ELECTRON MICROSCOPY OF THE GLIO-VASCULAR ORGANIZATION OF THE BRAIN OF *OCTOPUS*

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[Plates 6 to 25]

CONTENTS

The glio-vascular organization of the octopus brain has been studied by light and electron microscopy. The structure of the walls of the blood vessels has been described. Two types of neuroglia can be recognized, the fibrous and protoplasmic glia; also enigmatic dark cells. Most blood vessels in the neuropil are surrounded by extracellular zones containing collagen. These zones give off glio-vascular tunnels (strands) that penetrate the neuropil in a complex network. The extracellular zones and tunnels contain in addition to collagen, smooth muscle cells and fibrocytes. Glial processes surround the extracellular zones and incompletely partition them from the neuropil.

The small neuronal perikarya have no glial folds around them. The medium-size cells have thin glial sheets or finger processes related to their surfaces, which may indent the cells to form small trophospongia. The large neurons of the suboesophageal lobe have complex glial sheaths interspersed with extracellular channels. Both penetrate the neurons to form complex trophospongia. A new form of extracellular material has been observed in these extracellular channels. The occurrence of trophospongia in vertebrate and invertebrate neurons may be correlated with the absence of dendrites.

Special problems discussed include the nature of the trophospongial function, the question of fluid-filled extracellular zones and their possible function as lymph channels, and the presence in some of them of haemocyanin molecules identical with those in the blood vessels. Perhaps of special importance is the observation that the lobes of the octopus brain are permeated with extracellular tunnels containing smooth muscle fibres, but it still needs to be determined whether or not the muscle cells in the tunnels of the neuropil actively contract and massage the neuropil to facilitate metabolic and other exchanges.

Introduction

In the preceding paper Stephens & Young (1969) have reviewed the literature and made a detailed study, using light microscopy, of the glio-vascular organization of the cephalopod brain. Dilly, Gray & Young (1963), Gray & Young (1964) and Barber & Graziadei (1966) have studied synaptic organization in the brains of *Octopus and Eledone* with the electron microscope, but there have been no detailed studies on the glio-vascular relationships in the past by means of electron microscopy, although a description of the fine structure of the blood vessels of *Octopus* brain by Barber & Graziadei (1965, 1967*a*, *b*) has included brief reference to the neuroglia. Here attempts have been made to relate the observations to those of Stephens & Young. These include the fine structure of the different sorts of blood vessel and the different types of neuroglia, and the manner in which these are related to each other, to the neuronal perikarya and neuropil and to the extensive and well-defined system of extravascular channels (the glio-vascular strands) that are prominent in the brain of *Octopus*.

Methods

Electron microscopy

The median superior frontal, vertical, suboesophageal and optic lobes of *Octopus* were examined by two fixation methods. The lobes were removed and cut in small pieces (less than 1 mm thick) and placed for 3 h:

(1) In 1 % osmium tetroxide in saline buffered at pH 7·4 with veronal acetate. The pieces were dehydrated in ethanol, stained for 3 h in 1 % phosphotungstic acid and embedded in Araldite (see Gray 1964). The sections were either examined in the electron microscope without further staining or the contrast was enhanced by staining the section first with 0·8 % uranyl in absolute ethanol for 10 min and washing the grid for 20 s in a stream of absolute ethanol followed by a stream of distilled water and then staining with 1 % aqueous lead citrate (Reynolds 1963). This method gives excellent contrast and avoids lead precipitates.

(2) Pieces were placed in a mixture of 4 % formaldehyde (prepared from paraformaldehyde—see Pease (1964) and 0·5 % glutaraldehyde buffered with phosphate at pH 7·4 for 20 min (see Vaughn & Peters 1966). The pieces were then transferred to the 1 % osmium tetroxide solution for a further 3 h and dehydrated in ethanol. The pieces were block stained at the 70 % stage with 1 % uranyl acetate for 2 h and embedded in Araldite. Sections were further stained with 1 % aqueous lead citrate for 10 min. Some sections were stained with lead only, the block staining being omitted.

The first method gives good contrast, but variable quality of fixation. The second method usually gave reasonably good fixation of most structures but the mitochondria often appeared swollen.

Light microscopy

Phase-contrast microscopy of plastic sections up to 20 μm or more thick (see Gray 1961) cut by hand from the surface of the block was used to trim down to and study specific regions of the tissue.

Golgi material

Pieces of optic lobe were fixed for 24 h in the glutaraldehyde/formaldehyde solution mentioned above and then impregnated by a Golgi method (see Westrum & Lund 1966) for 7 days, dehydrated in ethanol and then cut into thin slices (less than 0·5 mm thick) with a razor blade. These were embedded in Araldite, which was allowed to cure in sheets about 1 mm thick. Suitably impregnated areas were observed and photographed by light microscopy and then sectioned for electron microscopy. The sections were stained in lead citrate to improve contrast of the non-impregnated material.

Observations

General

The detailed morphology of the median superior frontal, vertical, suboesophageal and optic lobes (together with the other lobes) of the brain has been described by Young (1969). Briefly, the first three lobes have the more usual invertebrate organization of a cortex containing the neuronal perikarya and a medulla consisting of fibre bundles and synapses which together, with other elements, constitute the neuropil. The optic lobe is much more complex. It has a cortex consisting of two sheets of neuronal perikarya with a layer of neuropil between, and a medulla which contains a neuropil and also numerous scattered islands of neuronal perikarya. Neuroglial cell bodies and their processes and blood vessels can be seen with the electron microscope in all the various zones of these lobes.

In the following description the glio-vascular organization of the neuropil will be considered first. The arrangement in general is the same for all the lobes. Then the organization of the environment surrounding the neuronal perikarya will be described. The smallest perikarya have no glial folds around them. However, glial wrappings can be detected around the larger ones of all of the lobes and in the suboesophageal lobe, where the perikarya are extremely large, there is a highly specialized and complex glial and vascular organization in the form of capsules round the individual perikarya.

The criteria for identifying blood vessels in sections are clear-cut—the internal incomplete layer of endothelium, basement membrane and pericyte layer (or multilayer of muscle fibres in the arteries). Extracellular zones are also easily identified. They are 'bounded' by a single membrane (i.e. that of the nearest cellular component) and they contain collagen and other extracellular material and lack intracytoplasmic organelles such as mitochondria, endoplasmic reticulum, microtubules, etc. Criteria for identifying neurons and glia and their ramifications cut in various planes are not so well established. Neuronal perikarya are usually unipolar and neuroglia usually multipolar and they contain different arrangements of organelles. Neurites may contain neurofilaments or microtubules and the synapses can usually be identified by the synaptic vesicles and membrane thickenings. Neuroglial processes lack these features and may contain the characteristic glial filaments. All too frequently, however, it is impossible to distinguish a neuronal from a glial profile in a section.

Glio-vascular organization of the neuropil

The structure has been illustrated diagrammatically in figures 1 and 2. In figure 1 a capillary is shown leading from an artery and leading into a large vein (all seen in longitudinal section). A vessel is here designated an artery when its wall contains layers of smooth muscle cells. Vessels with the smallest diameters are arbitrarily designated capillaries. Large vessels which correspond to those described as veins by Stephens &

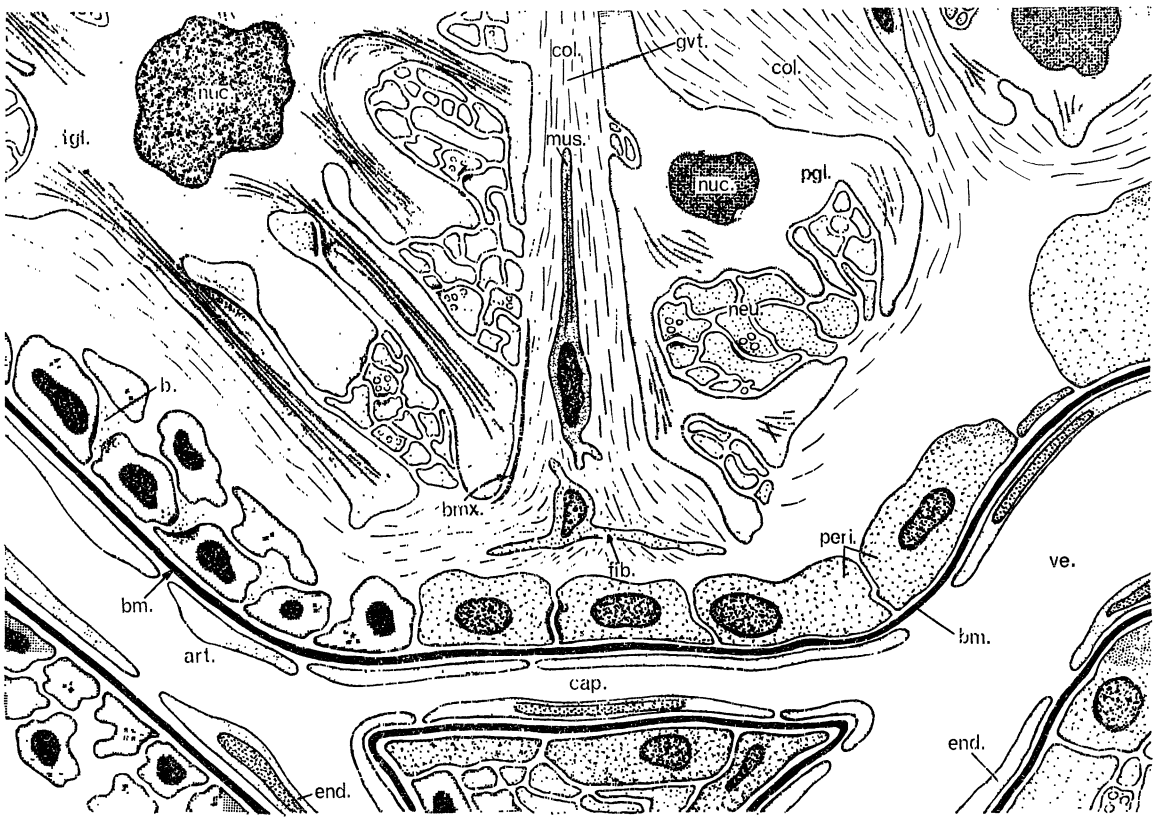

FIGURE 1. Diagrammatic section derived from electron micrographs showing glio-vascular relationships.

Young (1969) are also here designated veins. They lack a muscular wall and apart from size and certain specializations of the pericyte layer (p. 18) they have the same general structural organization as the capillaries.

Figure 1 shows the organization of the tissues around the blood vessels. The neuropil zones are much more extensive than shown here. They are compressed for clarity. Figure 2 shows a capillary (in transverse section) and the surrounding tissues.

The blood vessels

Figure 3, plate 6, shows a capillary in transverse section. The terminology will follow that of Barber & Graziadei (1965, 1967*a*, *b*). The lumen contains fine granular material, which at higher magnification can be seen to be haemocyanin molecules

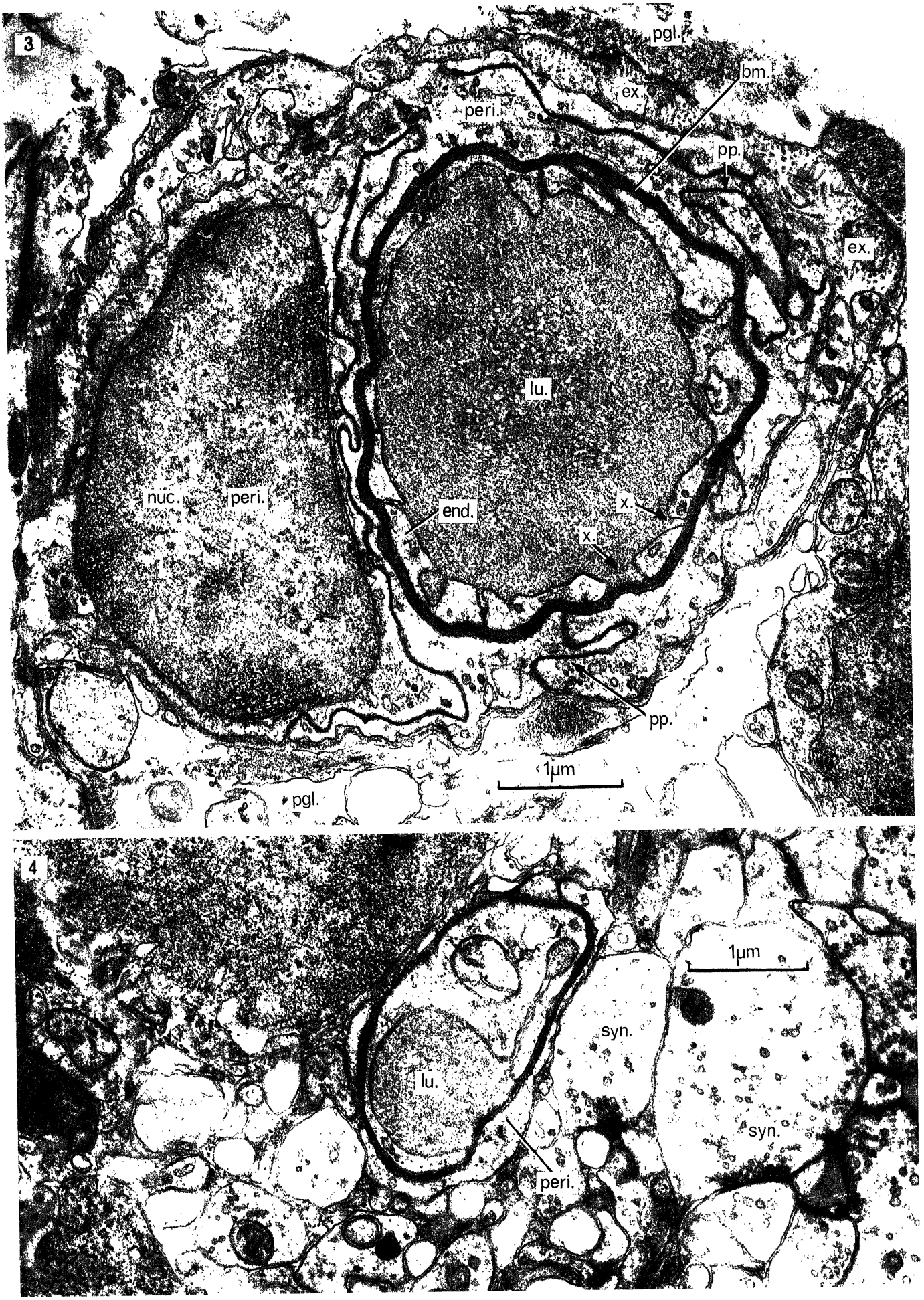

FIGURE 3. Cross-section of a capillary in the optic lobe.

FIGURE 4. Cross-section of a capillary and neighbouring synaptic zones in the optic lobe.

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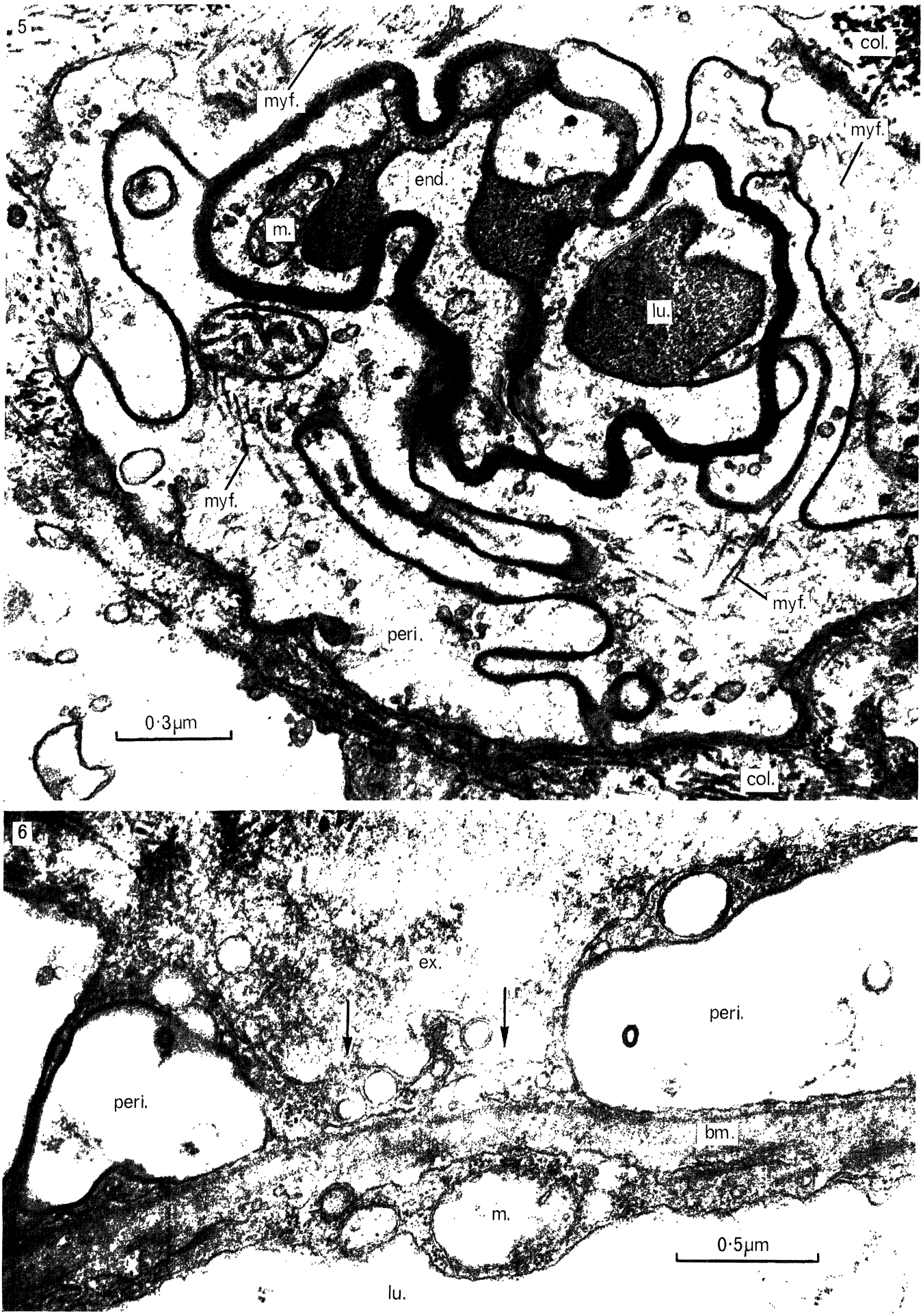


FIGURE 5. Cross-section of a capillary, the pericytes of which contain myofilaments (optic lobe).

