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# The Glio-Vascular System of Cephalopods

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# THE GLIO-VASCULAR SYSTEM OF CEPHALOPODS

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CONTENTS



The branches of the cerebral arteries run to the centre of each lobe of the brain and from there radiate outwards. The arteries are lined by endothelial cells, surrounded by pericytes. Outside these are large extracellular spaces containing collagen. These spaces continue as a system of 'glio-vascular' channels among the tissues. These channels contain collagen and other extracellular material and nuclei belonging probably to muscle cells and fibroblasts. This system permeates the neuropil and in the cell layers provides wrappings for the perikarya. The channels and extracellular material form tunnels of 'trophospongium' within the neuronal cytoplasm. Glial fingers also penetrate into these channels but are not well seen by light microscopy. The system of spaces among the tissues communicates with an elaborate set of branching 'lymphoid' channels. These collect into veins either in the membrane around the brain or at the centre of the optic lobe. The veins discharge to the pharyngo-ophthalmic vein and the brain is thus provided with a venous system partly isolated from the main haemocoele.

The true neuroglia cells are of two types. The protoplasmic glia cells have much-branched processes, especially in the neuropil. They have abundant end-feet attached to the outside of the perivascular channels and to the glio-vascular strands. They occur in the cell layers and their processes probably penetrate the neurons.

The fibrous glia have very long processes, mostly smooth and with little branching. The processes run in bundles accompanying the main tracts of nerve fibres in the neuropil and extending into the cell layers.

## 1. Introduction

Surprisingly little is known about the tissues that support and nourish the nerve cells of cephalopods. Van Lenhossèk (1896) described bushy glia cells in the plexiform zone of the optic lobes. He remarks that this was the first clear demonstration of branching neuroglia in an invertebrate. These formations were seen again by Cajal (1917) and Young (1962), but could not be found by Kopsch (1899) or Jakubski (1915). Cajal also described 'epithelial cells' surrounding the neurons of the outer granular layer of the optic lobe and 'astrocitos de tipo protoplasmico' throughout the lobe. These cells have been seen during the present work and will be called protoplasmic glia. They are characterized by their extensive branching. In addition, a further type of glia cell is

described, with very long processes, little branched. These will be called fibrous glia. They have not been described in detail before but are figured in the optic lobe in Young (1962).

A third type of glial tissue is the so-called fibrous astrocyte first described by Garaieff (1909) around the nerve perikarya and penetrating into them and later shown by Jakubski throughout the cell layer and neuropil. Bogoraze & Cazal (1944) were the first to realize the close relationship of this tissue to the blood vessels, and they described it as constituting a 'reseau glio-vasculaire', composed of 'formations vasculaires, de formations gliales et surtout de structures dont la nature vasculaire ou gliale est difficile à préciser'. This is an acute analysis of the problem of this enigmatic tissue. It is doubtful whether it is truly of 'glial' nature (whatever 'truly glial' may mean).

The problem of how the circulating blood nourishes the nervous tissue seems never to have been attacked in cephalopods. The structure of the vessels is known (see Barber & Graziadei 1965, 1967*a*, *b*) and the blood finally emerges into the large venous sinuses. There is no clear information about the capillary circulation or smaller veins. It is still not possible to solve this problem fully, but it will be shown that there is an elaborate system of tissue spaces throughout the neuropil and around the larger nerve cell bodies. These spaces communicate with a well-developed closed venous system. It is uncertain how these spaces become filled from the circulation and they will provisionally be called lymphoid channels. The various tissues described here as seen with the light microscope are described as seen by electron microscopy in the following paper by Gray (1969).

## 2. Methods

The greater part of the observations was made with material prepared by Golgi techniques, particularly using the Kopsch modification (Young 1962). This often stains blood vessels of all sizes and also the glia cells that will be described here. The network of glio-vascular channels and cells was stained by various modifications of the Bielschowsky, Hortega and other silver methods. The distribution of connective tissue was shown by Mallory and Masson staining after fixation in Bouin's fluid and by Laidlaw's and Wilder's techniques with formol-fixed material. Some animals were injected with suspensions of indian ink in sea water, through the cephalic aorta.

## 3. Blood vessels

The arterial supply passes mostly to the centre of each lobe and from there vessels proceed outwards. For example, large vessels run in the centre of the subvertical lobe, giving branches up and down within that lobe and others reaching up into the vertical lobes (figure 1, plate 1). These primary branches then divide repeatedly, mainly dichotomously, until they end as fine branches running out through the cell layers, many reaching to the surface. The courses of the vessels show various loops, and lateral anastomoses between vessels are common.

At the surface of the brain the vessels of many of the lobes (e.g. vertical lobe) join a network of veins within the cerebral membrane (figures 3, plate 1, and 23, plate 3)


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(see Barber & Graziadei 1967*a*). These veins ultimately discharge into the pharyngo-ophthalmic vein (Young 1969). Since they do not open into the large sinuses the cerebral circulation is thus isolated from venous pressure changes produced by compression of the large, open haemocoelomic spaces. In the optic lobes the venous drainage is only partly outwards to the membranes and mainly back towards the centre of the lobe. There is thus a fully developed set of veins leading to large venous channels at the centre of the lobe (figure 2, plate 1). The main arterial capillaries join the venous capillaries, which are more curled. The larger venous channels may broaden where two (or more) unite. The main veins at the centre of the lobe discharge not into the orbital sinus but, like the cerebral veins, to the pharyngo-ophthalmic vein.

Connected with the veins there is an elaborate system of intercellular spaces (figures 3, 4, plate 1). These form a fine network throughout the neuropil and a system of channels around the nerve cells. They can be filled with indian ink by injection through the aorta, but it is not certain that they are normally filled with blood from the arteries; blood-filled spaces have been recorded around the cells (for instance in the vertical lobe, Barber & Graziadei 1967*a*); but it may be that this elaborate system of channels receives fluid by passage through the capillary walls under pressure of the ventricular beat and then serves to return it to the veins. Such a system would have some parallel in the vertebrate lymphatic system.

It remains uncertain how the circulation proceeds, but these channels certainly play an important and previously unsuspected part. In addition to the larger openings into the veins, such as are seen in figure 3, injection sometimes shows narrow channels passing presumably between the endothelial cells and through the basement membrane and between the pericytes to the intercellular spaces (figure 5, plate 1). The normal direction of flow in these channels remains to be discovered.

It should be noted that this system is not the same as the set of 'glio-vascular channels' described by Bogoraze & Cazal and others and the relations of the two are discussed later. The system of 'lymphoid' channels is certainly very extensive and well defined, although not limited by any distinct membranes or set of cells. The system is often stained by Golgi methods (figure 6 and 7, plate 1). In the neuropil it appears as a set of fine branching channels, radiating around a larger vessel and apparently ending blindly (figure 6). In the cell layers it may appear as sheets of material, stained black, and connected by fine channels with the veins (see Young 1969).

The structure of the walls of the arteriolar vessels has been described by Barber & Graziadei (1965, 1967*a*, *b*). There is a discontinuous endothelium and a thick continuous basement membrane, around these a layer of pericytes, sometimes containing myofilaments, surrounded in turn by connective tissue, at least for the larger arteriolar vessels. The walls of the smaller vessels may consist only of discontinuous endothelium, basement membrane and pericytes.

