The Fine Structure of the Vascular System of Amphioxus: Implications in the Development of Lymphatics and Fenestrated Blood Capillaries *

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The blood and lymph vessels of a few mammals have been quite extensively studied by electron microscopy (3, 4, 5, 15, 22, 23, 24). By contrast, in lower animals even the blood vessels have been relatively neglected, to say nothing of the lymphatics. The few studies which have been made indicate that in reptiles (2), amphibians (31) and teleosts (15) the blood vessels are similar to those in the mammals. In the elasmobranchs, however, the interendothelial junctions appear less firmly closed, the basement membranes are more tenuous and the venous vessels are intermittently attached to the connective tissue by fine fibrils (11). These three features are strongly reminiscent of mammalian lymphatics and are probably associated with the low blood pressure of these fish, which is sometimes "negative" in the venous vessels (13, 30).

It is of great interest that even in the fairly primitive elasmobranchs, those which lack true lymphatics (32) still have fenestrated capillaries in some organs (11). There is evidence that the fenestrae allow the entry of large molecules and fluid into the venous limbs of capillaries (7, 8, 11), both in these animals and in the higher vertebrates, where they are very common in some regions. They may well supplement the lymphatic system, especially in relatively motionless regions, or where the lymphatics are infrequent. It appears, however, that the hagfishes lack fenestrae, but have some open junctions in their endocrine capillaries (9a). Thus their blood capillaries have some features in common with the lymphatics of higher vertebrates. (Neither open junctions nor fenestrae seem to be present in the cerebral capillaries of the myxines (27), but we have no information about the rest of their vessels.)

The invertebrates have had even fewer studies of their vasculature. Only the cephalopods (1) and earth-worms (17, 19, 20) have had any detailed description. The crustaceans have been briefly mentioned (19) and the leech's neural "endothelium" has been described (12), but it is evident that this is very unusual in site, structure and function. In their major vessels the cephalopods have pericytes with myofibrils, thick basement membranes and endothelium which is nearly continuous, but with a few open junctions. In the more peripheral vessels, the endothelial cells gradually come to lie further and further apart, until there are quite wide gaps 1–10 μ between them. However, the basement membranes are always present, as is a complete investment of pericytes – which come to lack the myofibrils. The pericytes have closed junctions which, though they are not "tight junc-

* This work is dedicated to the memory of *Nicole Granboulan*.

tions" (16), contain dense material which may present a considerable barrier to the passage of large molecules. The higher blood pressures and plasma protein concentrations in the cephalopods have obviously caused developments analogous to those in the higher vertebrates, but differing in detail. The situation in the more primitive earthworm is similar, but the endothelium is discontinuous even in the major vessels (17, 19, 20). (A point of nomenclature should be noted here: Workers on the earthworm have called the pericytes "endothelium", or "myoendothelium" and considered the endothelium to be "amoebocytes lying on the basement membrane", which they considered lay on the "lumenal side of the endothelium." It was pointed out by *Barber* and *Grazial-dei* (1), however, that the true amoebocytes are morphologically, and presumably functionally, distinct from the true endothelium since they contain many granules, more mitochondria, etc. Hence they considered that there was true endothelium – though often very discontinuous – inside the basement membrane, as in the mammals, and that it was pericytes which contained the myofibrils.)

In order to help bridge the gap between the invertebrates and the vertebrates, it was decided to study amphioxus, one of the most primitive of chordates. Its low blood pressure and plasma protein levels, and generally primitive development might be expected to be associated with vascular structural and functional peculiarities. These would not only be interesting in themselves, but, by their contrasts, might help to clarify what is found in the higher vertebrates. In particular, the way in which large molecules enter these vessels from the tissues would be of significance both for the study of the lymphatic system, and of the fenestrated blood capillaries.

The vascular structure of amphioxus detectable with the light microscope has been well reviewed by *Kampmeier* (21), whose description has been used as the basis for this paper. Briefly, there is a contractile ventral aorta, which pumps blood through the gill arches and nephridiae, which then flows into the pair of dorsal aortae. These merge on the stomach and intestine and supply the intestinal sinusoids, which run forwards on the "liver". Some of these converge into the contractile subintestinal vein, which supplies the "liver" sinusoids, which in turn flow into the hepatic vein, and thence into the ventral aortae. In addition to this branchioenteric circulation, the dorsal aortae supplies sinusoids to the segments of the body wall, which flow into the "cardinal" veins. (I shall use "central" or "major" vessel to include the aortae and the main veins; "peripheral" vessels refers to the rest of the vessels, which are basically sinusoids, i.e. they have wide, irregular, often flattened lumens, with – electron microscopically – gaps between the endothelial cells.) *Kampmeier* mentions a number of problems which have been answered in the present work, viz. the nature of the sinusoids, whether only the aortae have endothelial linings, and the nature of the "lymph" spaces. The way in which large molecules and particles enter the sinusoids of the gut and the vessels of the body wall has also been studied.

Materials and Methods

Twelve specimens of the amphioxus *Bronchiostoma moretonensis Kelly* (1966) (23) were studied through the kindness of Dr. *O. Kelly* of the Department of Zoology of the University of Queensland, who collected and sent them alive to me. Some were fixed at once. Others were anethetised with M.S. 222 (Sandoz, Switzerland), or simply placed

in sea water at 4 °C, which rendered them fufficiently insensitive and immobile for the experimental procedures used. In either case, upon being placed in sea water at 20 °C, normal mobility was restored and the animals continued to live for many hours until killed. Some animals had 0.005 ml of a 5% (w/v) solution of Indian ink (Pelikan, Gunther Wagner, Hannover; C11/1431 a) in Tyrode's solution micro-injected into the musculature of the body wall at 2 or 3 points; they were periodically stimulated to swim and killed after 3 hours. Others had ~ 0.01 ml of cod-liver oil sligthly stained with Sudan Black B introduced into the lumens of their intestines, via the beginning of the mid-gut or the anus. They were killed after 3 to 5 hours when all the oil had been evacuated.