FIGURE 6. This pericyte zone in the wall of a vein (medulla, optic lobe).

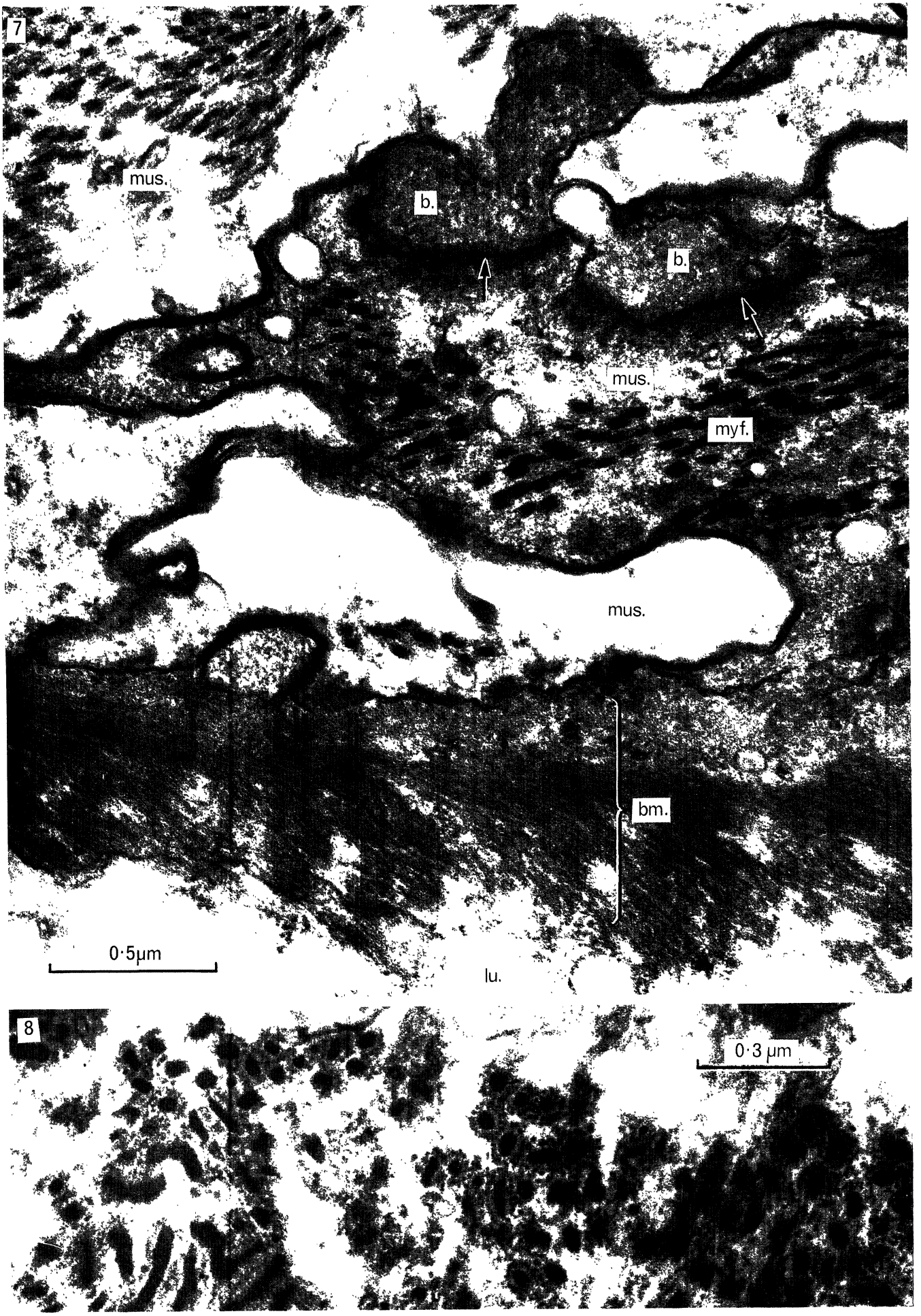


FIGURE 7. The muscular wall and basement membrane of an artery in the optic lobe.

FIGURE 8. High magnification micrograph of cross-section of myofilaments (see figure 7).

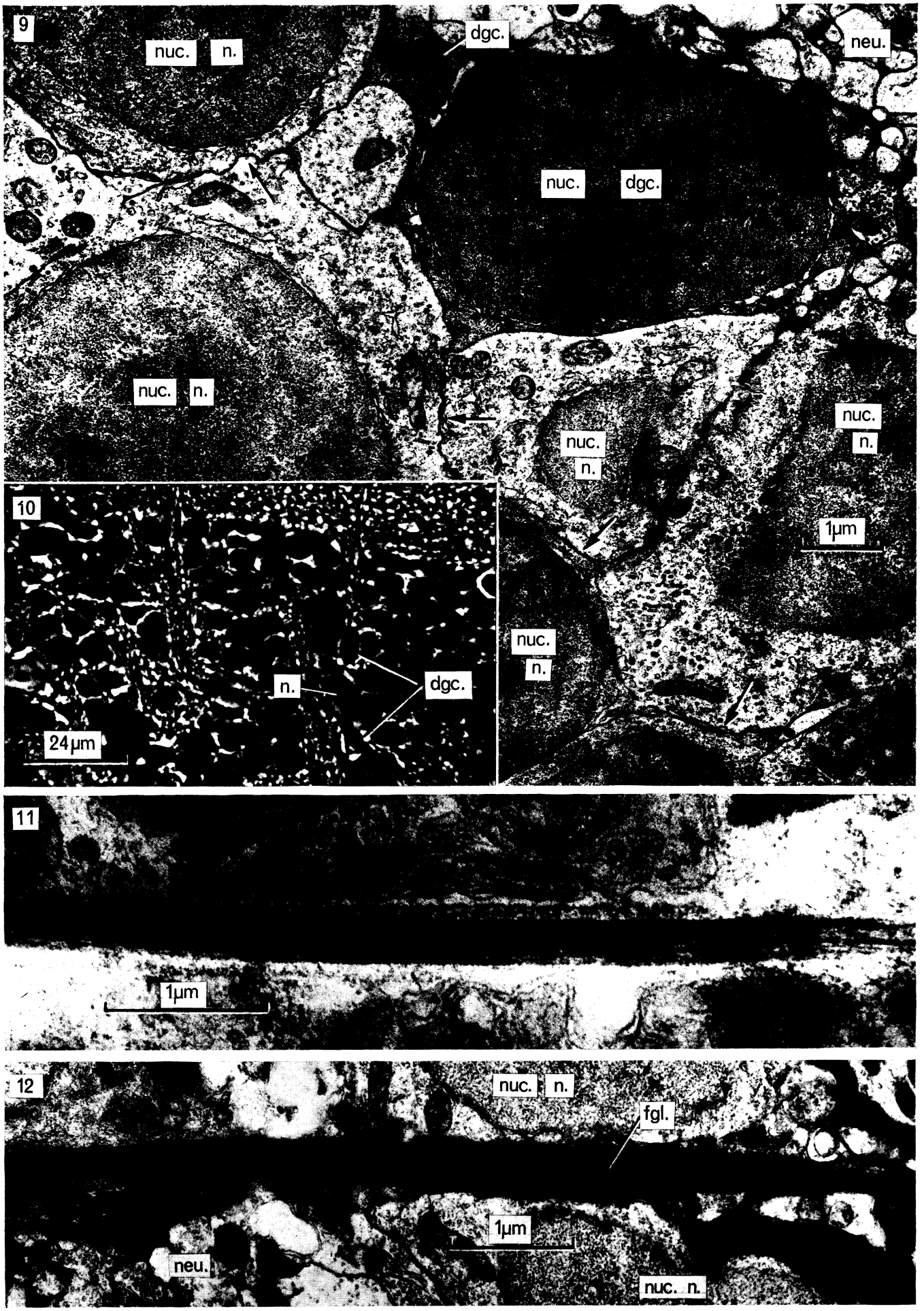


FIGURE 9. Section through neuronal cell bodies and a dark cell (optic lobe).

FIGURE 10. Phase-contrast light microscopy of plastic section through neuronal cell bodies and dark cells (inner granule zone of optic lobe).

FIGURE 11. Section of process of fibrous astrocyte from the cat's spinal cord (compare figure 12).

FIGURE 12. Section through process of fibrous glia in the optic lobe of the octopus (compare figure 11).

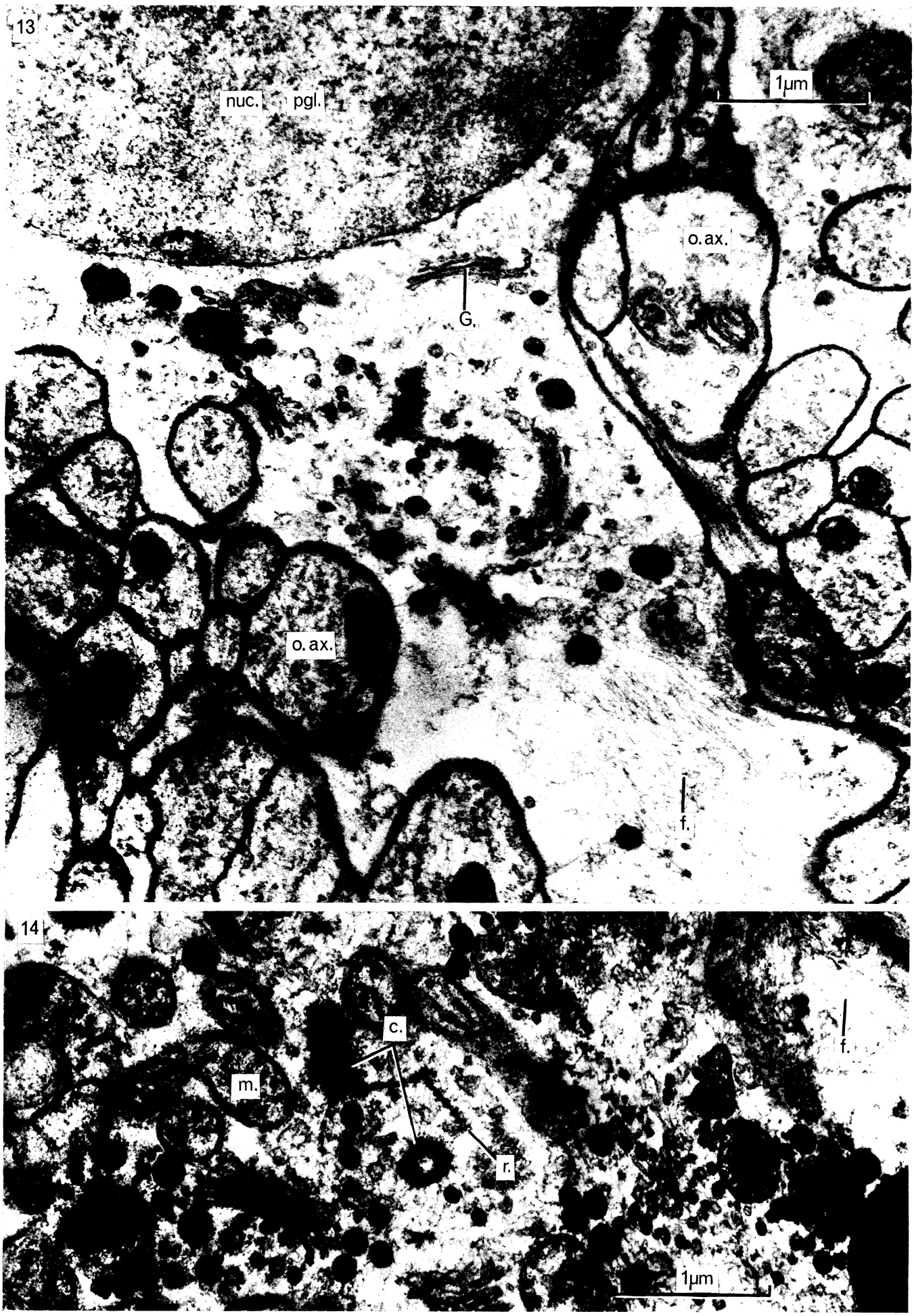


FIGURE 13. Part of a protoplasmic glial cell with adjacent optic nerve axons (external granule zone of optic lobe).

FIGURE 14. Cytoplasmic zone adjacent to the nucleus of a protoplasmic glial cell.

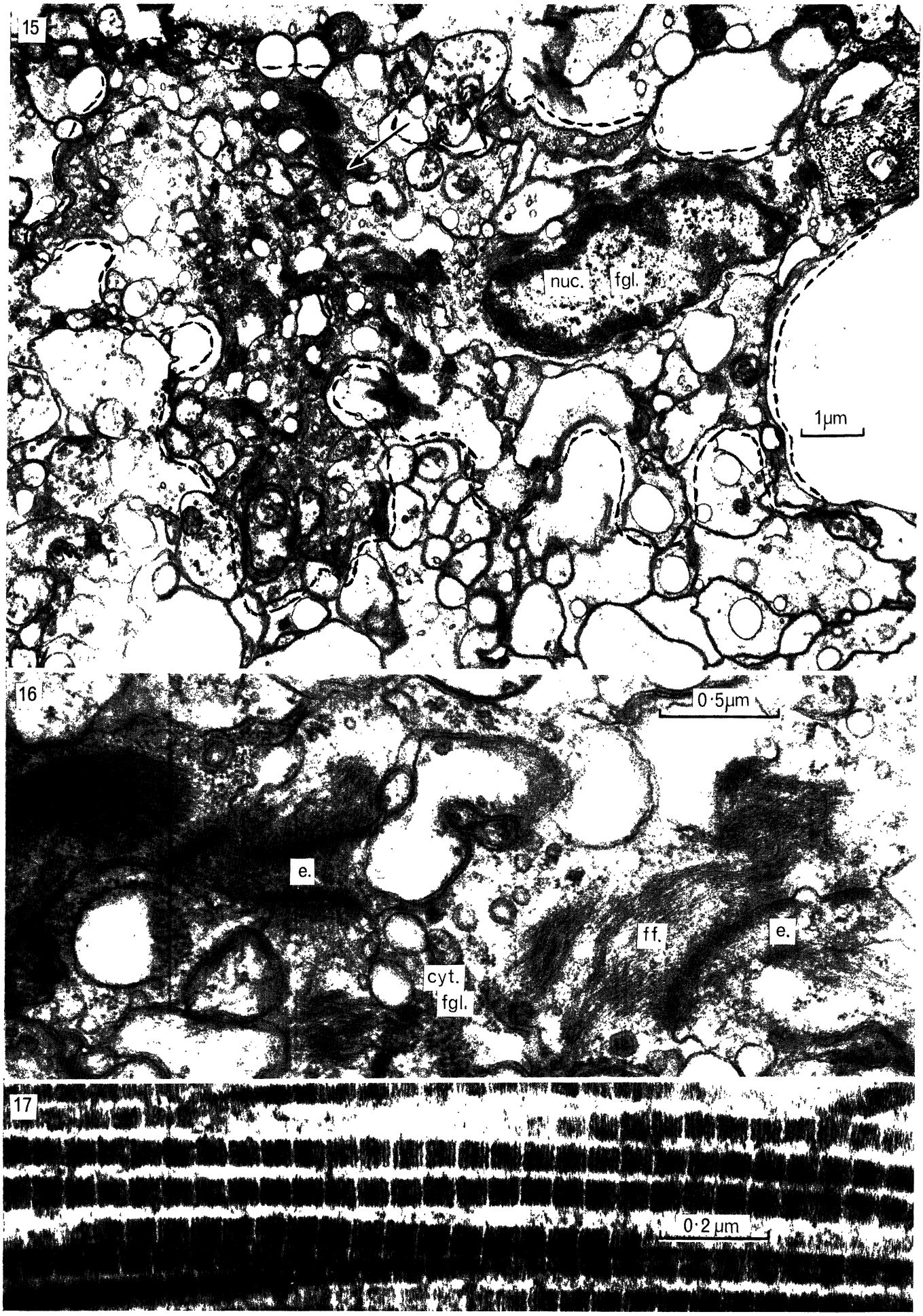
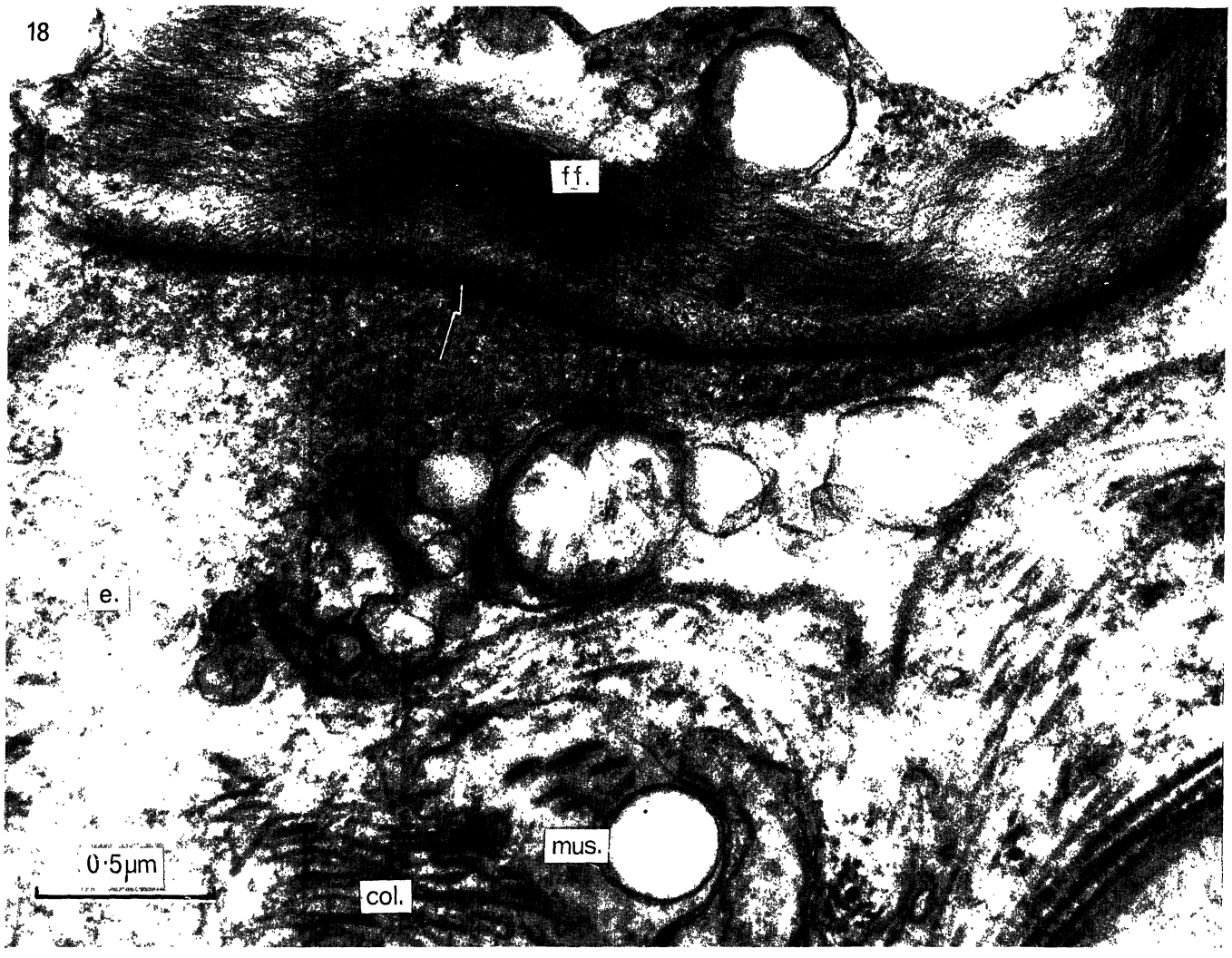


