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THE PHYLOGENY OF THE FINE STRUCTURE OF BLOOD VESSELS AND LYMPHATICS: SIMILARITIES AND DIFFERENCES

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ABSTRACT

As animals increased in size, various modifications had to come into being to carry nutrients and waste around the body. Different phyla solved the various problems differently; sometimes there was convergent evolution. In invertebrates the endothelial cells are often widely separated from each other; permeability is limited by the pericyte layer; the reverse occurs in vertebrates. In primitive chordates small peripheral vessels often consist only of the basement membrane, and even this may be partly missing; the more centrally one looks, the more the endothelial cells become continuous. Fenestrae appeared first in the agnatha, but only become common in the elasmobranchs. Increased size and activity necessitated still larger blood hydrostatic pressure and increased blood colloidal osmotic pressures to balance this. Since the permeability of the vessels could not be reduced, much more protein (and fluid) had to leak to the tissues. So the lymphatic system had to evolve. This is first seen in the torpedoes and fully evolved in the bony fishes. However, the small venous vessels of the elasmobranchs have openable inter-endothelial junctions and other structures very similar to those of the initial lymphatics.

Apart from the absence of fenestrae in lymphatics, or when this system is injected with a tracer, it is not always possible to tell them apart with the electron microscope. There are, however, various differences between them which will help to differentiate them.

The phylogeny of the vascular systems

The phylogenetic development of the vascular systems is a fascinating subject, because of the ways in which different groups of animals have solved the same problem. The phylogeny of the blood vascular system is covered in many standard texts (Grasse, 12, is the most detailed); seldom has that of the lymphatic system been reviewed. Kampmeier (16) has a very detailed account of the development of both systems from the macroscopic and light microscopic viewpoints, while Rusznyak et al. (19) and Yoffey and Courtice (21) deal briefly with that of the lymphatic system. As far as I am aware, the fine structural aspects of the phylogeny of either system has only been reviewed by myself (6,7). However, few physiological studies on permeability have been made in animals other than the higher vertebrates; fine structural evidence about primitive vascular systems largely consists of a few incidental studies on a few regions of a few species.

When an animal's body is small there are few problems; diffusion is sufficient and a circulatory system is unnecessary. Slightly larger bodies can be