All specimens were given an initial fixation in 4% glutaraldehyde in Millonig's fixative (26) for 12 hours; some were fixed whole (their dimensions were ~ 30–50 x 3 x 1 mm), others were cut transversely or longitudinally to aid fixation. All except those injected with carbon were given a 2 hour post-fixation in 2% Osmium tetroxide – which would have completely obscured the carbon – before being embedded. All blocks were stained with saturated Uranyl nitrate in absolute alcohol for 1 hour before embedding. They were then dehydrated and embedded in araldite by the usual methods. Some of the sections were stained with Lead citrate (28). Sections were examined in a Siemens Elmiskop I. Thick sections were also taken for light microscopy to identify the structures and to facilitate trimming to areas of interest. Examination of the blocks with a dissecting microscope was also very valuable in locating and sectioning vessels into which the carbon had entered. Vessels studied included: the dorsal and ventral aortae; the subintestinal, hepatic, lateral and cardinal veins, the smaller vessels supplying the gonads, pharyngeal arches and nephridia; the sinusoids of the intestine, diverticulum and body musculature; the "lymph spaces" of *Kampmeier* (21) and *Dubovik* (14), and the metapleural space.

Results

Light Microscopy

Since amphioxus neither has erythrocytes nor pigment in solution in the plasma, the blood vessels could not be seen in the uninjected living animal, nor in the unsectioned blocks. In the carbon-injected specimens, the material could be seen lying in a cloud around the injection sites and also in long narrow irregular tubes, usually projecting ventrally: these had the normal dissecting-microscopical appearance of sinusoids.

The various blood vessels, as described by *Kampmeier* (21), could be seen in the thick (1 µm) sections prepared for light microscopy; some of the "lymph" spaces could also be seen, but it will be shown later that these are almost certainly not true lymphatics. In particular, in those specimens given cod-liver oil, a diffuse, osmophilic-haze filled the dilated sinusoids around the intestine and, less markedly, around the mid-gut diverticulum. In the carbon injected specimens the carbon could be seen in the spaces between the muscle cells near the injection sites, and also in what appeared to be cross-sections of vessels up to 1–2 mm from the sites. The endothelium could be easily seen in the aortae and in some of the veins (e.g. the subintestinal, the hepatic and the lateral – or genital – veins), but it was much less evident in the more peripheral vessels and often could not be seen at all, especially in the sinusoids.

Electron Microscopy

Major Vessels. The dorsal and ventral aortae and the subintestinal, hepatic, cardinal and lateral veins were all very similar. The endothelial cells were like those of mammalian blood and lymph vessels (3, 4, 5, 15, 22, 24, 25), with the normal cellular organelles. One possible difference was that in amphioxus the endothelial cells were rather thicker, with more rough endoplasmic reticulum (Figs. 1–6). One very definite difference was the frequent presence of pronounced bands of quite thick fibrils in the cells (Figs. 1–3 and 6). These were adjacent to the abluminal edge of the cells, although occasional minor bands were seen elsewhere, and usually ran circularly. Since they were only seen in the vessels known to be contractile (21), they probably cause this. They showed some ill-defined evidence of cross-striations, but not as much as in the "pericytes" of the earthworm (20). In cross-section (Fig. 3) they could be seen to have dense centres with less-dense, filamentous material on the exteriors.

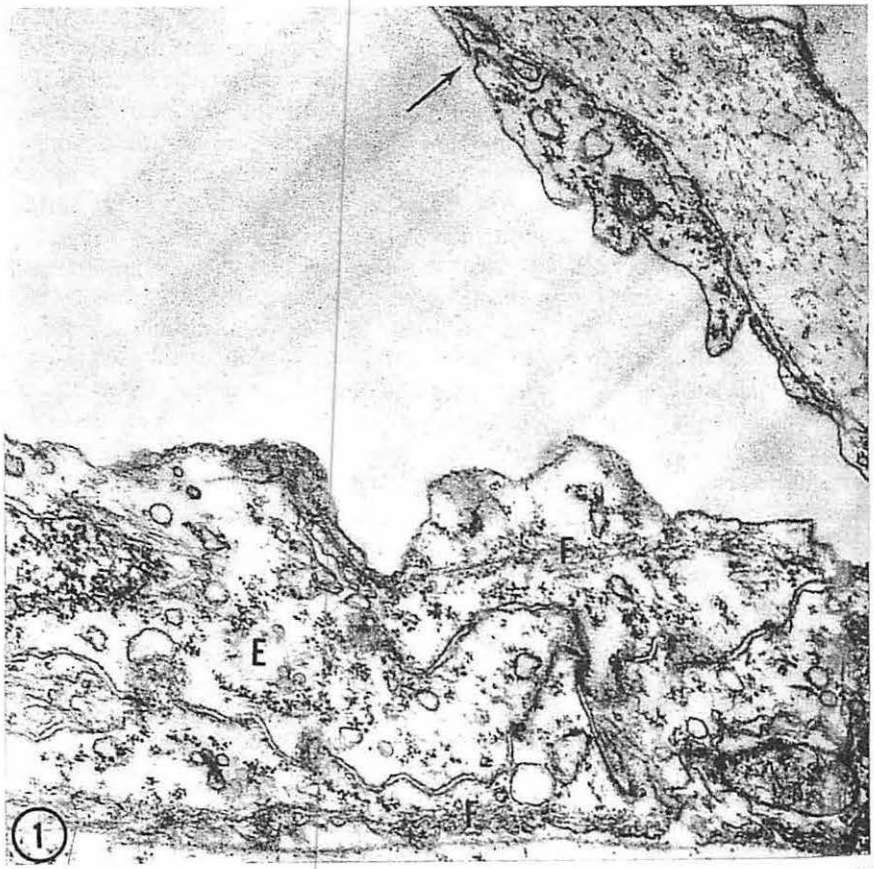

Fig. 1 Ventral aorta. Some of the endothelium (E) is three cells deep, some is only one. Fibrils, identified as myofibrils (F) are present in some cells. There are a number of closed junctions with zonulate present, and one open junction (arrow). The basement membranes are visible, and some of the surrounding connective tissue. × 9,000.

