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The innervation of the cerebral arteries in the rat: an electron microscope study

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From the beginning of this century numerous attempts have been made to study the nerves accompanying the cerebral vessels and to assess their significance in the regulation of blood flow to the brain. A review of the literature is given by Lassen (1959).

It is generally agreed that the major cerebral arteries are accompanied by nerve fibres, but it is not certain whether this applies also to the vessels within the cerebral tissue. The primary purpose of the present investigation, therefore, was to see whether both the extracerebral vessels and the intracerebral vessels were accompanied by nerve fibres. Here, a fresh approach was made to the problem by using the more definite identification of nerve fibres made possible by the resolution of the electron microscope and by examining complete transverse sections of the arteries.

MATERIALS AND METHODS

The rat (Wistar strain) was chosen for this investigation because its major extracerebral arteries were not too large for cutting complete transverse sections. Arteries were fixed *in situ* so that a normal relationship between vessel, leptomeninx and brain was maintained. The animal was perfused with buffered osmium tetroxide solutions according to the method described by Palay, McGee-Russell, Gordon & Grillo (1962). Artificial respiration was not applied. The brain was dissected carefully and ice-cold fixative dripped on to the surface of the vessels.

Segments of the basilar artery and the middle cerebral artery just beyond their points of origin were selected for further processing. A thin slab of cerebral tissue was taken with each portion of vessel and finally trimmed to blocks 3 mm × 2 mm. Attempts to make smaller blocks resulted in a stripping off of the leptomeninx.

The tissue was dehydrated in ascending grades of alcohol, stained in a 1 % alcoholic solution of phosphotungstic acid and infiltrated in Araldite.

Central arteries supplying the basal ganglia of the rat were taken as examples of the intracerebral vessel. This was done for the sake of comparison as intracerebral vascular nerves were illustrated in similar vessels in other species by Penfield (1932).

The arteries were preserved by perfusing the animal with buffered osmium tetroxide solution as in the previous experiment. A horizontal incision was made through the basal ganglia and the fixative dripped on to the cut surfaces. Areas for further processing were selected with the aid of a dissecting microscope.

In later experiments, hypothermic animals (rectal temperature 25 °C), were perfused with ice-cold 5 % gluteraldehyde in Palade-sucrose buffer at pH 7·3–7·4. The areas selected for examination were osmicated for 2 h and then dehydrated and

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infiltrated in Araldite as described previously. Sections were cut with a Cook and Perkins microtome. They were mounted on formvar-coated grids and examined under an A.E.I., E.M. 6 electron microscope.

Tissue preserved with gluteraldehyde was post-stained with uranyl acetate and lead citrate (Reynolds, 1963).

OBSERVATIONS

The extracerebral arteries

Two arteries were selected from this group—the basilar artery (diameter, 0·3 mm approximately) and the middle cerebral artery (diameter, 0·2 mm approximately). Both vessels have a similar structure (Fig. 1). The lumen is lined by a single layer of

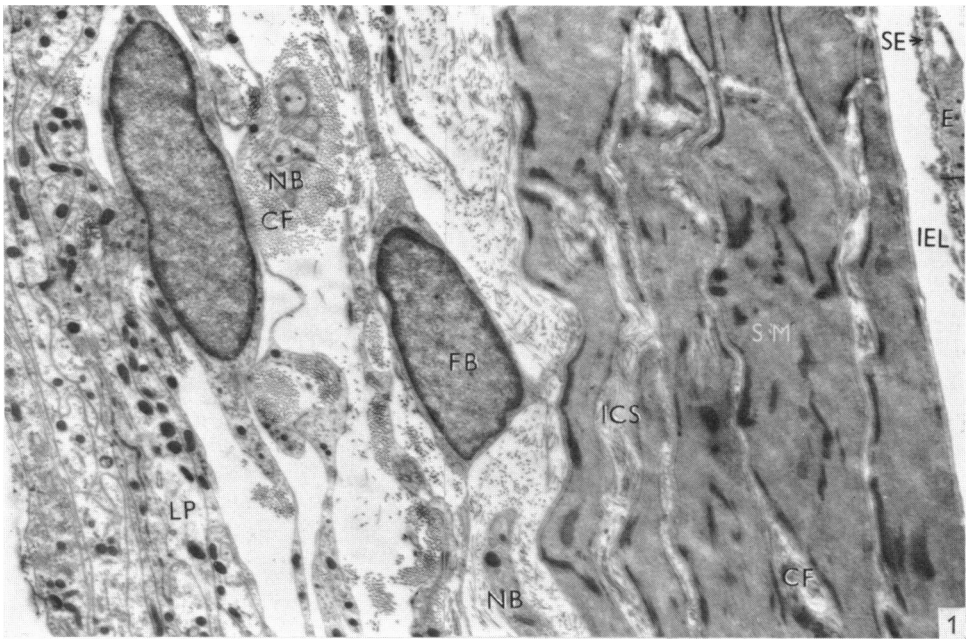


Fig. 1. A montage of electron micrographs of the basilar artery of the rat to show the structure of the arterial wall. The flattened endothelium (E) is separated from the internal elastic lamina (IEL) by a subendothelial space (SE). The tunica media is formed by smooth muscle cells (SM) separated from each other by the intercellular space (ICS) which contains collagen fibres (CF). In the tunica adventitia are bundles of nerve fibres (NB), fibrocytes (FB) and collagen fibres (CF). External to this layer is the closely knit leptomeninx (LP). ×4,500

endothelial cells. These cells are separated from the internal elastic lamina by a sub-endothelial space containing islets of elastic tissue and collagen fibres. The entire elastic tissue content of the arterial wall is usually concentrated into a single band—the internal elastic lamina—which lies between the tunica intima and the tunica media.

