

The Fine Structure of Some Blood Vessels of the Earthworm, *Eisenia foetida**

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ABSTRACT

The fine structure of the main dorsal and ventral circulatory trunks and of the subneural vessels and capillaries of the ventral nerve cord of the earthworm, *Eisenia foetida*, has been studied with the electron microscope.

All of these vessels are lined internally by a continuous extracellular basement membrane varying in thickness (0.03 to 1 μ) with the vessel involved. The dorsal, ventral, and subneural vessels display inside this membrane scattered flattened macrophagic or leucocytic cells called amebocytes. These lie against the inner lining of the basement membrane, covering only a small fraction of its surface. They have long, attenuated branching cell processes.

All of these vessels are lined with a continuous layer of unfenestrated endothelial cells displaying myofilaments and hence qualifying for the designation of "myoendothelial cells." The degree of muscular specialization varies over a spectrum, however, ranging from a delicate endowment of thin myofilaments in the capillary myoendothelial cells to highly specialized myoendothelial cells in the main pulsating dorsal blood trunk, which serves as the worm's "heart" or propulsive "aorta." The myoendothelial cells most specialized for contraction display well organized sarcoplasmic reticulum and myofibrils with thick and thin myofilaments resembling those of the earthworm body wall musculature. In the ventral circulatory trunk, circular and longitudinal myofilaments are found in each myoendothelial cell. In the dorsal trunk, the lining myoendothelial cells contain longitudinal myofilaments. Outside these cells are circular muscle cells. The lateral parts of the dorsal vessels have an additional outer longitudinal muscle layer.

The blood plasma inside all of the vessels shows scattered particles representing the circulating earthworm blood pigment, erythrocrurin.

INTRODUCTION

Three longitudinal trunks of vessels—the dorsal, the ventral, and the subneural—form the main blood circulatory pathways in the middle body segments of the earthworm. The dorsal vessel is the only longitudinally oriented pulsating trunk (Bahl, 1921; Johnston, 1903; Johnston and Johnson, 1902; Linville, 1907; Prosser, 1950).

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The earthworm blood circulatory system has several interesting characteristics. It is a closed system with an extensive capillary bed (Prosser, 1950). The blood pigment is the heavy molecule iron compound, erythrocrurin, which is in solution in the plasma (Svedberg, 1933). The endothelial cell is generally considered to be an essential element in the vertebrate circulatory system. However, in the earthworm, the existence of endothelial cells in the main circulatory trunks has been questioned (Lankester, 1865; Bergh, 1900, 1902; Haffner, 1928). Indeed, there is considerable confusion in the literature regarding the nature of the lining of earthworm blood vessels. Some of the viewpoints are briefly summarized.

Bergh (1890, 1900, 1902) suggested that the

blood circulatory system of the earthworm was developed from contractile elements and that endothelial cells appear later only in capillaries and small vessels where metabolic transfer between blood and tissue occurs. He denied the existence of an endothelial cell layer in the main circulatory system. Many other authors also doubted the existence of an endothelial cell in the main blood vessels (Lankester, 1865; Haffner, 1928). They considered that a non-cellular connective tissue layer (Leydig's "intima" or "cuticle") (Retzius, 1905; Bergh, 1900) forms the innermost limiting layer of the blood vessel. On the other hand, some other investigators described endothelial cells in these same vessels (Ude, 1896; Johnston, 1903). Vejdosky (1905) denied the concept of a non-cellular intima and believed that an inner longitudinal muscle cell layer forms the actual endothelial layer. He proposed the term "vasothelial myoblast" for this layer.

It may be that the significance of these uncertainties hinges in part on one's definition of an endothelial cell. For purposes of this paper, the definition of an endothelial cell used by Bennett, Luft, and Hampton (1959) is espoused. Thus defined, any cell lining a blood or lymphatic channel is properly termed an endothelial cell, whether or not it has specialized structural or functional features which might distinguish it from other types of endothelial cells. Further clarification of the controversies emerges from a recognition in this study of the true nature of the "cuticle" or "intima" noted by some authors to line earthworm blood vessel.

In this paper the fine structure of the dorsal, ventral, and subneural vessels and of the capillaries in the ventral nerve cord has been studied by means of the electron microscope with special reference to the points mentioned above. It will be shown that all of these earthworm vessels are lined with endothelial cells which are specialized, some more than others. The specialization noted here is the presence of myofilaments, implying contractile function. Endothelial cells so characterized are called *myoendothelial cells*. Some of the myoendothelial cells are highly specialized, with a complex muscular endowment. Others are more simple. Moreover, it is shown that earthworm blood vessels are lined with an extracellular basement membrane which lies inside the lumen (centripetal) from the endothelial cell. Although this basement membrane has been described previously, its nature has not been recognized until this present study.

Materials and Methods

Earthworms of the species *Eisenia foetida* were placed on ice in an extended position for a few minutes in order to quiet them. Cold (4°C.) fixative consisting of equal parts of 5 per cent OsO₄ and of *s*-collidine buffer (pH 7.4) (Bennett and Luft, 1959) was injected into the coelomic cavity. After about 5 minutes, portions of the dorsal, the ventral, and the subneural vessels and of the ventral nerve cord were removed, cut into bits, and placed in fresh fixative for 30 minutes to 4 hours at 4°C. The material was rapidly dehydrated through a series of graded ethyl alcohols and embedded in a mixture of 90 per cent *n*-butyl methacrylate and 10 per cent methyl methacrylate. The blocks were sectioned with glass knives on a Porter-Blum microtome and were observed by means of an RCA EMU-2C electron microscope fitted with compensated objective, 50 μ objective aperture, and special stabilized lens power supply, or with a Siemens Elmiskop I with partially activated double condenser, 200 μ condenser aperture, and 50 μ objective aperture.

Permanganate fixation (Luft, 1956), with subsequent araldite embedding (Glauert and Glauert, 1958) and phosphotungstic acid treatment were also used for special purposes.

OBSERVATIONS

General Features.—All of the earthworm blood vessels described in this paper have certain features in common. The plasma contained in their lumina contains suspended particles of the iron-containing, hemoglobin-like, oxygen-carrying blood pigment, *erythrocruorin* (Figs. 1, 2, 4, 7, 8, 13). All of these vessels are lined with a well defined internal basement membrane (*b*) which intervenes between the suspended erythrocruorin particles and the inner surfaces of the endothelial cells. Surrounding this basement membrane in all of the vessels is a continuous complete layer of endothelial cells, more or less specialized. And finally, an external basement membrane around the outer circumference of the endothelial cells is absent or very tenuous. All the vessels except the capillaries display, in addition, scattered stellate "amebocytes" with intricate tenuous processes lying inside the lumen of the vessel, inside and against the inner surface of the basement membrane (Figs. 7, 8, 11, 14, 16).

Erythrocruorin Particles.—The lumina of all of the blood vessels contain plasma in which appear numerous small particles of uniform size about 17 μ in diameter. Some of the particles have a dense cortex about 5 to 6 μ thick, surrounding a less dense core. Quadrate and polyangular particles have also been observed (Figs. 1 and 2). These

particles are identified as representing molecules of the red blood pigment of the earthworm, erythrocrucorin.

Basement Membrane.—Lining each of the earthworm blood vessels, just inside the endothelial cell and separating the inner endothelial plasma membranes from the blood pigment particles, is an extracellular coating component detectable as a fluffy but nearly homogeneous slightly dense layer of variable thickness (Figs. 1, 2, 4, 7, 8, 10, 11, 13 to 16, *b*). In material fixed with permanganate, this coating is of low density near the cell membrane but of greater density, 15 to 20 $m\mu$ away from the cell membrane (Fig. 4), where it appears as a dark line running parallel to the cell surface. This layer can also be recognized in material fixed in osmium tetroxide, where it displays much the same appearance and dimensions, though showing somewhat more density than after permanganate fixation. Thin extensions of this material are found between the imbricated surfaces of endothelial cells at areas of mutual contact as far as the region of the desmosomes (Figs. 5 and 6).

