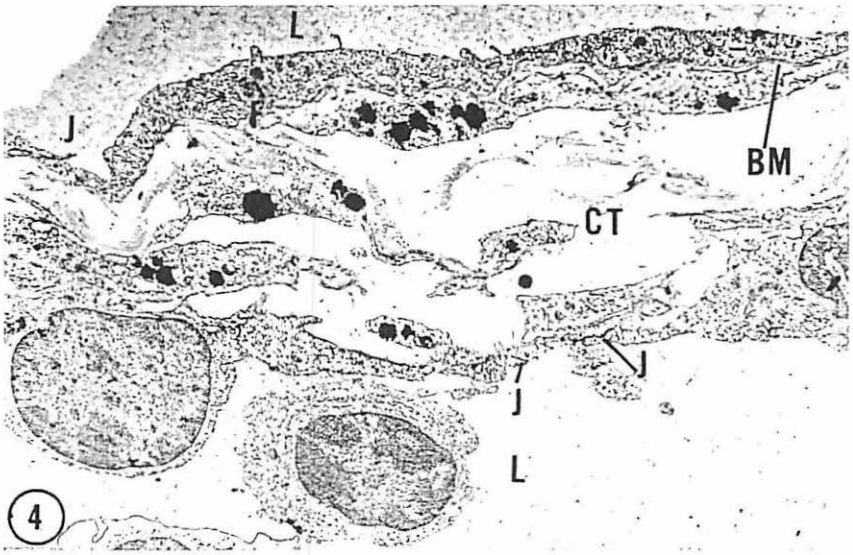
Fig. 2 As for Fig. 1. Chylomicra (C) are visible in the lumen (L), in the endothelial cells (E) and in a partly open junction. These last appear to have some difficulty in passing the basement membrane (BM). While the junction appears partly closed in this section, it may have been recently open, or may be completely patent in another plane; certainly chylomicra have passed along it. 40,000x.

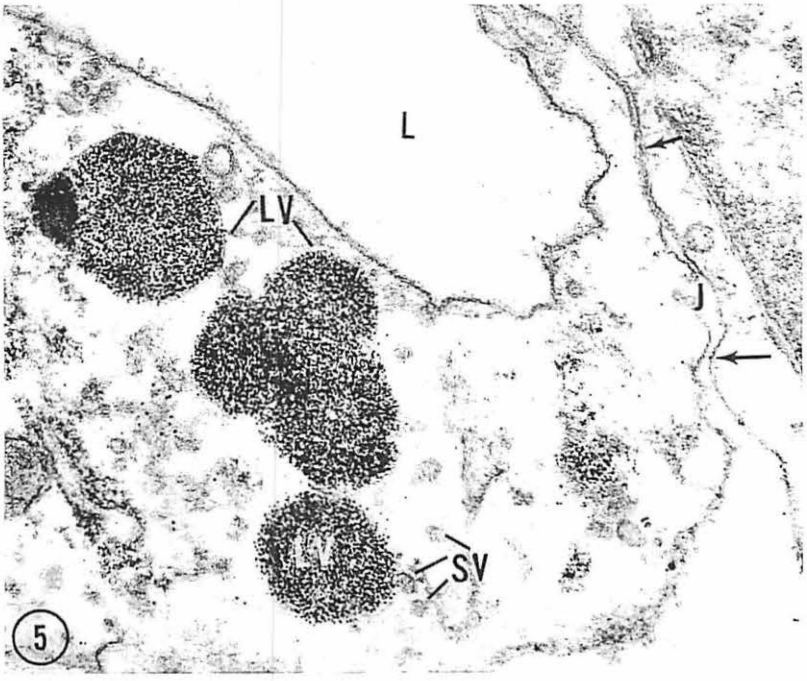
Fig. 3 As for Fig. 1. Chylomicra (C) are visible in a narrow lumen (L) and entering the endothelium via a large indentation (I). 60,000x.

Fig. 4 Mouse aortic lymph node, 24 hours after ferritin had been injected into a hind foot-pad. Two lymphatics (L) are shown, together with connective tissue elements (CT) between them. A number of closed junctions (J) are visible between the endothelial cells. Some accumulations of ferritin (F) in large vesicles and some dark lipid deposits are present. The well developed basement membranes (BM) are just discernable at this magnification. Methacrylate, lead stained, 7,000x.