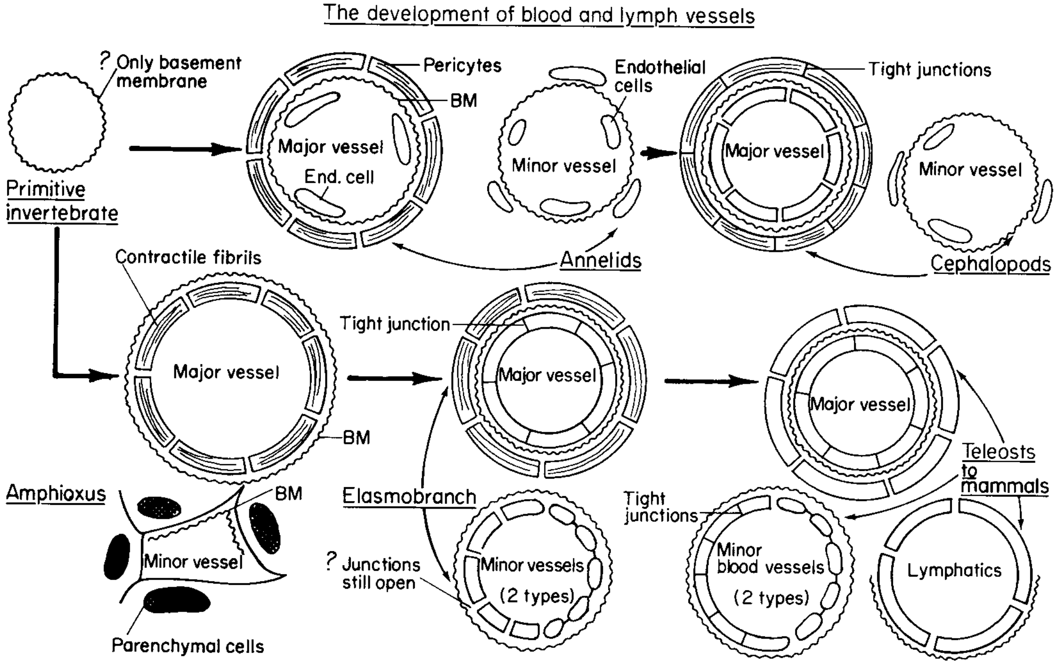

Fig. 1. A summary of the main fine structural phylogenetical differences. The hagfish (agnatha) lie between amphioxus and elasmobranch. (Ref. 7).

served by simple hearts which stir the fluid in the open body compartments. However, increases in size and increases in activity bring problems. Increase in size necessitates a blood circulatory system to distribute nutrients and remove wastes to and from the tissues.

For the blood vascular system in the larger animals, one of the main problems is how to get enough pressure to push the blood around fast enough through the many fine tubes which are necessary to distribute it adequately yet not to let this pressure rupture them. This is solved in capillaries by the basement membrane and in larger vessels by fibrous and elastic tissue and muscle. The basement membrane is secreted by the endothelium. In the more primitive chordates (which include the vertebrates) and in all the invertebrates these are isolated cells not joined together *(Figs. 1-3)*. In the most primitive chordate, amphioxus, the minor vessels may even lack the basement membrane over part of their circumferences (5; *Fig. 4*). The blood vascular system is directly continuous with the tissue channels. To some extent, this is true even in mammals--via

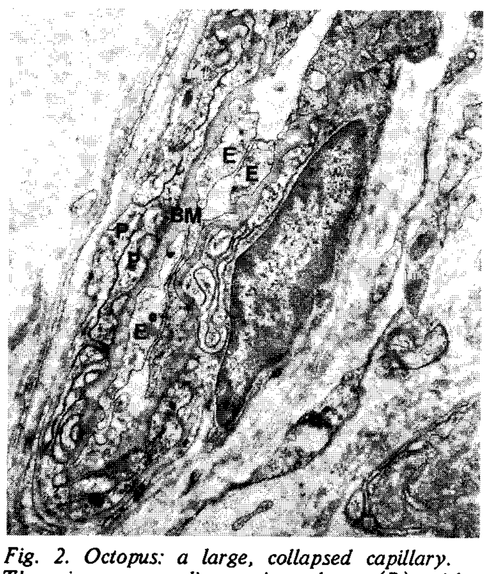

Fig. 2. Octopus: a large, collapsed capillary. There is a surrounding pericyte layer (P), with close junctions, outside the basement membrane (BM) and some isolated endothelial cells (E) with large gaps between them. x10,000. (Courtesy of Dr. J. Browning, ref. 2).

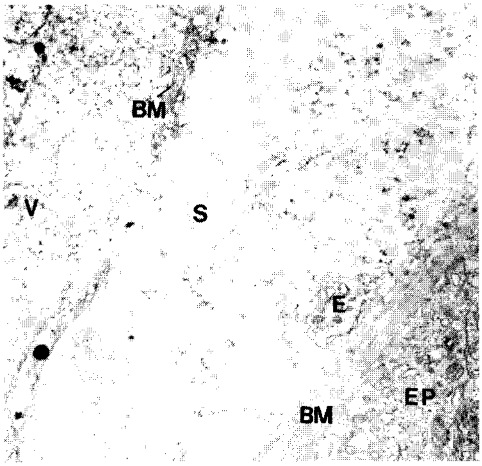

Fig. 3. Amphioxus: a sinusoid (S) is shown, with a thick basement membrane (BM) between it and the epithelium (EP) of the gut. It is continuous with the subintestinal vein (V). Both vessels have occasional, isolated endothelial cells (E). x2,500. (Ref. 5).

the closed (and any open) junctions and the fenestrae; in amphioxus vertebrates it is particularly evident. Not even a complete basement membrane separates the two sets of vessels. In the more advanced chordate, the vertebrate agnatha, the basement membranes are more complete but are often tenuous (9,14,15,17; *Fig. 5*).