This internal basement membrane gives a positive reaction when treated with the periodate-Schiff method of Hotchkiss (1948) and McManus (1946) (Fig. 12).

Capillaries.—The capillaries of the ventral nerve cord have a complete wall of unfenestrated endothelial cells which make close contact with their neighbors. Thus they resemble vertebrate capillaries of Type 1 as classified by Bennett, Luft, and Hampton (1959). At certain regions of mutual contact, desmosomes or specialized attachment structures can be recognized, similar to those in vertebrate capillaries noted by Moore and Ruska (1957) and by Bennett, Luft, and Hampton (1959) (Figs. 5 and 6). Elsewhere, the plasma membranes of cells at surfaces of contact are separated by an interval of about 7 to 10 $m\mu$, in which basement membrane material can be recognized (Fig. 2). The desmosomes appear to surround each cell completely like a girdle or belt. They are not violated by gaps, nor is the endothelial cytoplasm anywhere pierced by resolvable pores. The plasma membranes of the endothelial cells in regions apart from the desmosomes display two parallel peaks in density about 2.5 $m\mu$ thick with an intervening light material of about the same thickness (Figs. 2 and 3, arrows). Thus it displays a structure corresponding to the unit membrane of Robertson (1957).

Vesicles which are assumed to be involved in pinocytosis (Lewis, 1931; Palade, 1953, 1956 *b*;

Bennett, 1956 *a*, 1957; Bennett, Luft, and Hampton, 1959) have been observed in the endothelial cell cytoplasm, but they occur less frequently than they do in the vertebrate capillary. These vesicles approach and often fuse with plasma membranes of both the lumen surface and the outer surface of the endothelial cells. One also finds irregularly shaped closed profiles of endoplasmic reticulum with Palade's granules (Fig. 6), (Palade, 1956 *a*, 1955; Palade and Siekevitz, 1956 *a*, *b*).

It is interesting to note that the cytoplasm of the endothelial cells contains a small number of filamentous structures about 25 $m\mu$ in diameter. They form small parallel groups of filaments but are not grouped distinctly into fibrils. Their size and density suggest that they are myofilaments (Figs. 5, 6). This interpretation implies that these earthworm capillary endothelial cells can be classified as myoendothelial cells, though of a relatively slightly specialized type.

The internal basement membrane of these capillaries is about 30 to 40 $m\mu$ thick (Figs. 1, 4, and 5). Outside the capillaries of the ventral nerve cord one sees no basement membrane, though glial cell processes are in intimate contact with the endothelial cells (Figs. 1, 5).

Subneural Vessel.—The subneural vessel is a longitudinal channel running in the capsule of the ventral nerve cord. The structure of its wall is similar in many respects to that of the capillaries described above.

The endothelial cells of the subneural vessel contain more abundant myofilaments than do the capillary endothelial cells (Figs. 7, 8). These filaments are about 25 $m\mu$ in diameter. The paramyosin periodicity described by Hanson (1957) was not detected in the material used in this study, either in the body wall musculature or in the myofilaments of the endothelial cells.

Infolding of the surface membranes is frequently observed in these myoendothelial cells. A row of vesicular profiles is often associated with the deepest portions of inward foldings of plasma membrane, in a manner similar to that described by Moore and Ruska (1957), by Palade (1956 *b*), and Bennett, Luft, and Hampton (1959) (Fig. 10).

Desmosomes similar to those observed in the capillary endothelium are also present in the subneural vessel (Fig. 7).

The endothelial cell surface facing the lumen is covered by a basement membrane of the same thickness as that in the capillary (Figs. 7 to 9). On the lumen side of the basement membrane cell

processes less than 100 $m\mu$ thick can sometimes be observed. Granules of variable size and density can be observed in the cytoplasm of these processes. These cells seem to correspond to the amebocytes or *wandstaendige Blutzellen* of Haffner (1928). Blood pigment particles are scattered between these cells and the basement membrane (Fig. 8).

Neither a basement membrane nor a distinct muscular layer can be observed outside the endothelial cell layer of the subneural vessel. Thus the outer surface of the endothelial cells is surrounded directly by connective tissue space (Figs. 7, 8).

Ventral Vessel.—The ventral vessel is formed by well developed myoendothelial cells.

A rather thick, 0.5 to 1 μ , inner layer, similar to a basement membrane, covers the surface of the cells lining the lumen (Figs. 11, 13). At a certain distance from the cell surface this layer shows a dense demarcation line, and the density then gradually decreases towards the lumen (Figs. 11, 13). The inner limit of this layer is poorly defined. It corresponds to Leydig's "intima" described by Retzius (1905), or to the "cuticle" of Bergh's terminology (Bergh, 1900).

The cytoplasm of the myoendothelial cells contains myofilaments grouped into fibrils with some degree of regularity forming an inner longitudinal and an outer circular arrangement. Myofibrils running in both directions can be seen in the same cell (Fig. 11). In some micrographs the fibrils show cross-striations which may correspond to the regularly arranged endoplasmic reticulum existing in the interfibrillar cytoplasm (Fig. 11). In the lateral part of the vessel wall, well developed longitudinal myoendothelial cells can be observed. These also lie in contact with the internal basement membrane lining the lumen (Fig. 13). In general, myofilaments occupy the part of the cytoplasm adjacent to the basement membrane. The remainder of the cytoplasm contains cell organelles such as mitochondria, vesicles of various sizes, endoplasmic reticulum, and small granules (Fig. 11).

On the peritoneal side of the lining cell layer there is a basement membrane layer resembling that lining the lumen (Fig. 11, *cn*).

Inside the vessel, located on the cuticular layer, ameboid cells can be observed. One can recognize large cell bodies and many slender processes where the cytoplasm is very attenuated. These cells cover only a small part of the total inner surface of the vessel (Fig. 13).

Dorsal Vessel.—It is generally known that the dorsal vessel of the earthworm is a pulsating channel with a very well developed musculature in the wall.

The innermost continuous layer of the vessel wall is a basement membrane ("cuticle") about 1 μ in thickness (Figs. 14, 15). Outside this layer lie highly specialized myoendothelial cells corresponding to the inner muscular layer. The myofilaments in these myoendothelial cells are oriented longitudinally. Basement membrane material often extends between the different processes of the longitudinal myoendothelial cells (Fig. 15, arrow).

The myofilaments in these cells are grouped into myofibrils with tubular endoplasmic reticulum occupying the interfibrillar sarcoplasm (Fig. 14). A row of circular profiles of endoplasmic reticulum can be traced from the interfibrillar endoplasmic reticulum to the surface cell membrane (Fig. 14).

Outside the longitudinal myoendothelial cells there is a well developed circular muscle layer in which the myofibrils and interfibrillar endoplasmic reticulum are well organized. This arrangement is similar to that described in the body wall musculature by Hanson (1957). In the cells examined in the present study the myofibrils occupy a part of the cytoplasm adjacent to the inner longitudinal muscle cell layer. The rest of the cytoplasm is filled with mitochondria (Fig. 15).

The lateral portion of the dorsal vessel has an outer longitudinal muscle layer (Fig. 14).

Amebocytes are found inside the basement membrane of the dorsal vessel. They are similar to those already described inside the subneural and the ventral vessels (Figs. 14, 16).

DISCUSSION

The ameboid cells noted in this study within the lumina of blood vessels inside the lining basement membrane have been described by many authors. There are several interpretations of the nature of these cells. They have been variously regarded as the nuclei of the "vasothelial myoblasts" (Vejdovsky, 1905), as amebocytes or "*wandstaendige Blutzellen*" (Haffner, 1928), as endothelial cells (Ude, 1896; Johnston, 1903), or as blood cells (Bergh, 1900). These cells within these vessels have the following characteristics: They do not appear to be firmly attached to the basement membrane or cuticle; they cover only a small portion of the vessel surface; the cytoplasm contains granules of various sizes and densities; they do not seem to

be endothelial cells, but rather to be macrophages or blood cells (however, whether these cells are derived from endothelial cells or are of a different developmental origin is still in question).