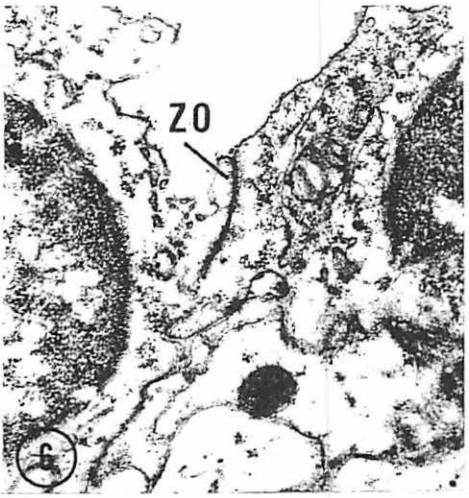
Fig. 5 As for Fig. 4. Many ferritin molecules are visible, in the endothelium, in large vesicles (LV) and in small ones (SV). There are some molecules apparently lying free in the cytoplasm, but these are probably artefacts of fixation. Some other molecules (arrows) are visible in part of ajunction (J), which is mostly a zonula adhaerens. 65,000x.

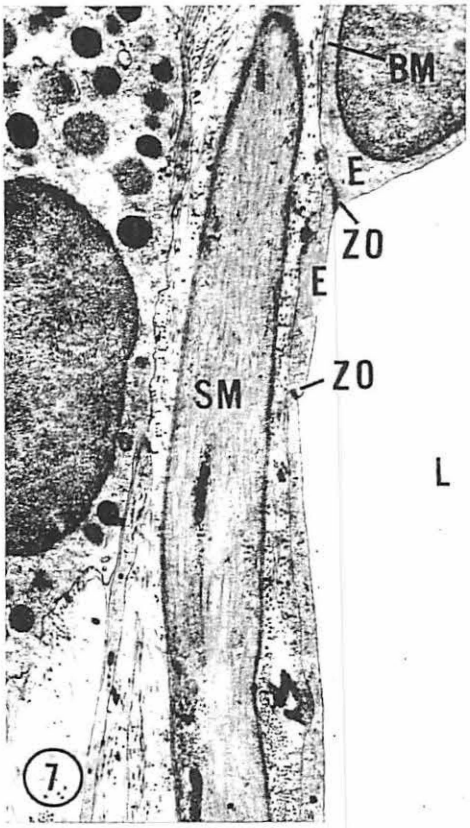
The thoracic duct is somewhat different from the other large lymphatics. Again the junctions are all closed, with almost universal zonulae adhaerentes and occludentes (Figs. 10–12). At the junctions the endothelial cells are often prolonged into the lumen as "ruffle pseudo-podia" (Fig. 12). Rarely, these projections rejoin the cells to enclose large, fairly empty vesicles. The basement membrane is often almost invisible, but there

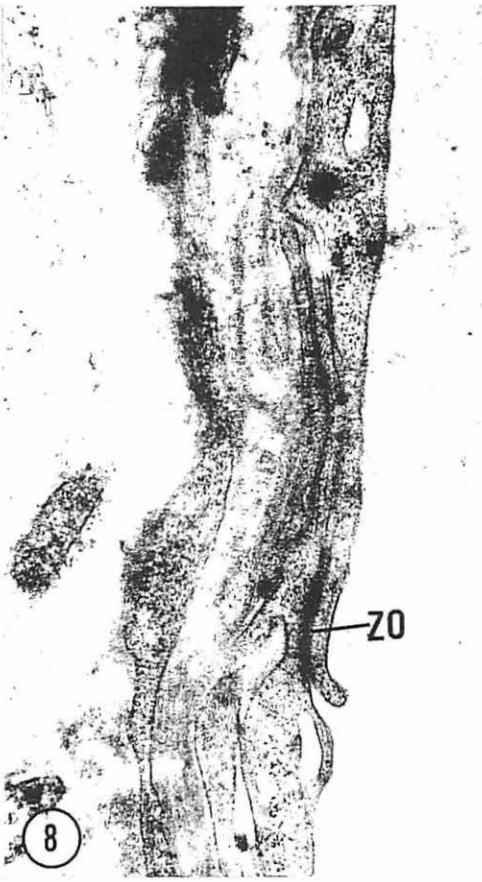


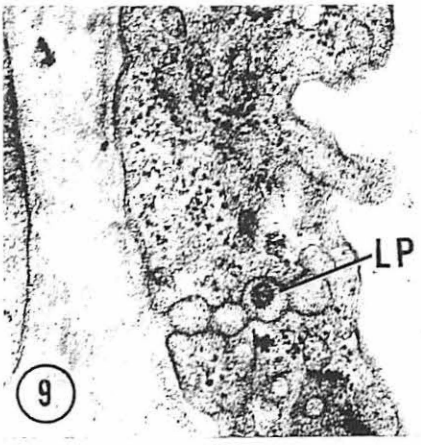


is a well developed elastic lamina which is somewhat fenestrated. External to this is a well developed muscular layer.