FIGURE 15. Section through a fibrous glial cell seen at low magnification. Region indicated by arrow is enlarged in figure 16 (optic lobe medulla).

FIGURE 16. Enlarged portion of the fibrous glial cell shown in figure 15.

FIGURE 17. Octopus 'collagen'.



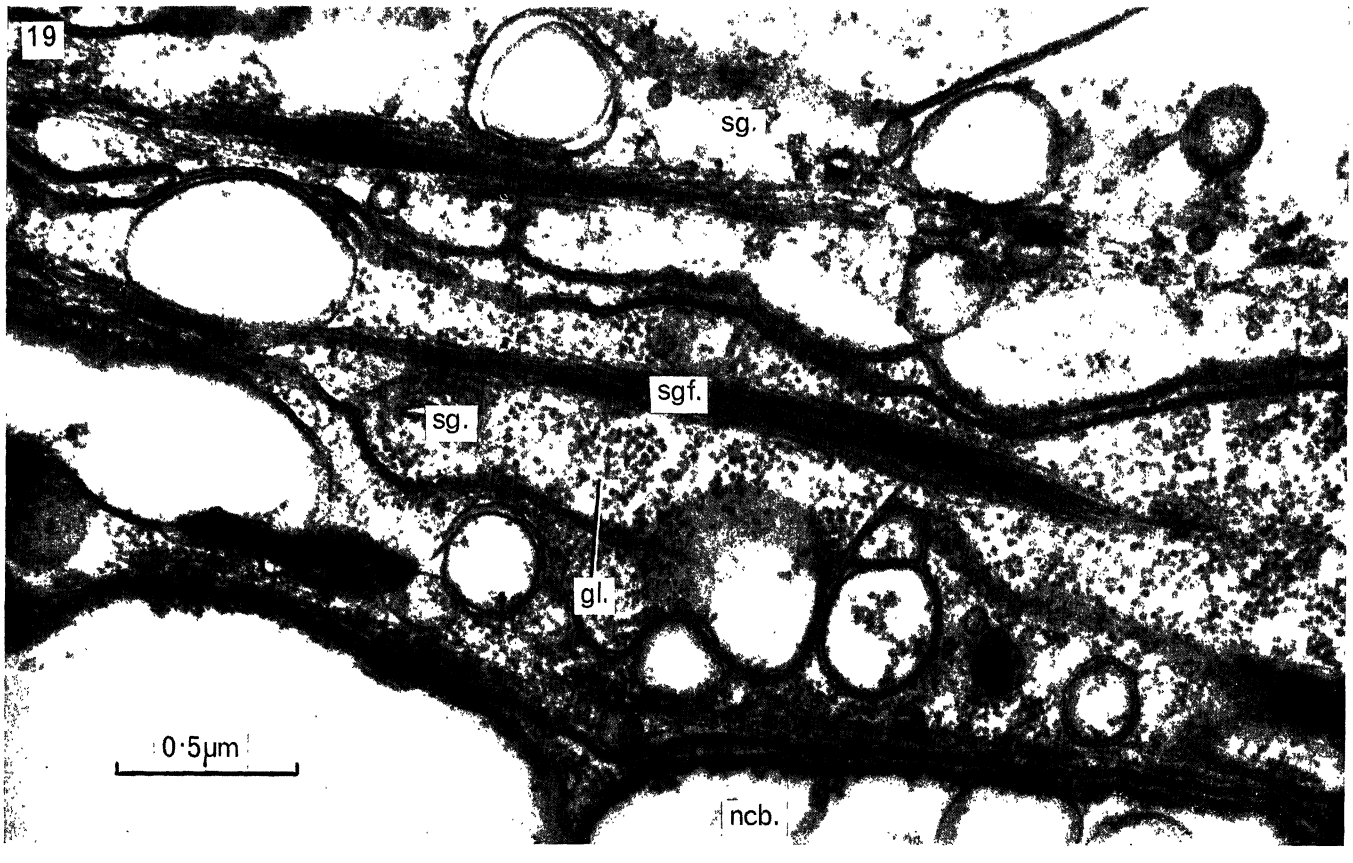


FIGURE 18. Part of fibrous glial cell (above) where it is apposed to an extracellular zone. The surface membrane shows specializations (medulla, optic lobe).

FIGURE 19. Glial folds forming sheath round large neuron cell body (cortex of suboesophageal lobe).

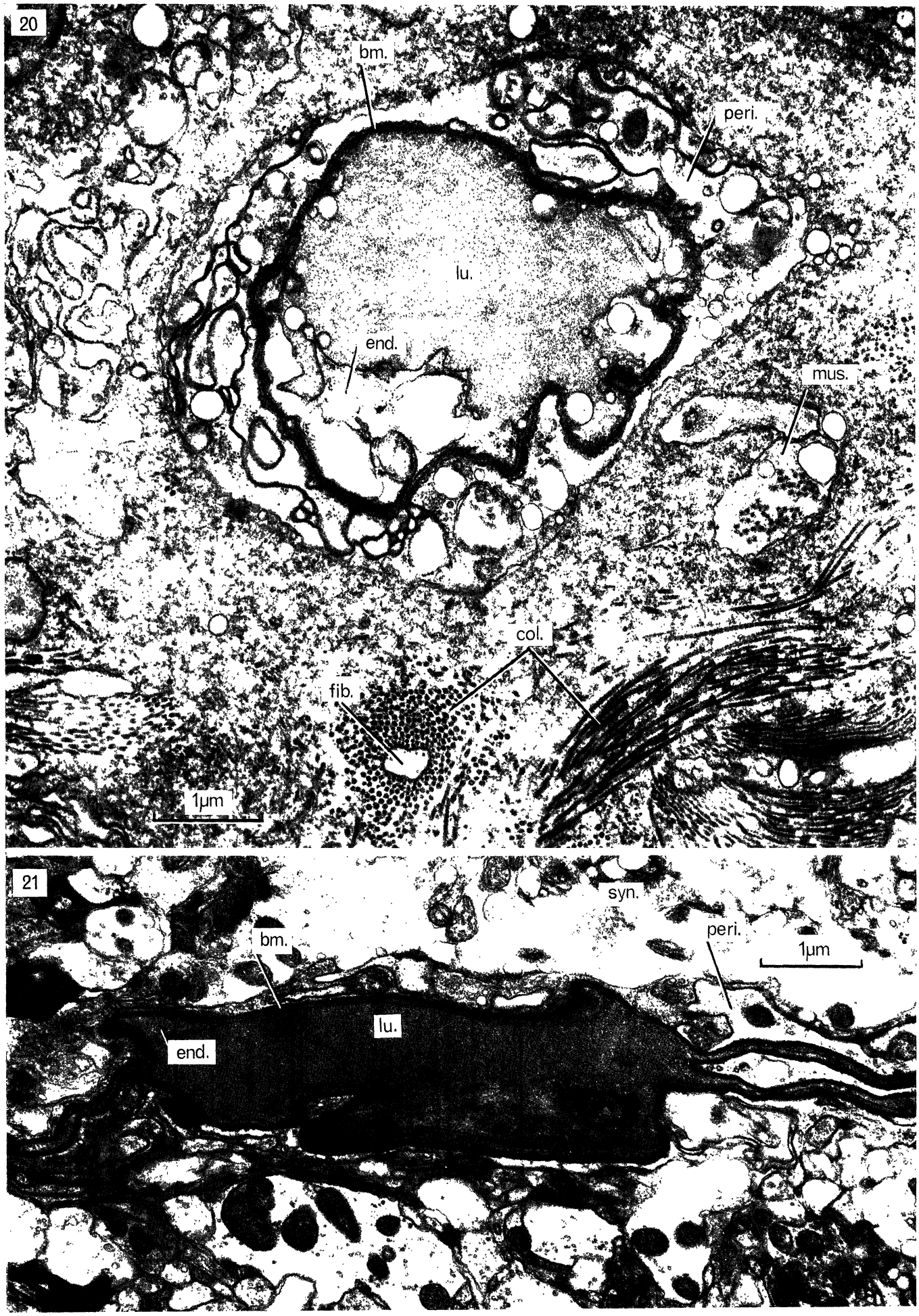


FIGURE 20. Cross-section of capillary lying in an extensive extracellular zone. The zone contains collagen, fibrocyte and muscle processes (medulla optic lobe).

FIGURE 21. Longitudinal section of capillary in plexiform zone of cortex of optic lobe.

(p. 22) of the blood. Amoebocytes are also occasionally encountered in sections (Barber & Graziadei 1965). The lumen is lined by endothelial cells which sometimes contain microtubules, the endothelial lining is incomplete in places (x). Here the basement membrane, an uninterrupted sheet of dense granular material (see pp. 18 and 21) which lies immediately outside the endothelium, comes into direct contact with the blood. A layer of pericyte cells lies outside the basement membrane. There may be one or several pericytes depending on the size of the vessel. As far as can be judged from the micrographs there

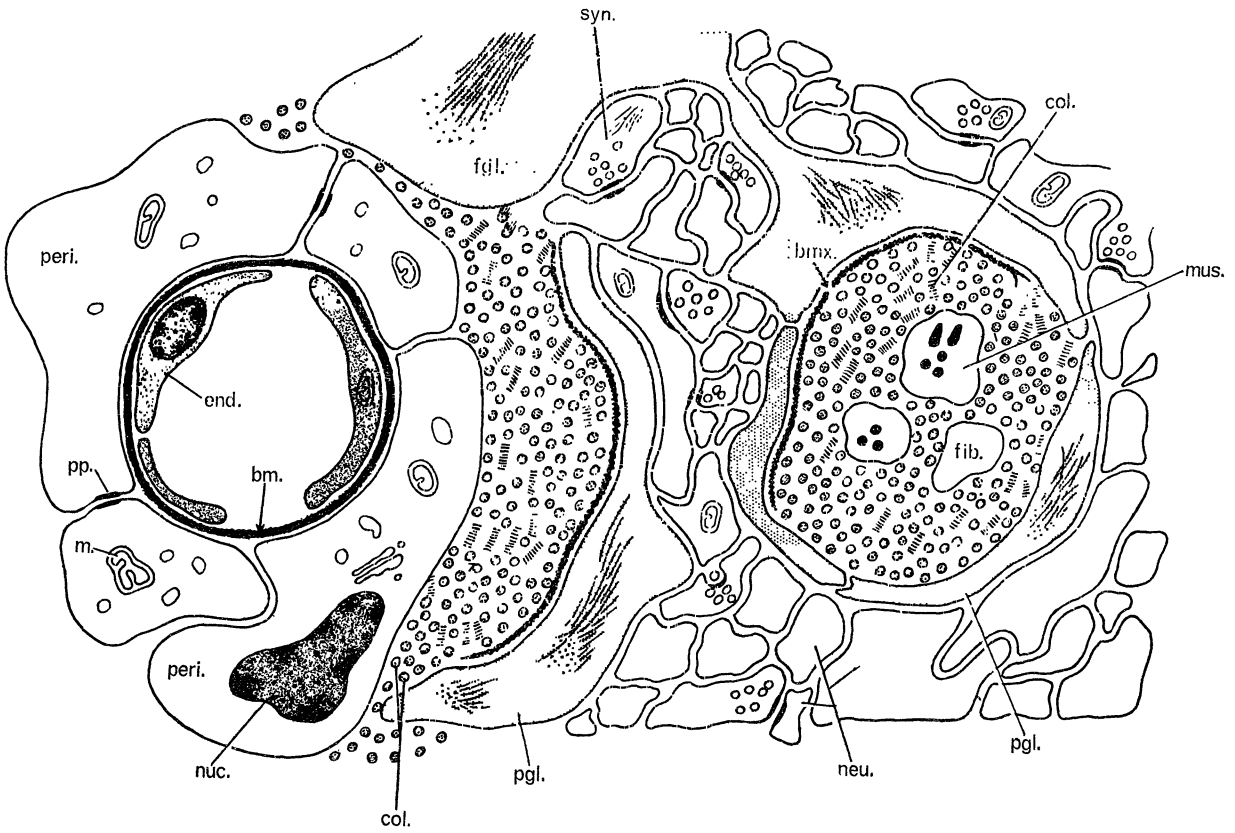


FIGURE 2. Diagrammatic section derived from electron micrographs showing glio-vascular relationships. Sections through a capillary (left) and a glio-vascular tunnel (right) are included.

is always only a single layer, that is to say each pericyte has its innermost surface directly apposed to the luminal basement membrane (luminal, since a second different type of basement membrane will be referred to below).

The apposed surfaces of the pericytes are crenated and projections interdigitate with one another. Specialized contact zones, regions where the cleft between apposed pericyte membranes contains dense material (first described by Barber & Graziadei 1965, 1967*a*), can be observed. It is suggested here that they should be termed 'pericyte plaques'—a non-commital term suitable until we know something about their function. Certainly as pointed out by Barber & Graziadei, they are not tight junctions. The apposed pericyte surface membranes are everywhere separated by a distinct cleft (including zones containing pericyte plaques) 100 to 200 Å or more across.

Mitochondria and, more frequently, clusters of vesicles up to 1000 Å or more in

diameter occur in the pericyte cytoplasm. Occasionally the pericytes of certain blood vessels contain groups of rods interpreted by Barber & Graziadei as myofilaments (figure 5, plate 7—see Discussion).

The cross-sectional diameters of the lumina of these small vessels ranges from less than 0·5 μm up to 6 to 8 μm. The thickness of such vessels when the pericyte wall is included may range from 2 to 10 μm.

The much larger collecting vessels considered to be veins have so far only been studied in detail in the hilar region of the optic lobe. They show the same basic organization as the capillaries, but the lumina are much wider and may reach 20 to 30 μm or more. The pericytes have not been observed to contain myofilaments. At certain points the pericytes appear granular and are extremely thin (less than 1000 Å) (arrows, figure 6, plate 7). These will be considered further in the Discussion.