The subneural vessels and the capillaries have an endothelial cell layer which resembles closely that of vertebrate capillaries, Type 1 of Bennett, Luft, and Hampton (1959). The cytoplasm of these endothelial cells contains vesicles which are presumably involved in pinocytosis, as similar vesicles appear to be in the vertebrate capillary (Lewis, 1931; Palade, 1953; Bennett, 1956 *a*, 1957; Palade, 1956 *b*; Moore and Ruska, 1957; Alksne, 1959). However, these vesicles occur much less frequently in the earthworm than they do in the vertebrate. Whether this is due to a difference in mechanism of active transport or in rate of metabolism is not known at present.

The filamentous structures which can be seen frequently in the endothelial cell cytoplasm of the subneural vessel and less frequently in the capillary endothelial cell have the same dimensions and the same density as the myofilaments of the body wall muscle (Hanson, 1957; Hanson and Lowy, 1957). They run parallel to each other, as myofilaments generally do. In view of these facts the filamentous structures in the endothelial cytoplasm are considered to be myofilaments, even though no regular periodicity characteristic of paramyosin (Hanson and Lowy, 1957) has been detected in longitudinal sections of these filaments.

The fact that the endothelial cell itself may contain myofilaments, as reported in this study, is considered to be a morphological basis for contractile properties of the endothelial cell in the earthworm.

Retzius (1891, 1905) described the existence of muscle cells in the walls of small vessels and of large contractile vessels of polychaetes, and Parker (1923) supported this observation. However, both authors thought that these cells represented Rouget cells on the outside of the vessels. Moreover, they denied the existence of endothelial cells in these channels.

The myofilaments in the circular muscle layer of the dorsal vessel show regular myofibrils and have well organized interfibrillar endoplasmic reticulum. The organization of the endoplasmic reticulum is somewhat similar to that of the sarcoplasmic reticulum in vertebrate striated muscle (Bennett, 1956 *b*, 1959; Porter, 1956; Porter and Palade, 1957).

It is shown here that endothelial cells can show varying degrees of morphological specialization with respect to contractile function. Those of the capillary show only slight specialization; those of the dorsal vessel are most highly specialized and are outfitted with well organized myofilaments, myofibrils, and sarcoplasmic reticulum. Intermediate degrees of specialization are also found. Thus a spectrum of myoendothelial cells can be recognized representing differences in degrees of specialization for contractile function.

In those invertebrates in which the respiratory pigment is in solution in the plasma the large size of the pigment molecule tends to confine it to the circulatory system (Prosser, 1950). The basement membrane inside the capillary forms a barrier which prevents the blood pigment from leaving the vessel or from making contact with the endothelial cells. In this respect the basement membrane appears to have a function analogous to the red blood cell surface membrane. Svedberg and Eriksson (1933) reported that the particle weight of earthworm erythrocrucorin is about 2,500,000. Svedberg (1933) suggested that each particle consists of 72 units of molecular weight 34,500 and has 144 iron atoms. This is about 36 times as large as hemoglobin, which has 2 units of molecular weight 34,500 and 4 iron atoms. If we accept Richter's (1958) and Farrant's (1954) data on the size of the iron component of ferritin and assume that particles observed in earthworm blood vessels have spherical or cubic form, the particles are about 20 to 40 times as large as the iron component of ferritin. Svedberg and Eriksson (1933) postulate that the radius of the earthworm erythrocrucorin particle would be about 9.3 $m\mu$. This is in fair agreement with the measured diameters of the particles observed in this study (about 17 $m\mu$).

In this study it is shown that the so called "cuticle" or "intima" of the larger earthworm blood vessels (Retzius, 1905; Bergh, 1900), is in reality an internal basement membrane. It differs only in thickness from the internal basement membrane of earthworm blood capillaries. Bennett, Luft, and Hampton (1959) have pointed out that most vertebrate blood vessels (those of Type A) have an external basement membrane outside the endothelial cells. Except for position and thickness, this vertebrate basement membrane resembles the inner one lining earthworm blood vessels. The recognition of an internal basement membrane lining blood vessels is not entirely novel in this

study, however, as Bennett, Luft, and Hampton (1959) noted a very thin basement membrane on the inner surface of capillaries in the lamina propria of the intestine of the rat. This lining basement membrane in rat intestinal capillaries is much more delicate than the one lining capillaries in the earthworm nerve cord. Moreover, it bridges intracellular pores or fenestrations, which have not been noted in the earthworm blood vessels.

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EXPLANATION OF PLATES

Legend for all Figures

<i>a</i> , amebocyte	<i>gr</i> , granules
<i>at</i> , desmosome or attachment body	<i>i</i> , indentation of cell membrane
<i>b</i> , basement membrane	<i>l</i> , vessel lumen
<i>bc</i> , basement membrane of the nerve cord	<i>lm</i> , longitudinal myofilament
<i>bp</i> , blood pigment (erythrocrucorin)	<i>m</i> , mitochondria
<i>c</i> , cross-striation in circular myofibrils	<i>mf</i> , myofilament
<i>ca</i> , capsule of ventral cord	<i>n</i> , nucleus
<i>cm</i> , circular myofilaments	<i>o</i> , outer longitudinal muscle
<i>cn</i> , connective tissue	<i>p</i> , plasma membrane
<i>cu</i> , thick basement membrane layer (cuticular layer)	<i>s</i> , cross-section of the myofilament
<i>e</i> , endothelial cell	<i>t</i> , mesothelial cell
<i>er</i> , endoplasmic reticulum (or sarcoplasmic reticulum)	<i>w</i> , cellular portion of vessel wall
<i>g</i> , glial cell	<i>y</i> , demarcation line
<i>gf</i> , glial filaments	<i>z</i> , longitudinal section of myofilament
<i>go</i> , Golgi apparatus	