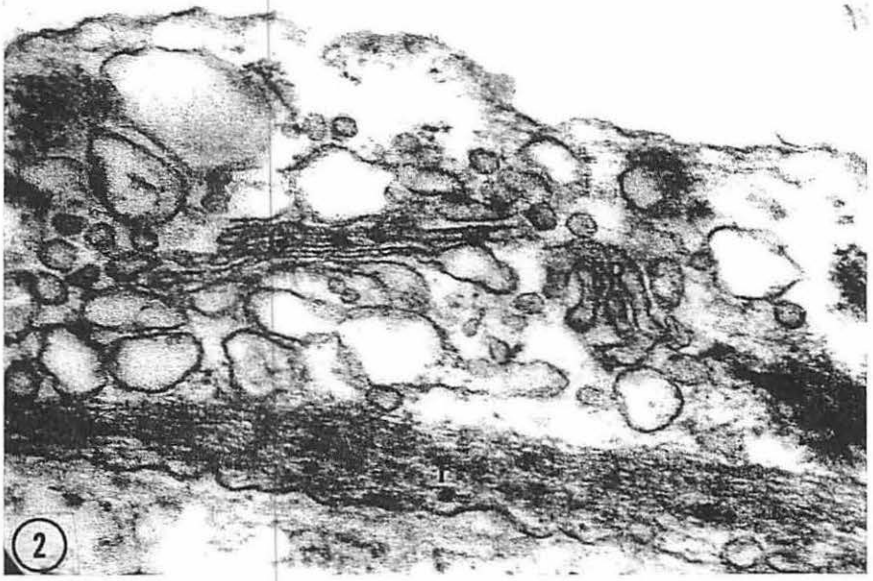

Fig. 2 Ventral aorta, showing the cellular organelles, including Golgi apparatus (G), endoplasmic reticulum (ER), various sized vesicles, ribosomes and a band of fibrils (F). ×65,000.

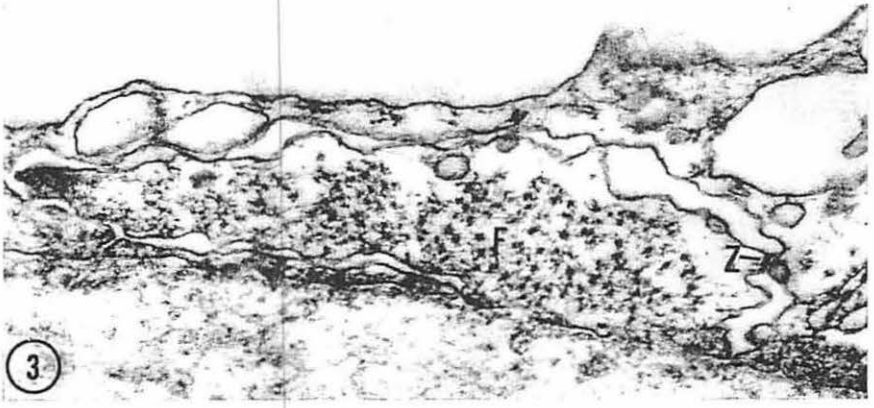

Fig. 3 Dorsal aorta, showing a collection of fibrils in cross-section (F) and a partly open junction, with a zonula (Z). ×30,000.

In the aortae the endothelium was sometimes 3 or 4 cells deep (Fig. 1); sometimes it was only one cell deep, as was usual in the veins. The intercellular junctions were usually fairly straight and simple, with some zonulae adherentes and occludentes (Figs. 1 and 3), but such zonulae were often lacking (Figs. 1, 4 and 5). Hence, while most junctions were closed, some were open over all or parts of their lengths (Figs. 1, 3 and 4), as is found in mammalian lymphatics in active tissues (3, 4, 5) and in injured mammalian post-capillary venules and capillaries (25).

The endothelial cells rested on a basement membrane (Figs. 1–6). Exterior to this was a variable amount of connective tissue. Sometimes this was very dense, with closely packed fibres, which often exhibited the cross striations of collagen (Fig. 4). At other times it was much looser and thinner (Fig. 3). There were no pericytes.

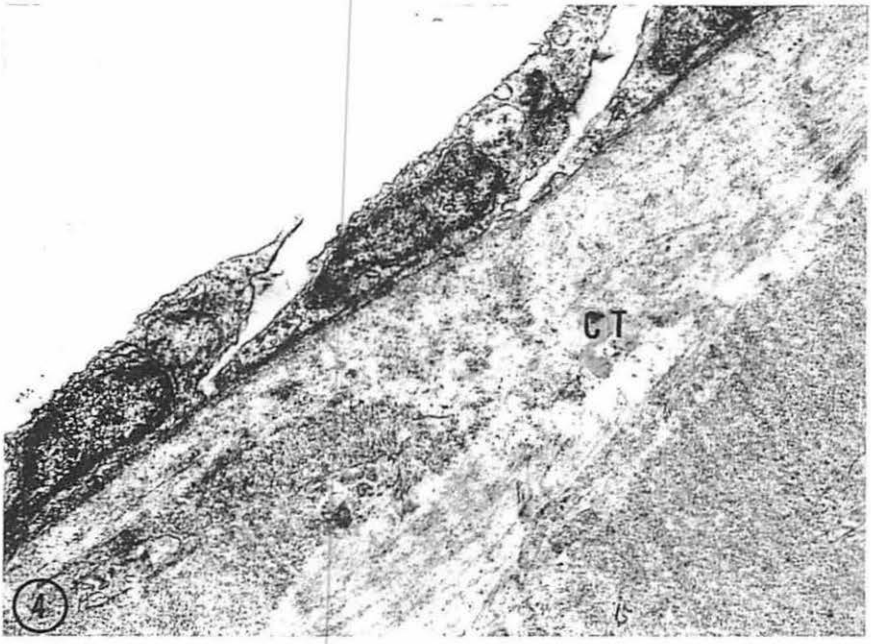

Fig. 4 Dorsal aorta. There are two open junctions and much surrounding connective tissue (CT). ×7,000.