A description of arterial structure and its innervation has been given by Barber & Graziadei (1967*b*) so only certain aspects will be described here. The usual endothelium is present and the basement membrane is often extremely thick (1000 Å or more) and may contain fibrous material (figure 7, plate 8). Immediately outside the basement membrane are found several layers of smooth muscle cells. Both the thick and thin myofilaments are clearly recognizable (figure 8, plate 8). In many places (figure 7) the apposed membranes of adjacent muscle cells are separated by the usual 100 to 150 Å cleft. However, at some points (*b*) there are appreciable extracellular zones containing fine granular material. These are probably continuous with the extracellular spaces outside the muscle wall of the artery (compare figure 1). Where the surface membrane of a muscle fibre faces one of these extracellular zones (arrows, figure 7), it contains dense granular material along the cytoplasmic surface. Baxter & Nisbet (1963) and Nisbet & Plummer (1966) have described apparently similar structures in gastropod cardiac muscle. These zones do not penetrate between the innermost layer of muscle cells lining the basement membrane. Here the cells are all directly apposed across 100 to 200 Å clefts. Observations so far suggest that these narrow clefts are directly continuous with the larger extracellular zones just referred to.

The enigmatic dark cells

The dark cells have apparently not been described before, either by light or electron microscopy. Figure 10, plate 9, shows several of these cells seen by phase-contrast light microscopy of plastic sections in the inner granular layer of the optic lobe. They are surrounded by numerous small neuronal perikarya and some large ones. By electron microscopy these cells also appear dark or dense. The dense nucleus (figure 9, plate 9) can be seen surrounded by a rim of dense cytoplasm which extends off to the left and bifurcates. High magnification shows that the dense appearance of the cytoplasm results from the presence of numerous granules each about 150 Å in diameter. The granules also occur in the processes giving them the same dense appearance. The granules are thought to be ribosomes (*a*) because of their size and (*b*) because they can sometimes be seen attached to membranes of the endoplasmic reticulum. Such membranes are, however, relatively scarce in these cells. A group of neuronal perikarya (granule cells)

can also be seen in figure 9. In these the cytoplasm is relatively pale, because of the sparser distribution of ribosome clusters. At present it cannot be decided whether the dark cells should be classed as neuroglia or mesenchymal cells.

Structure of the neuroglia

Two morphologically distinct types of multipolar neuroglial cells can be seen with the electron microscope. These are the protoplasmic glia and the fibrous glia.

The protoplasmic glia is the well-known type described by Stephens & Young (1969) and others. An example is shown in section in figure 13, plate 10. The nucleus is moderately dense and the cytoplasm generally very pale because of a lack of organelles especially ribosomes (compare the neuron cytoplasm in figures 36 and 37, plate 20). A few Golgi units are present and some membrane-bound granules. Fine glial filaments are present in an arm of the cytoplasm. Bundles of axons (of retinal origin) can be seen lying in the glial folds. The pale cytoplasm lacking in ribosomes and containing bundles of fine filaments makes a striking resemblance between these cells and the protoplasmic astrocytes of the mammalian central nervous system (see Gray 1964). As Palay (1965) has pointed out, the mammalian protoplasmic astrocytes have fine bundles of glial filaments (in contrast to the coarser tightly packed bundles found in the fibrous astrocytes—see below). Occasionally numerous mitochondria occur near the Golgi bodies, together with a pair of centrioles (figure 14, plate 10).

Stephens & Young (1969), in the preceding paper, describe for the first time giant multipolar cells observed in Golgi preparations. Because of their long thin unbranching fibre-like processes they have named them fibrous glia. In spite of their size they would probably never have been detected with the electron microscope, such is the sampling problem, had it not been for the insistence by Young (numerous personal communications) that they should be there.

A low-power view of a section thought to be such a fibrous glial cell is shown in figure 15, plate 11. The irregular outline of the cell is shown with a dashed line. The nucleus lies to the right. A region of this section (arrow) is shown at higher magnification in figure 16, plate 11. The cell is characterized by bundles of tightly packed glial fibrils here initially cut in transverse section. A peculiarity of this type of cell is that it has extracellular channels penetrating deep within its folds. Two such channels are labelled. They contain fine granular material. It will be seen that dense zones occur at regions of the glial membrane apposed to the extracellular zones. One such region is shown in figure 18, plate 7, at very high magnification. The glial fibrils can be seen above running close to but apparently not inserted into the surface membrane (in contrast with a desmosomal arrangement, although on the left the impression is that a few fibrils do run towards the membrane). A dense zone of the fine filamentous or granular material lines the cytoplasmic side of the surface membrane. A dense band of extracellular material (arrow) (basement membrane?) lies just outside the surface membrane in the extracellular zone, which can be seen containing the granular material mentioned above. Figure 18 supports the view that these channels are extracellular for here the channels can be seen in continuity with a zone which is without doubt extracellular since it contains

collagen. In addition to these extracellular zones groups of axons are sometimes enclosed within the folds of these fibrous glia.

As described by Stephens & Young (1969) the processes of these fibrous glia run often for long distances through the brain tissue. One is shown sectioned longitudinally in figure 12, plate 9—a long thin process almost filled with a bundle of glial fibrils. For comparison a process of a fibrous astrocyte from the spinal cord of the cat is shown in figure 11, plate 9. Both were fixed and stained by the same method.

No special location has been found for the dark cells or protoplasmic astrocytes: they occur in all the regions examined. The fibrous glia were mostly observed in the medulla of the optic lobe near the hilum, where presumably they are most abundant. Here the axons form aggregates of the optic tract, and Stephens & Young (1969) found that such glia tend to be associated with tracts. Another fibrous glial cell was seen in a section of part of the tract running into the vertical lobe from the median superior frontal lobe.

Mention should be made here of another cell type that occurs in the hilum and medulla of the optic lobe. This is the subpedunculate tissue (see Young 1969), consisting of chains of cells of unknown function.

The glio-vascular relationship of the neuropil

Having examined the fine structural cytology of the various elements involved, we can now consider the way they are interrelated.

When plastic sections of the neuropil are examined with phase-contrast light microscopy, the blood vessels can easily be identified. When sectioned longitudinally the endothelial nuclei show an elongated appearance: the pericyte nuclei are in contrast more rounded. Usually the vessels (especially the larger ones) are partly or completely surrounded by clear zones and these lead into channels which run off at right angles or obliquely to the blood vessels and form a complicated anastomosing three-dimensional network throughout the lobes of the brain. At first sight these side channels might be mistaken for fine branches of the blood vessels, but close examination with the light microscope shows that neither the endothelial nor pericyte nuclei occur in them. This is the system described by Bogoraze & Cazal (1944) as glio-vascular channels.

The channels are easily identified by electron microscopy and confirmed not to be branches of the blood vessels, but tunnel-like extensions of the extracellular zones that partly or completely surround all but the smallest blood vessels (figures 1 and 2). In the section shown in figure 3, plate 6, such a perivascular space is confined to the upper right of the vessel, whereas in figure 20, plate 13, there is an extensive zone completely surrounding the blood vessel. These zones contain (in addition to two cellular components mentioned below) two main extracellular elements, fine granular material and collagen-like connective tissue fibres (figure 20). An example of the fibres is shown in high magnification in figure 17, plate 11. Here the fibres are cut longitudinally and shown a macro-period of about 560 Å with a number of sub-bands. This material will henceforth be referred to as collagen, although detailed studies of the periodicity, arrangement of the bands, amino acid analysis and X-ray diffraction studies are necessary before this material can be pronounced identical with vertebrate collagen (see Gray 1959; Smith & Treherne 1963).

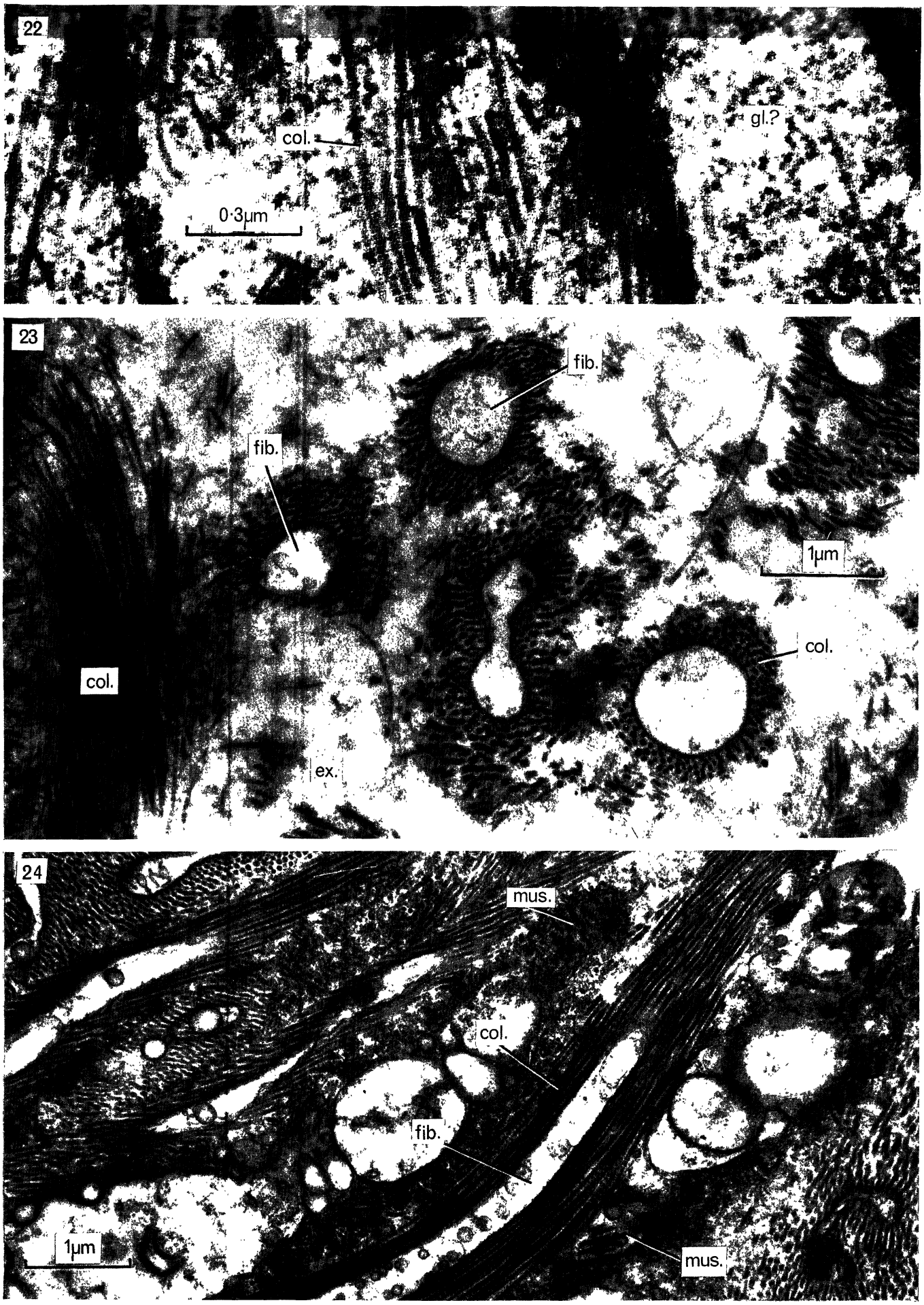


FIGURE 22. Fine collagen-like fibrils and glycogen-like granules. Both occur in an extracellular zone in the cortex of the suboesophageal lobe.

FIGURE 23. Cross-section of fibrocyte processes with associated collagen (medulla, optic lobe).

FIGURE 24. Longitudinal and transverse sections of fibrocytes with associated collagen. Cross-sections of muscle fibres are also seen (medulla, optic lobe).

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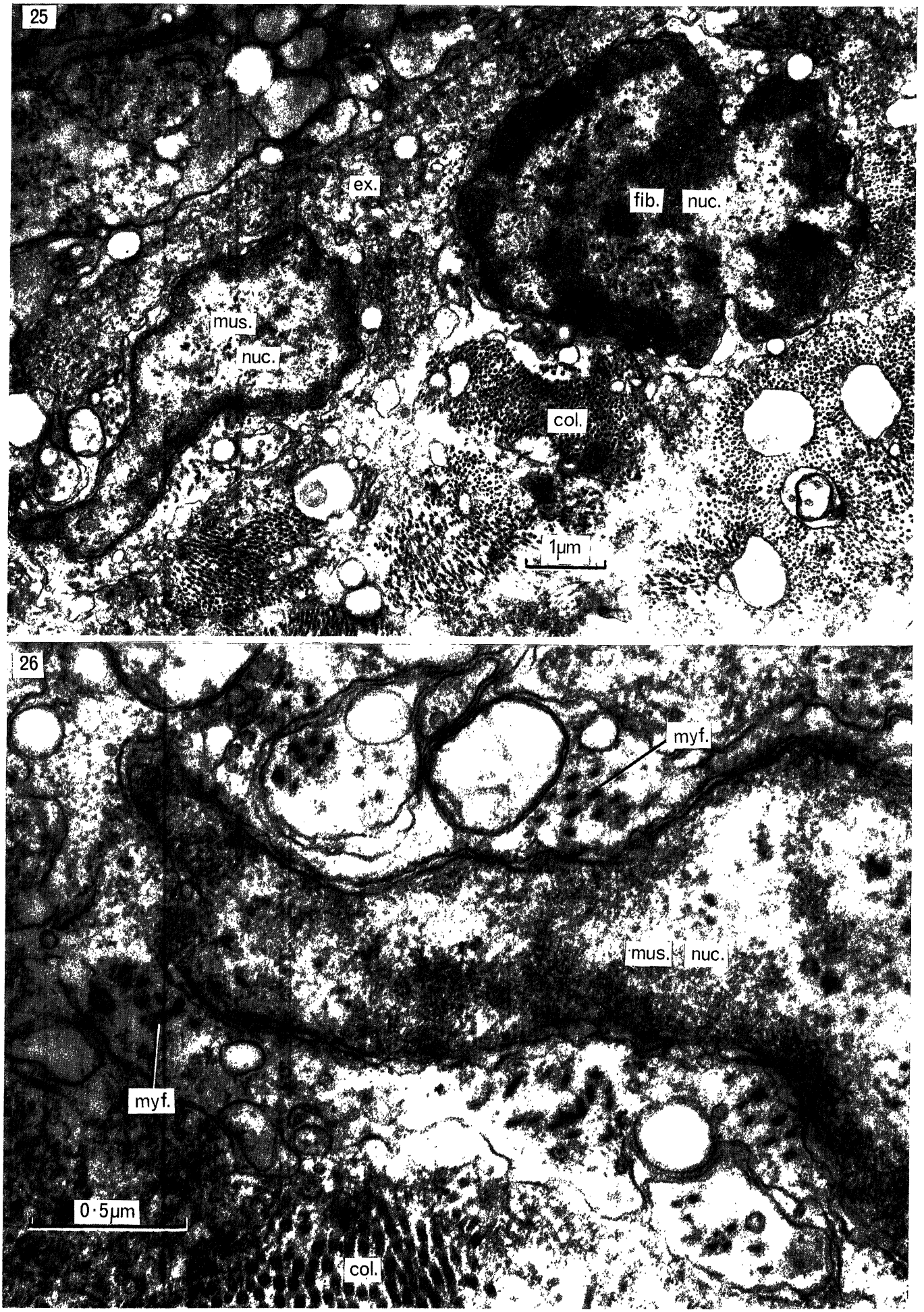
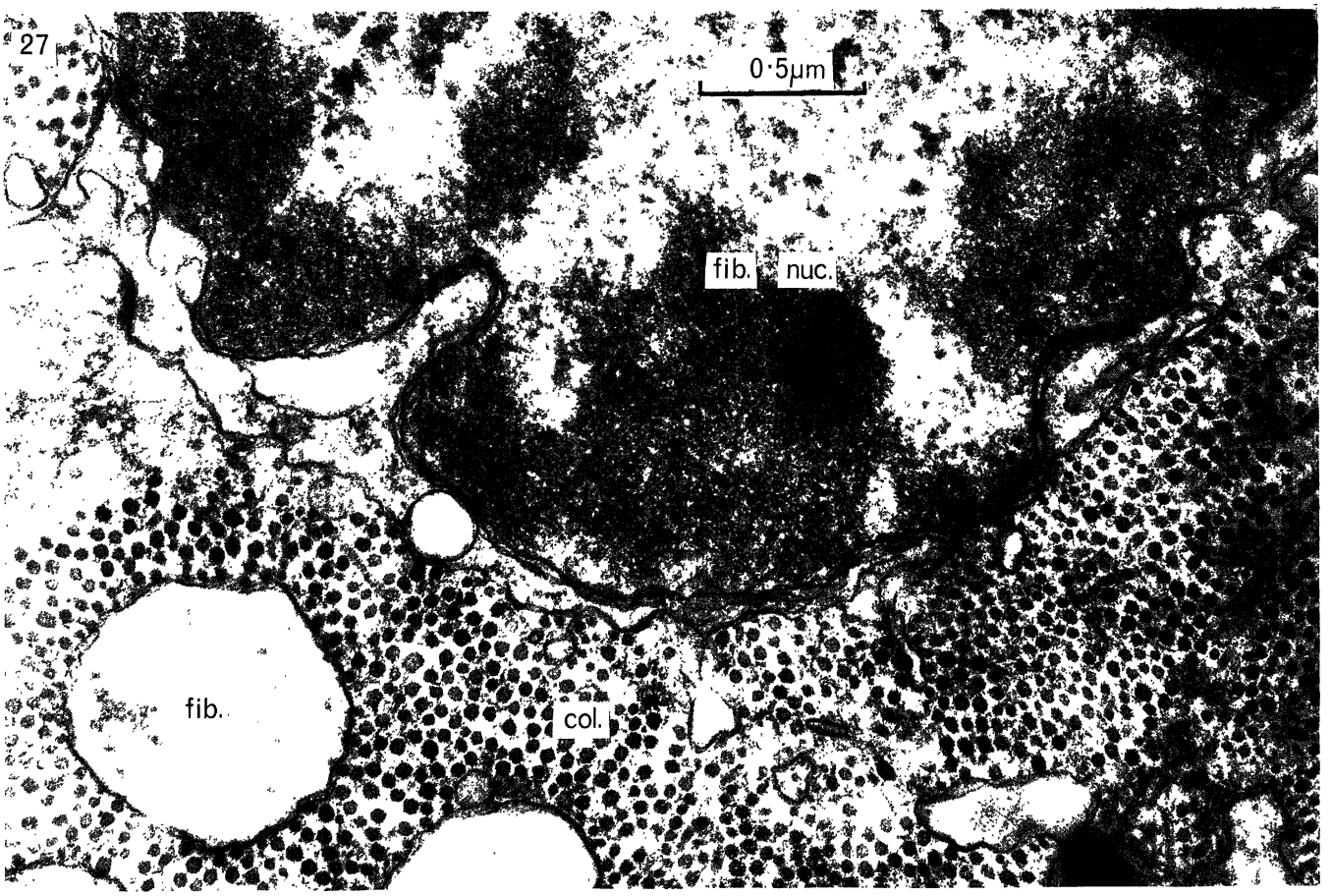


FIGURE 25. Section through a smooth muscle cell (left) and a fibrocyte (optic lobe medulla).
FIGURE 26. Enlarged portion of the muscle cell shown in figure 25.