Some features of the pericytes can be seen in the Golgi preparations. They appear as bands around the larger vessels, sometimes ending as flattened branched leaflets (figure 8, plate 1). In the smaller vessels the cells form extended webs over the surface and nuclei are seen at intervals (figure 9, plate 1).

In some preparations stained with Cajal's sublimate, gold–chloride method, systems of




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fibres running spirally round and along vessels have been seen (figure 10, plate 1). The nature and significance of these bands is not clear. They may be muscular. Bands seen in the same preparations running longitudinally are presumably ridges of the endothelial cells (figure 11, plate 1).

The endothelial nuclei have a smoothly oval outline and are densely packed with fine granules. They are as much as 20 $\mu$m long and 5 $\mu$m wide, but some are smaller. The pericyte nuclei are also flattened ovoids, but rather smaller (up to $15 \times 5 \times 3$ $\mu$m). They have a characteristic appearance with large scattered chromatin granules. They may adopt various shapes other than a simple oval (figure 12).

Accompanying the pericytes in the material around the vessels is much collagen and other extracellular material (figures 12, 13, plate 2). Presumably this is produced by fibrocytes, but these have not been specifically identified with the light microscope.

## 4. The three types of glial cell

Three further systems can be recognized in the cell layers and neuropil and some or all of them presumably fulfil the functions of carrying materials between the vessels and the nervous tissues. It is not easy to know whether all the types should be included in the category 'neuroglia', a term that is in any case somewhat vague even in vertebrates and still more so among invertebrates. We shall therefore apply it to all three types found here. They will be categorized as (1) the glio-vascular tissue (Bogoraze & Cazal 1944); (2) protoplasmic glia ('astrocitos de tipo protoplasmico', Cajal 1917); (3) fibrous glia (here fully described for the first time but figured in the optic lobes in Young (1962). A further category of enigmatic 'dark cells' has been seen by electron microscopy (Gray 1969).

## Description of plate 1

Figure 1. Sagittal section of vertical and subvertical lobes to show main vessels. Golgi.

Figure 2. H.S. optic lobe, showing arteries, veins and capillary circulation. Golgi.

Figure 3. T.S. pedal lobe after injection through the aorta of indian ink, formol fixation and staining with v. Gieson's method. The veins are filled and communicate with a system of 'lymphoid' channels in the cell layers.

Figure 4. T.S. pedal lobe after injection of indian ink and staining as figure 3. The veins and some of the lymphoid channels are filled in the neuropil and cell layer.

Figure 5. T.S. of a medium-sized vessel (*bl.*, ? a vein) of the optic lobe after injection as in figure 3. The ink is escaping through channels in the wall of the vessel and enters a system of spaces (*sp.*) around the small neurons.

Figure 6. Network of lymphoid spaces in vertical lobe communicating with a vessel. Golgi.

Figure 7. Details of network of lymphoid spaces in dorsal basal lobe. Golgi.

Figure 8. Medium-sized vessel to show pericytes. Golgi.

Figure 9. Small vessel, showing pericyte. Golgi.

Figure 10. Small vessel of optic lobe, showing spiral thickening. Sublimate, gold chloride.

Figure 11. T.S. vessel of optic lobe, showing ridged endothelium. Sublimate, gold chloride.



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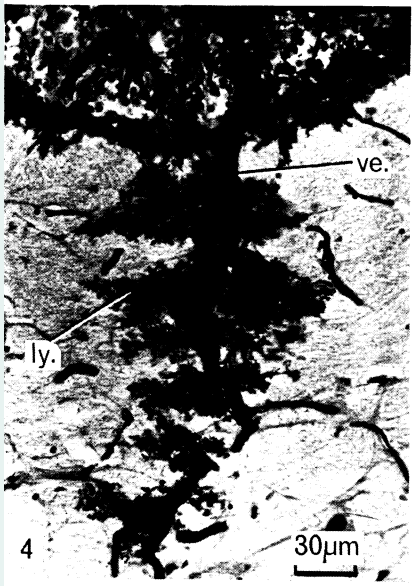