The development of the heart itself shows examples of differences in evolution. In the invertebrates the contractile fibres appear in the pericytes, outside the basement membrane. In almost all of the vertebrates this pattern was also followed, with the single layer of pericytes becoming multiple layers (in the heart and veins); this was also followed in the collecting lymphatics and lymph hearts (below). However, in amphioxus (the most primitive of the chordates) it is the endothelial cells which develop contractile fibres, but only form a single layer--inside the basement membrane. Curiously, the endothelium of both the blood and lymph vessels of the mammals still possesses the ability to contract (reviewed: 8).

The second major problem is how these vessels could be permeable enough to allow the various substances (from gases to macromolecules) to enter and leave the blood system, without vast amounts of whole blood escaping to the tissues. So a relatively impermeable barrier of cells developed in the walls of the vessels--with permeable devices in them: tight, closed and open junctions (using the terminology defined in ref. 8), vesicles and fenestrae. However, this solution to the problem has been found in different ways (*Fig. 1*). The chordates (including vertebrates) place the relatively impermeable layer on the inside of the vessels, viz. the endothelium; by contrast, invertebrates place it on the outside of the vessels, viz. the pericytes (*Fig. 2*)! Curiously, the leeches developed their relatively impermeable barrier from the endothelum (just as in the vertebrates); this is sometimes even fenestrated (13)--a most surprising example of convergent evolution!

In the more primitive chordates (e.g., amphioxus - Ref. 5, *Figs. 3,4, and the hagfish--Ref. 9; Fig. 5*) the endothelial cells in the smallest blood vessels are separated by gaps which are microns wide. As the vessels become larger, the endothelium becomes more and more continuous until, in the central vessels, it is as continuous as in the

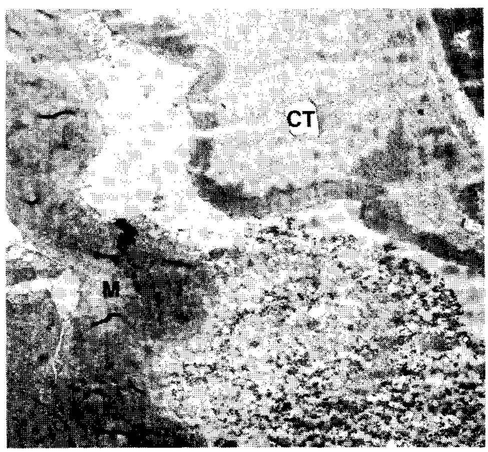

Fig. 4. Amphioxus (which had carbon injected into its blood vessels): A peripheral "vessel" lies between the muscle (M) and the connective tissue (CT) of the body wall. No basement membrane can be seen and certainly no endothelium. x2,000. (Ref. 5).

Fig. 5. Hagfish: An open junction (J) is seen between two endothelial cells in the neurohypophysis; elsewhere in this region the cells are often tightly joined. No fenestrae were present. x45,000. (Ref. 9).

mammals. Even in sharks (elasmobranchs) the venous endothelial junctions are openable (10; *Fig. 6*).

In the annelids the junctions between the pericytes, while usually 7.5 to 20nm wide (reviewed: 7), are sometimes as wide as 1 micron (18). In the cephalopods (reviewed: 7) the junctions are about 20nm wide, and ferritin molecules can pass through them (2,11)--although they possibly also traverse the pericytes via vesicles. (Haemocyanin molecules are too big to traverse these junctions, as are carbon particles--2.) The tissue channels in cephalopods are slightly smaller and less numerous than in mammals (3).