The smooth muscle cell is the only cell type within the media. In the basilar artery the media is made up of four or five cell layers and is about 10 μ thick, whereas in the

middle cerebral artery it is built up of two or three cell layers and is about 5·5 μ thick. The cells are characterized by myofilaments and dense bodies. In addition to the usual cytoplasmic organelles they show numerous vesicular infoldings of the plasmalemma. Each cell is surrounded by a basement membrane and except at points of membrane contact they are separated by intermuscular spaces containing collagen fibres. Intercellular bridges have not been observed.

The tunica adventitia lies between the media and the leptomeninx and is 2–7 μ thick. It contains fibrocytes, collagen fibres and nerve bundles. The attenuated fibrocyte cell-processes meet each other to form a more or less continuous sheet encircling the vessel. Some of them come into simple apposition with the cells of the leptomeninx, while others establish contact with the outer layer of smooth muscle cells of the media. It has not been possible to make a clear distinction between the adventitial fibrocytes and the cells of the pia mater as they appear to be morphologically identical. For purposes of description, therefore, the outer layer of adventitial cells lining the spaces containing cerebrospinal fluid has been regarded as the pia mater.

The leptomeningeal cells give rise to numerous cytoplasmic processes which interdigitate to form a compact layer—the arachnoid mater. The processes become loosely knit as they approach a blood vessel. The spaces thus formed contain collagen fibres.

The most striking and significant feature concerning the nerve fibres related to the cerebral arteries is their restriction to the outer, adventitial coat of the vessels (Fig. 1). No nerve fibres have been found within the media in an intensive search in over 125 sections of the basilar and middle cerebral arteries.

The nerve fibres have a similar form and pattern of distribution yet their numbers differ in the two vessels. In assessing the density of the nerve plexus surrounding the vessels two conditions have had to be satisfied. First, only complete transverse sections of the arteries have been examined. This ensures the inclusion of all accompanying nerve fibres, because the available evidence points to nerves having a longitudinal course along the vessels. Next it has been necessary to make a correction for the segments of the arterial wall obscured by grid bars. When formvar-coated 100-mesh/in grids are used the number of bars hiding each section is reduced to four, and whenever possible a more accurate count has been made by examining serial sections.

On this basis the basilar artery is found to be accompanied by about sixteen nerve bundles containing around 160–180 nerve fibres altogether. Six to ten nerve bundles are observed around the middle cerebral artery containing approximately 70–100 nerve fibres.

Figure 2 is a diagrammatic reconstruction of a transverse section of the basilar artery made from a study of seven ultrathin serial sections and shows the distribution of the accompanying nerve bundles. These fasciculi are of varying sizes and shapes. The large groups usually occupy the periphery of the adventitia and may occasionally be encircled by pial cell-processes. Two very big bundles occupy almost symmetrical positions on the side of the vessel nearer the brain. Their approximate positions are indicated in Fig. 2. Small groups lie in the vicinity of the outer layer of smooth muscle cells, while bundles of intermediate size are scattered throughout the adventitia. The form of the nerve groups is extremely variable, and occasionally flattened or chain-like arrangements have been observed.

The majority of the nerve bundles in the tunica adventitia are composed only of non-myelinated nerve fibres and have the usual relationship to the Schwann cells (Fig. 3). In this series a few bundles containing single myelinated fibres with the characteristic lamellar arrangement have also been observed in the adventitia of the middle cerebral vessels (Fig. 4), but none are seen around the basilar artery. This differs from the observations made by Pease & Molinari (1960). In their investigation the nerves accompanying pial vessels have always lacked myelin.