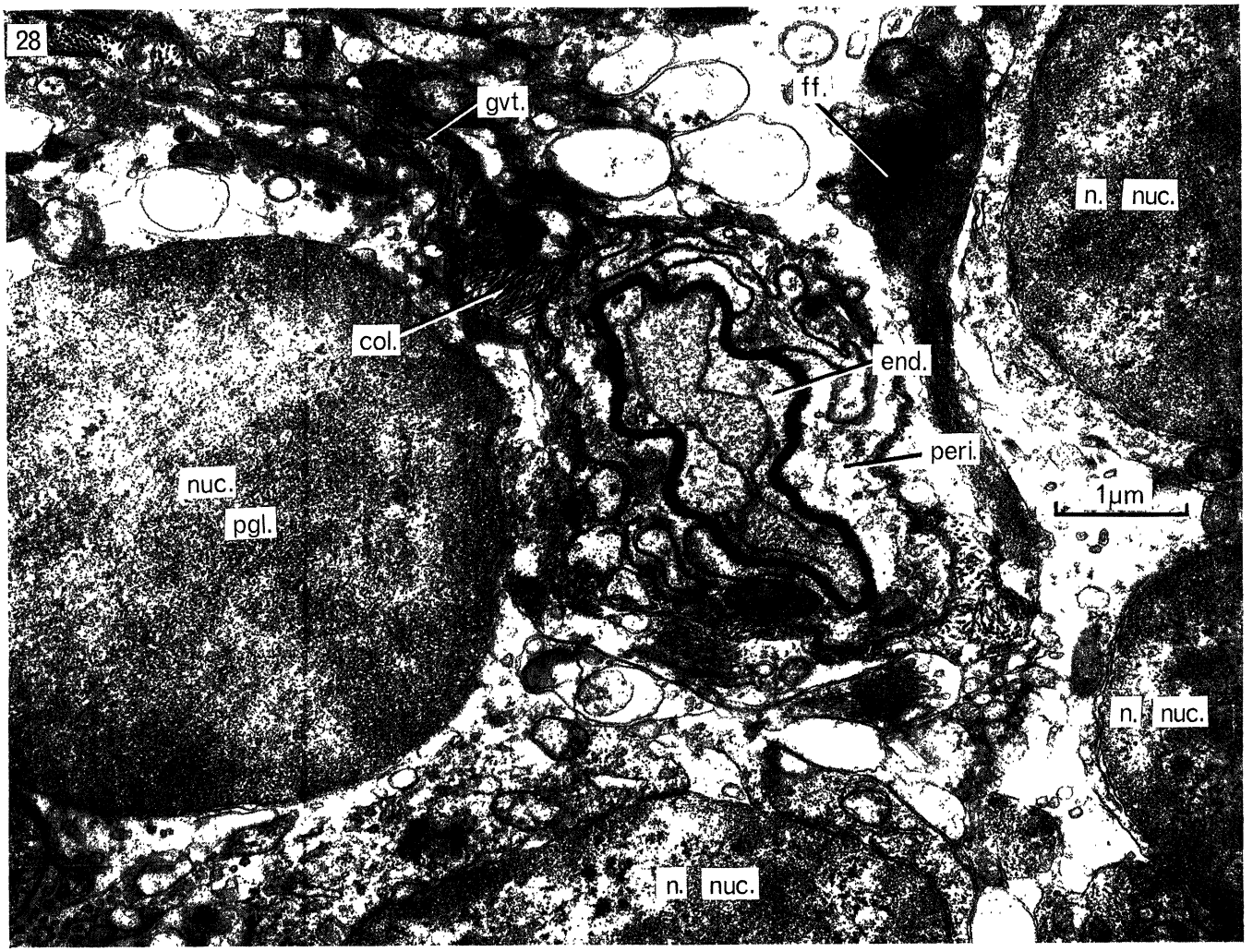
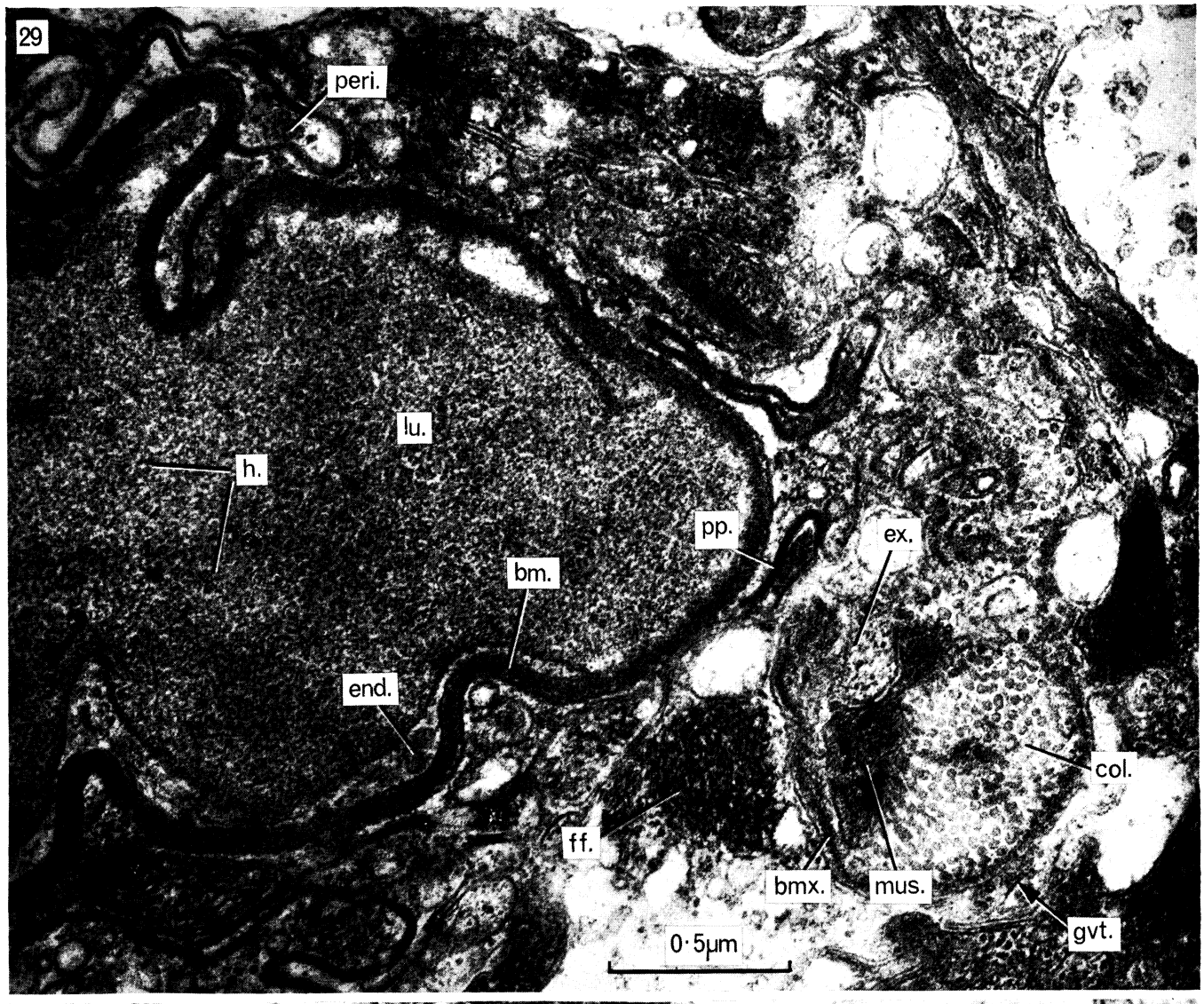


FIGURE 27. Enlarged portion of the fibrocyte shown in figure 25.

FIGURE 28. Cross-section of a capillary with a glio-vascular tunnel extending away above and to the left (medulla optic lobe).



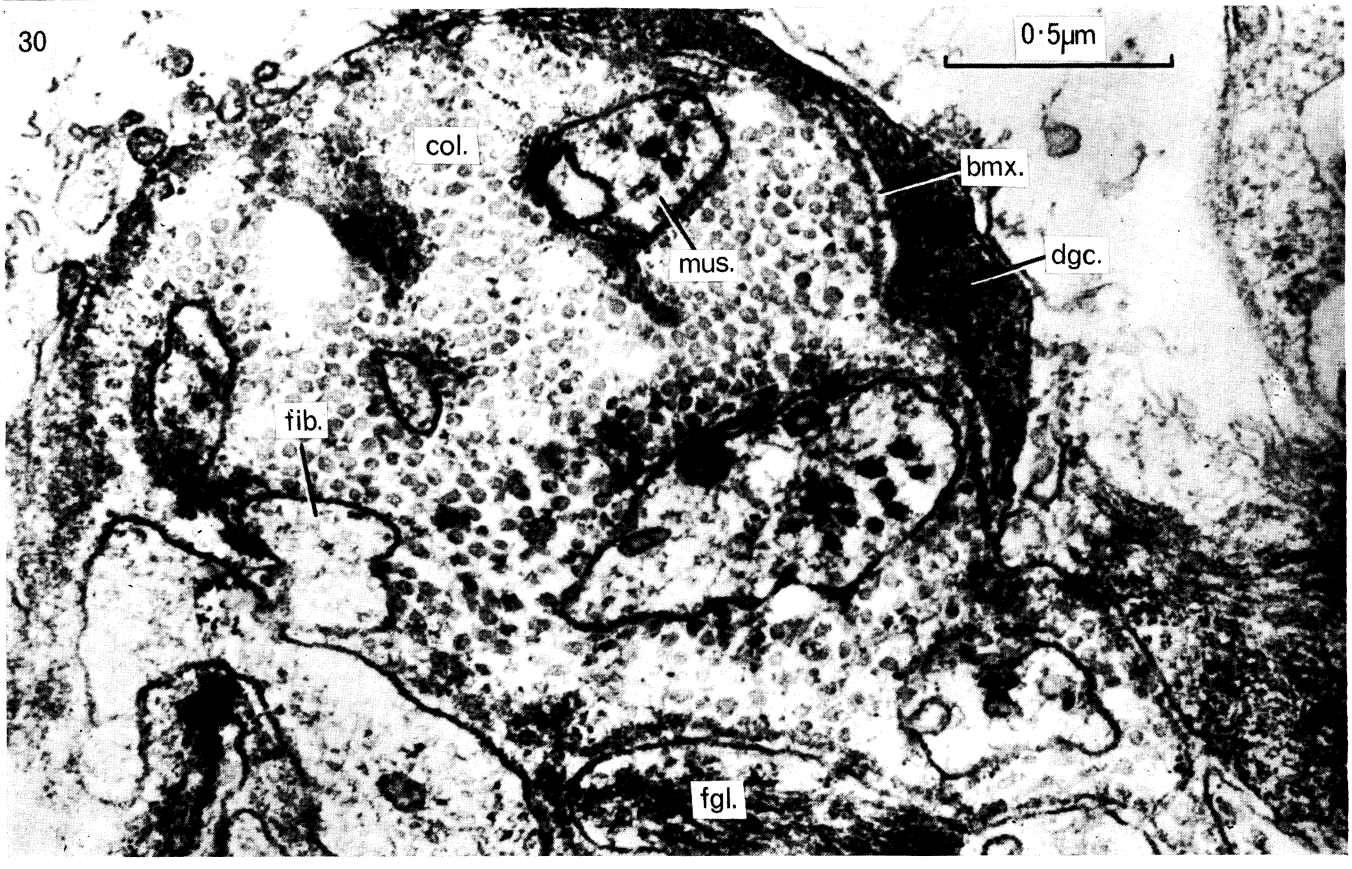
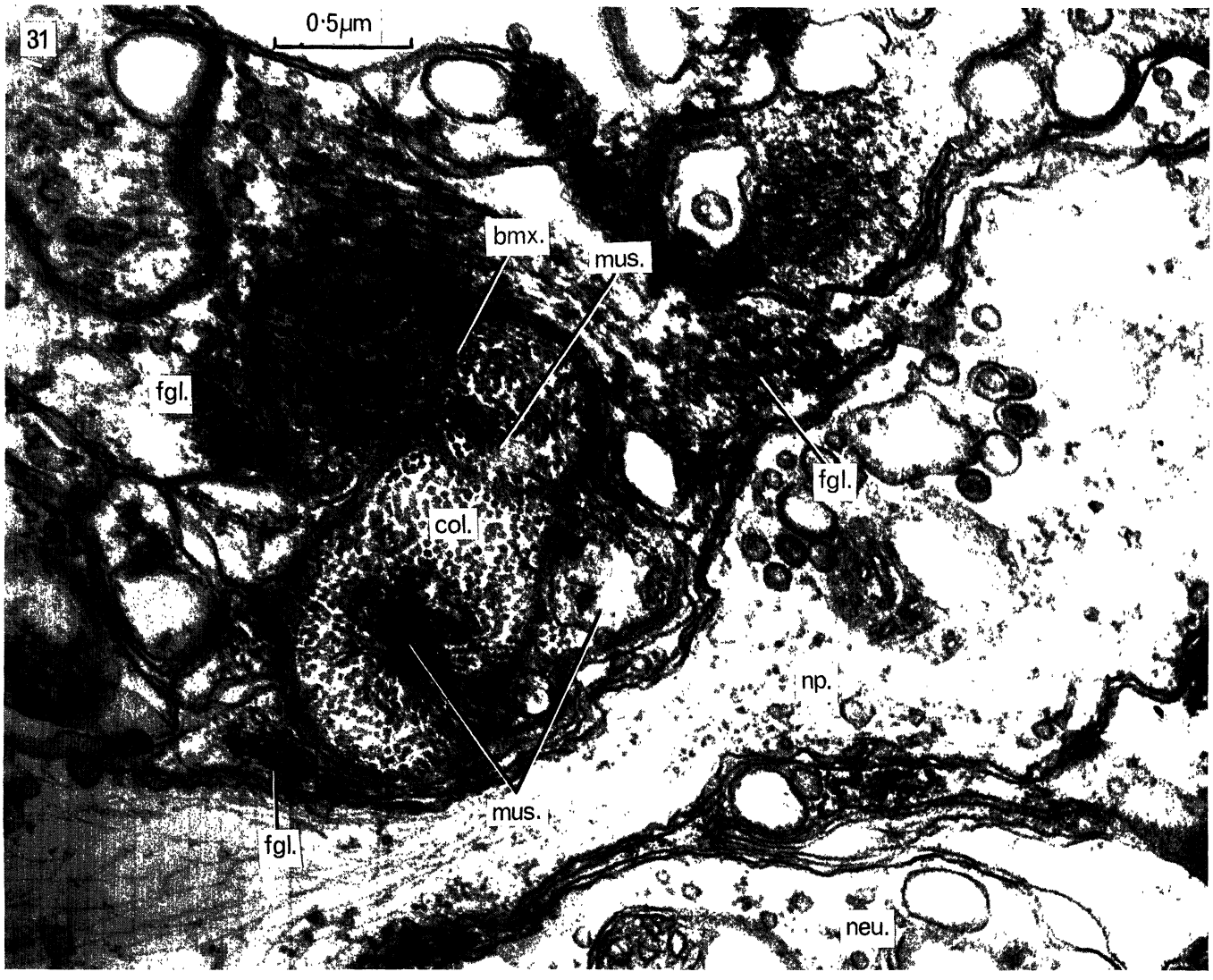
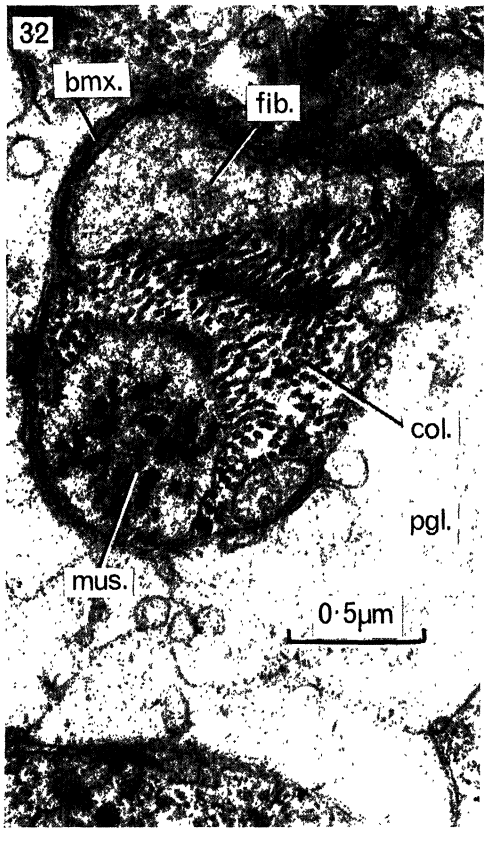


FIGURE 29. Cross-section of part of a capillary with adjacent glial and muscular processes (optic lobe).