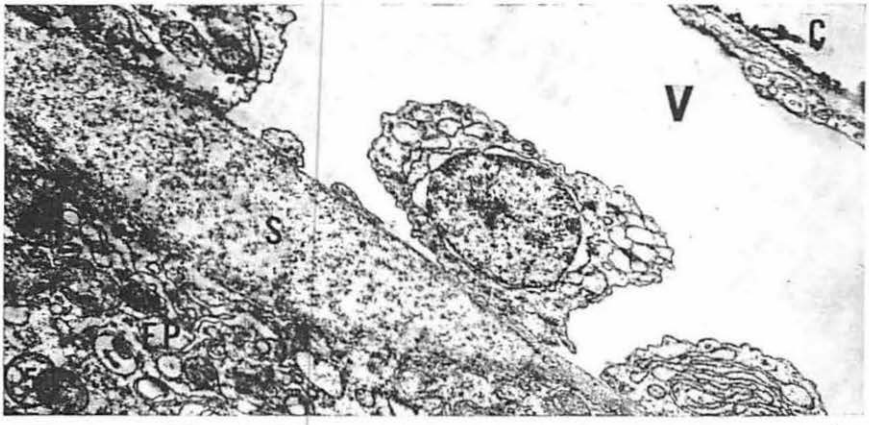

Fig. 5 Hepatic vein. The vein (V) has rounded endothelial cells, with quite large gaps between them. On one side is a sinusoid (S) containing lipoproteins with the epithelium of the gut (EP) next to it; on the other side is the coelom (C), with its lining cells rather damaged, but still showing their typical electron-opacity after Osmium fixation and Uranyl nitrate staining. The various basement membranes are visible. ×5,000.

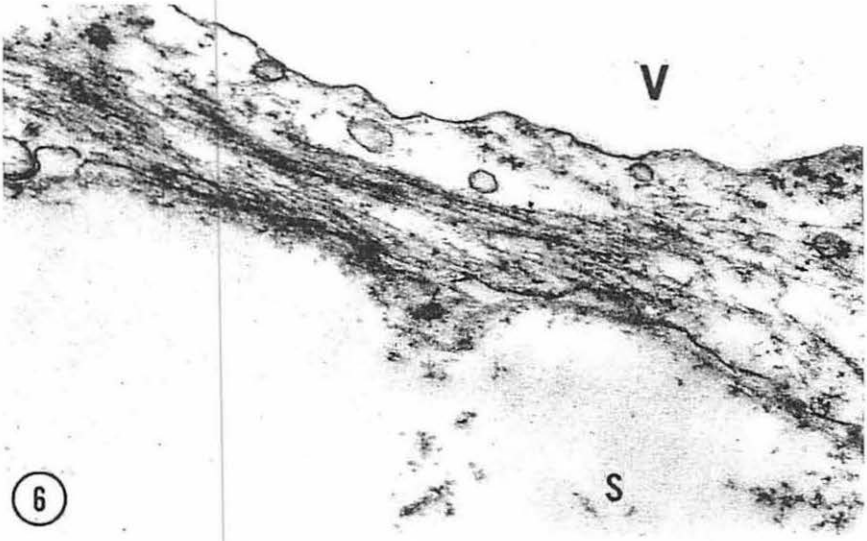

Fig. 6 Subintestinal vein (V), showing myofibrils, a closed junction, the basement membrane and an intestinal sinusoid (S) with some lipoproteins. × 30,000.

Sinusoids. These really included all the other vessels, even those corresponding to minor arteries and veins, for they all had wide gaps between the endothelial cells. There was a continuous gradation from the almost continuous endothelium of the aortae, to the peripheral vessels which were formed simply by the spaces between the parenchymal cells – sometimes without even a basement membrane (Figs. 7–11). Usually there was a basement membrane which enclosed a large, irregular space, which could sometimes be seen joining a more major arterial or venous vessel (Fig. 7). However, especially in the body wall musculature, the basement membrane was sometimes lacking adjacent to the muscle cells (Figs. 10 and 11). The size, shape and direction of such vessels were hence determined by the dispositions of the cells and the more solid elements of the connective tissue. In spite of this, it was obvious that such vessels did have fairly definite, if perhaps temporary, boundaries. The carbon and lipid particles often seemed to nearly fill the lumens, but were stopped abruptly where the cells joined a basement membrane, or came very close to each other, or where two basement membranes united (Figs. 10 and 11). Presumably the segmental appearance of the vessels in the body wall (21) is a reflection of the segmental arrangement of the myotomes and hence of the "vascular" spaces between them. In fact such sinusoids seemed remarkably similar to the "prelymphatic pathways" described in mammalian connective tissue (10).

Some of the basement membranes were just related to the vessel, with connective tissue outside them, others were shared with other cells, e.g. the epithelial cells of the gut or of the diverticulum (Figs. 7 and 9). The membranes themselves were composed of fine fibrils, and were often not as compact as in the mammalian blood vessels, but were frequently thicker – especially those next to the epithelial cells of the gut (Figs. 7 and 9).

The vessels with basement membranes often had occasional endothelial cells lying inside the basement membranes, but separated from each other by 50 or more μm (Fig. 7).