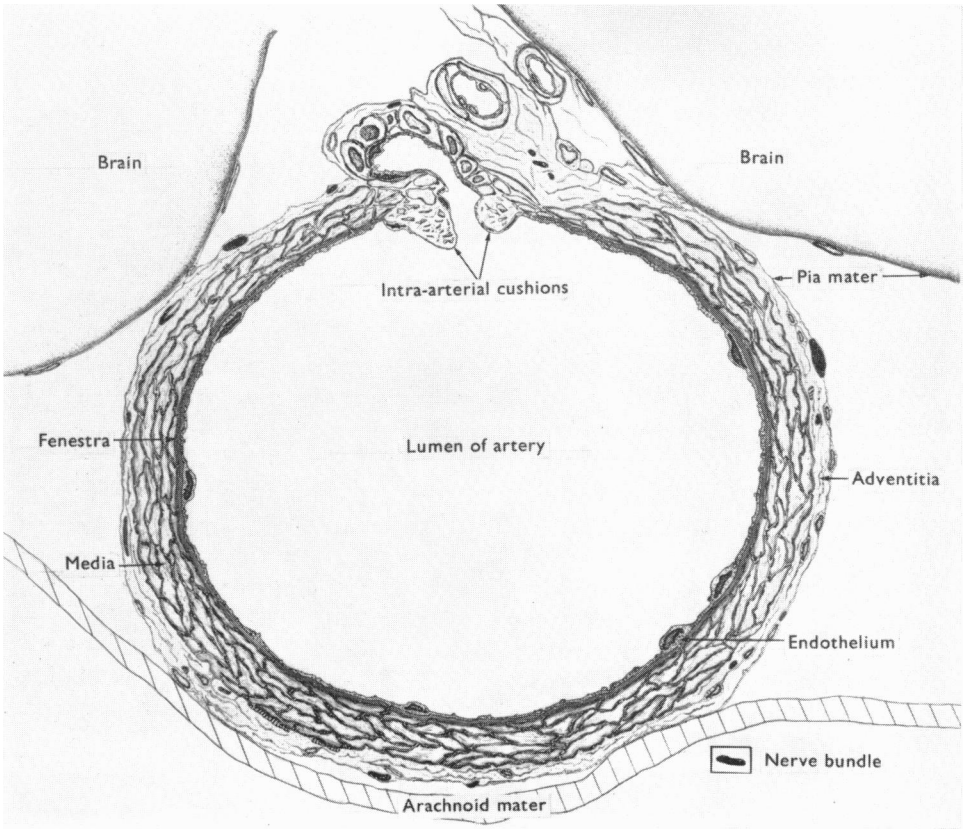


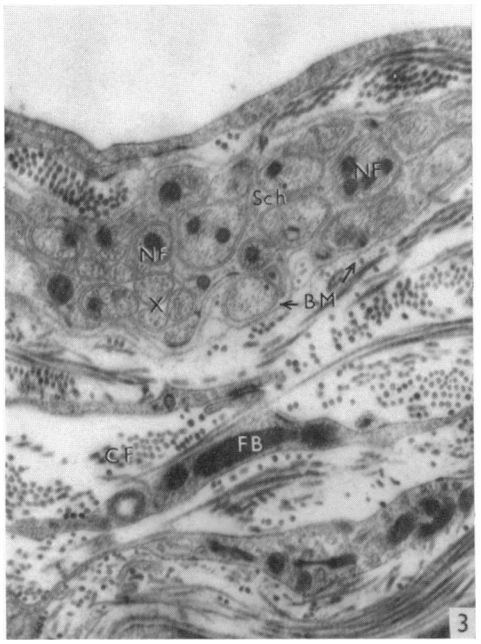
Fig 2. A reconstruction of a transverse section of the basilar artery of the rat showing the distribution of the nerve bundles.

Each nerve bundle is made up of an extremely variable number of neurites. In the sections examined the smallest bundle contains three fibres and the largest about thirty fibres. The diameter of these fibres range from 0·15 to 1·1 μ. The internal structure of the fibres does not vary from the structure of autonomic nerve fibres described by Gasser (1958), Elfvin (1958) and Richardson (1960). Figure 3 shows a typical example.

The intracerebral vessel

Penetrating arteries arise from the major distributing vessels lying in the subarachnoid space and have a short extracerebral course before entering the brain substance. As the innervation of the vessels within and outside the brain are being compared, it is essential to decide which part of these penetrating vessels can be defined as extracerebral and which part intracerebral and where, in fact, is the likely level of transition.

An attempt to answer these questions has been made by examining complete transverse sections of the penetrating arteries at varying depths.



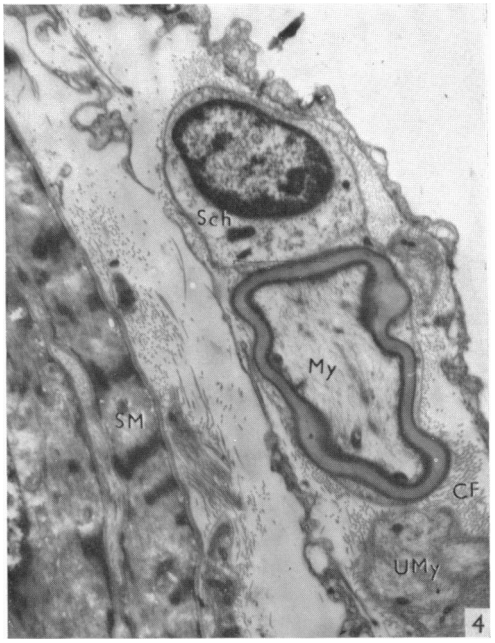
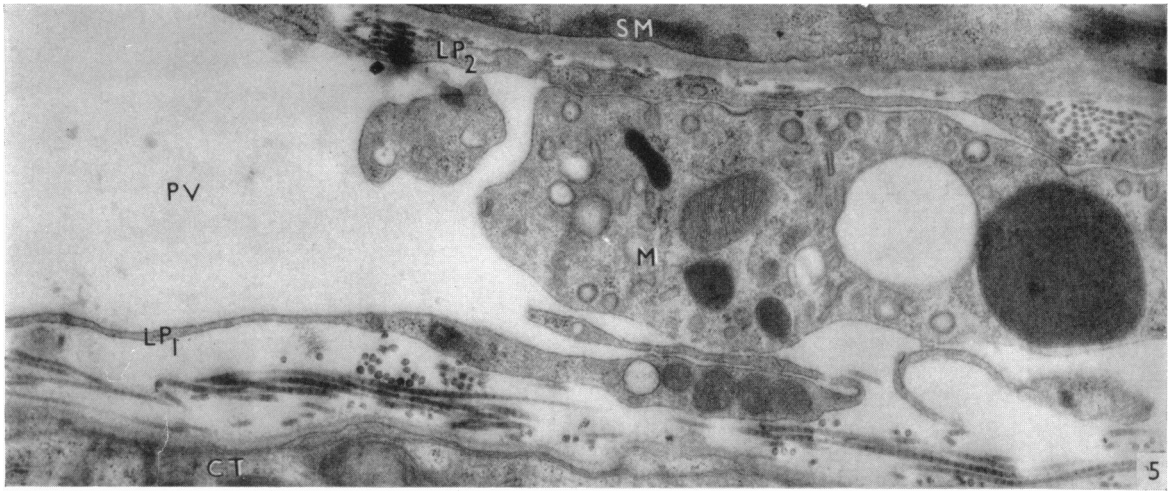