FIGURE 30. Cross-section of a glio-vascular tunnel. Protoplasmic and fibrous glia lie below and a dark process is seen on the right (optic lobe).





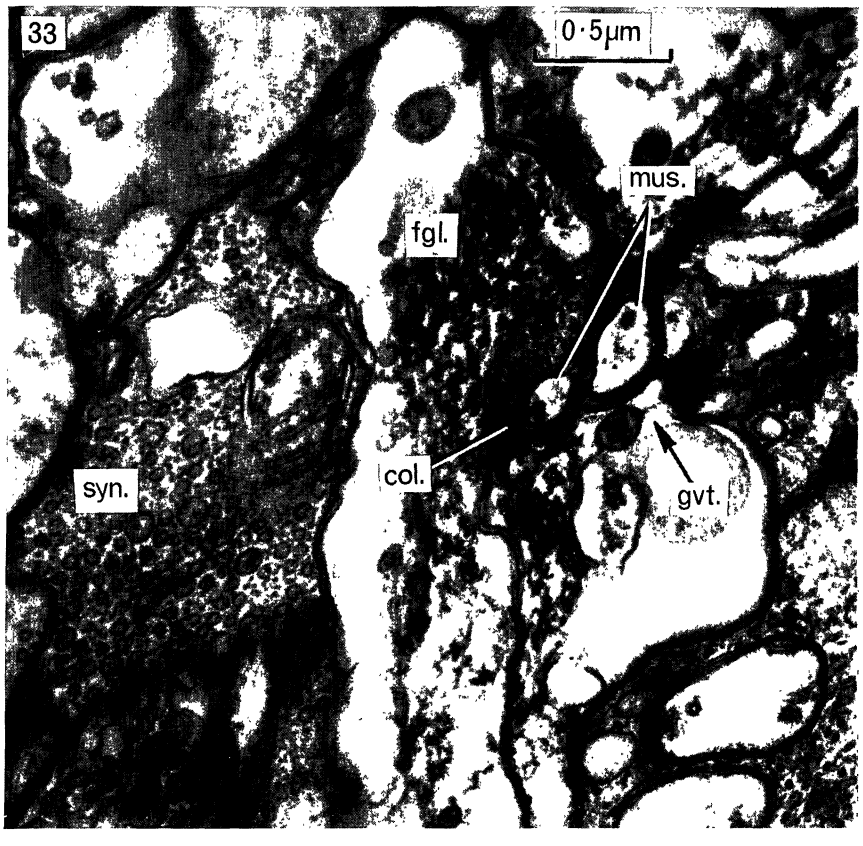


FIGURE 31. Cross-section through small glio-vascular tunnel. It is divided into compartments by a basement membrane (medulla suboesophageal lobe).

FIGURE 32. Cross-section through a small glio-vascular tunnel (optic lobe).

FIGURE 33. Cross-section through a very small glio-vascular tunnel. It contains two muscle fibres each with only one thick myofilament.

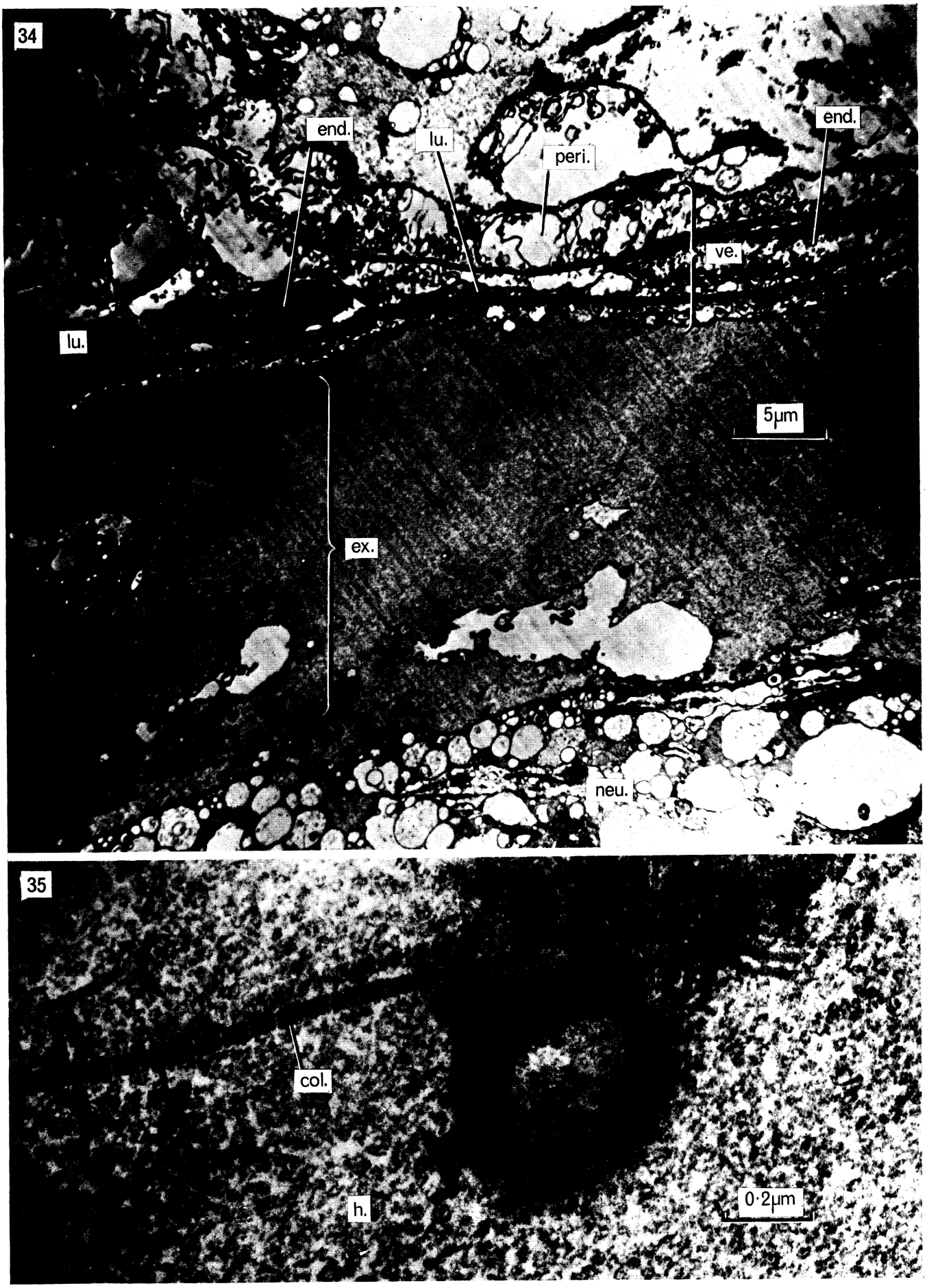
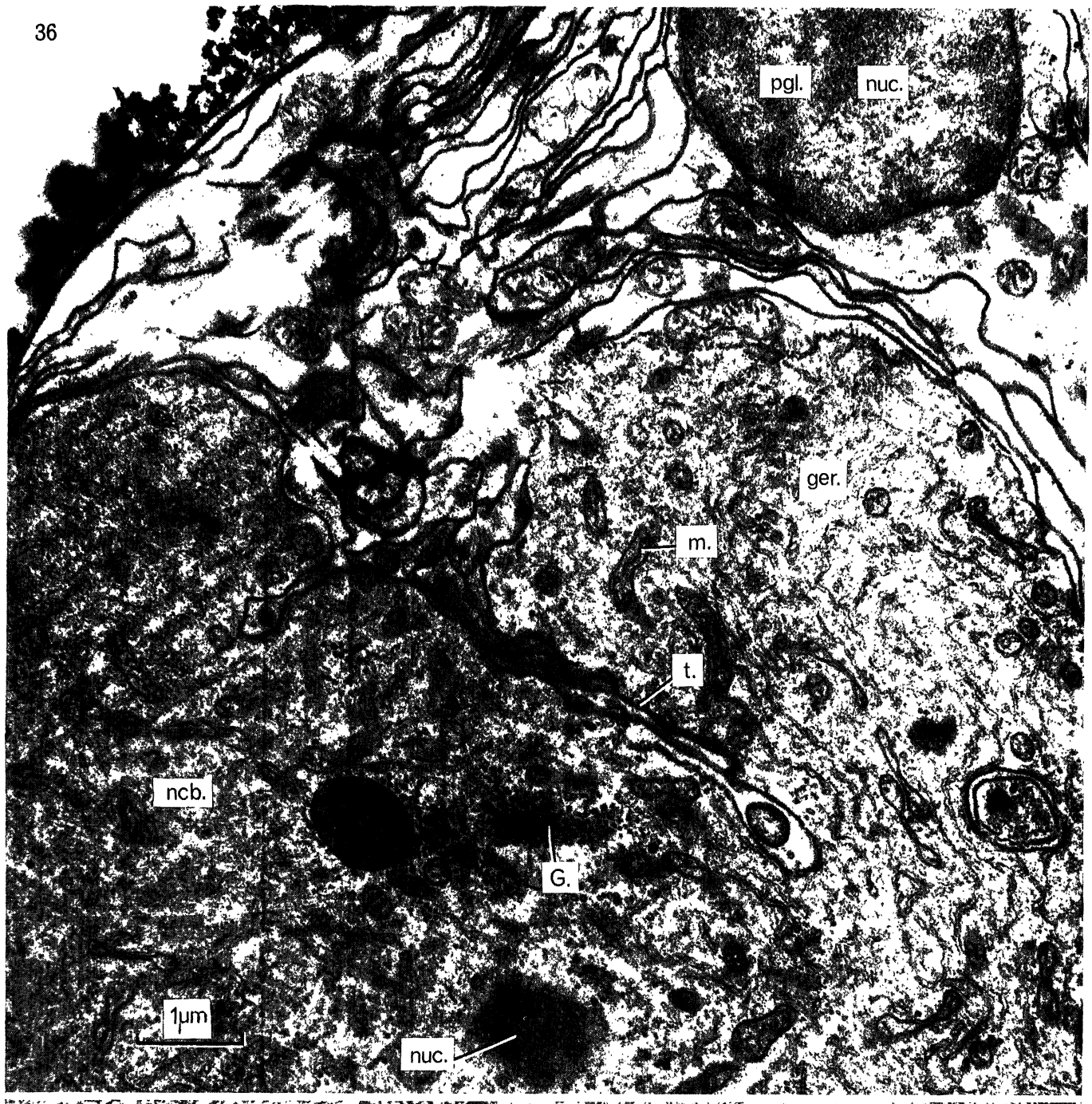


FIGURE 34. Longitudinal section through a vein situated adjacent to a large extracellular channel which contains haemocyanin (low magnification, medulla of optic lobe).

FIGURE 35. Extracellular zone containing a collagen fibre and haemocyanin molecules.



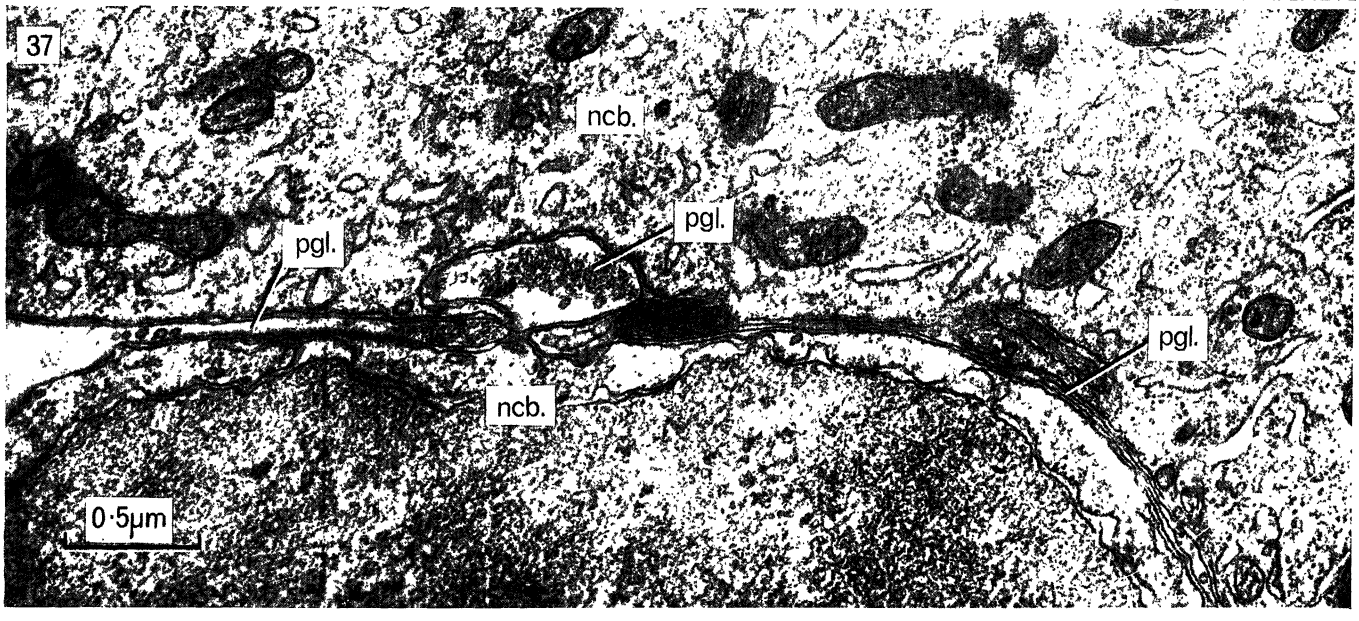
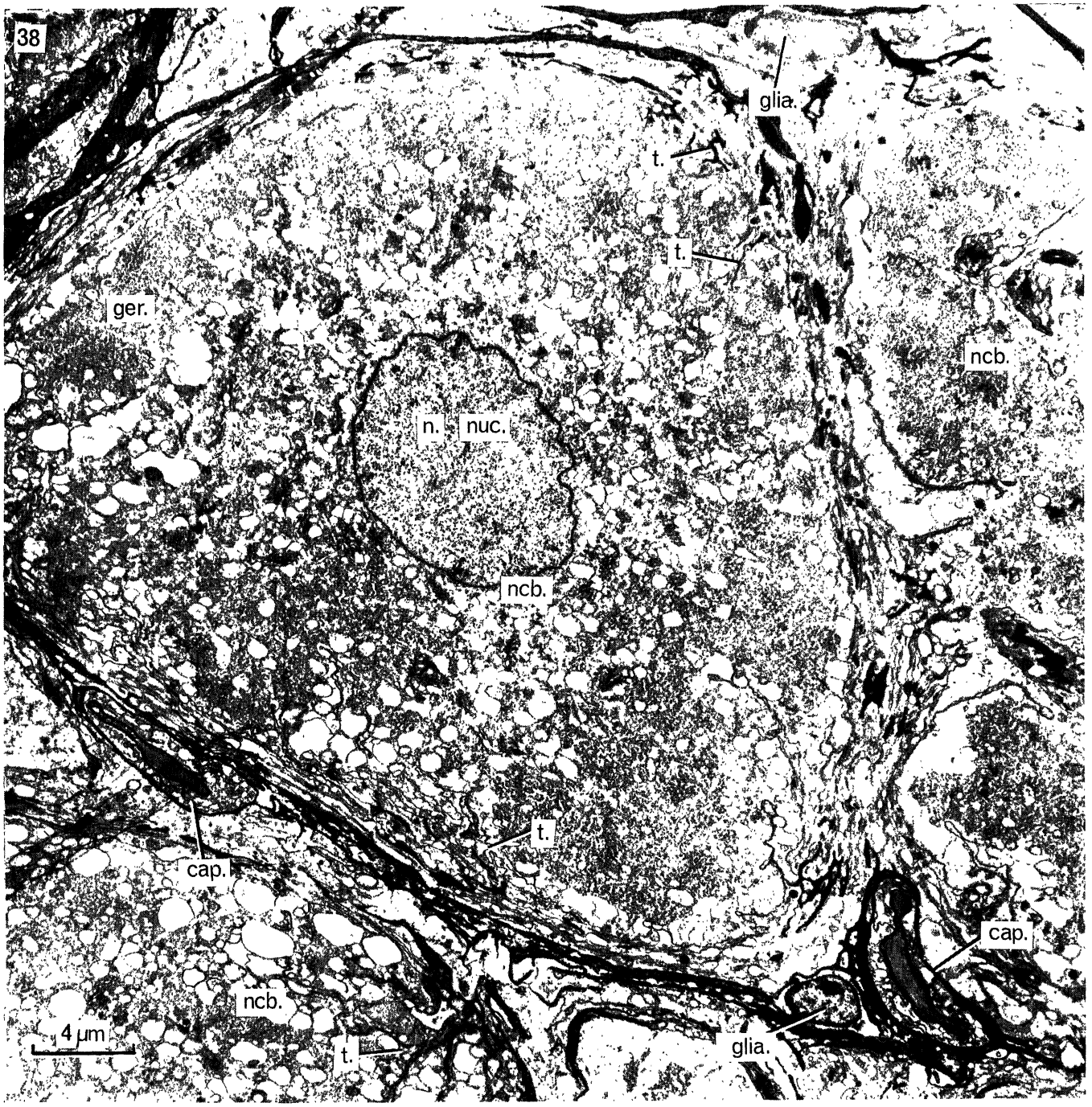


FIGURE 36. Small trophospongial invagination in medium-sized neuron in outermost zone of optic lobe.