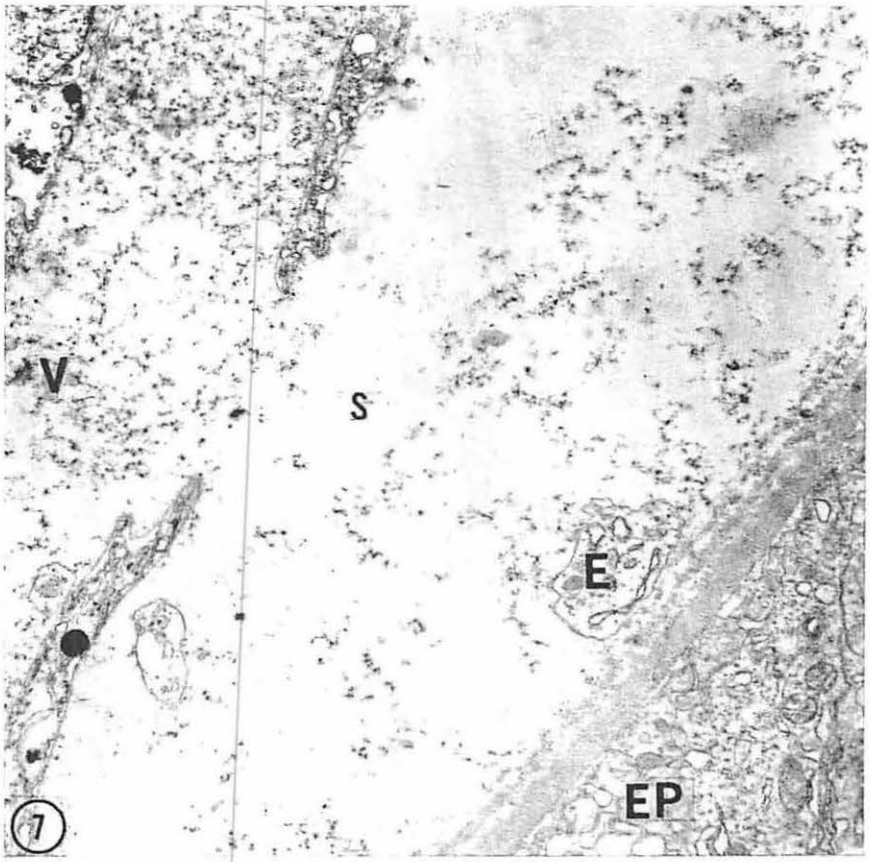

Fig. 7 Subintestinal vein (V), showing its continuity with a sinusoid (S); both contain many lipoproteins. The sinusoid is separated from the gut epithelium (EP) by the thick basement membrane. There is a solitary endothelial cell (E) visible on the epithelial side of the sinusoid. ×3,000.

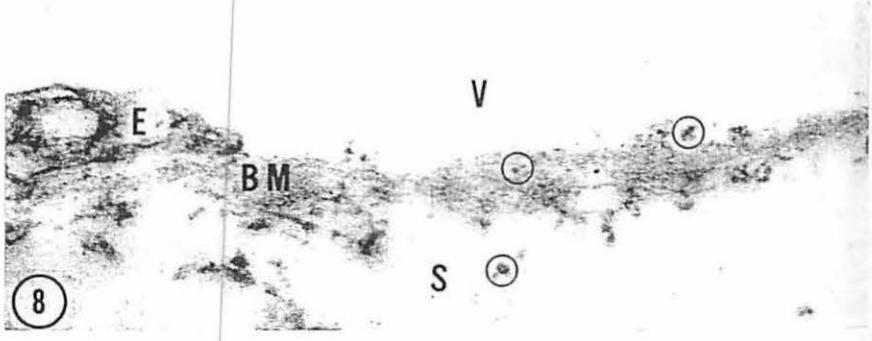

Fig. 8 Lipoprotein molecules (circled) can be seen in an intestinal sinusoid (S), in the basement membrane (BM) and in a vein (V). A portion of an endothelial cell (E) is visible, but the walls of both vessels mostly consist of basement membrane. ×50,000.

Some cells were flat and seemed well attached, others were more rounded and seemed less firmly fixed, but did not resemble amoebocytes (1). The more important vessels had more and more endothelial cells, until they were so close that it just seemed that the vessel had many open junctions (Figs. 5, 7 and 8).

There is then, a continuous gradation in the vessels. The contractile vessels, especially the aortae, have almost continuous endothelium, sometimes multilayered, with intracellular fibrils and thick connective tissue surrounds. The lesser vessels gradually loose the intracellular fibrils and have less connective tissue in their walls. Their junctions

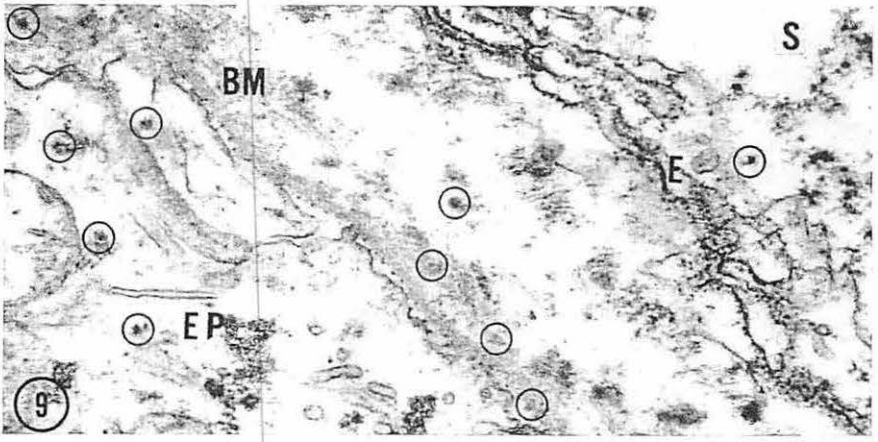

Fig. 9 Lipoproteins (circled) can be seen in and between the gut epithelial cells (EP), next to and in the basement membrane (BM) beneath a rather loosely attached endothelial cell (E), and in a sinusoid (S). In the latter they show an irregular, fibrillar external coat. ×40,000.