Fig. 3. An electron micrograph of one of the large nerve bundles accompanying the basilar artery. The bundle consists of a group of unmyelinated nerve fibres (NF) surrounded by a Schwann cell (Sch). At X are four nerve fibres within a single mesaxon. The basement membrane (BM) separates the nerve fibres from the fibrocyte cell-processes (FB) and the collagen fibres (CF). $\times 20,000$.

Fig. 4. A single myelinated nerve fibre (My) with its Schwann cell (Sch) lying adjacent to a group of unmyelinated nerve fibres (UMy) in the wall of the middle cerebral artery of the rat. CF = collagen fibres; SM = smooth muscle cell. $\times 6,000$.

The vessel (diameter, 20–45 μ) is built up of the usual three coats (Fig. 6). A fragmented layer of elastic tissue or a thick basement membrane separates the tunica intima from the tunica media. The media, approximately 0·8–2·0 μ wide is composed of 1 or 2 layers of smooth muscle cells and contributes most to the thickness of the arterial wall. The tunica adventitia is extremely thin (0·3 μ, approximately) and usually contains a single layer of fibrocytes which by previous interpretation can be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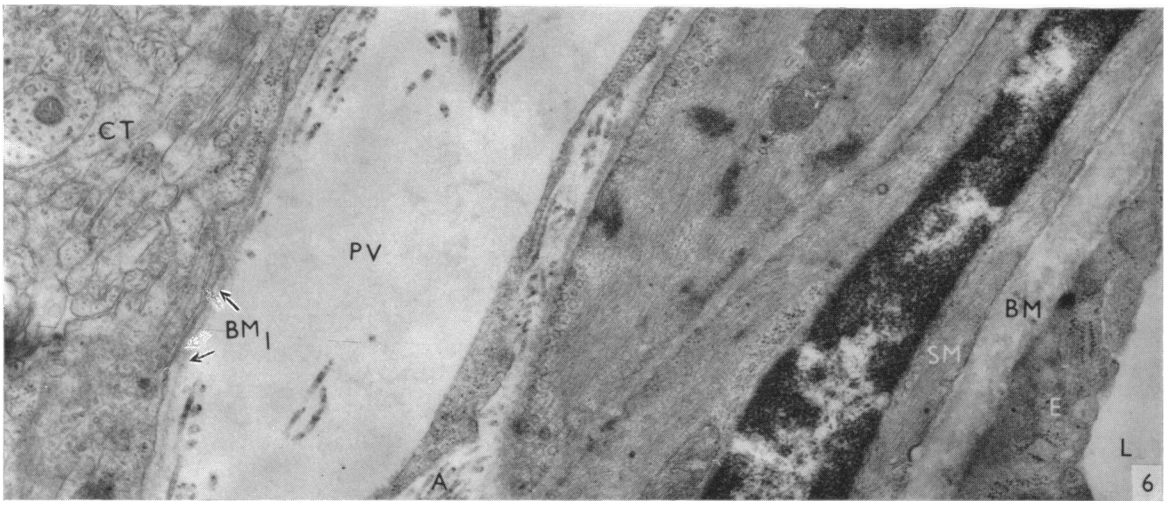
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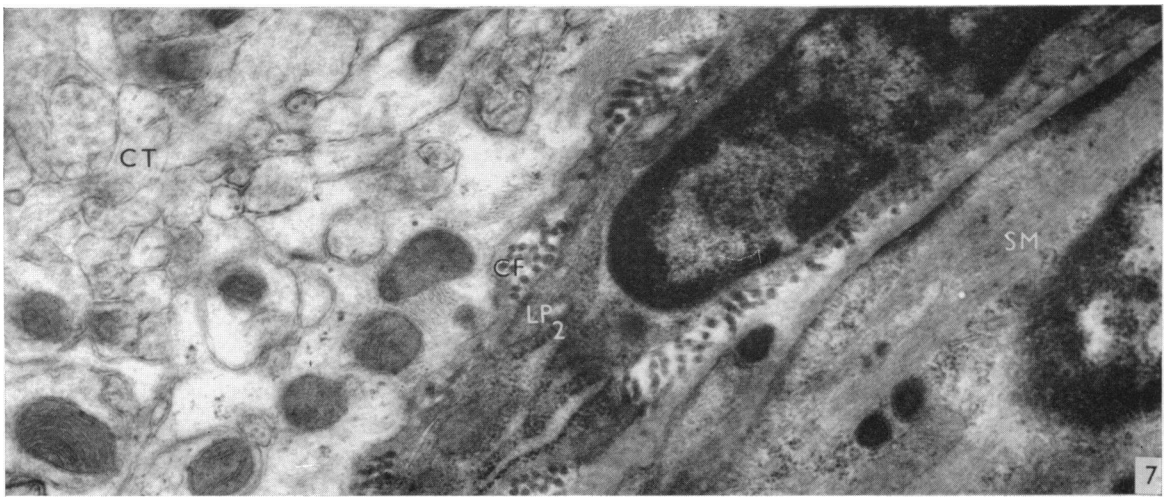
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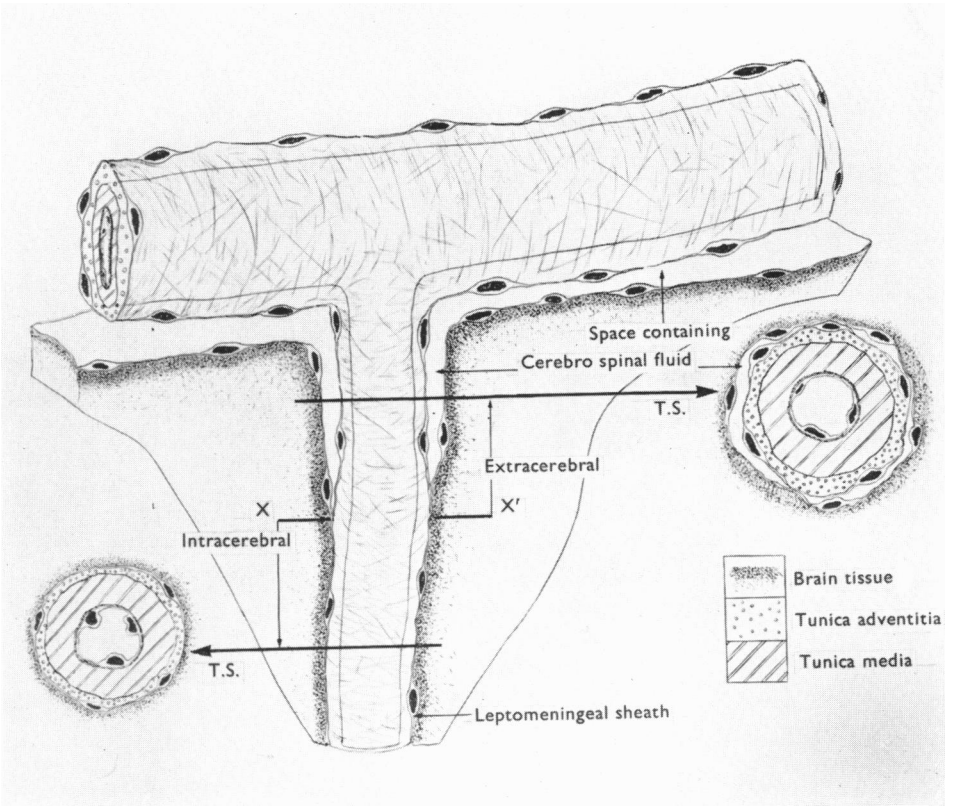
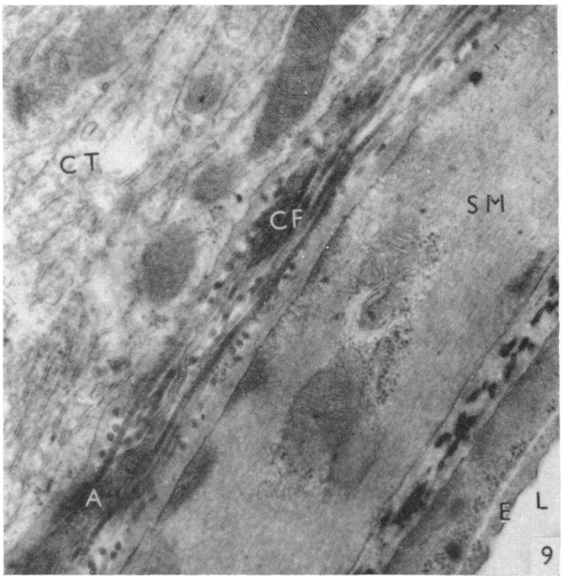