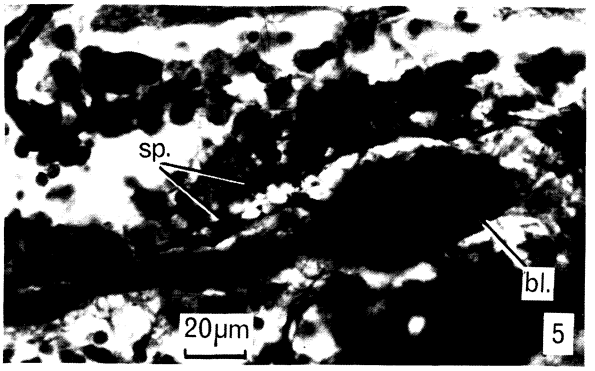


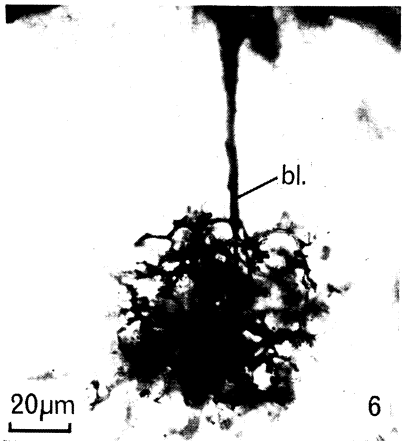


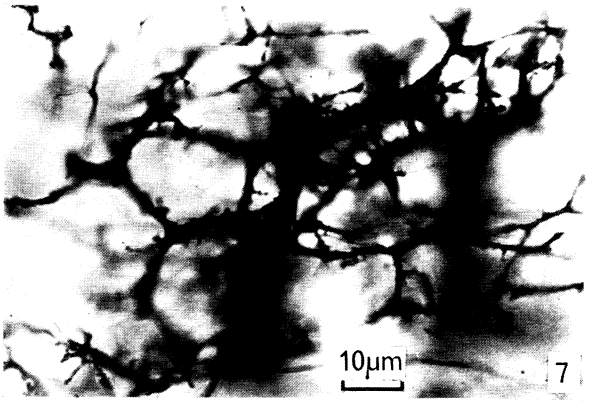


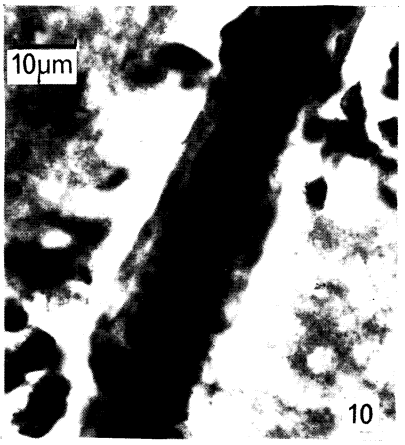



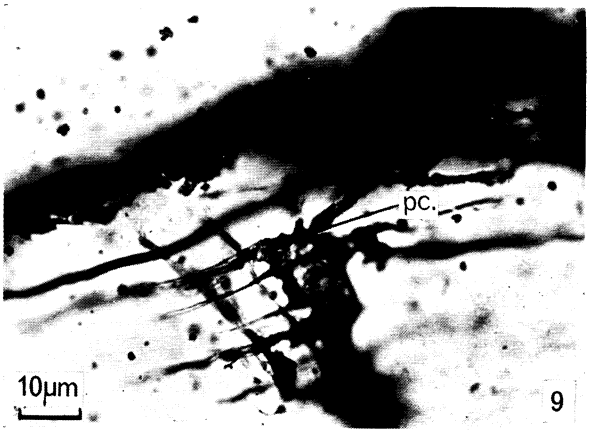


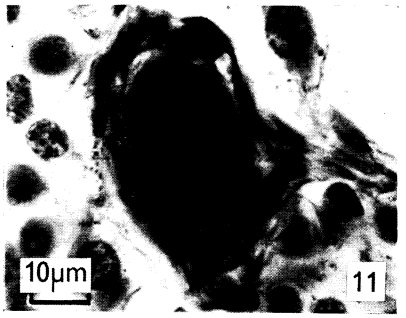


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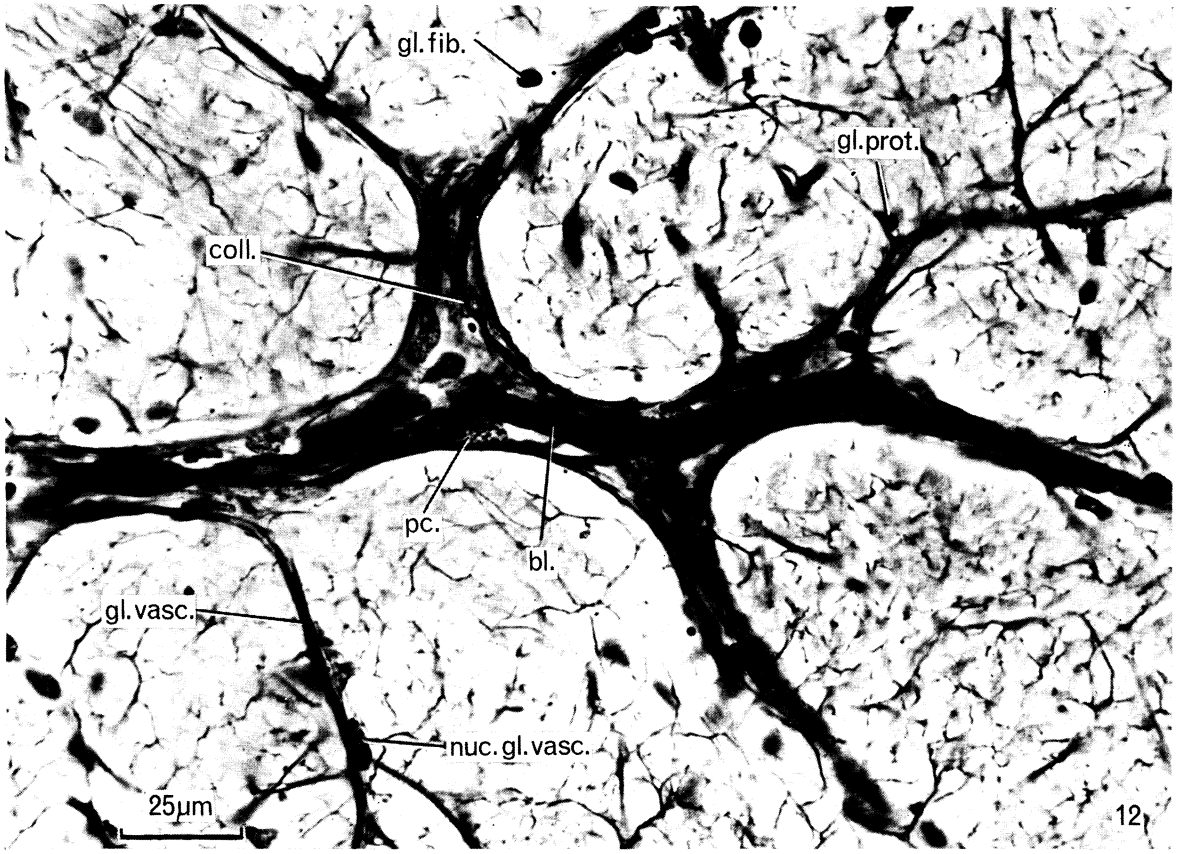