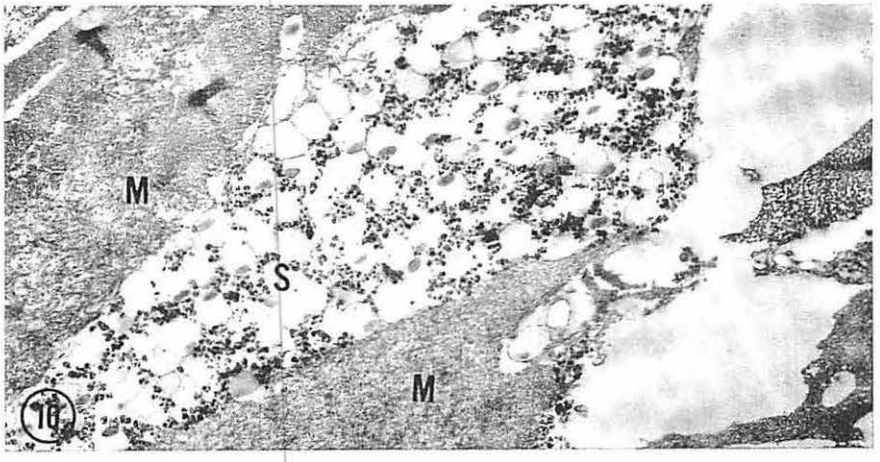

Fig. 10 Injected carbon particles can be seen in a tissue space (S), between two muscle cells (M), in the body wall. Under the dissecting microscope this appeared as an irregular vessel, about 1 mm. long, continuous with other vessels. There is no endothelium, nor basement membrane, visible. ×20,000.

less often have zonulae and become more frequently open, until every cell is separated and is finally placed many micra from its neighbour. The most minor vessels frequently lack even a continuous basement membrane and are continuous with the connective tissue spaces. No doubt the extents and locations of such continuities vary with time, movements, and the metabolic activities of the different regions.

The Passage of Particles and Large Molecules into the Vessels

Carbon in the Body Wall. It was evident that the injected carbon was in any spaces in the tissues adjacent to the injection-sites (Figs. 10 and 11). These were continuous with the sinusoids, which were eventually continuous with the main veins. Where continuous basement membranes were lacking, to say nothing of continuous endothelial linings, it was more or less arbitary where one drew the line between the particles being

Fig. 11 A vessel, with injected carbon between the muscle (M) and the connective tissue of the body-wall (CT). ×2,000.

outside or inside a vessel. In a sense the whole of the sol portion of the connective tissue ground substance constitutes a continuous series of vessels, whose dimensions and connections vary with time.

Lipoproteins in the Intestinal Wall. Cod-liver oil is fairly unsaturated; preliminary *in vitro* experiments confirmed that it reacts with Osmium tetroxide to yield an electron-dense product. It was found that the administered oil was endocytosed by the epithelial cells of the intestine and the posterior part of the diverticulum, and was liberated near the bases of the epithelium (Fig. 9) as small particles (20–40 μm). These very closely resembled mammalian lipoprotein molecules (3) and so were tentatively identified with them.

The lipoproteins were seen against the epithelial basement membrane, inside it, and in the sinusoids outside it (Figs. 7–9). Once they had passed through this membrane they often acquired a fuzzy coat of filamentous material (Figs. 7–9); this could be interpreted either as membrane material which had adhered to them, or as the normal plasma proteins which precipitated on them during fixation. The plasma proteins are in low concentrations, and little evidence of them was seen on the endothelial plasma membranes, in the lumens of the vessels elsewhere, or in the intestinal sinusoids in non-oil-fed specimens. It was hence considered that the basement membrane origin of this material was more likely.

The lipoproteins in sinusoids seemed to pass into the intestinal vein and its major contributaries in three ways. At times there was a complete gap in both the basement membrane and the endothelium of the veins which overlayed the outer aspects of the sinusoid (Fig. 7). Much more frequently the basement membrane was intact, but the venous endothelial junctions were widely open; the lipoproteins could be seen against and in the membrane, and in the open junction and lumen of the vein (Fig. 8). A third possible path was via the vesicles in the venous endothelium after a similar passage through the basement membrane anywhere along the cells. It is difficult to arrive at quantitative estimate of the amounts passing via these three paths, but since vesicular transport is so slow in comparison with that via open junctions (4, 6) it would seem that this is unlikely to be of much significance. While the open junctions where the basement membrane is also lacking are infrequent, it may be that the absence of the retarding influence of the membrane may more than make up for this; on the other hand, the basement membranes are not as dense as in mammals and they may offer far less resistance, in spite of their relatively greater thicknesses.

Fenestrae

No fenestrae were seen in any of the vessels, including those of the intestine diverticulum, gonads and nephridia – all of which might be expected to possess them by analogy with those of vertebrates, including elasmobranchs (11).

Lymphatics

No evidence of true lymphatics was seen. This was in spite of the most careful investigation, with both the light and electron microscopes, of the regions where *Dubovik* (14) affirmed and *Kampmeier* (21) suggested that they may occur. In particular the regions around the dorsal aortae, the notochord, the neural cord and the dorsal fin ray

were studied in detail. At times spaces were seen in such locations, but the electron microscope revealed them as simple spaces between the cells – often without a basement membrane lining. They were thus no different from the vessels of the body-wall musculature. It was considered very probable that they were continuous with the general spaces in the connective tissues, which were eventually continuous with the blood vascular system. This continuity was shown by their occasionally containing particles of carbon or lipoproteins, in the specimens containing these.

The spaces in the metapleural folds were lined by cells very similar to those lining the major vessels, but without the intraendothelial fibrils. The presence of lipoproteins and carbon, in the treated animals, indicated that the spaces were continuous with both the arterial and venous blood vessels – as was suggested by *Zarnik* (34). Thus they correspond to blood sinuses, rather than lymphatics or coelomic spaces.