Fig. 8. A diagram representing the changes in the relation between a penetrating vessel, the leptomeninx and the neuropil. The perivascular space is in communication with the space containing cerebrospinal fluid and terminates at the level marked XX^1. The vessel above this level is defined as extracerebral and the vessel below this is intracerebral. Transverse sections of the extracerebral and intracerebral portions of the vessel are shown.

Fig. 5. An electron micrograph to show the relationship between the cerebral tissue (CT) and the proximal segment of the penetrating artery. The vessel is separated from the cerebral tissue by a perivascular space (PV) and is therefore regarded as extracerebral. This space is lined on the outside and the inside by a single layer of leptomeningeal cells (LP_1 and LP_2) and occasionally, as in this specimen, contains a monocyte (M). SM = smooth muscle cell. (Gluteraldehyde fixation). $\times 36000$.

Fig. 6. A section through the deeper part of the proximal segment. As in the previous figure, the perivascular space (PV) separates the vessel from the neuropil (CT) but at this level the outer limit of the space is defined by the basement membrane (BM_1) lining the cerebral tissue. The structure of the penetrating artery is illustrated. The smooth muscle cells (SM) are separated from the endothelium (E) by a basement membrane (BM). The adventitia (A) is extremely thin. (Gluteraldehyde fixation.) $\times 35,000$.