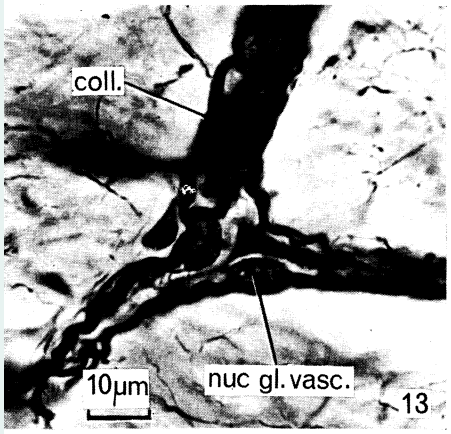


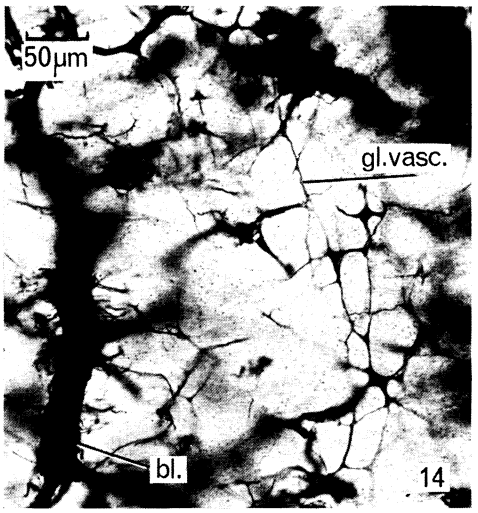


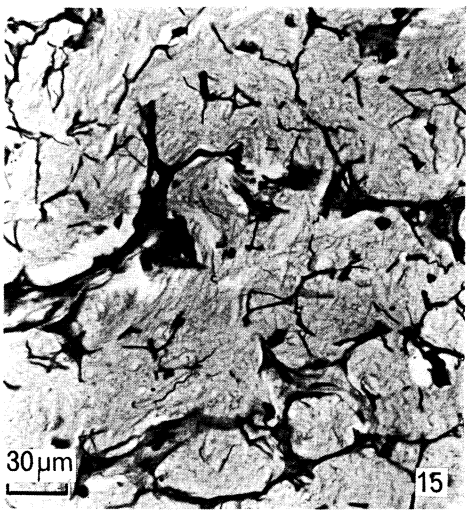


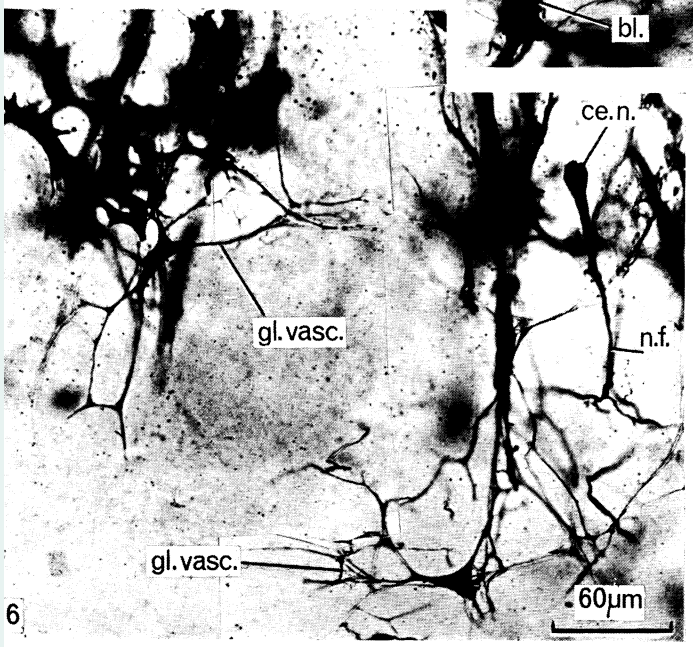


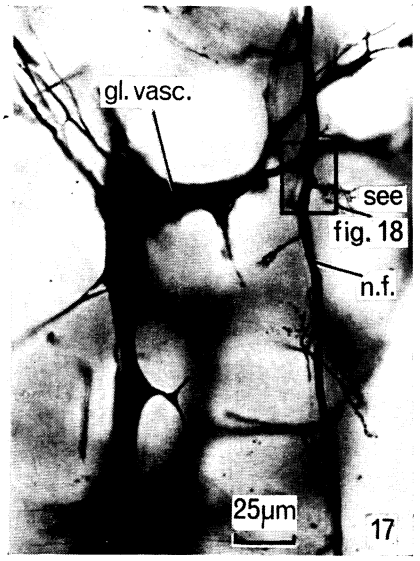


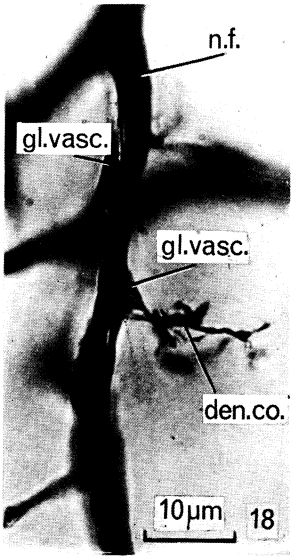

### 5. Glio-vascular tissue

Throughout the central nervous system and stellate and other ganglia there is a set of straight channels, distinct from the lymph channels (figures 12, 14, plate 2). These 'glio-vascular channels' are clearly also entities distinct from the branched protoplasmic glia, but like them they are attached to the perivascular spaces of the vessels. They often leave the vessels at right angles and may run straight for many tens of microns without branching. The glio-vascular channels occasionally carry a cell body and nucleus, from which four or five strands proceed in different directions (figure 16). Each of these strands in turn runs straight for a long distance before branching and eventually joining with another one or with a larger perivascular space. The finer branches of the tissue accompany finer branches of the neurons (figures 17, 18). The larger neuronal trunks lie within sheaths of this tissue. The finest dendritic collaterals in the neuropil may be accompanied by corresponding glio-vascular collaterals (figure 18), suggesting that the latter are performing some function of nourishment or support.

The appearance produced by this system is indeed of a 'supporting framework' throughout the neuropil and cell layers. The system is associated with much collagen (figure 15, plate 2, and figure 21, plate 3), which is connected with that around the larger vessels, but the system is not simply a set of collagen fibres. The strands are certainly not all cannulated but many are so. Some have terminal branches that end free in the tissues. The individual trunks of the tissue show irregular coarse granules, sometimes arranged as rows and giving no clear sign of a lumen. Where the strands join the main trunks it is clear that they are at least in part a continuation of the perivascular extracellular space (figure 12, plate 2). Their nuclei may adopt various shapes. At places where three strands join they may be triangular. They can be distinguished from the protoplasmic and fibrous glial nuclei by their larger size and coarsely granular chromatin.

Since this is clearly the tissue described by Bogoraze & Cazal as glio-vascular tissue this name will be retained. The system presumably functions for transport as these authors suggest. It is not clear whether any of the cells in this system are fibrocytic or muscular. It is probably associated with the muscle fibres seen in the neuropil with electron microscopy (Gray 1969). Nuclei that perhaps belong to muscle fibres are seen in figure 20, plate 3, associated with glio-vascular strands. The best interpretation is probably that the strands are canalized at least in part and that their finest branches

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### Description of plate 2

Figure 12. Small branching blood vessels of neuropil of pedal lobe, showing pericytes and glio-vascular channels and nuclei. Hortega.

Figure 13. Glio-vascular channels with collagen (*coll.*). Hortega.

Figure 14. Straight glio-vascular channels, also a vessel with attached glial end feet. Golgi.

Figure 15. Collagenous framework in neuropil of pedal lobe. Masson.

Figures 16, 17. Portions of the glio-vascular system. Golgi.

Figure 18. Part of field of figure 17, showing collateral dendrite (*den. co.*) of a nerve fibre (*nf.*) with a branch of the glio-vascular tissue accompanying it (*gl. vasc.*). Golgi.



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serve to exchange fluid with the tissues. In some places there is contact between these straight fibres and branches of the system of tissue spaces described above. They are, however, very distinct systems as can be seen in figure 44, plate 5.

This glio-vascular tissue is abundant in the cell layers (figures 21, 22, plate 3). It is connected with the system of spaces around the cells (figure 24). It is here especially associated with collagen and other fibrous extracellular material (Gray 1969). This makes a framework connecting with the marginal system of vessels and fibres that lies between neuropil and cell layer (Jakubski 1915) and also with the surface membrane of the brain (figure 23). Each nerve cell body is surrounded by a web of fine fibrils (figures 25, 26). Some of these are certainly collagenous and belong to this glio-vascular system. Others are branches of the protoplasmic and fibrous glia cells, which also occur in the cell layers (see below). The exact relationship of the various types of glia in the cell layers to each other and to the neuronal cell bodies has still to be determined. There is certainly a great deal of collagenous tissue in the form of bundles and sheets of fibres around the cells and penetrating within them (figure 26). The relation of this system to that of the other sorts of glia remains to be determined.

The small cells of the vertical lobe may appear as if embedded in a stained matrix (figure 27). The nature of this is uncertain but perhaps represents the system of blood spaces. Barber & Graziadei (1967*a*) have shown that these small cells may be in direct contact with blood, without the intervention of other layers. Bogoraze & Cazal (1944) show a somewhat similar appearance and attribute it to an 'astrocyte protoplasmique satellite' (their figure XIII*d*).

### *Protoplasmic glia*

Staining with Hortega's method shows that attached to the blood vessels of the neuropil are cells with much branched, spreading processes (figures 28, 29, plate 4). These are clearly the 'protoplasmic astrocytes' of Cajal and of Borogaze & Cazal and will be called

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### Description of plate 3

Figure 19. Branches of the lymphatic system of channels (*ly*) for comparison with the neighbouring glio-vascular tissue (*gl. vasc.*). Golgi.

Figure 20. Nuclei of various types in neuropil, including probable muscle nuclei. Hortega.

Figure 21. Collagen and glio-vascular system of the cell layers and neuropil of pedal lobe. Hortega.

Figure 22. Collagen and glio-vascular channels surrounding neurons in the cell layers. These channels communicate with the larger vessels. It is not clear whether they are occupied by the protoplasm of glio-vascular cells or are simply spaces, stained by silver. Hortega.

Figure 23. Collagen of the glio-vascular channels and venous channels in the membrane around the brain. Masson.

Figure 24. Glio-vascular tissue from cell layer of suboesophageal lobe. Showing fibres and sheets partly surrounding the cells. Golgi.

Figures 25, 26. Fibres (probably of collagen) around suboesophageal neurons and penetrating within them. Bielschowsky.