Permeable Paths through the Endothelial Barrier

The Junctions. When the junctions are open, large particles can pass through them, e. g. the chylomicra and lipoproteins in Peyer's patches (Fig. 2). (This is the same method by which most of these particles enter the lacteals in the intestinal villi – 1, 16.)

The closed junctions are too narrow for chylomicra or lipoproteins (Fig. 1). However, ferritin molecules are seen in the closed junctions (Fig. 5). They are not, of course, nearly as numerous as they are in open junctions (3, 4), but some 5–10 are seen in most of the long closed junctions if ferritin is present in the lumen. They are often seen, external to a zonula occludens, contained in a zonula adhaerens. None are observed in a zonula occludens. Other workers have made similar observations on blood vascular and mesothelial junctions (6, 9, 10, 12). It is unlikely that the molecules can traverse a zonula occludens. Therefore they probably reach these positions by passing through a part of the junction which does not possess such a zonula, or by crossing the cells in vesicles.

The vesicles. Ferritin and lipoprotein molecules are seen in small ($\sim$ 70 mμ) vesicles attached to both luminal and abluminal plasma membranes of the endothelial cells, and in vesicles lying free within the cells (Figs. 5, 9). It is very probable that many of the molecules which are seen lying external to the cells have traversed them in these bodies – especially in vessels where there are no open junctions. (This has been demonstrated in many other vessels [2, 3, 4, 9, 10, 15]). The only alternative explanations to vesicular transport would be that the molecules passed down the closed junctions, or travelled freely through the cytoplasm of the cells.

By serial-sectioning, it has been shown elsewhere that many of these connective tissue molecules lie far from any junctions and frequently appear to be emering from vesicles (2). In addition, it has been noted that many junctions contain zonulae occludentes, which appear to be impermeable to such large molecules. While some free ferritin molecules are seen in the cytoplasm (Fig. 5), it is now considered that this appearence is probably caused by artefacts produced during fixation (2, 3, 4). In the present experiments and in others (1, 2), lipoprotein molecules are not seen in closed junctions, nor free in the cytoplasm, yet they also are seen in vesicles and external to the endothelium. Thus the vesicles are the only paths left to account for this slow passage of large molecules through the endothelial barriers. Unfortunately, the continuous slow release of the ferritin molecules from the foot pads, and of the lipopro-

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Fig. 6 As for Fig. 4, but an inguinal node. A junction has a well developed zonula occludens (ZO). 80,000x.

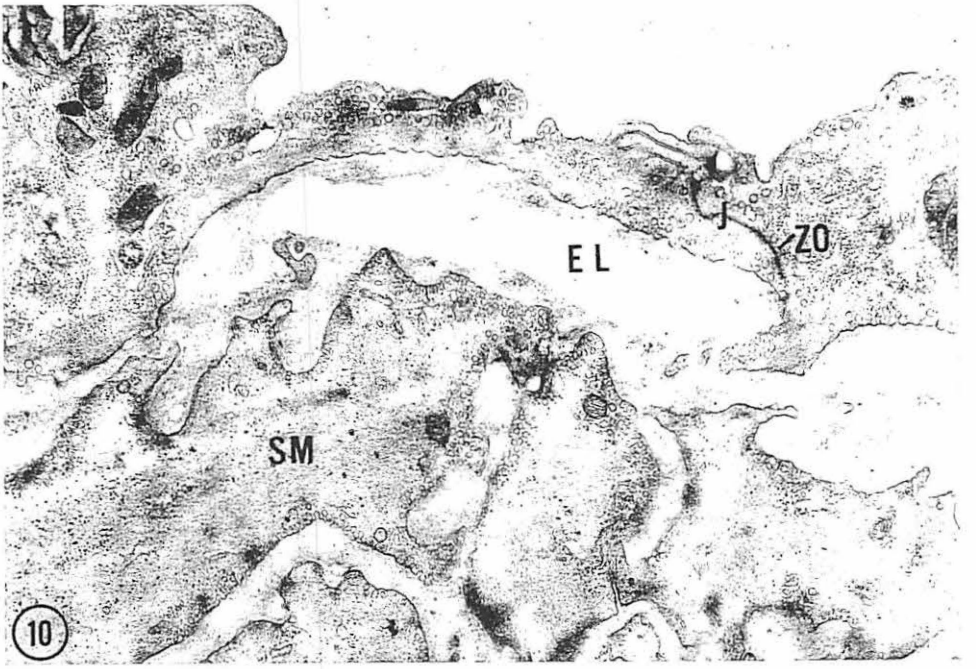
Fig. 7 A large lymphatic in the mesentery of a guinea pig. Two closed junctions, with zonulae occludentes (ZO), are visible. There is a well developed basement membrane (BM) and much surrounding connective tissue, including a smooth muscle cell. Epon, PTA and lead stained, 11,000x.