Fig. 7. An illustration of the close relationship between the penetrating artery and the neuropil (CT). The two structures are not separated by a perivascular space and the vessel is considered to be truly intracerebral. LP_2 = leptomeningeal cell; CF = collagen fibres; SM = smooth muscle cell. (Gluteraldehyde fixation.) $\times 21,000$.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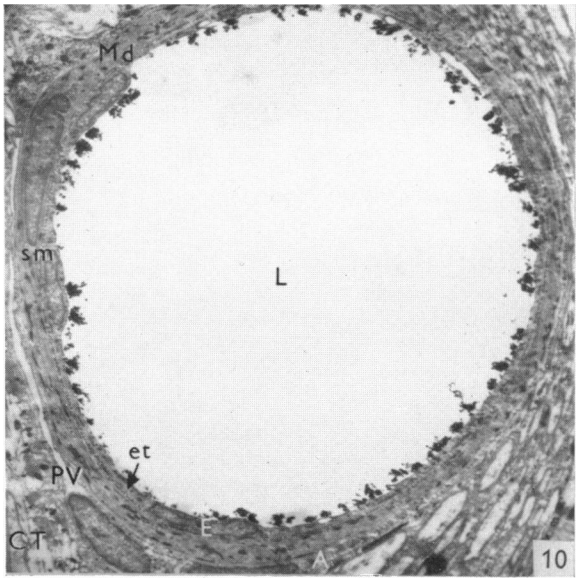
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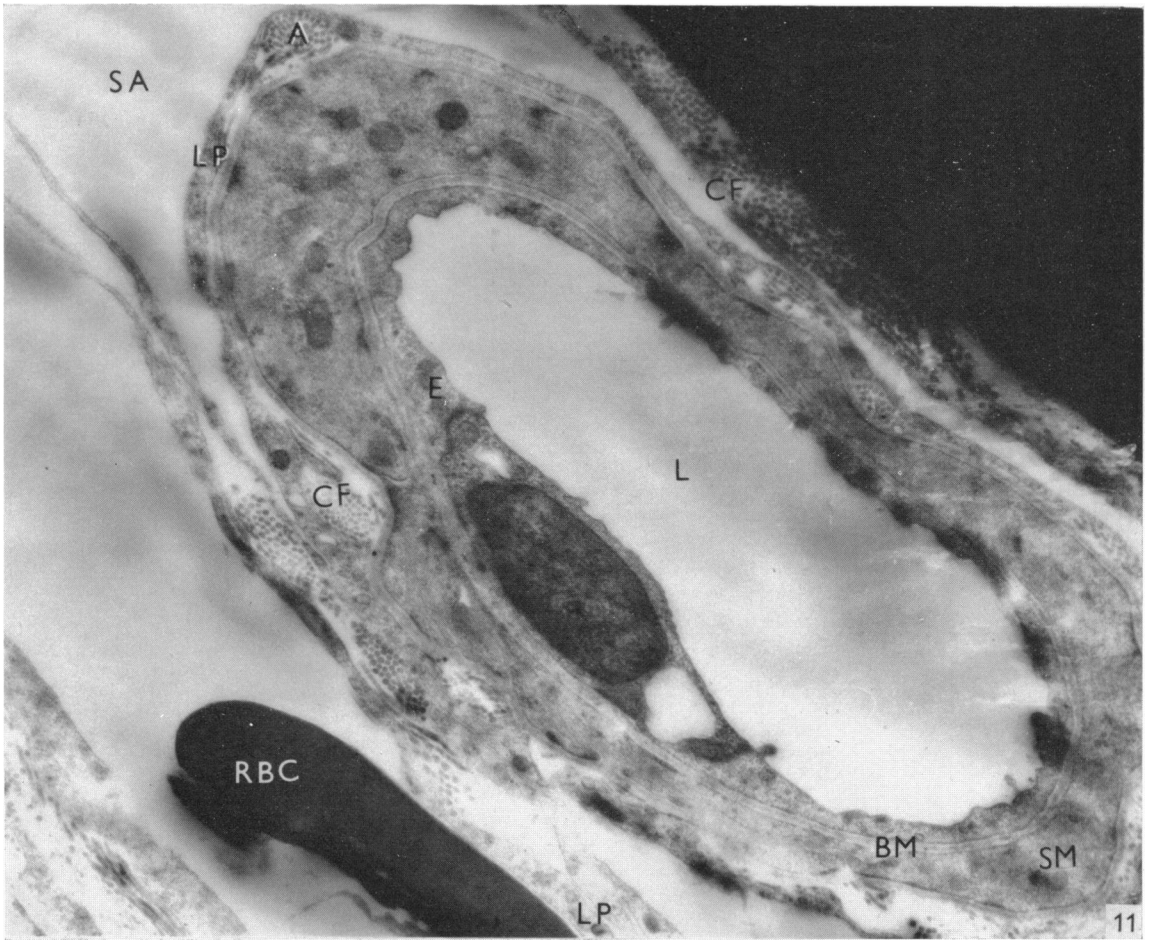
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regarded as an extension of the leptomeningeal sheath of the parent vessel. Collagen fibres appear between the sheath and the smooth muscle of the media.

A well-defined space separates the penetrating vessel from the surrounding cerebral tissue (Figs. 5, 6). On the inside the space is limited by the leptomeningeal sheath of the vessel. On the outside it is marked by different structures depending on whether the cross-section is through superficial or deeper portions of the artery.

In the superficial part the outer limit is defined by a single layer of leptomeningeal cells—the pia mater (Fig. 5). This cell layer disappears in the deeper parts and leaves the basement membrane of the cerebral tissue as a boundary (Fig. 6). The change seems to be a gradual one as a single cross-section may sometimes show both arrangements. Collagen fibres are scattered between the basement membrane of the cerebral tissue and the pial cells but at sites free of cells the collagen lies within the space, in the vicinity of the basement membrane. Similar spaces are observed around seven central arteries of the basal ganglia in four rats. Their limits are always clearly defined. There is no disruption of the leptomeningeal cells or their processes. Nor are there tears in the astrocyte cell-membranes as described by Nelson, Blinzinger & Hager (1961). These spaces are therefore regarded as true perivascular spaces.