Figure 27. Small neurons of vertical lobe embedded in intercellular material of uncertain nature (*i.c.*). Golgi.



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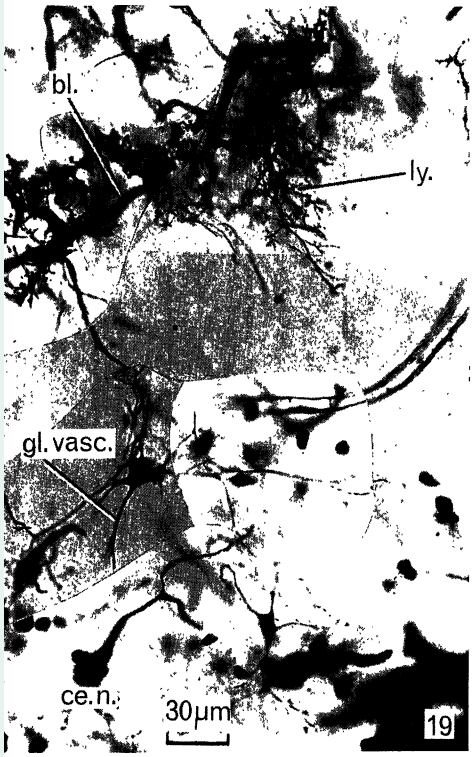