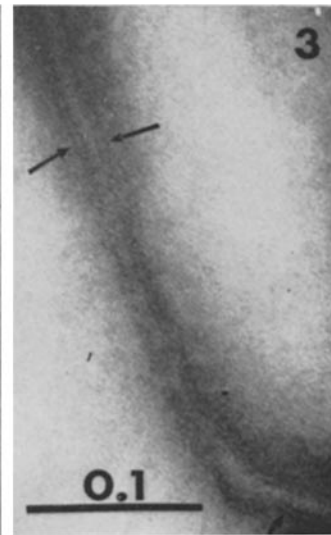
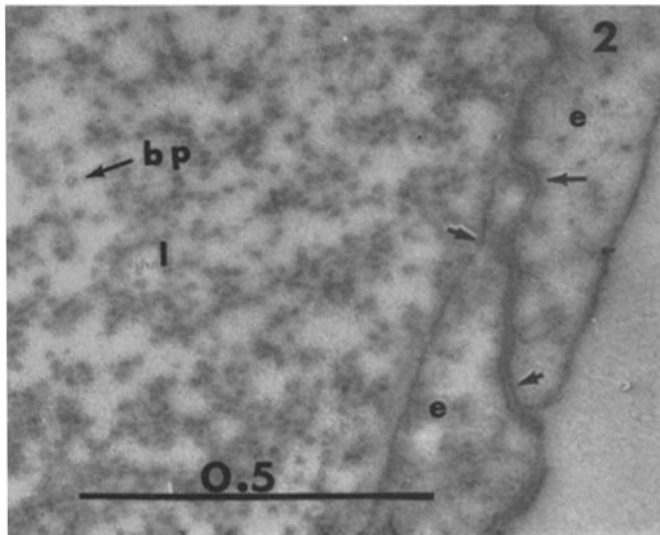
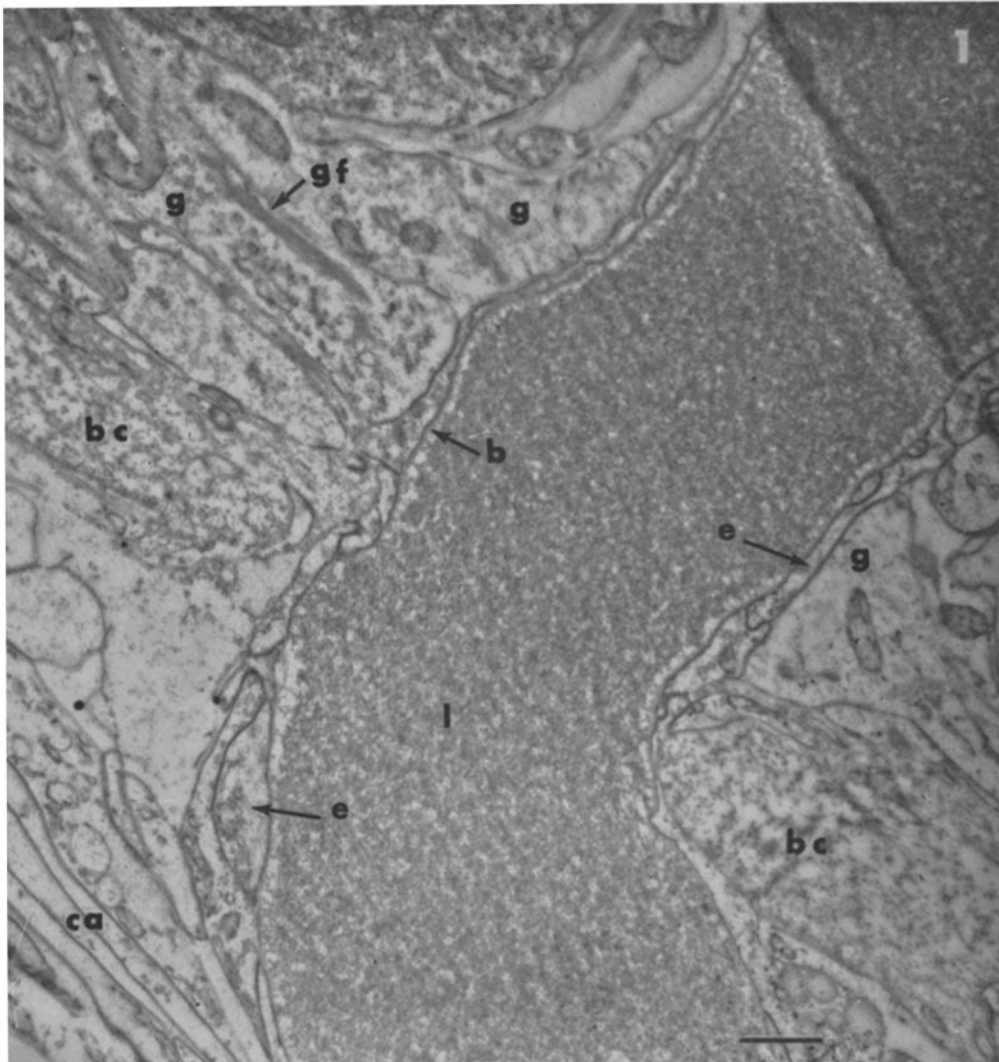
PLATE 359

FIG. 1. A low power electron micrograph of a longitudinal section of a small blood vessel penetrating the capsule (*bc*, *ca*) of the ventral nerve cord. A complete endothelial cell layer (*e*) covers the lumen along the vessel. A thin basement membrane (*b*) covers the endothelial cell surface toward the lumen. The plasma is filled with blood pigment particles (erythrocrucorin molecules).

In the ventral nerve cord many glial cell processes (*g*) containing glial filaments (*gf*) attach to the endothelial cells directly. $\times 11,000$.

FIG. 2. A high power electron micrograph of a part of the capillary lumen and endothelial cell layer. The capillary lumen (*l*) is filled with small erythrocrucorin particles about $17\text{ m}\mu$ in diameter. Some of them have rectangular profiles and show a less dense core (*bp*). The endothelial cell junction is characterized by intimate contact of adjacent cells welded together at desmosomes. A narrow interval about $7\text{ m}\mu$ in width separates the opposing plasma membranes at regions in which no desmosomes are present. The endothelial surface membranes show characteristic unit membrane structure (arrows). $\times 93,000$.

FIG. 3. A high power electron micrograph of a part of apposed plasma membranes of capillary endothelial cells. Two peaks of density in each of the apposed plasma membranes are clearly seen (arrows). $\times 240,000$.

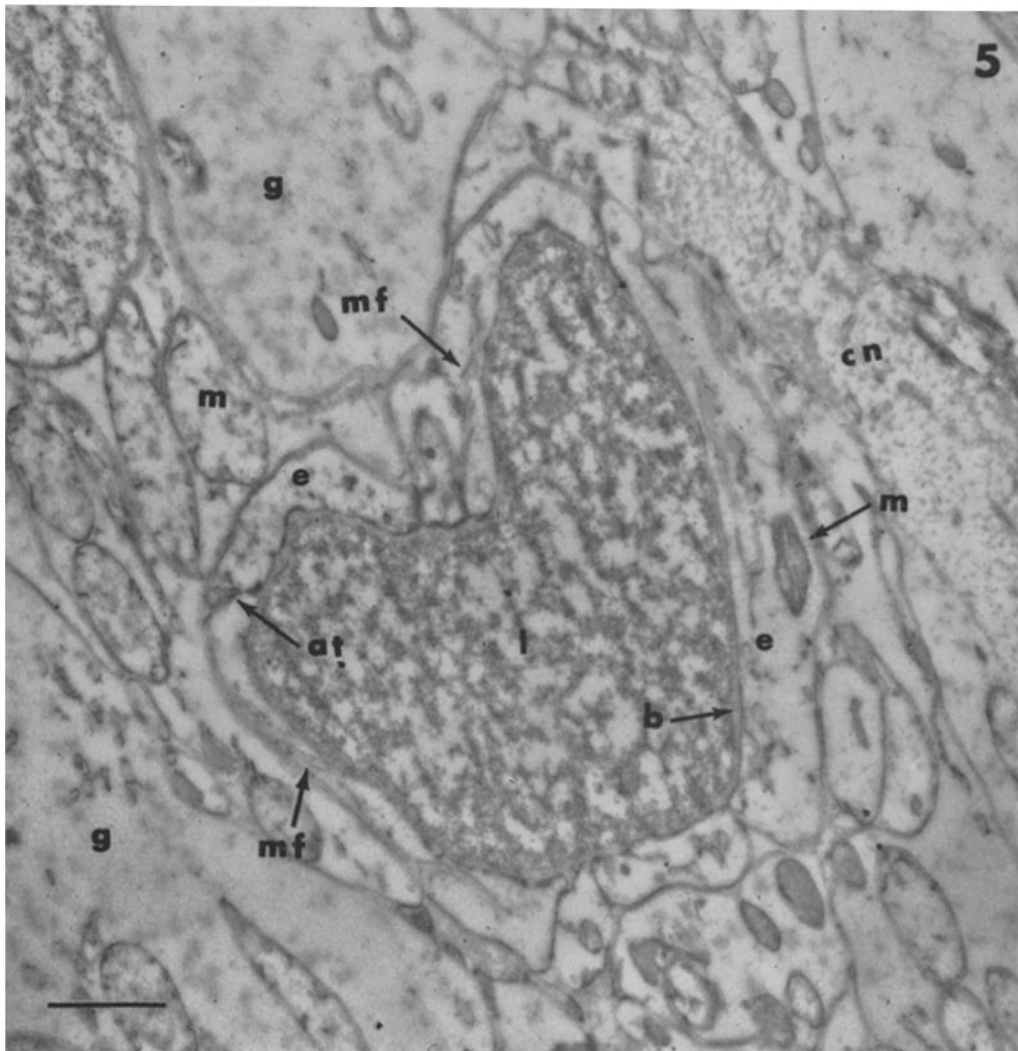
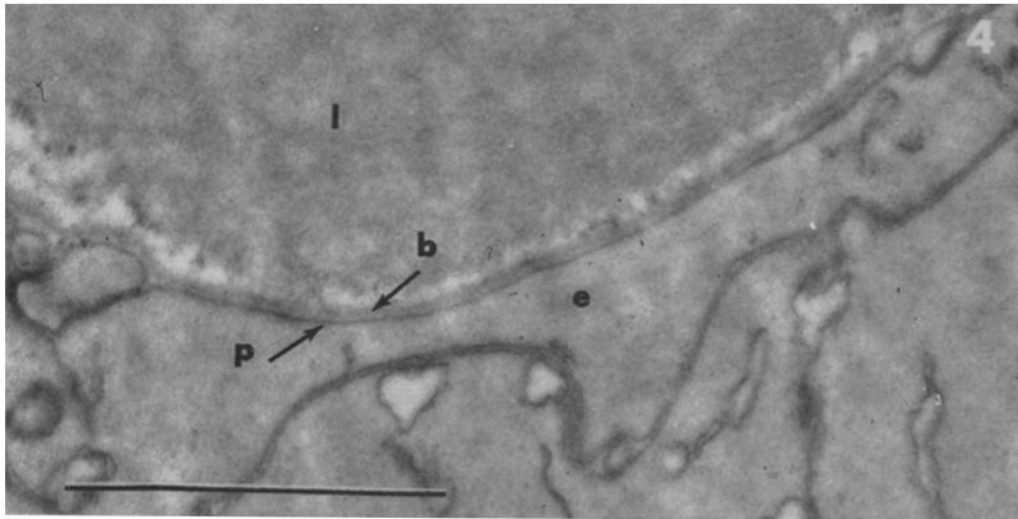