FIGURE 37. Surface region of medium-sized neuron with glial lamellae and glial indentation (medulla, optic lobe).



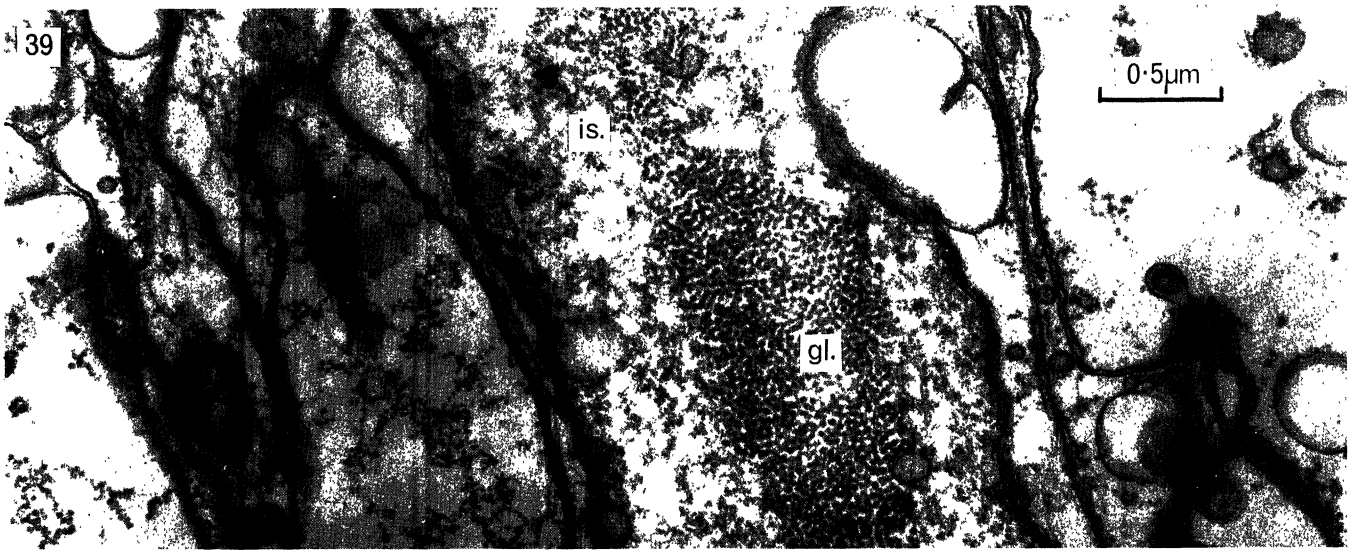


FIGURE 38. Section taken at low magnification through the large neuron cell bodies in the cortex of the suboesophageal lobe.

FIGURE 39. An axonal initial segment of a large neuron of the suboesophageal lobe. The axon contains glycogen.

Some of the smaller vessels may have no appreciable extracellular zones outside the pericyte layer. In figure 4, plate 6, for example, a presynaptic knob can be seen in direct apposition to a pericyte surface with only a 100 to 150 Å intercellular cleft intervening. In the cortex of the optic lobe many of the fine vessels lack the extracellular sleeve. Figure 21, plate 13, shows such a vessel in longitudinal section with synaptic and glial profiles directly apposed to the pericyte layer.

Examination of the lateral glio-vascular tunnels shows that they contain four main elements, the fine granular material, collagen fibres, fibrocytes and elongated cells thought to be smooth muscle cells (see figures 1 and 2). All these are also present in the perivascular extracellular zones.

The cell bodies of the fibrocytes and muscle cells are best studied in the hilar region of the medulla of the optic lobe, where extracellular zones are extensive and packed with collagen fibres (figure 25, plate 15). Here both a fibrocyte and muscle cell can be seen. The former is identified because of its close association with collagen (part is enlarged in figure 27, plate 16) and absence of myofilaments. The latter (part enlarged in figure 26, plate 15) contains bundles of regularly spaced myofilaments. The same criteria can be used to identify cross-sections of their processes, which, of course, cannot usually be followed to the cell body in the plane of section. In figure 23, plate 14, presumed fibroblast profiles are cut transversely and in figure 24, plate 14, some are cut longitudinally. Note the absence of myofilaments and the close association of collagen. Figure 20, plate 13, shows a muscle process in cross-section and illustrates the lack of association with collagen, although the fine extracellular granular material is commonly found around such processes.

Both fibrocytes and muscle cells occur in the tunnels as well as in the perivascular spaces. The tunnels may be as much as 6 μm or as little as 0·2 μm in diameter. One is shown in longitudinal section branching out from the perivascular zone of a small blood vessel (figure 28, plate 16). Both regions contain collagen. In figure 29, plate 17, a small tunnel is seen near a blood vessel and in figures 30 to 33, plates 17 and 18, cross-sections of tunnels can be seen in the neuropil. They all contain one or more muscle fibres identified by their myofilaments, and fibrocyte processes and the rest of the lumina of the tunnels are packed with collagen fibrils. The walls of the tunnels are formed from both protoplasmic and fibrous glia (identified by their glial filaments).

The glia also form an outer boundary around the perivascular spaces of the smaller blood vessels. The cell bodies of the glia either lie against these (figure 28) or applied to the tunnels (as part of the glial lining) or they can be situated out in the neuropil, and have elongated processes that form 'end-feet' on the perivascular spaces or the tunnels (figures 29 to 33). 'Dark' cell processes also have a similar relationship. Figure 30 shows such a process lining part of a tunnel with fibrous glial processes lining other parts.

A dense lamina can sometimes be seen within the glial sheath and intimately related to the outermost collagen fibres of the tunnels (and perivascular zones). Since this lamina is extracellular it is here regarded as a basement membrane (figures 1, 2, *bmx*). It is seldom complete however and seems only to line parts of the tunnel wall (see figure 30, plate 17 and figure 32, plates 18). Sometimes (figure 31, plate 18) it partitions the tunnel into separate tubes. As mentioned above, some of the tunnels are extremely small

(figure 33). Here a tunnel has a cross-sectional diameter of only 0·8 μm. It contains densely packed collagen and contains two muscle fibres each with only one 'thick' myofilament. Note the sheath of glial processes and outside this, neuronal processes and synapses.

Haemocyanin in extracellular zones

Examination of the larger vessels in plastic sections with phase-contrast light microscopy often reveals the luminal material to have a translucent homogeneous appearance, and electron microscopy shows that this results from the presence of aggregations of loosely packed haemocyanin molecules in the fixed material. Whilst the large vessels in the hilum of the optic lobe were being studied certain large vein-like channels were observed with phase microscopy to contain the same translucent material. However no endothelial lining or pericyte layers could be detected (compare the blood vessel above it). The same channels were examined with the electron microscope by re-embedding the section and cutting ultrathin sections of it.

Such channels (*ex*) (figure 34, plate 19) were seen to contain haemocyanin molecules indistinguishable in morphology and packing density from that observed within blood vessels. Figure 35, plate 19, shows the ring shape of the haemocyanin molecules, which are about 300 Å in diameter (see van Bruggen & Weibenga 1962; Barber & Graziadei 1965). The spaces containing haemocyanin are continuous with the other collagen containing extracellular zones and tunnels. Figure 35 shows a collagen fibre surrounded by haemocyanin molecules. The significance of haemocyanin in extracellular channels will be considered in the Discussion.

Glio-vascular organization round neuronal perikarya

Three main arrangements can be recognized which are related to the size of the neuron.

Small neurons

These small so-called granular neurons are present in astronomical numbers in all the lobes described here (and others not dealt with here, see Young 1969) except the suboesophageal. They are unipolar neurons with perikarya ranging from 5 to 8 μm in diameter. The surface membranes of the neurons are directly apposed across a 100–150 Å cleft (see arrows, figure 9, plate 9) and the flat facets resulting from mutual apposition during ontogeny give the perikarya polygonal profiles in section. Glia and blood vessels are scattered throughout the granule cell masses. In summary the granule perikarya are not encased or contacted by glial folds (although occasionally a glial cell will be adjacent) and generally speaking collagen extracellular spaces and lateral tunnels are reduced or absent in the cortical zones where the granule cell bodies mostly occur.

Medium-sized neurons

These cells have perikarya ranging from 10 to 20 μm in diameter and the ones studied here were found either on the inner border of the cortex of the vertical lobe, or the outer border and the medulla of the optic lobe.

These perikarya have thin sheets or finger processes of glia (usually the protoplasmic variety) covering parts or most of the cell surface (figure 37, plate 20) and separated by the usually 100 to 150 Å cleft. The glia usually consist of a single layer, although several layers are sometimes present. The glial process (or processes) may only slightly indent the perikaryal surface as in figure 37 or very occasionally on these medium-size neurons send small trophospongial extensions into extracellular tunnels of the perikaryon (figure 36, plate 20). In this figure the neuron is seen in section lying just below the surface of the optic lobe (see Dilly *et al.* 1963). Above is a protoplasmic glial cell and its numerous folds form part of a subsurface glial palisade characteristic of the surface of the optic lobe. In other words, the numerous folds shown here are not specially related to the neuron surface, because other medium-size neurons situated slightly deeper in the cortex have only the one or two apposed glial folds mentioned above.

The large neurons of the suboesophageal lobe

These unipolar neurons have perikarya from 20 to 100 μm in diameter, situated in the cortex of the lobe (figure 38, plate 21—compare the size of the cell body of a glial cell in the bottom right corner). In this figure the nuclear profile looks small in relation to the size of the perikaryon because the section has passed through the edge of the nucleus. The cytoplasm contains masses of granular endoplasmic reticulum forming the cytoplasmic basis of the Nissl substance seen by light microscopy, Golgi apparatus, numerous agranular vesicles, bundles of neurofilaments and clusters of glycogen granules. Pigment granules and other globular structures (see Young 1932) occur but these and the other organelles mentioned still require a detailed study with the electron microscope.

By careful orientation of the block it was possible to cut sections running longitudinally through the trunks of these cells to show their continuity with the perikaryon. Wigglesworth (1960) using light microscopy, has described accumulations of glycogen, in the hillock regions of insect neurons, so this possibly was investigated in the octopus. Only three of twenty-five initial trunk regions examined were found to contain glycogen, however (figure 39, plate 21).

Numerous small blood vessels (figure 38) run between the perikarya, which at this low magnification can be seen to be covered in a lamellated sheath. The sheath contains very dense zones and these can be seen at higher magnification to be extracellular and inserted between cellular glial lamellae (see below). Trophospongial invaginations of the perikaryal surface can be seen in several places, but are not conspicuous at this low magnification.

A part of the wall of a blood vessel and a perikaryal sheath is shown at higher magnification in figure 40, plate 22. Extracellular zones containing dense material can be seen surrounding the pericyte layer and between the glial lamellae. Possibly all these extracellular zones extending from the blood vessel to the neuron form one continuum, but serial sectioning is necessary to decide this point.

The extracellular zones contain fine collagen fibres (seen mostly in cross-section in figure 40, plate 22) and a new type of material consisting of fine and coarse granules. The coarse granules are arranged in a series of plates that can be seen in section (figure 41, plate 22) embedded in the fine granular material. It is these materials plus the fine

collagen fibres that can be seen in the sheath as dense zones in figure 38. Very occasionally less densely packed fine granules resembling glycogen were found amongst the fine collagen fibres (figure 22, plate 14). Not infrequently the basement membrane of the blood vessels of this region of the lobe contains dense granules (figure 42, plate 23) just like those forming the plates of the extracellular zones. These extracellular granulated materials have apparently not been described before and remain an enigma at present. Possibly they are glycogen, but to the author's knowledge glycogen in the form of granules has never been described by electron microscopists as occurring extracellularly.

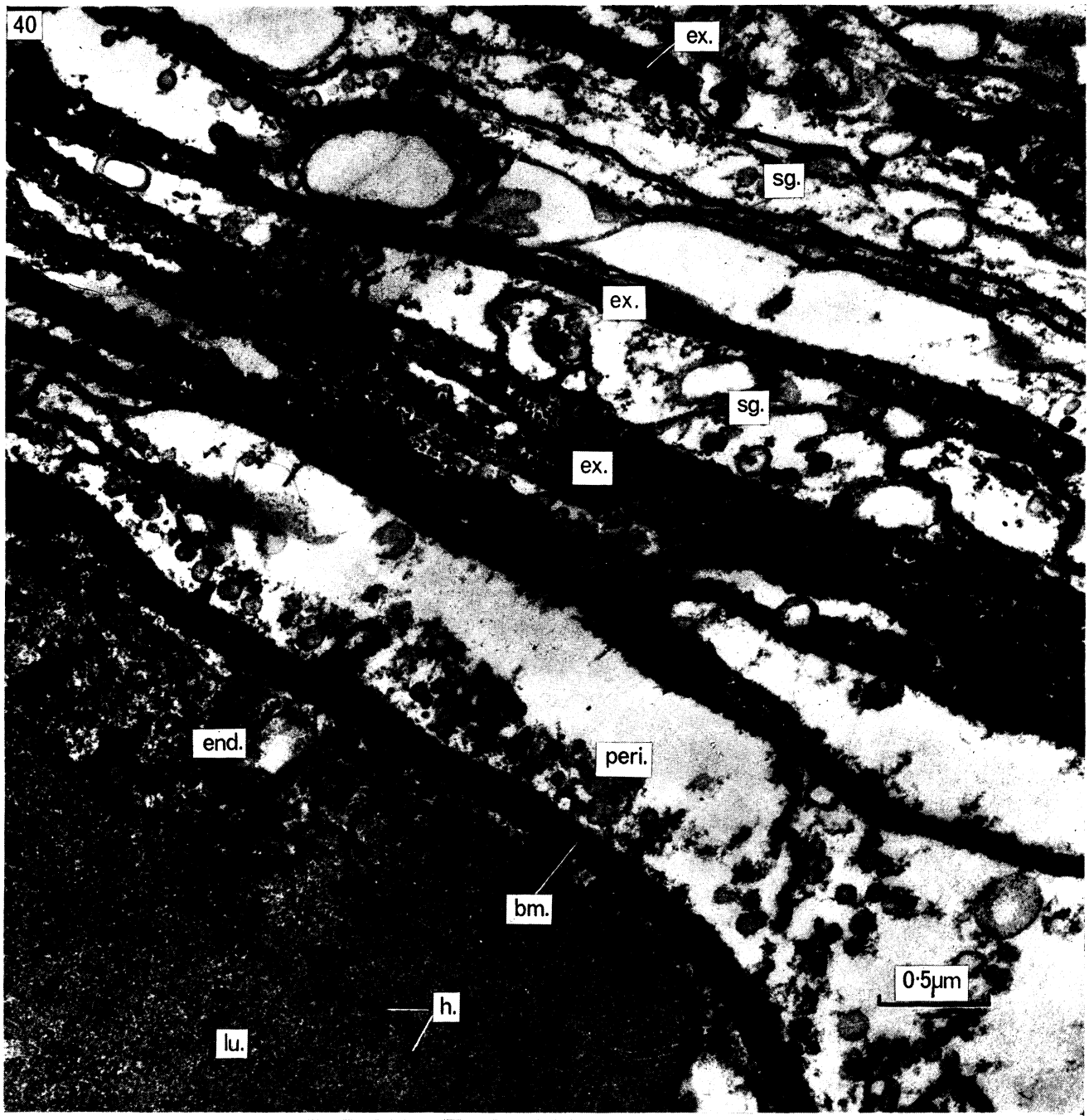
The glial lamellae are often packed with intracytoplasmic glycogen granules and may not contain bundles of glial filaments (figure 19, plate 12 and figure 40, plate 22). These glia resemble protoplasmic glia except that they contain more glycogen granules and their processes are more flattened to form series of lamellae. Perhaps they should be considered in a separate category. Stephens & Young (1969) were never able to impregnate them for light microscopy. Perhaps the high glycogen content interferes with the staining techniques. The trophospongial intrusions into the neurons may take the form of single channels (figure 45, plate 24) or, as in the section taken just below the neuron surface, show radiating extensions from a central channel (figure 44, plate 24). Both glial finger processes and extracellular material are invaginated to form the trophospongium seen in longitudinal section in figure 45 and in transverse section in figure 43, plate 23. The glial processes contain glycogen and glial filaments and the extracellular zone the granular organization mentioned above. Note that the glial folds or finger-like extensions are always apposed to the neuronal membrane while the extracellular zone forms the central core, insulated from the neuron surface by the glia. Collagen fibres have not so far been found accompanying the granular material in the extracellular zones of the trophospongia. Stephens & Young (1969) have observed collagen in the larger trophospongia, however, in preparations stained for light microscopy.