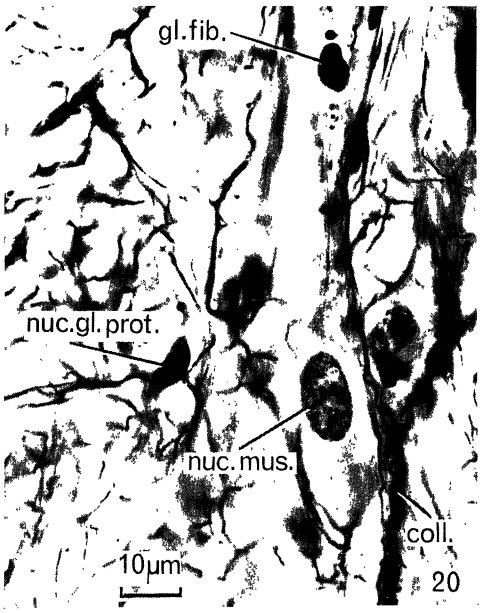


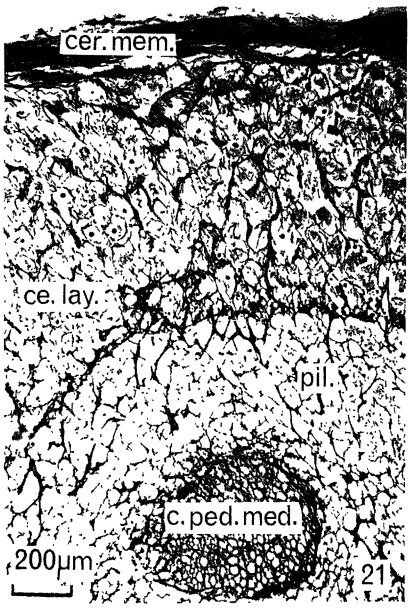


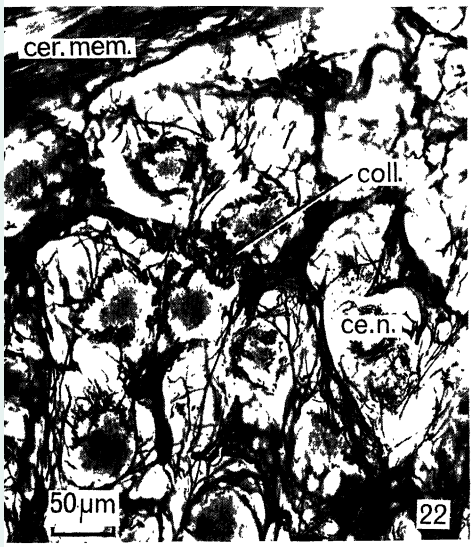


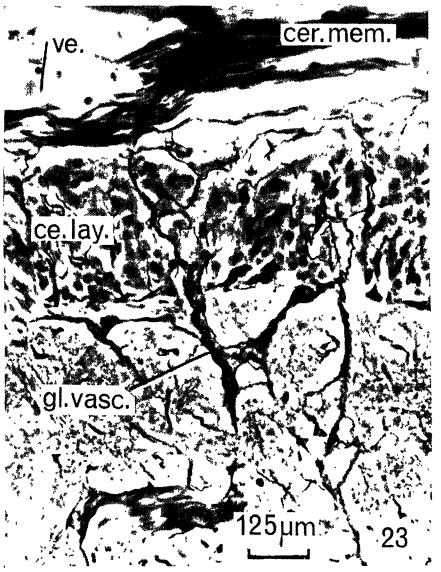


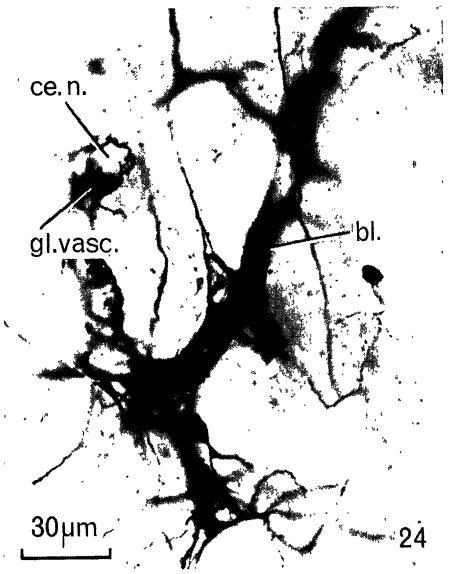


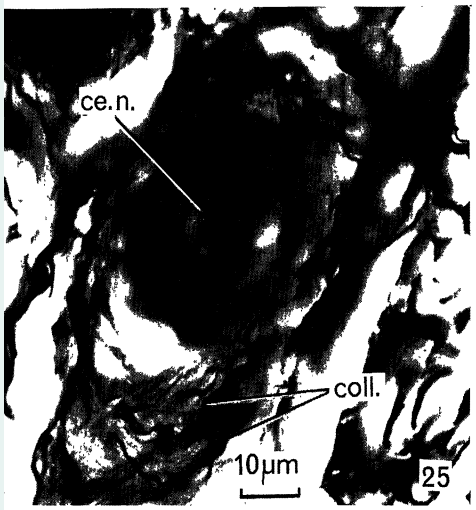


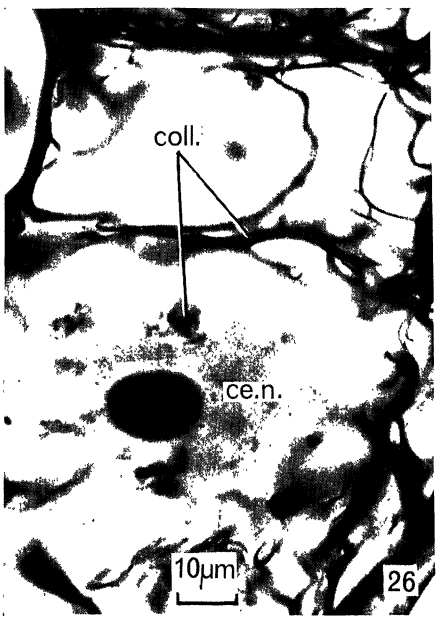


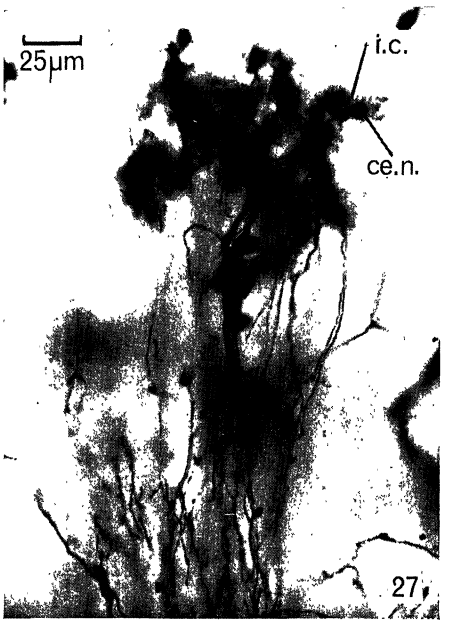


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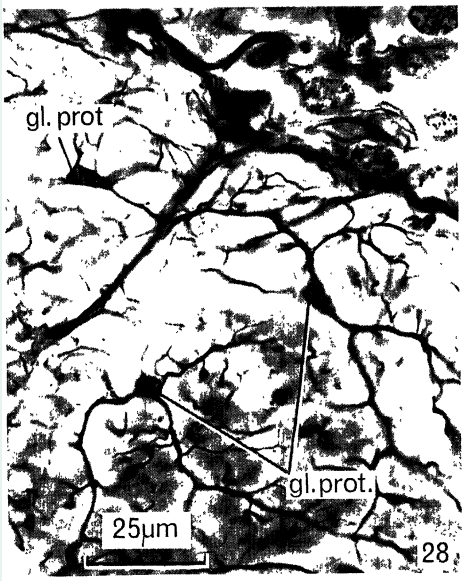


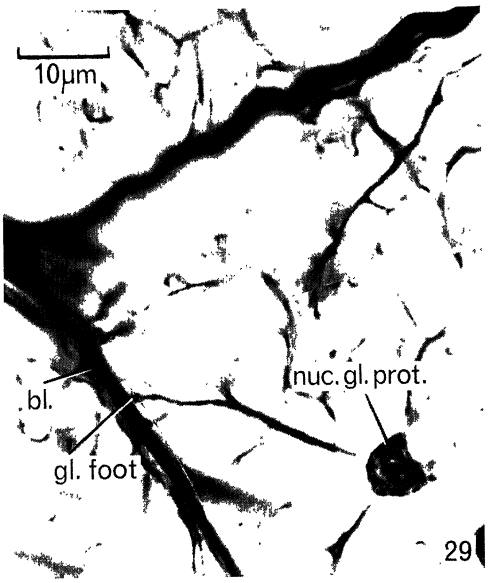



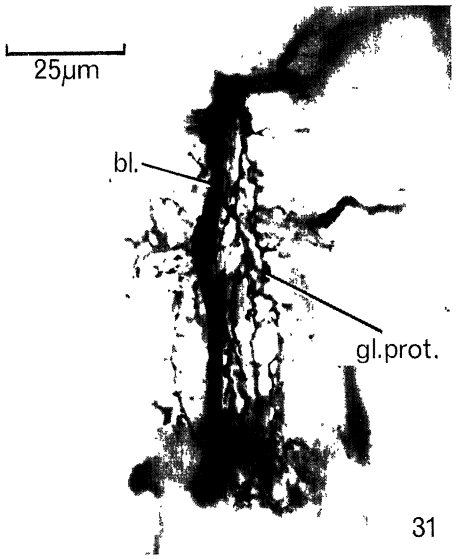


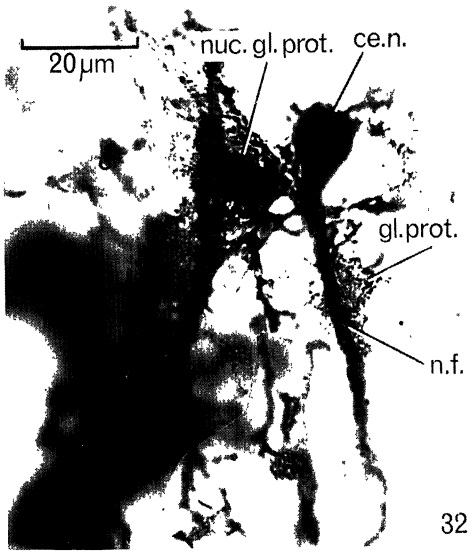



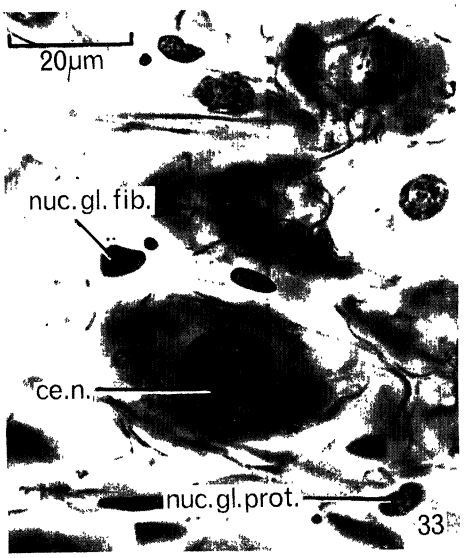





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here 'protoplasmic glia'. They have end feet attached to the blood vessels and to the glio-vascular strands. Their finest branches among the cells and neuropil are probably not shown by the Hortega method. The Golgi method shows more complete branching structures, which may be the same cells (figure 30, plate 4). In these preparations it is not easy to distinguish these branching cells from the branching lymphoid tissue spaces. Indeed it may be that the spaces penetrate these cells as they do the fibrous glia (see below) and hence adopt a similar shape. In the plexiform layer of the optic lobe the Golgi method shows branching systems that have been called glia since the work of Lenhossék (1896). As figure 31 shows, they are closely attached to blood vessels at the inner edge of the plexiform zone and their branches radiate outwards between the incoming optic nerve fibres. These structures have some resemblance to the protoplasmic glia but they have not been stained with the Hortega method so their nature remains uncertain.

Figure 32 shows the protoplasm of a cell of a different shape, wrapping around the trunk of a large nerve cell of the vertical lobe. This is also presumably a protoplasmic glia cell.

The protoplasmic glial cells have characteristic nuclei, generally darker staining than those of the pericytes and glio-vascular cells but with much smaller masses of chromatin (figures 28, 29). The nuclei are about $8 \times 4$ $\mu$m at most, smaller than those of the pericyte–glio-vascular system. They have also a characteristically irregular outline and shape, depending upon the origins of the main trunks of the cell. Thus they may be triangular or square, or of irregular shape with four, five or more points, each directed towards a major branch.

### 6. Fibrous neuroglia

In addition to the two types of glial cell already described the neuropil of all lobes of the brain contains fibrous glial cells. Some of them are huge cells with a central mass of diameter 100 $\mu$m, and fibres reaching for hundreds of microns from the cell body (figure 35, plate 4, and figure 36, plate 5). There may be 50 or more of such fibres, all arising from the centre and branching little, except for a few twigs towards the ends (figure 37).