Fenestrated endothelium

Fenestrae represent a solution of another problem. Sometimes large local exchanges of fluid or macromolecules are needed, yet there must be only a small net leakage of these molecules to the tissues. This is achieved by fenestrae very permeable to all classes of molecules and yet maintaining high colloid osmotic pressures and so facilitating the return of tissue fluid (and the macromolecules it sweeps along) to the blood at the venous capillaries (8). Fenestrae have not been found in any invertebrates other than the leeches (13). The earliest vertebrate in which they have been found is the hagfish (14,17) in the kidney; however, they were not found in other areas of the hagfish such as the thyroid and the neurohypophesis where they are common in vertebrates (9). They are, however, common in the endothelium lining the blood capillaries in the mucosa of the small intestine of elasmobranchs (sharks)--where they readily permit the passage of lipoproteins into the vessels (10). There are no lymphatics in the gut of elasmobranchs (16); when carbon particles, too large to penetrate the fenestrae, are injected into the mucosa they are not removed at all (10). Fenestrae are common in the viscera and some other regions of sharks (10), and in all more developed vertebrates.

The lymphatic system

However, a further problem arose: the increased sizes and activities of the higher vertebrates necessitated an even greater hydrostatic pressure in order to move the blood to carry oxygen to the tissues fast enough to sustain greater muscular activity. This had to be balanced by increased plasma protein concentrations. The permeability of the vessels could not be made any less, because this would mean insufficient exchange of

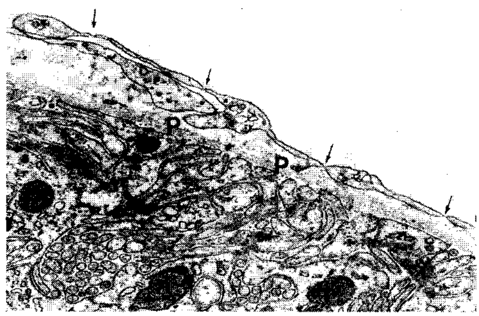

Fig. 6. Shark: A number of fenestrae (arrows) are visible in the endothelium of a capillary in the kidney. A junction is widely open over most of its length and is undoubtedly completely open in a plane other than that of the section. There are a number of endothelial projection (P) into the interstitial tissue. The basement membrane is poorly developed. x12,000. (Ref. 10).

the smaller nutrient and waste molecules. Thus even more macromolecules (and some fluid osmotically held to them) would leak out of the vessels. Hence a way had to be found to remove these excess protein and fluid molecules and return them to the blood. This was the lymphatic system.

There has been considerable argument about how early the lymphatic system came into being. Ando-Yoshia (1) held that true lymphatics were present in cephalopods. However, Smith (20) showed that the latter's results were caused by the seawater he injected causing many of the blood vessels to contract and hence not be filled by the dye it contained; these were then erroneously termed 'lymphatics.' Similarly, electron microscopy and tracer experiments indicate that reports of lymphatics in animals more primitive than the elasmobranchs (reviewed: 16) are fallacious (reviewed: 6,7). Even in the elasmobranchs lymphatics probably only occur in the torpedoes; reports of them in other primitive forms can be explained (6) by a downstream spasm of the veins (probably caused by rough handling) coupled with a considerable uptake of fluid from the tissues (partly caused by a great reduction in tissue pressure upon hauling them up from the deep).

It is of great interest that the venous vessels in elasmobranchs possess many of the attributes of initial lymphatics of mammals (10). They *(Fig. 6)* have openable junctions between the endothelial cells, a tenuous basement membrane and connective tissue fibrils attached to endothelial projections (which permit the endothelial cells to be moved apart by tissue movements). The functions of the lymphatic system are therefore probably performed, in primitive vertebrates, by openable endothelial intercellular junctions in the venous capillaries which are opened by swimming activity. These can no longer be allowed to be opened once the blood pressure increases as it does in the teleosts. Hence at this point a true lymphatic system had to be developed. This is present (over a relatively small portion of the body) in the torpedoes (which are also elasmobranchs).