Clearly, unlike *Dubovik's* (14) belief, amphioxus does not have a closed system of lymphatics separate from the blood-vascular system: indeed, it does not have true lymphatics at all – in the mammalian sense. There is only one set of vessels, which are continuous with the tissue spaces. Whether one calls these "blood" vessels or "blood-lymph" vessels is a matter of definition.

Discussion

Vascular Structure

The surprising light microscopical observations of earlier workers (21) have been largely confirmed. It can be seen indeed that the vascular system of amphioxus does consist of peripheral sinusoids, often only spaces between the cells, which gain in basement membranes, endothelium and closed junctions, as the major vessels are approached in either an arterial or a venous direction. This is the more surprising in that it has become usual for the electron microscope to reveal very thin, continuous, sheets of cells, wherever they might have been expected, but were not detectable by light microscopy. It can be seen, however, that the situation in amphioxus is similar to that in the more primitive invertebrates (1, 14, 17, 20) – at least regarding the discontinuous endothelium.

One may speculate that the isolated endothelial cells in the sinusoids are responsible for the formation of the basement membranes, except where there are other cells available to do this, e.g. the thick membrane clearly formed by the intestinal epithelial cells. Since endothelial cells show considerable powers of movement in growing capillaries, it might well be possible for them to travel along the peripheral vessels in amphioxus, forming the basement membranes. Such a concept would conform to the suggestion that in mammals the membrane is made by the endothelial cells (25). At all events, in amphioxus there appear no other cells available to form it in many regions.

It is highly likely that the contractile endothelium, with the prominent fibrils in the cells, is the forerunner of the vertebrate heart. (Similarities between endothelial cells and smooth muscle cells, and between these and cardiac muscle cells have frequently been noted.) It is of interest that in the annelids, cephalopods and crustacea (1, 17, 19, 20), which are highly developed invertebrates but well-removed from the vertebrate line of development, it is the pericytes which have developed contractility. Unfortunately no studies have been made of the more primitive examples of the invertebrates, especially those near the divergence of the Protostomia and the Deuterostomia.

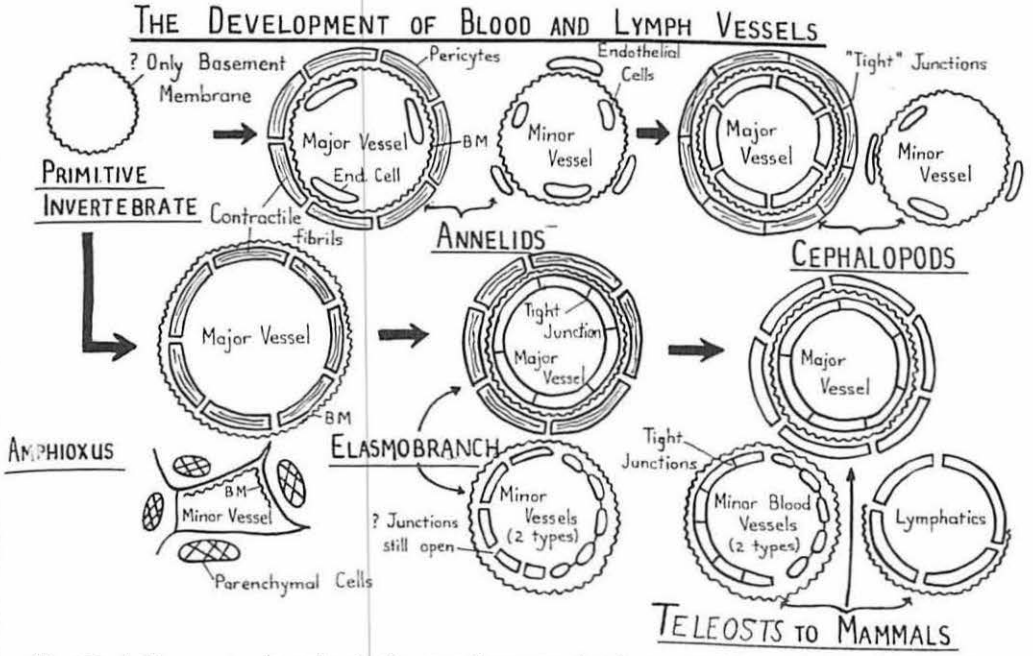

Fig. 12 A diagram to show the similar, yet divergent, development of the vascular systems in the vertebrates and invertebrates. The relations of the open capillary junctions, fenestrae and lymphatics are also indicated.

One can similarly easily imagine that the sinusoids gradually evolved into the completely endothelialized blood and lymph vessels of the higher vertebrates (Fig. 12). Sinusoidal vessels only remain in a few specialised regions, e.g. the liver, bone marrow, etc. Again the higher invertebrates, such as the cephalopods (1) show the evolvement of completely endothelialized vessels, while even the annelids still have many sinusoids (17). It would seem that sinusoids represent the primitive condition, but again fine structural evidence from primitive invertebrates is lacking.

In amphioxus there were found no true lymphatics, i.e. separate from the vascular system. Of course one can arbitarily separate certain spaces, with or without basement membranes, and call them lymphatics (14, 21). This can now be seen to be based solely on their anatomical positions and on phylogenetical implications; it has no structural nor, as far as can be seen, functional basis.

Similarly, no fenestrae were seen. These and the lymphatics are the only ways by which large molecules are thought to be removed from the tissues (5, 7, 8, 13, 29, 33). Thus amphioxus must have some other arrangement for this purpose. From the experiments with carbon and lipoproteins it is evident that this is by the particles and large molecules passing into the blood vessels, primarily via the open junctions and the large gaps between the endothelial cells. Such a passage has obvious similarities with the way in which large molecules, etc., pass into the mammalian lymphatics (3, 4, 5, 9). By contrast, in injured mammalian post-capillary venules and capillaries, plasma passes out of the vessels via the open junctions (25).