### Description of plate 4

Figures 28, 29. Protoplasmic glial cells from neuropil of suboesophageal lobes. Hortega. Note end feet on vessels (*gl. foot*).

Figure 30. Branched protoplasmic glia cell attached to blood vessel. Subvertical lobe. Golgi.

Figure 31. Plexiform zone of optic lobe. Branched system previously called protoplasmic glia but probably branches of the vessel (*bl.*), at the inner end of the zone (above). Golgi.

Figure 32. Protoplasmic glia cell at margin between cell layer and neuropil of the vertical lobe. The glial protoplasm wraps around the cell body and process of a neuron. Golgi.

Figure 33. Two sorts of glial nuclei and fibres of glia and collagen in the cell layers of suboesophageal lobe. Hortega.

Figure 34. Large nerve cell body from suboesophageal lobe with fibres penetrating within it (*f.*). It is uncertain whether these are all collagenous or some glial. Hortega.

Figure 35. Large fibrous glia cell from subvertical lobe. Golgi.


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The fibres have in the main a smooth outline. Their diameters are about 1 $\mu$m and do not vary greatly. At intervals there are small collections of granules and these may dilate the fibres to bulbs as much as 5 $\mu$m in diameter. They may occur along the length of the fibre or at its end. Occasional short collateral branches with bulbs attached occur, but it is a striking characteristic of the branches of these fibres that they do not divide as do those of the protoplasmic glia.

These fibrous glial cells have been seen only in Golgi preparations. There is no sign of them in the Hortega preparations even when the protoplasmic glia is well stained. In these latter preparations there are, however, nuclei that can almost certainly be ascribed to the fibrous glia. They are small ($7 \times 4$ $\mu$m), have a regular oval outline and very fine granular chromatin (figure 38, plate 5). They are thus quite unlike the nuclei of either the pericytes, glio-vascular nuclei or protoplasmic glia. They usually occur alone in the neuropil, away from the vessels or glio-vascular strands. There are usually some granules staining darkly with silver at each end of the nucleus.

Where the neuropil is penetrated by tracts the fibrous glia form bundles accompanying the bundles of nerve fibres (figures 39, 40). Thus in the lateral inferior and superior frontal lobes they run in bundles as do the nerve fibres (figure 41). In the median inferior and superior frontal lobes they form interweaving bundles. There is thus little doubt that the special function of this type of glia is related to that of nerve fibres, rather than cells. Nevertheless, the fibrous glial fibres certainly extend into the cell layers, for instance in the vertical lobes. Some of the smaller fibrous glial cell bodies lie within the cell layers.

The neuropil of the median superior and inferior frontal lobes also contains smaller cells with little-branched fibres (figure 42). These may be in relation to the smaller bundles of nerve fibres.

There is abundant fibrous glia in the optic lobe. In the plexiform zone the cells lie mainly in the tangential plane (Young 1962, fig. 8). Bundles of glia fibres accompany the radial tracts of nerve fibres in the region deep to the plexiform zone. At the centre of the lobe the bundles of glia fibres follow the tracts of nerve fibres between the cell islands (Young 1962, fig. 13).

## Description of plate 5

Figure 36. Large fibrous glia cell from subvertical lobe. Golgi.

Figure 37. Detail of fibrous glia fibres, mostly unbranched and with occasional swellings. Golgi.

Figure 38. Nuclei from suboesophageal lobe, including one presumed to be of fibrous glia. Hortega.

Figure 39. Large fibrous glia cells from the brachial lobe. Golgi.

Figure 40. Large fibrous glia cells in vertical lobe, with some fibres extending inwards to the neuropil and others outwards into the cell layers. Golgi.

Figure 41. Sagittal section of lateral superior (*fr. s.l.*) and lateral inferior frontal lobe (*fr. i.l.*), to show the fibrous glia following the course of the bundles of nerve fibres. Golgi.

Figure 42. Median superior frontal showing small fibrous glial cells (Golgi–Cox).

Figure 43. Fibrous glial cell and related glio-vascular tissue from the magnocellular lobe. Golgi.

Figure 44. Region of subvertical lobe showing fibrous glia, glio-vascular tissue and lymphoid spaces all together.



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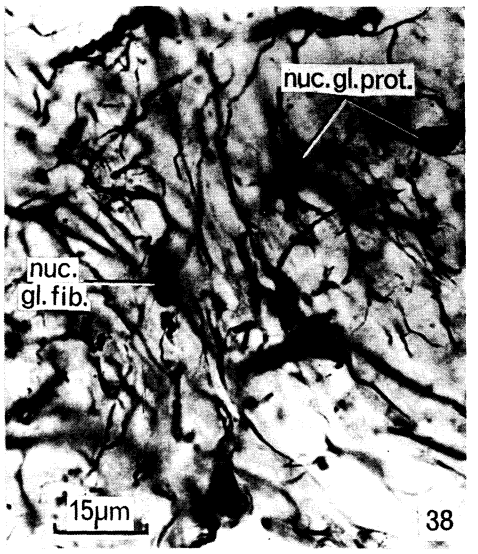