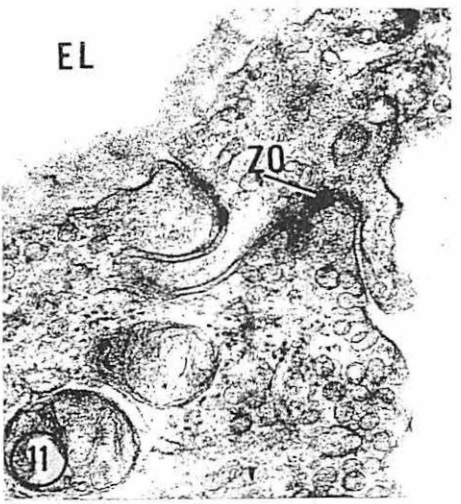
Fig. 8 As for Fig. 7. A complex junction with a zonula occludens is visible, as is a prominent basement membrane. 17,000x.

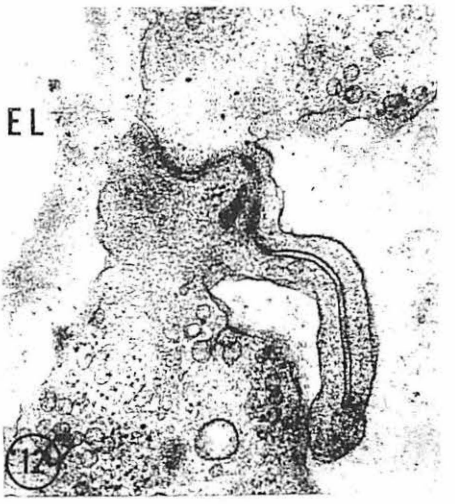
Fig. 9 As for Fig. 7, but after lipoproteins had been produced. One (LP) is seen in one of a chain of small vesicles. Such chains are very rare; fusion of two or three vesicles is common. 55,000x.

teins from the intestines, made it impossible to get any idea of the lengths of time involved in this process. (Such estimates have been made elsewhere – 2.)

Many ferritin molecules are also seen in large vesicles (0.1–1 μ – Figs. 4, 5). It is probable that such bodies arise by the coalescence of many small vesicles and the adherence of their contents (2, 3). While it is possible that any individual large vesicle may have been formed by the simultaneous ingestion of many particles, this is unlikely







to account for many of these bodies (2); such an occurence has not been observed in the present investigation. It is likely that the large vesicles will remain in the cells for months (2).

Chylomicra are sometimes seen entering the cells, and contained in them, in large vesicles (Fig. 1–3). These large vesicles are different from those mentioned above in that they are formed en masse, not by coalescence. Some chylomicra are also seen on the cell's abluminal surfaces, often emerging from large vesicles. It seems that they can traverse the cells in these bodies (Fig. 1). This has been shown to occur in other investigations (1, 2).

Discussion

It is evident that as the lymphatic vessels get larger they become less and less like the small lymphatics, and more and more like the equivalent venous vessels. Their endothelial intercellular junctions tend to become universally closed, with frequent zonulae of both kinds. Their basement membranes become more developed, as do the connective tissue and smooth muscular elements in their walls; elastic tissue also appears. The thoracic duct displays all these features in their most developed forms, except that the basement membrane again becomes tenuous or absent. Its functions seem to be taken over by the prominent internal elastic lamina. This is seen also to occur in the great arteries and veins (11, 17, 21).