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FIG. 4. Electron micrograph of earthworm blood capillary fixed with permanganate and embedded in araldite. The basement membrane (*b*) inside the capillary is seen more distinctly than in osmium-fixed material. It is of low density near the endothelial cell plasma membrane (*p*) but is markedly more dense about 15 $m\mu$ from the cell membrane, where it appears as a dark line running parallel to the cell surface. $\times 50,000$.

FIG. 5. A cross-section of a capillary in the ventral nerve cord. A desmosome can be observed at endothelial cell junction (*al*). The endothelial cell cytoplasm contains myofilaments (*mf*). The basement membrane inside the capillary (*b*) can also be seen. $\times 16,000$.



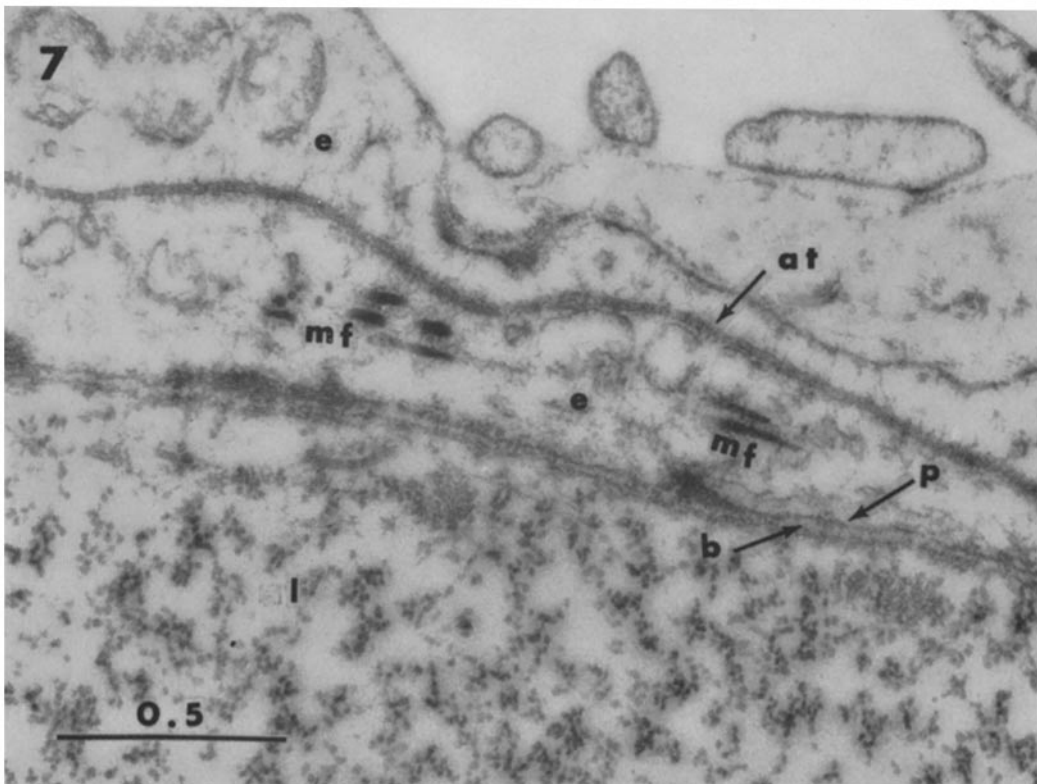
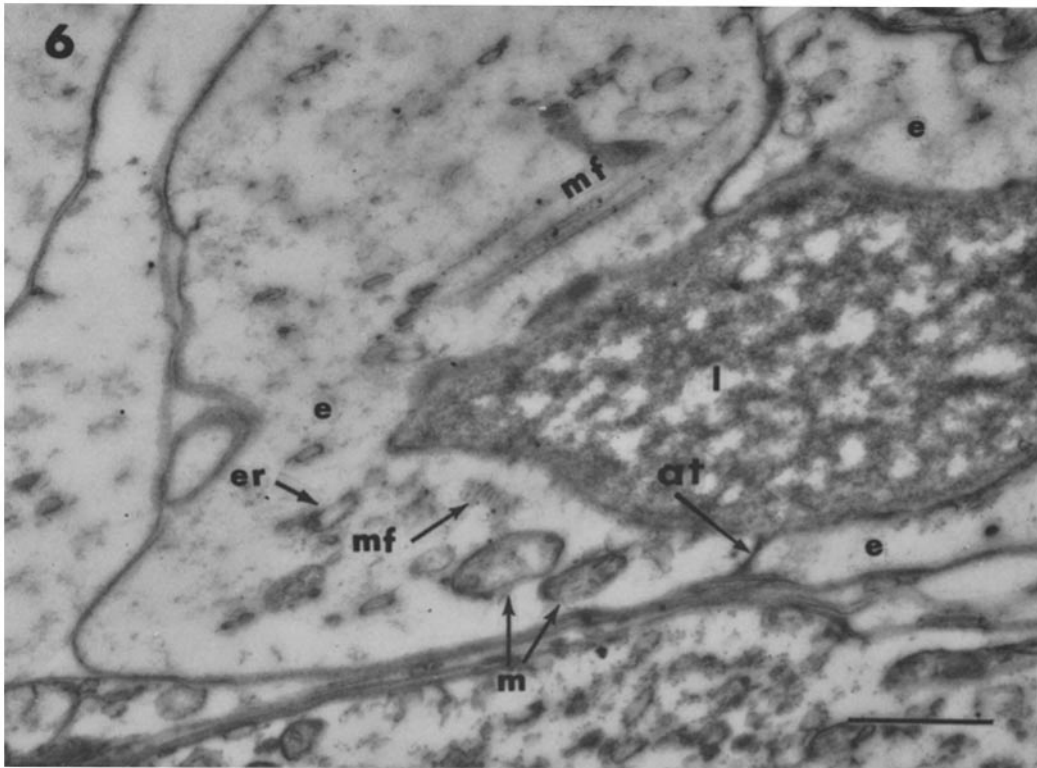
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FIG. 6. An electron micrograph showing part of a capillary myoendothelial cell which contains myofilaments (*mf*) running parallel to each other. $\times 20,000$.