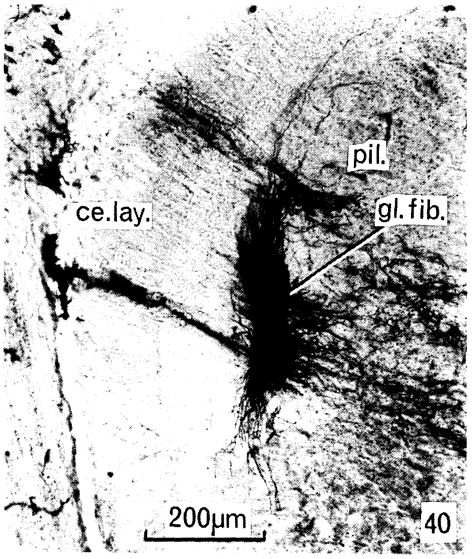


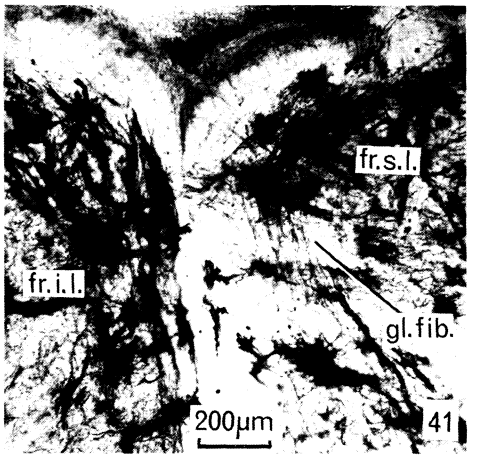



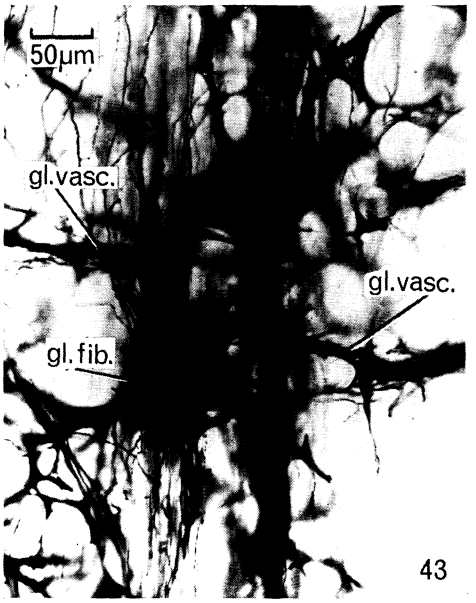


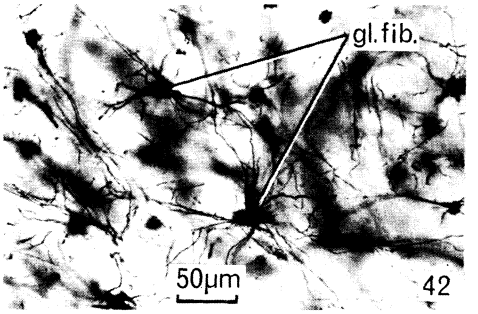


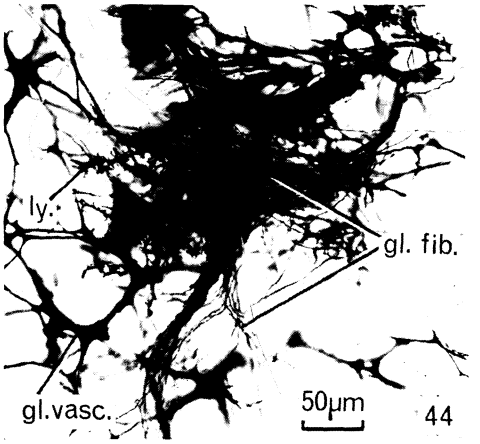


(*Facing p.* 8)



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The relation of these cells to the blood vessels remains uncertain. They are often very close to the glio-vascular channels (figure 43) and branches of the lymphoid intercellular spaces often appear round the centre on to which the glial fibres converge and mix up closely with them. The centre of each of these fibrous glia cells is nearly always a very darkly stained mass in the Golgi preparations. Probably this mass is composed not only of the glial cell body but of stained vascular and lymphoid spaces as well. Electron-microscopy shows that in these fibrous glial cells there is a system of channels within the cytoplasm. These are presumably the lymphoid channels of the present account (Gray 1969).

## 7. Glia in the cell layers

Among the cell bodies there are abundant pericytes and cells and fibres similar to those of the glio-vascular tissue of the neuropil. In addition, there are nuclei similar to those identified as belonging to the protoplasmic and fibrous glia (figure 33, plate 4). The details of the glia in the cell layers have not been seen, since the processes are not usually as well stained with either Hortega or Golgi methods as they are in the neuropil. In figure 32 there are processes apparently of protoplasmic glia wrapping around the cell body and axon. Fibres are seen penetrating into channels within the cytoplasm of the larger neurons (e.g. figure 34), but it is not clear whether these include glial fibres as well as collagen. The nuclei in figure 33 labelled as belonging to fibrous glia have the shape of those so named in the neuropil and, like the latter, they are accompanied by an argentophil body. Unfortunately their processes have not been seen in any of the preparations.

## 8. Discussion

The non-neural tissues of the cephalopod nervous system are evidently complex and not altogether easy to compare with those of other groups. The blood can pass directly from the arteries through a closed system of capillaries to veins. The latter collect either in the outer membranes around the brain or, in the optic lobe, to an elaborate set of veins returning to the hilum (see Young 1969). The capillaries do not form an elaborate network in close relationship to every part of the tissue. Instead there is a system by which material is transported to and from the neurons. The afferent part of this system is formed by the perivascular channels and glio-vascular system. The status and function of the latter are still not entirely clear. It is a system of spaces of characteristic shape, described by Garaieff (1909), Jakubski (1915) and Bogoraze & Cazal (1944). These spaces have now been shown to contain collagen, non-collagenous extracellular fibres, fibrocytes, muscle cells and perhaps blood (Gray 1969). It is reasonable to suppose that fluid enters them, either directly or through the capillary walls, and is carried to the tissues. Fine branches of the glio-vascular system reach the synaptic points in the neuropil (figure 18, plate 2). This glio-vascular tissue seems to be peculiar to cephalopods, though no doubt it is a development of intercellular materials found in other molluscs (see Amoroso, Baxter, Chiquoine & Nisbet 1964).

Return of fluid from around the cells takes place by a set of fine, branched 'lymphoid' channels, opening into the veins. These can be filled by injection but the appearances




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seen in figures 3 and 4, plate 1, suggest that this filling was by reflux under excess pressure. These channels may normally serve to return fluid that has been forced through the capillary walls by the ventricular pressure and carried through the perivascular spaces and glio-vascular system. In some places the fluid in these intercellular spaces comes into direct relation with nerve cell bodies or nerve fibres (Barber & Graziadei 1967*a*; Gray 1969). Often, however, glial material intervenes.

Branched 'protoplasmic glia' have been described previously and the cells so named here are similar to those of Cajal (1917) and Bogoraze & Cazal (1944). The structures called glia cells in the plexiform zone of the optic lobe by van Lenhossék (1899) and Young (1962) may, however, be branched vascular channels (p. 7). The large fibrous glia here described are a type not seen before and not the same as the 'astrocytes fibreux' of Bogoraze & Cazal, which are clearly part of the glio-vascular system.

The protoplasmic glia cells as they appear with silver stains are branched, 'spidery' structures, because the stain is concentrated on the glial fibres. The whole glial cell probably forms sheaths around the nerve cell bodies and nerve fibres, as it does in insects, crustacea and annelids (Wigglesworth 1959, 1965; Hámori & Horridge 1966; Kuffler & Potter 1964). Indeed under suitable circumstances this sheathing form can be clearly seen (figure 32, plate 4).

The large fibrous glia do not stain with Hortega's stain, although they contain more glial fibrils than the protoplasmic glia. The two types do not perhaps differ fundamentally, the fibrous ones being mainly related to fibre tracts, the protoplasmic ones to cell bodies, and neuropil.

There are thus three main systems in the nervous system other than the neurons and blood vessels. These three are the glio-vascular channels, the branching lymphoid channels and the glia. All three can be seen together (but of course none of them optimally) in a Golgi preparation in figure 44, plate 5. Materials probably usually flow from the vascular capillaries through the glio-vascular channels to the glia and neurons and from there back via the lymph channels to the veins.

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## List of abbreviations

| | | | |
|---|---|---|---|
| *art.* | artery | *gl.vasc.* | glio-vascular channel (straight) |
| *bl.* | blood vessel | *i.c.* | intercellular material |
| *ce.lay.* | cell layer | *ly.* | lymphoid channels |
| *ce.n.* | nerve cell | *n.f.* | nerve fibre |
| *c.ped.med.* | middle pedal commissure | *nuc.gl.fib.* | nucleus of fibrous glial cell |
| *cer.mem.* | cerebral membrane | *nuc.gl.prot.* | nucleus of protoplasmic glial cell |
| *coll.* | collagen | *nuc.gl.vasc.* | nucleus of glio-vascular tissue |
| *den.co.* | dendritic collateral | *nuc.mus.* | nucleus of muscle fibre |
| *f.* | fibres penetrating cell | *pc.* | pericyte |
| *fr.i.l.* | lateral inferior frontal lobe | *pil.* | neuropil |
| *fr.s.l.* | lateral superior frontal lobe | *sp.* | spaces filled with blood |
| *gl.fib.* | fibrous glia | *subv.* | subvertical lobe |
| *gl.foot* | glial end foot | *v.* | vertical lobe |
| *gl.prot.* | protoplasmic glial cell | *ve.* | vein |

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