Vascular Function

The passage of plasma out of injured mammalian blood vessels is obviously a result of the outwards hydrostatic pressure acting alone, with the inwards osmotic pressure nullified by the wide opening. The entrance of fluid (and the large molecules swept along with it) into the lymphatics is usually ascribed to an inwardly directed hydrostatic pressure difference (33). Recently it has been shown than an osmotic pressure difference, produced by the higher concentrations of large molecules inside the lymphatics, than in the connective tissue, is of great importance (9), especially since other findings indicate that the hydrostatic pressure difference may usually be directed outwards rather than inwards (18). (No doubt transient, but probably important, variations in these hydrostatic pressures are caused by movements, etc.).

In amphioxus the forces causing the movement of large molecules from the tissues to the vessels are unknown, but may be guessed at. Neither the hydrostatic nor the colloidal osmotic pressure of the blood is high, nor could they be in the sinusoids. They may be expected to approximately balance (13, 33) – indeed, with the tenous walls of the sinusoids, they would have to. The infrequent, but relatively powerful, swimming movements of the animal could be expected to increase temporarily the tissue pressures enough to force material, such as the carbon, into the sinusoids in the body wall and other regions. In the gut it is likely that fluid enters the animals via the epithelium and carries the lipoproteins released there through the basement membrane and into the intestinal sinusoids.

Vascular Phylogeny

Since it is evident that fluid and large molecules pass into the vessels of amphioxus through the open junctions, it would be easy to imagine that this is how the lymphatic system developed evolutionally, and that therefore one should call these blood-lymph vessels in amphioxus. Similarly, in the hagfish the endocrine capillaries are nearly fully endothelialised, but there are many open junctions (9a). However, the elasmobranchs have fully endothelialised vessels with apparently closed, though loosely held, junctions (11). Here it might be expected that the apparently closed junctions would prevent material entering the vessels. This would make it difficult to understand how this mechanism could be redeveloped when the true lymphatics finally separated-off from the veins in the more developed torpedoes and the teleosts (11, 21, 29, 32, 33).

It is possible that in the elasmobranchs, which have a low blood pressure, occasional powerful muscular contractions may force material through the "loosely closed" junctions into the capillaries and smaller venous vessels in the body-wall musculature. This is unlikely to happen in the teleosts with their higher blood pressures and firmer junctions, and hence these may have necessitated the development of the lymphatic system. Unfortunately, at present it is not known if muscular contractions can force material through the junctions into the venous capillaries of the elasmobranch body wall. If so, this would preserve the ability of these junctions to be easily opened and thus facilitate the development of the lymphatic system. I.e. in teleosts the junctions in the newly separated lymphatics would retain this ability to open and allow passage, while those in the blood vessels would become more tightly closed to resist the higher blood pressures.

However, even in the elasmobranchs, many regions must be sufficiently distant from the body wall so that muscular concentrations can have little effect. It may be that this

is the reason that fenestrated blood vessels have developed in these animals, since there is considerable evidence that fenestrae will remove large molecules and fluid from the tissues into the venous capillaries (7, 8, 11). It is of interest that in amphioxus, and in the hagfish (9a), there are no fenestrae. One presumes that the low hydrostatic and osmotic pressures in these lower chordates permit the existence and functioning of permanently open junctions to remove large molecules and collections of fluid from the tissues.

Conclusion

Such a process of the development of the fine structures (Fig. 12) and the functions of the blood and lymph systems, including the fenestrae, seems very attractive. It would be from discontinuously lined sinusoids to fully lined vessels, both as one passed from minor to major vessels and from the primitive animals to the more highly evolved ones. This occurs in both the invertebrates and the vertebrates – presumably in response to higher blood pressures. In those vertebrates with fully endothelialized capillaries, but with low blood pressures, strong muscular contractions would still be able to force collections of fluid and large molecules into the venous capillaries via the loosely held junctions. In regions remote from the body wall musculature, fenestrae developed to allow the uptake of large molecules into the fully endothelialized capillaries. When the blood pressure rose so much that material could no longer be forced into the venous capillaries (especially as the junctions would have to be more strongly united to stand the pressures) the fenestrae would remain. However, especially in the body-wall musculature with its non-fenestrated capillaries, another path could have to be developed – retaining the open junctions. This was the lymphatics, which were still linked to the functional state of the tissues in that their uptake improves with muscular contractions. Thus they could come to be secondarily important in the gut too, with the contractions of the villi. This would allow the gut epithelium to produce the large chylomicra, which could not pass through the fenestrae. Attractive as such a scheme may be, our lack of evidence makes this no more than a very tentative hypothesis at present. There is, however, suggestive structural, functional and phylogenetical evidence in favour of all parts of it.

Summary

Major and minor vessels were studied with the electron microscope, in amphioxus, *Branchiostoma moretonensis, Kelly* 1966. The major vessels had almost continuous endothelium, with probably contractile myofibrils, a basement membrane and collagenous supporting tissue. There were no pericytes. The minor vessels consisted of sinusoids, with the endothelial cells often being separated from each other by many micra. Only the basement membranes defined the walls of the vessels; often even these were lacking over parts of the walls. The vessels were thus continuous with the tissue spaces. No true lymphatics were seen.

When carbon was injected into the body wall, some of it lay in the tissue spaces, but this was continuous with some in true vessels. Some animals had cod-liver oil injected into the gut. This was endocytosed by the epithelial cells and appeared at their base as particles very similar to mammalian lipoproteins. These crossed the epithelial basement membrane to enter the sinusoids, and they eventually passed to the veins either via areas where these communicated with the sinusoids, or by passing through the mutual basement membranes and through open junctions in the venous endothelium.

The significance of these findings for an understanding of the evolution of the vertebrate lymphatic system and fenestrated blood capillaries is discussed.

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