FIG. 7. A high power micrograph of a part of the subneural vessel wall. Two endothelial cells (*e*) are overlapping each other. A desmosome (*di*) is observed binding two endothelial cells to each other.

Myofilaments (*mf*) $25\text{ m}\mu$ in diameter can be observed in the cytoplasm of one of these myoendothelial cells. The surface of the cell is covered by a thin basement membrane. Phosphotungstic acid-treated material. $\times 62,000$.

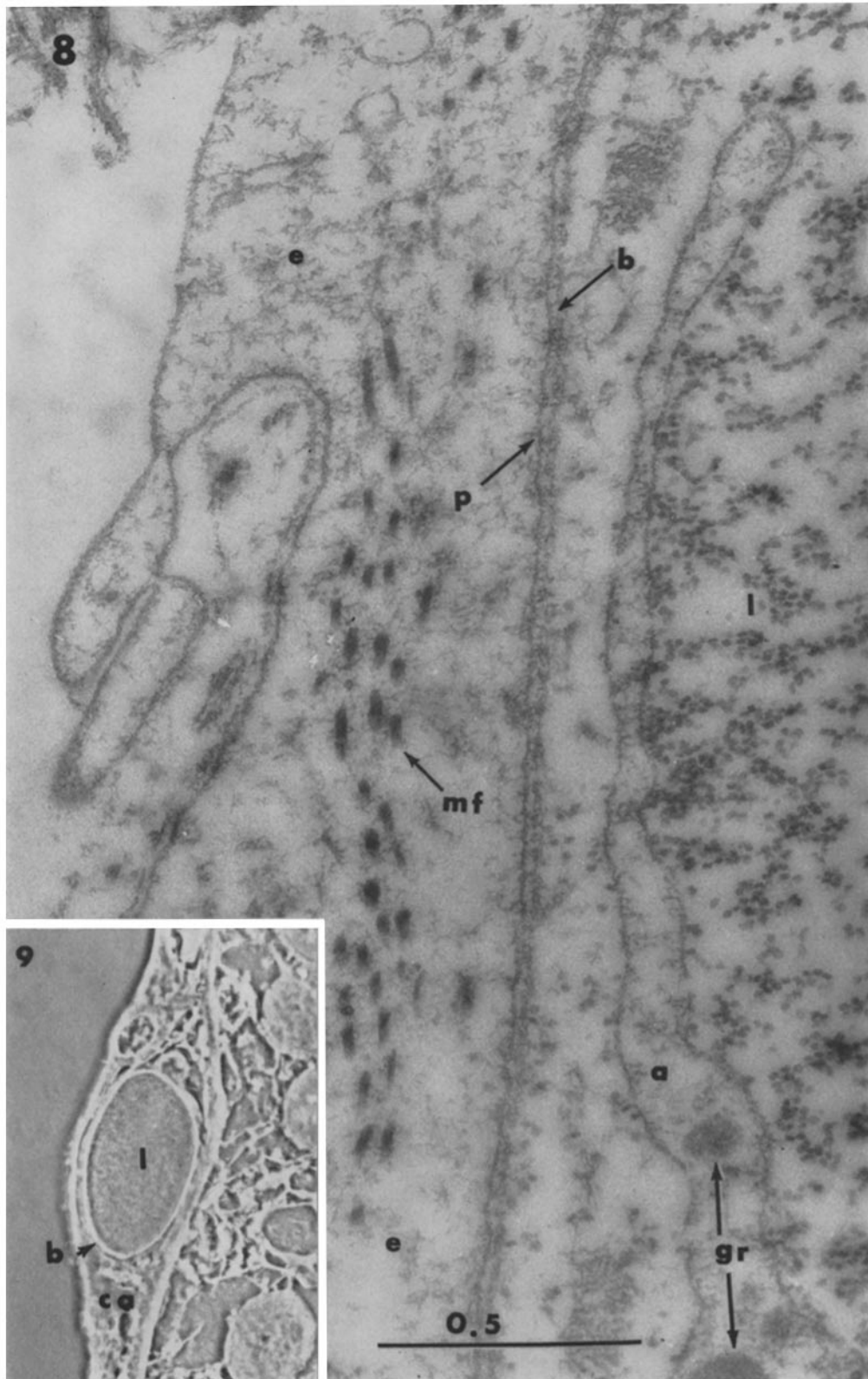


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FIG. 8. A high power electron micrograph of a part of a subneural vessel wall. The myoendothelial cell (*e*) contains a group of myofilaments (*mf*). A thin basement membrane (*b*) about 30 to 40 $m\mu$ thick covers the cell surface toward the lumen (*l*). An attenuated cell process (*a*) of an amebocyte, containing large dense granules (*gr*), can be observed in the vessel. Blood pigment particles are scattered between this cell process and the basement membrane. Phosphotungstic acid-treated material. $\times 82,000$.

FIG. 9. A phase contrast photomicrograph of a cross-section of the subneural vessel showing the basement membrane inside the vessel. $\times 400$.



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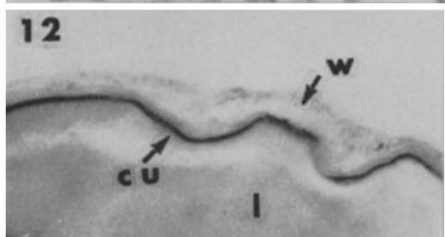
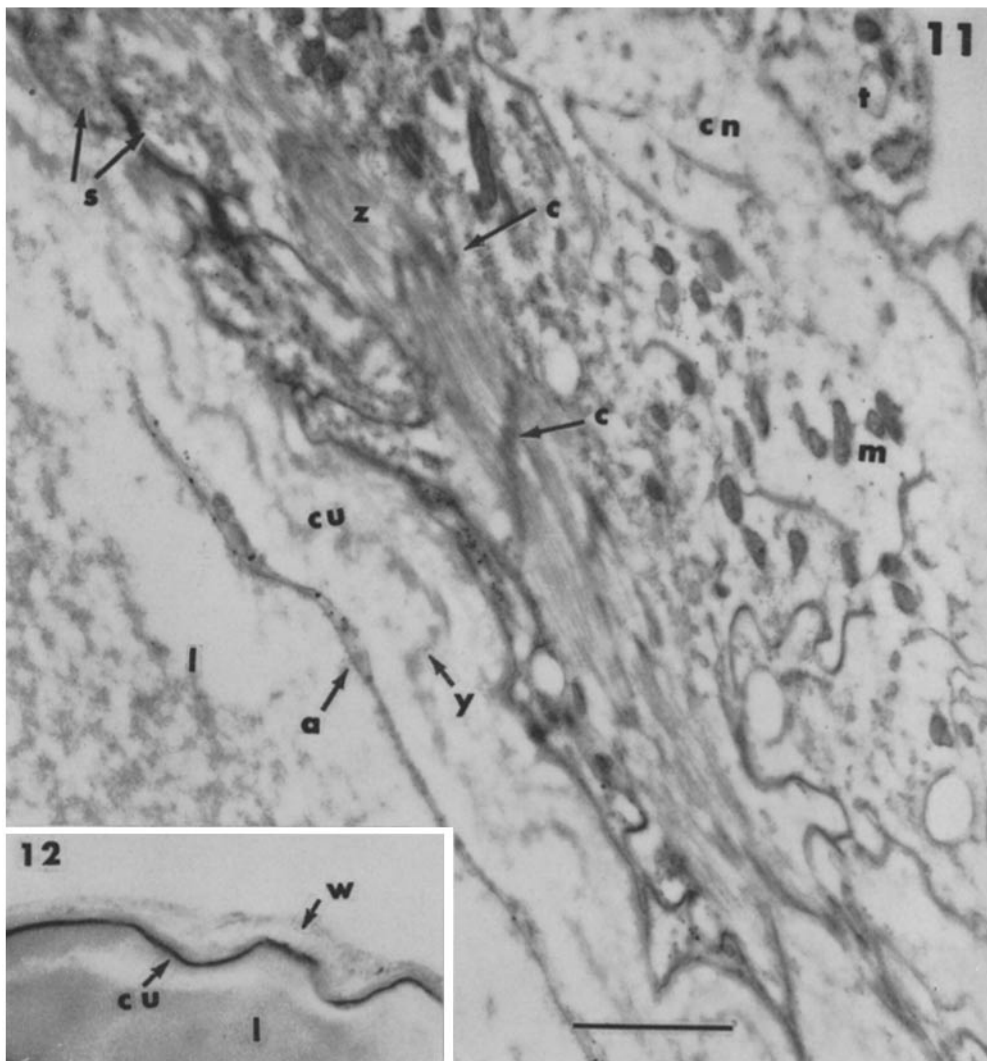
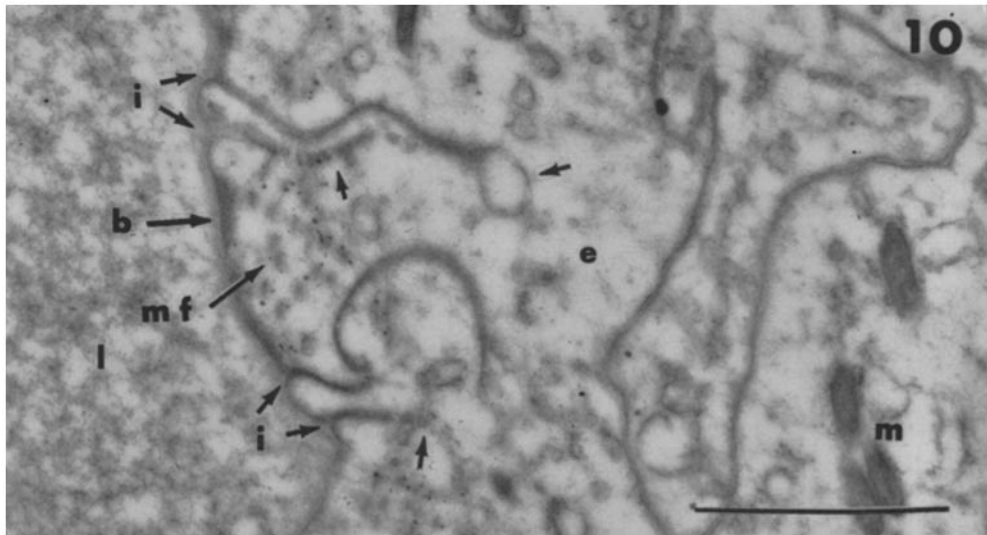
FIG. 10. High power electron micrograph of a part of a myoendothelial cell of the subneural vessel showing many deep infoldings of the surface membrane (*i*). A row of vesicular profiles is often associated with the troughs of infolded recesses in the plasma membrane (arrows). $\times 35,000$.