There are no trabeculae traversing the perivascular space though rarely a monocyte may be found (Fig. 5). The space is not of uniform width. In the present series the largest gap between neuropil and vessel is about 8 μ. The space is reduced as the artery goes further into the neuropil. Ultimately the space is obliterated with the coming together of the leptomeningeal sheath of the artery and the cerebral tissue (Figs. 7, 9). Figure 8 is a diagrammatic representation of the changing relationship between a penetrating vessel and the neuropil. There is no direct relationship between the vessel and the neuropil where there is a perivascular space. A close relationship is achieved only with the obliteration of this space. The level of transition from extracerebral to intracerebral, therefore, is defined as the termination of the perivascular space, which may or may not coincide with the beginning of the cortical surface. It is proposed that the penetrating artery beyond this point of transition be taken as intracerebral.

Intracerebral arteries selected on the above basis have been examined for nerve fibres. The tunica adventitia is the layer of special interest, as it is here (Penfield, 1932; Chorobski & Penfield, 1932) that nerves are said to be demonstrable.

Figures 7 and 9 illustrate segments of the adventitial coat from two intracerebral

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Fig. 9. A portion of an intracerebral arteriole showing the contents of the adventitia (A) and its relationship to the neuropil (CT). CF = collagen fibres; SM = smooth muscle cell; E = endothelium; L = lumen. (Gluteraldehyde fixation.) ×19,000.

Fig. 10. A complete transverse section of a penetrating artery. The vessel is directly related to the neuropil (CT) except at PV. The adventitia (A) is extremely thin. The media (Md) is composed of a single layer of smooth muscle cells (SM) and is separated from the endothelium (E) by a fragmented elastic tissue layer (et). Along the lumenal border is a precipitate of osmium. ×1,850.

Fig. 11. A cross-section of an extracerebral arteriole from the subarachnoid space (SA). The tunica media is made up of a single layer of smooth muscle cells (SM). The adventitia (A) is limited externally by a single sheath of leptomeningeal cells (LP). The leptomeninx, tunica adventitia and the tunica media do not contain any nerve fibres. L = lumen of vessel; E = endothelium; BM = basement membrane; CF = collagen fibres; RBC = red blood cell. ×15,000.

arteries. This layer contains collagen fibres and leptomeningeal cell-processes. Complete transverse sections of twelve intracerebral arteries (Fig. 10) from five rats have been examined for nerves but none are found.

From these observations it is clear that there is a difference in the distribution of nerves around extracerebral arteries and intracerebral arterioles. The extracerebral segments of the penetrating arteries are also free of nerve fibres but the more surprising observation is the absence of nerves in small extracerebral arterioles in the subarachnoid space. A cross-section of a vessel from the cisterna pontis is illustrated in Fig. 11 and its approximate position in relation to the basilar artery is shown in Fig. 8. So far only a few of these vessels having a diameter of about 10–20 μ have been examined but none has been found to contain any longitudinally running nerve fibres.

DISCUSSION

In this study care has been taken to distinguish between the extracerebral and intracerebral segments of the penetrating artery and also to define a probable level of transition.

The extracerebral part has been recognized by the presence of a well-defined perivascular space which separates the artery from the cerebral tissue. In its intracerebral course the vessel is not surrounded by a space and is related directly to the neuropil.

All the vessels have been examined in transverse section as this seems the most logical way of visualizing all the longitudinally running nerve fibres. If there are no nerve fibres in a complete cross-section, it is very likely that the artery concerned is not innervated. Until now most of the information regarding the nerves accompanying the cerebral arteries has been obtained by examining whole tissue preparations under the light microscope, but due to limitations in technique it has not been possible to define clearly the relationship between the nerve fibres and the constituents of the arterial wall, nor has it been possible to estimate the density of the accompanying nerve plexus. There have been a few attempts to examine transverse sections of the cerebral arteries under the optical microscope (Clark, 1934; Christensen, Polley & Lewis, 1952) but the recognition of the related nerves seems to have been largely dependent upon the doubtful specificity of stains employed and not on morphological characteristics. Moreover, it is not possible to estimate the number of fibres in a single bundle because a large proportion of the neurites are about 0·1 μ in diameter and are therefore beyond the limits of optical resolution. These difficulties are overcome by examining the sections under the electron microscope. Due to the high resolution of the instrument, it is possible to differentiate nerves from other cell types with greater accuracy and also to count the number of neurites within a single bundle.

This study has confirmed the presence of nerve fibres around the major extracerebral arteries. The most impressive feature about the distribution of these nerves is their restriction to the tunica adventitia. No nerve fibres are seen within the tunica media in over 125 sections of the cerebral arteries examined under the electron microscope. Similar observations have been made in the cerebral arteries in the cat and monkey (Pease & Molinari, 1960) and in man (Dahl & Nelson, 1964) and also in the thoracic aorta of the rat (Pease & Paule, 1960) and in the femoral artery of the mouse (Rhodin, 1962). The only exception seems to be the specialized sheath artery of the spleen. In

this vessel the muscle cells are shown to be richly provided with nerve endings (Rhodin, 1963).