In the bony fishes (teleosts) a true lymphatic system is found. As one ascends vertebrate development, only minor (quantitative) alterations are found in the fine structure of the vessels of this system. There are changes in fine structure in the lymph hearts and the contractile connecting lymphatics; however, these are aside from the present considerations.

It is interesting that even in mammals the endothelial junctions echo the blood vascular junctions of very primitive vertebrates. In the periphery many junctions are openable, becoming more and more closed as one passes to the great vessels. Again, as one passes centrally the walls of the collecting lymphatics and the trunks contain more and more tissue elements (although not as many as those of veins).

Fine structural differences between mammalian lymphatics and blood vessels

It is usually possible to differentiate between blood and lymphatic vessels with the light microscope. This is provided that one can see the lymphatics at all!; they all too frequently collapse when tissues are cut unless ligatures are placed around the periphery of the block before it is removed or freezing techniques are used. While both classes of vessels can always be seen by electron microscopy, the much thinner sections mean that much less depth of tissue is viewed and identification is much more difficult. It must be said at the outset that at times no-one can tell for certain whether a given vessel is a blood vessel or a lymphatic. Hopefully, immunological or histochemical markers will be developed. Claims for these have been made, but there is at present much controversy about their validity.

If one sees a fenestra, one can be certain that the vessel contains blood; they never occur in lymphatics. However, they are only present in blood vessels in certain regions. The only other certain method is to inject the local lymphatic system with a tracer. It must be visible in the light microscope so that one can be sure that one has injected the local lymphatic plexus--seeing it filled with tracer by vital microscopy (or two suitable tracers must be used, mixed together). Alternatively, one may inject a tracer composed of large particles into a body cavity (e.g., the peritoneum) from which it can only be removed by the lymphatic system. In the gut, naturally occurring tracers--chylomicrons--can also be used. Failing these aids, one can only guess to which class a given vessel belongs.

The criteria for identifying mammalian lymphatics have been reviewed (8). The initial lymphatics are generally larger than blood capillaries or post-capillary venules (but remember that a section may pass through a small portion of a bend of a vessel). The initial lymphatics usually have very irregular outlines and are frequently rather collapsed. Their basement membranes are usually tenuous. Contrary to what is often said, their endothelium is actually rather thicker than that of blood capillaries (4,8), but appears thinner because their diameters are so often greater. The initial lymphatic endothelium is often paler than blood endothelium and may have open junctions; but both these features are also common in injured blood vascular endothelium!, and injury can occur during handling. The initial lymphatics have endothelial projections into the interstitial tissue to which fibrils are attached. The initial lymphatics may contain erythrocytes but usually fewer than in blood capillaries. The protein in their lumens is usually less concentrated than in capillaries; however, it may vary in both of these from fixation artifacts, and that in the initial lymphatic lumen varies during the initial lymphatic cycle (8).

At all times, the tissues must be handled carefully. The lymphatics are best kept as they were by ligating on all sides of the tissue (before one removes it and on the inside of the incisions) or using freezing.

ADDENDUM

It has just been brought to my attention that recent work (largely by Vogel, but supported by evidence from other workers--even as long ago as Favaro in 1906) strongly questions the very existence of a lymphatic system in teleosts. There is very powerful evidence that all fishes (elasmobranchs as well) have a secondary blood circulation fed by many arterio-artery anastomoses from the major arteries of the primary circulation. These anastomoses are very easily put into spasm (e.g. by any handling unless large amounts of vaso-dilators are used). Hence, the secondary circulation then contains only cell-free plasma; for this reason it has been called a "lymphatic system." This is almost certainly wrong and I regret that I, as well as almost all authors, have been blinded by tradition in this way. Reviews of this work may be found in references 22 and 23.

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