FIG. 11. A cross-section of a part of the ventral vessel wall. A thin process of an amebocyte (*a*) containing granules of various sizes and densities can be seen in the lumen (*l*). A thick basement membrane layer (*cu*) covers the cell surface lining the lumen. A dark demarcation line (*y*) can be observed in this layer.

The lining myoendothelial cells are highly specialized, containing myofilaments grouped into myofibrils. Longitudinal myofibrils (*s*) can be seen as cross-sections of myofilaments in a part of the cytoplasm which is adjacent to the lumen, and circular myofibrils can be seen as longitudinal sections of myofilaments (*z*) in outer parts of the cytoplasm. Fibrils running in both directions can be detected in the same cell. A cross-striation can be seen in the circular myofibrils (*c*).

The rest of the cytoplasm contains cell organelles such as mitochondria, endoplasmic reticulum, and small granules. $\times 21,000$.

FIG. 12. A light micrograph showing a cross-section of the ventral vessel showing a strong periodate-Schiff reaction in the basement membrane layer inside the vessel. The conspicuous prominence of this layer has led some authors to speak of it as a "cuticle" or "intima." $\times 1,200$.

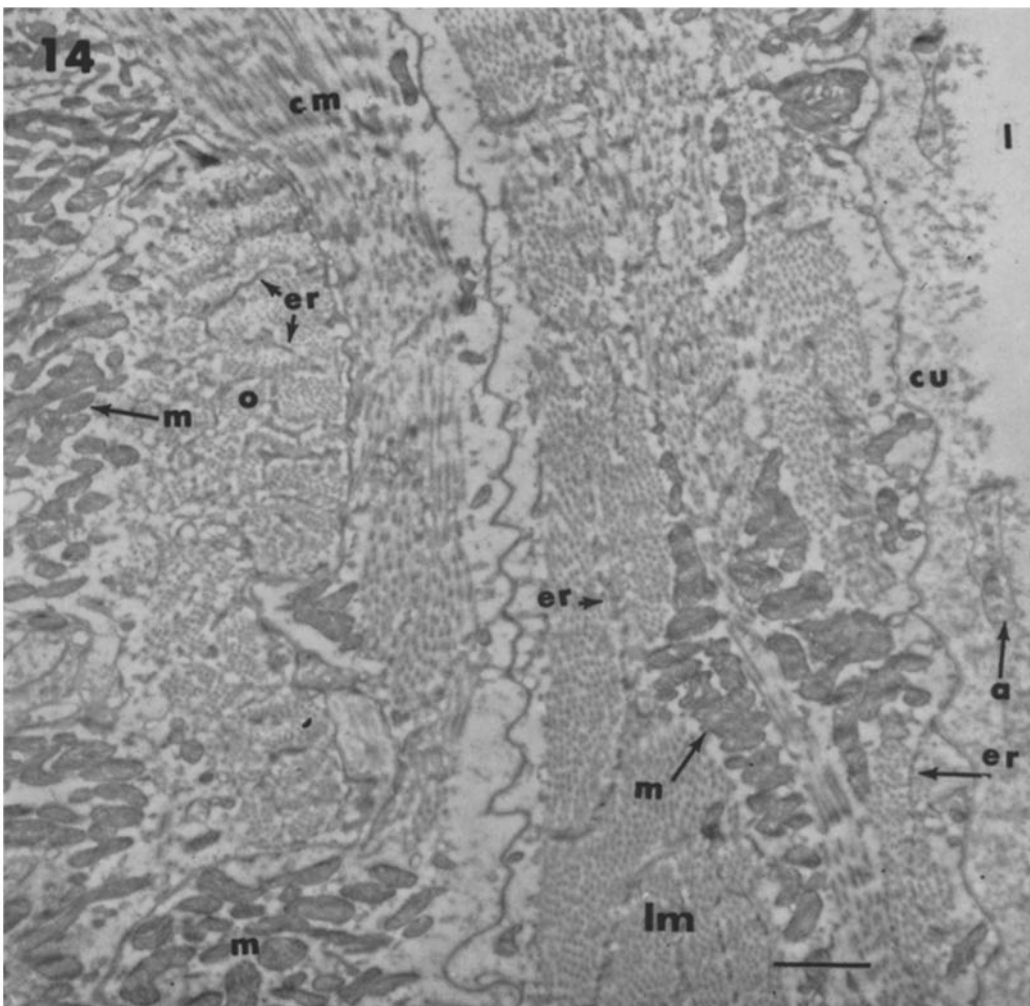
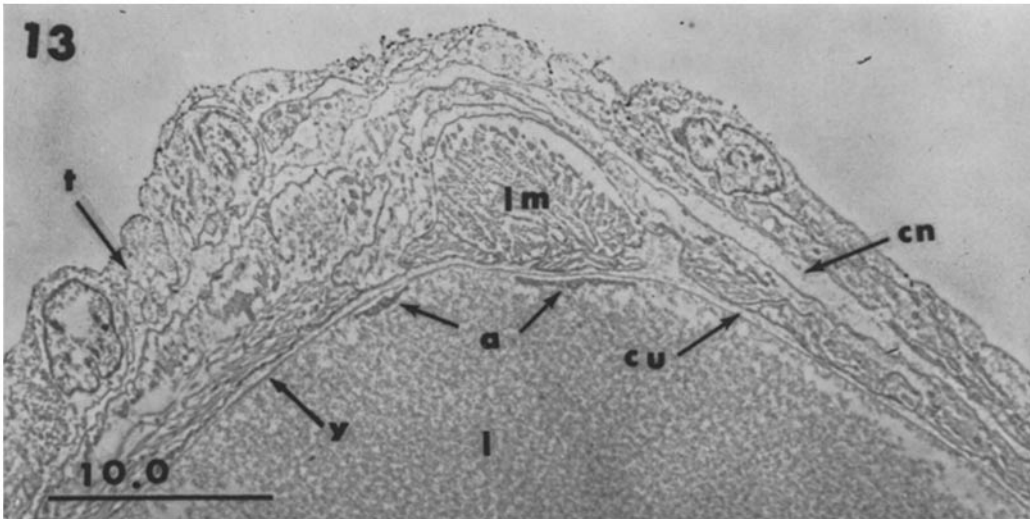