A different arrangement has been observed in the viscera. The nerve bundles are distributed among the smooth muscle cells and are thought to release transmitter substances at intervals along their length (Caesar, Edwards & Ruska, 1957; Richardson, 1958, 1960; Taxi, 1961). Discrete nerve endings have also been described (Richardson, 1962; Thaemert, 1963; Merrillees, Burnstock & Holman, 1963). It would therefore appear that the nerves accompanying the cerebral vessels may be subserving a different function or that they may be having special points of contact with the outer layer of smooth muscle cells of the media.

The majority of the nerve fibres in the adventitia are non-myelinated. They are grouped in fascicles of varying size with the larger bundles occupying a peripheral position. Their structure is similar to non-myelinated fibres in other parts of the body (Gasser, 1958; Elfvin, 1958) and they are associated with the Schwann cells in the typical fashion. Some of the neurites are more or less completely enveloped by the Schwann cell cytoplasm. Others are only partly invested, allowing the Schwann cell basement membrane to cover the exposed surface of the nerve fibres.

One or two myelinated fibres have been observed in the adventitia of the middle cerebral artery. This finding is not unexpected, as myelinated fibres have been demonstrated previously under the light microscope (Huber, 1899; Clark, 1934) and this has recently been confirmed under the electron microscope by Dahl & Nelson (1964).

Serial sections of the basilar artery have been examined and it is found to have 160–180 nerve fibres arranged in sixteen bundles. The middle cerebral artery is accompanied by about eight bundles.

A few small extracerebral vessels in the subarachnoid space and a dozen intracerebral vessels have also been examined in transverse section. These vessels have a structure similar to arterioles in other parts of the body (Moore & Ruska, 1957; Zelander, Ekholm & Edlund, 1962) and are not accompanied by nerve fibres. This is not in agreement with the findings of Clark (1929, 1934), Penfield (1932), Chorobski & Penfield (1932) and Humphreys (1939). These authors claim that the arteries entering the central nervous system are innervated.

The first and more obvious explanation for this discrepancy would be to relate it to a species difference. This possibility cannot be ruled out as a comparative study has not been made but it may be of some significance that Penfield (1932) has been unable to find any evidence of a morphological difference in the innervation of intracerebral arteries in cat, dog, monkey (*Macacus rhesus*), chimpanzee and man. Secondly, in previous investigations intracerebral vascular nerves have been demonstrated only by metallic impregnation methods—a series of modified silver techniques—and has yet to be confirmed by any other neurohistological staining procedure. Refinement of composition and reaction of fixative, which are now known to play a key role in differentiating nerve fibres from argyrophilic connective tissue (Samuel, 1953; Richardson, 1960), have not been taken into account and it seems likely that the stained strands in these investigations may not have been nerve fibres.

The significance of the present findings may be assessed in relationship to vessels described as arterioles in other parts of the body. According to the present view, the

term 'arteriole' is reserved for vessels primarily concerned in the maintenance of peripheral resistance. Such vessels owe this property to its dominant constituent, the smooth muscle cell, which is thought to possess an inherent or basic level of constriction wrought by two distinct mechanisms—nervous and chemical (Lamport, 1955).

A neural mechanism of control can be suggested only for arterioles accompanied by nerve fibres. Recent electron microscope studies have shown this to be true for the arterioles of the ear (Appenzeller, 1963), the pancreas (Lever & Esterhuizen, 1961; Zelander, Ekholm & Edlund, 1962), the spleen (Rhodin, 1963) and the choroid (Feeney & Hogan, 1961).

In contrast, there are no nerve fibres around the intracerebral and extracerebral arterioles so far examined. Similar observations have been made in the retinal arterioles in man, monkey and rat (Hogan & Feeney, 1963). Thus it seems likely that the calibre of the extracerebral and intracerebral arterioles is not modulated by a neural mechanism as proposed by Chorobski & Penfield (1932), Clark (1934) and Humphreys (1939), but that in all probability it is determined by the tone of the vessels controlled by a chemical mechanism as originally suggested by Roy & Sherrington (1890).

The major extracerebral arteries, on the other hand, are surrounded by a dense nerve plexus. Little seems to be known about their fate at present.

In this connexion, Pease & Molinari (1960) have expressed the view that the smooth muscle of the media is not subjected to a direct nervous stimulation. This cannot be ruled out, as bulbous neurites packed with vesicles and mitochondria have been observed near the outer layer of smooth muscle cells (Samarasinghe, 1963). Dahl & Nelson (1964) have reported specialized sensory endings comparable to Meissner's corpuscles.

SUMMARY

1. The distribution of nerves in the wall of the extracerebral and the intracerebral arteries in the rat has been studied under the electron microscope.

2. It is suggested that the penetrating artery extending beyond the termination of the perivascular space be defined as intracerebral and up to that point as extracerebral.

3. In the large extracerebral arteries nerve fibres are found only in the tunica adventitia. There is no evidence of nerve fibres within the media or in the intima. The majority of the nerve fibres are unmyelinated and are invested by Schwann cells in the typical fashion. There are one or two myelinated nerves around the middle cerebral arteries.

4. The intracerebral vessels are not accompanied by any nerve fibres. The tunica media is built up of one or two layers of smooth muscle cells and structurally they are similar to arterioles in other parts of the body.

5. Extracerebral arterioles, with a single layer of smooth muscle cells, do not have any nerve fibres around them. Thus it appears that neither the extracerebral nor the intracerebral arterioles are under the influence of a neurogenic controlling mechanism.

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