The various factors responsible for the opening of the junctions in the small lymphatics have been discussed elsewhere (2, 3). Prominent among them is the poor support of the junctions of these vessels because of their infrequent zonulae and tenuous basement membranes. The frequent zonulae and the thicker, more resistant, walls of the large lymphatics obviously help to keep their junctions closed. They do this in spite of the quite high transient pressures and frequent movements to which these vessels are subjected.

It is evident that this absence of open junctions must be the reason why the large lymphatics have such low permeabilities to the large molecules. Some large molecules do, however, traverse the endothelium, or remain within it in large vesicles. It has been estimated that only about 3 % of the large molecules in the lymph are lost during its passage through a lymph node (13). It would appear very probable that this slow continuous loss occurs, as it does in other vessels (2, 9, 10, 14, 15, 18), by the molecules traversing the cells in the small vesicles; although a very small amount may pass down the closed junctions – as has also been reported at other sites (6, 9, 10, 12).

Fig. 10 Rat thoracic duct. There is a well developed, fenestrated, elastic lamina (EL). There is some evidence of an endothelial basement membrane, but it is quite tenuous. A junction is shown; it is mostly zonula adhaerens, but also has a z. occludens (ZO). Some of the smooth muscle (SM) can be seen. Araldite, lead stained. 16,000x.

Fig. 11 As for Fig. 10. There is a junction, mainly zonula adhaerens, but with a z. occludens. The elastic lamina is shown, but there does not appear to be a basement membrane. 45,000x.

Fig. 12 As for Fig. 10. The endothelial cells have projections on both sides of the junction; one is almost fusing with its cell again. This is quite common in the thoracic duct. Again no basement membrane is visible. 40,000x.

It is also highly likely that the relatively high permeabilities of the large lymphatics to small molecules (13, 20) is due to their passage down the closed junctions as occurs in other vessels (5, 6). This loss of small molecules will, of course, be especially great when the intralymphatic hydrostatic pressure is high. This will occur during contractions of the "lymphangiome" and, particularly, if there is a raised venous pressure, e. g. if the outlet of the lymphatic is obstructed. It is this difference between the permeabilities of the large lymphatics to the different sizes of molecules which causes the lymph to be concentrated as it passes centrally (20).

It is well recognised that one of the principal functions of the lymphatic system is to transport large molecules to the venous system. This function is obviously possible because of the closed junctions of the large vessels. Another important function of the lymphatic system is to carry away large amounts of fluid and smaller molecules when they collect rapidly and tend to flood the tissues, e. g. during muscular activity. It is evident that the high permeability of the large lymphatics to such molecules must mean that only a small proportion of them are actually carried as far as the venous system by the largest lymphatics. Many must pass out from the lymphatic system en route, but they will not leave the vessels to any great extent while they are still within the flooded region because of the high external hydrostatic pressures. Instead, they will escape into the unflooded regions lying centrally along the routes of the large lymphatics. Here the small molecules will readily be able to enter the venous system, because they will disturb the hydrostatic/osmotic pressure relationships. Thus the large lymphatics still transport the small molecules to the venous system, but they enter it indirectly through the connective tissues as well as by direct lymphatico-venous communications.

Summary

The fine structures and permeabilities of some large lymphatics were investigated. It was found that large lymphatics tend to have few or no open junctions, many zonulae adhaerentes and occludentes, and well developed basement membranes. They also have prominent connective tissue elements and smooth muscle cells in their walls. The thoracic duct, however, has a poor basement membrane, but a quite well developed elastic lamina.

Ferritin and lipoprotein molecules passed through the cells in small vesicles, which sometimes coalesced to form large ones. Ferritin was also found in closed junctions, even in zonulae adhaerentes, but not in zonulae occludentes. Chylomicra appeared sometimes to traverse the cells in large vesicles, as well as passing along any open junctions.

The closed junctions of the large lymphatics make the vessels poorly permeable to large molecules. Thus these are transported with little loss, which is essential for the functioning of the lymphatics as a transport system.

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The Use of Polaroid Film in Lymphography

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Lymphology 1 (1969), 25–27

The use of Polaroid radiography in various areas of radiology is well known and has been described in the recent literature with special reference to urological[1] and neurosurgical[2] procedures. The application of the Polaroid technique to lymphangiography has been justified at the Hospital of the University of Pennsylvania as a means to determine the position of the catheter needle in the lymphatic channel.

Materials and methods

Since January, 1968, Polaroid radiographs have been routinely obtained at the Hospital of the University of Pennsylvania at the beginning of the lymphographic procedure to ascertain the position of the catheter needle. The technique used in per-

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