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FIG. 13. A low power electron micrograph of a cross-section of the ventral vessel. It has well developed longitudinal myofibrils (*lm*) in the lining myoendothelial cells. Two thin amebocyte processes are observed in the lumen (*a*). These are located on the inner surface of the basement membrane and cover only a small portion of the vessel face. $\times 3,000$.

FIG. 14. A low power electron micrograph of a cross-section of the lateral portion of the wall of the dorsal vessel, showing inner longitudinal myoendothelial cell (*lm*), and the circular (*cm*) and outer longitudinal (*o*) muscle layers. Each has well organized myofibrils and interfibrillar endoplasmic reticulum (*er*). $\times 13,000$.

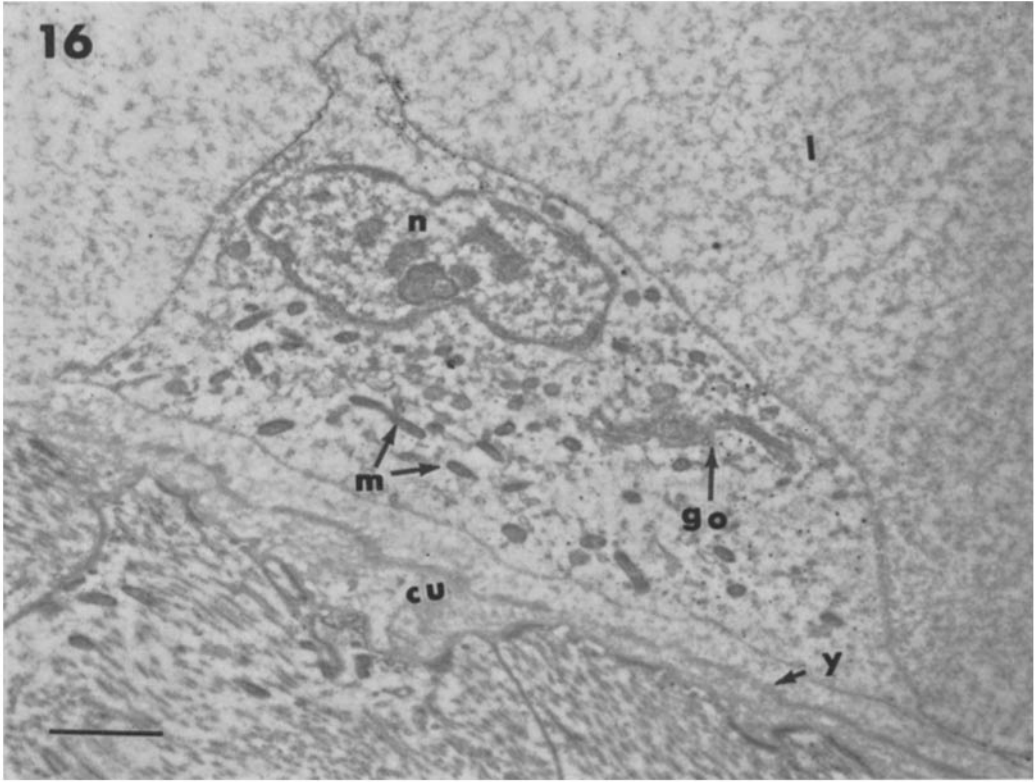
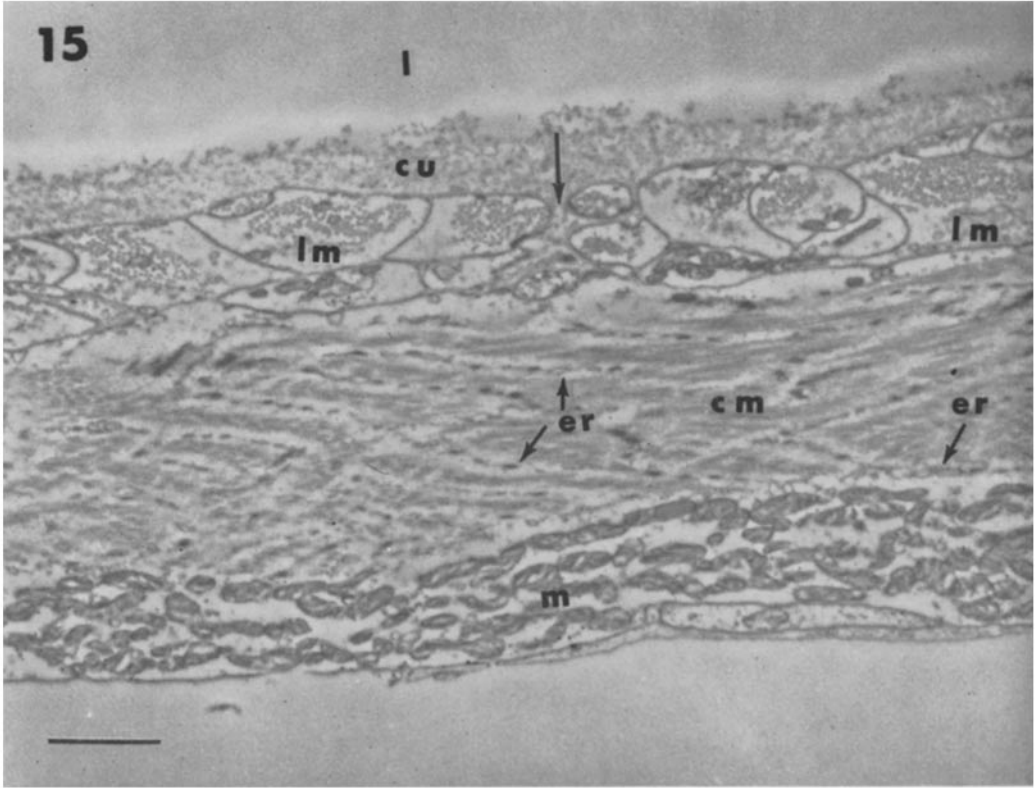


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FIG. 15. A low power electron micrograph of a cross-section of the dorsal vessel wall showing inner longitudinal myoendothelial (*lm*) and outer circular (*cm*) muscle layer. The circular muscle has well organized myofibrils and interfibrillar endoplasmic reticulum (*er*). These components occupy a part of the cytoplasm adjacent to the inner longitudinal myoendothelial layer. The rest of the cytoplasm contains mitochondria (*m*). Amebocytes are not displayed in this picture. The basement membrane extends between the different processes of the longitudinal muscle cell (arrow). $\times 13,000$.

FIG. 16. An amebocyte in the dorsal vessel. It is located on the cuticular layer (*cu*). Blood pigment (erythrocrucorin) particles are scattered between this cell and the basement membrane. $\times 13,000$.



(Hama: Earthworm blood vessels)