A note on electron microscopy of Golgi material

Although an invaluable technique in the study of the nervous system, the mechanisms of the Golgi method and the way it impregnates various and isolated elements are far from understood. In the preceding paper Stephens & Young (1969) have used this method to study the neuronal and glio-vascular organization. They observed that in preparations where the blood vessels tended to be preferentially impregnated, one or perhaps all of the elements involved—the endothelial cells, pericyte layer or glial layer could take up the stain. Electron microscopy of the Golgi material confirmed this view. Figure 46, plate 25, shows the endothelium impregnated: figure 47, plate 25, the pericyte zone and figure 48, plate 25, almost certainly shows a glial process outside the pericyte zone. This technique has not been applied extensively but it will clearly prove valuable in correlating light- and electron-microscopic observations (see Blackstad 1965).

Discussion

Barber & Graziadei (1965, 1967*a*, *b*) described the innermost layer of cells in the blood vessels as endothelium. In sections it often looks like a lining layer, the outermost



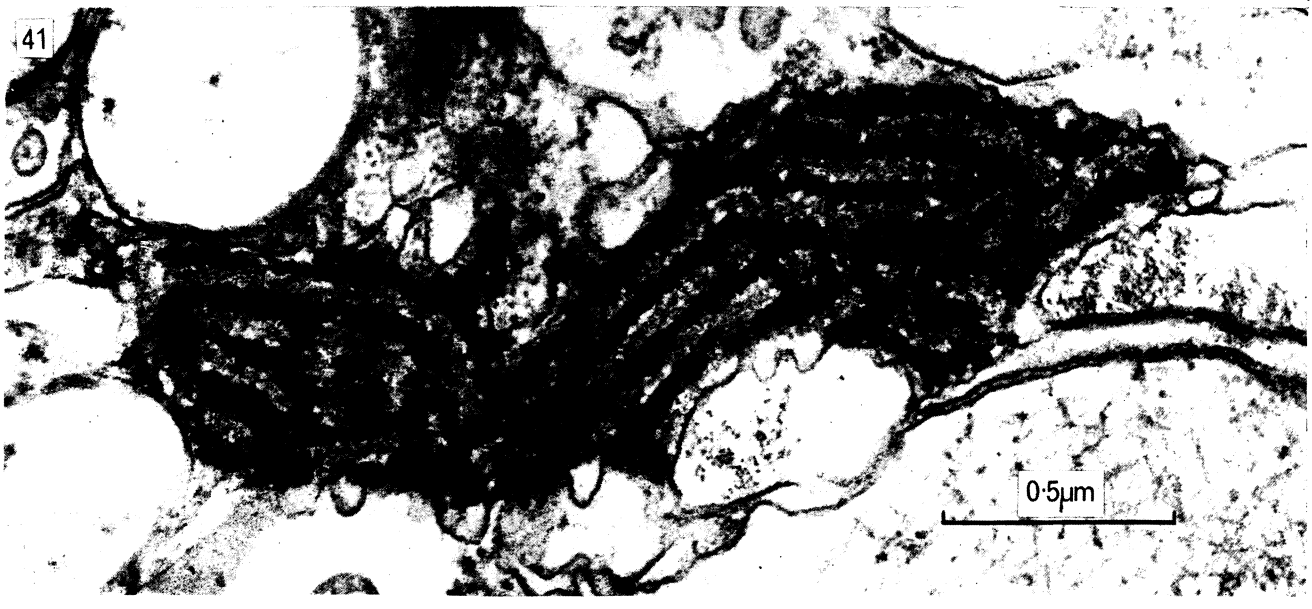


FIGURE 40. Portion of blood vessel (cross-section) and glial folds and extracellular zones forming part of the environment of a large cell body of a neuron of the suboesophageal lobe.

FIGURE 41. Section through the peculiar extracellular material encountered in the cortex of the suboesophageal lobe.

(*Facing p.* 24)

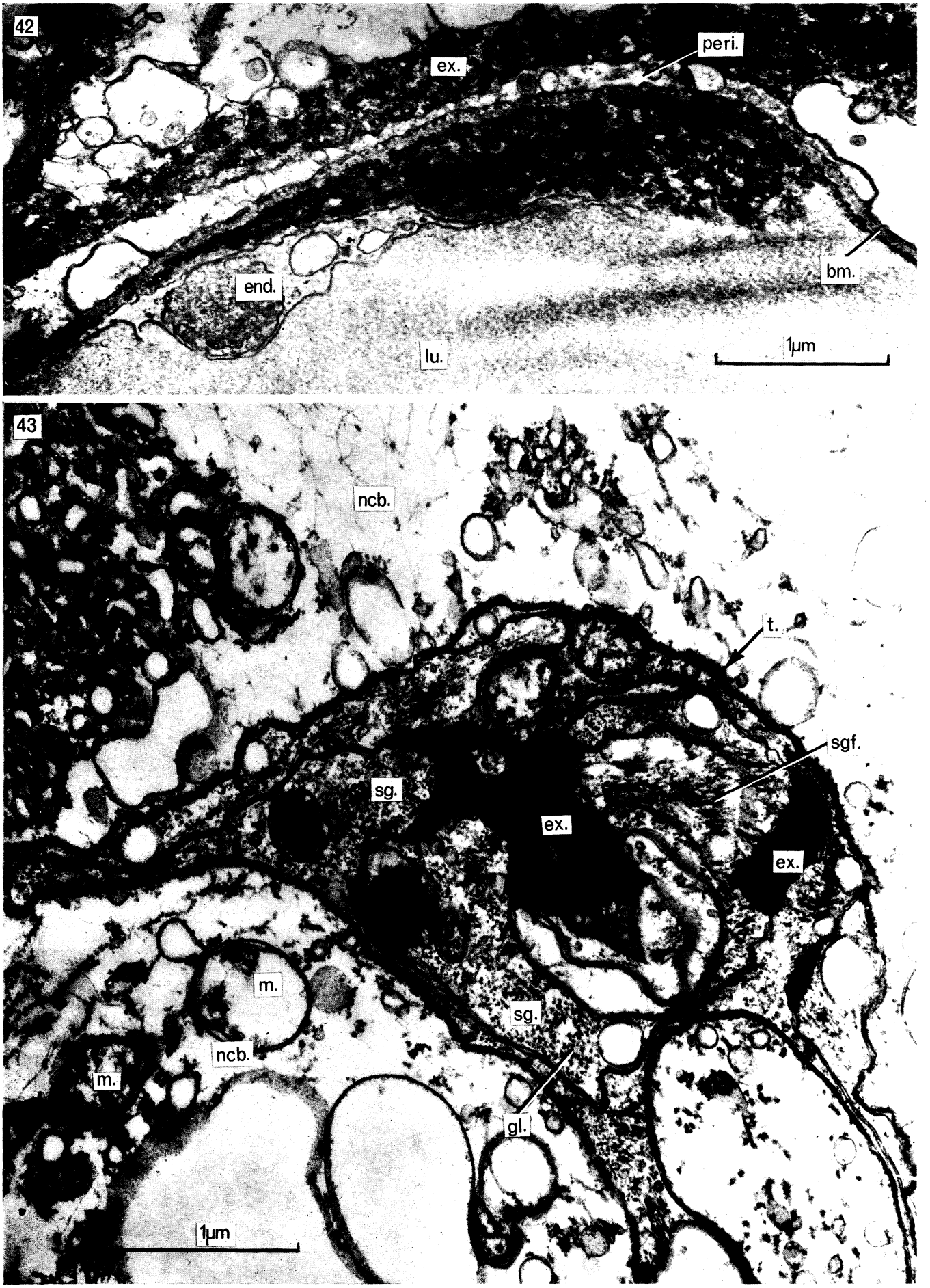


FIGURE 42. Part of the perimeter of a small vein. The basement membrane contains additional extracellular material (see figure 41).

FIGURE 43. Cross-section through a trophospongial invagination into a large neuron cell body of the suboesophageal lobe.

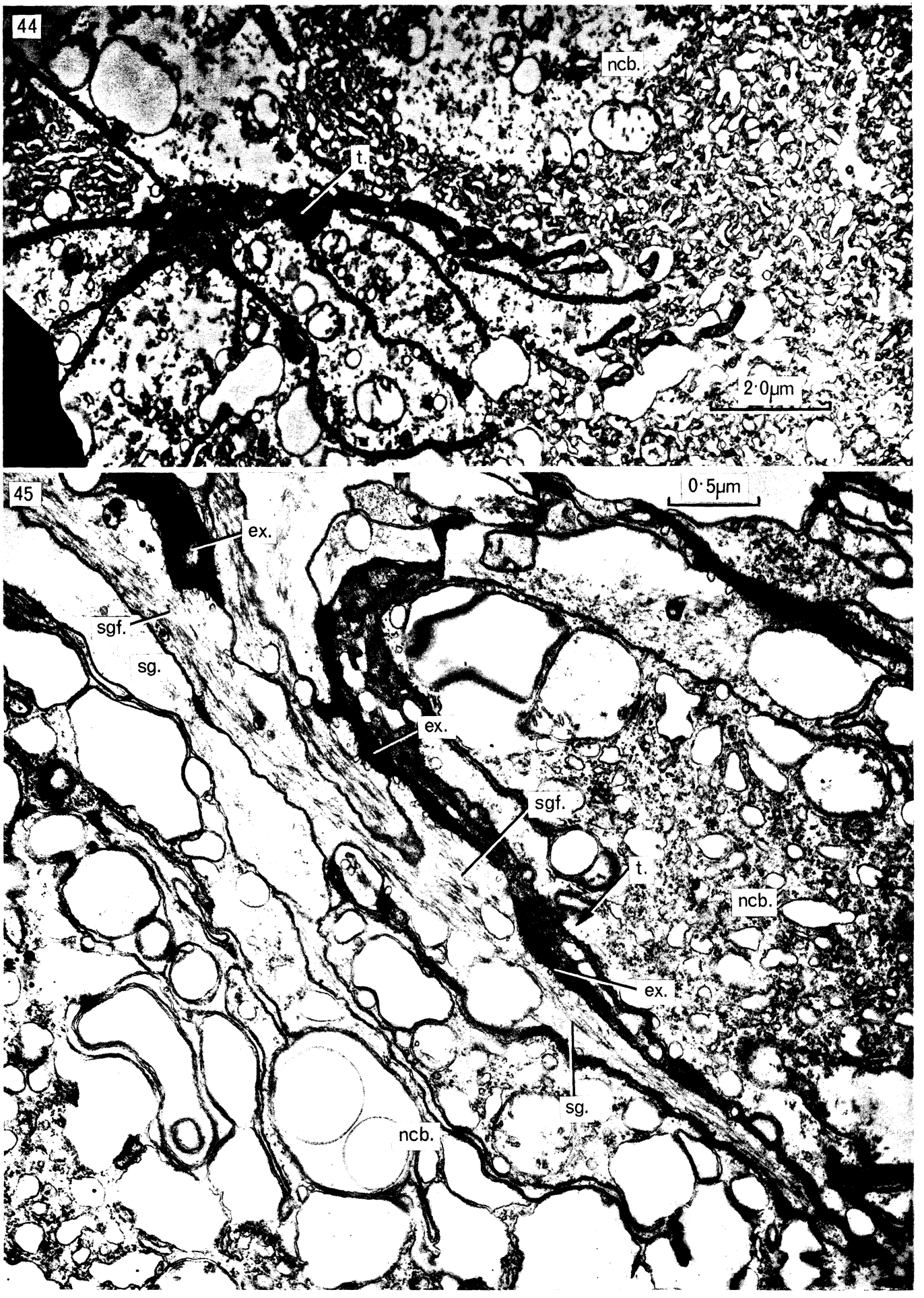
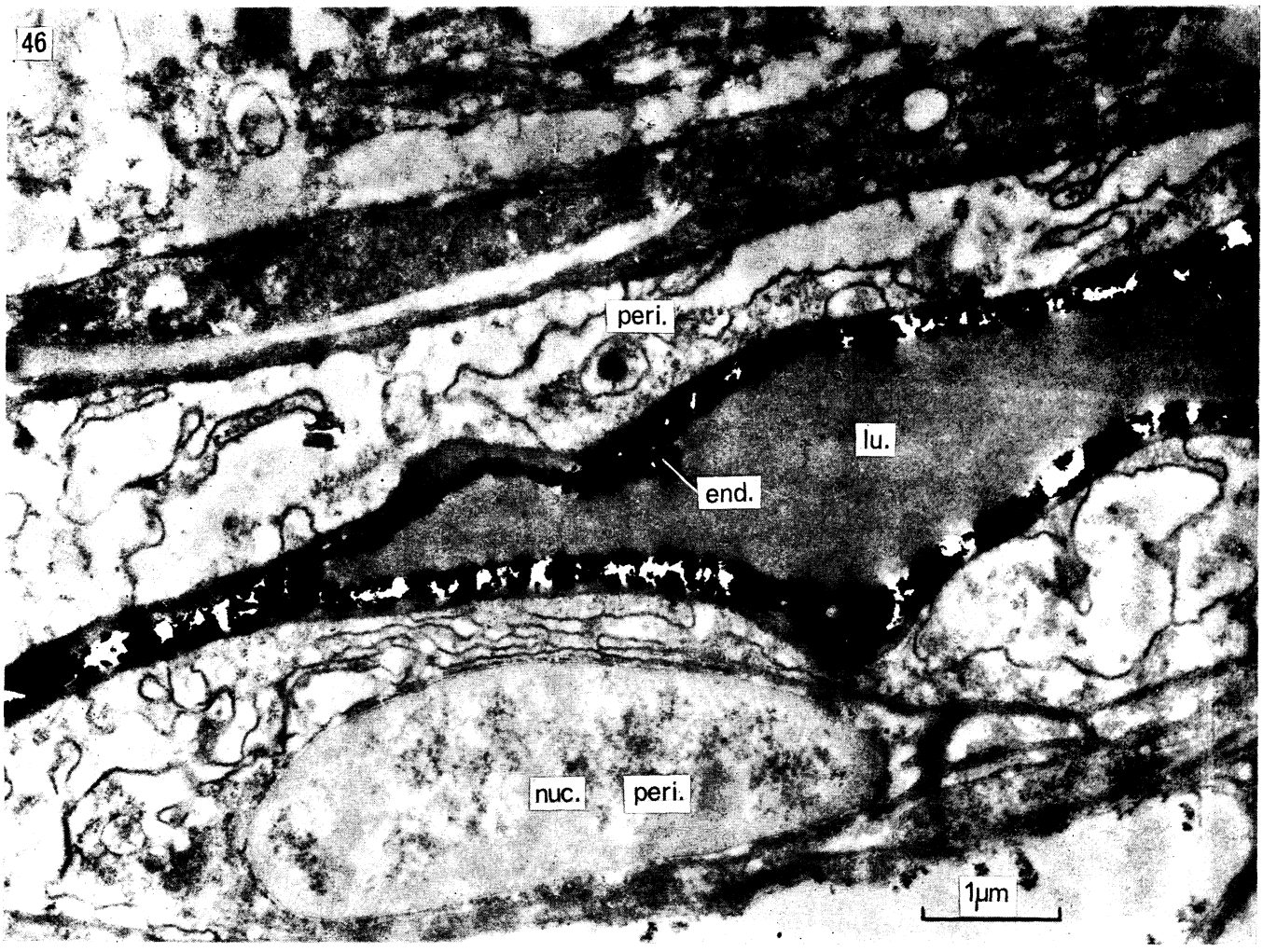
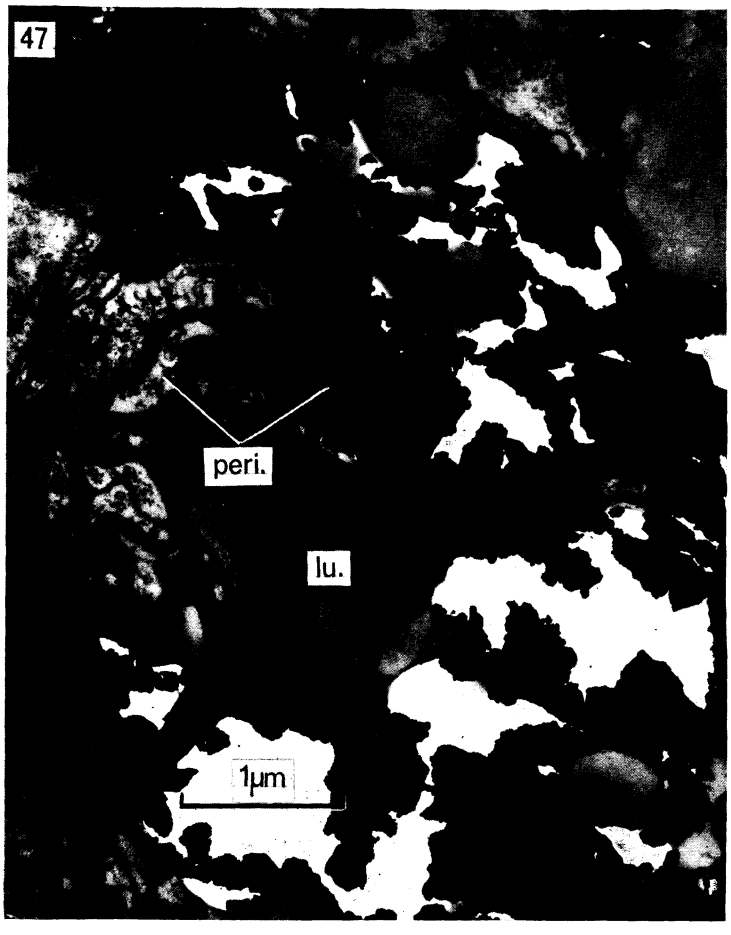


FIGURE 44. Low-power cross-section through a trophospongium which has numerous side channels (neuron of suboesophageal lobe).

FIGURE 45. Longitudinal section of a trophospongium of a large neuron (suboesophageal lobe).





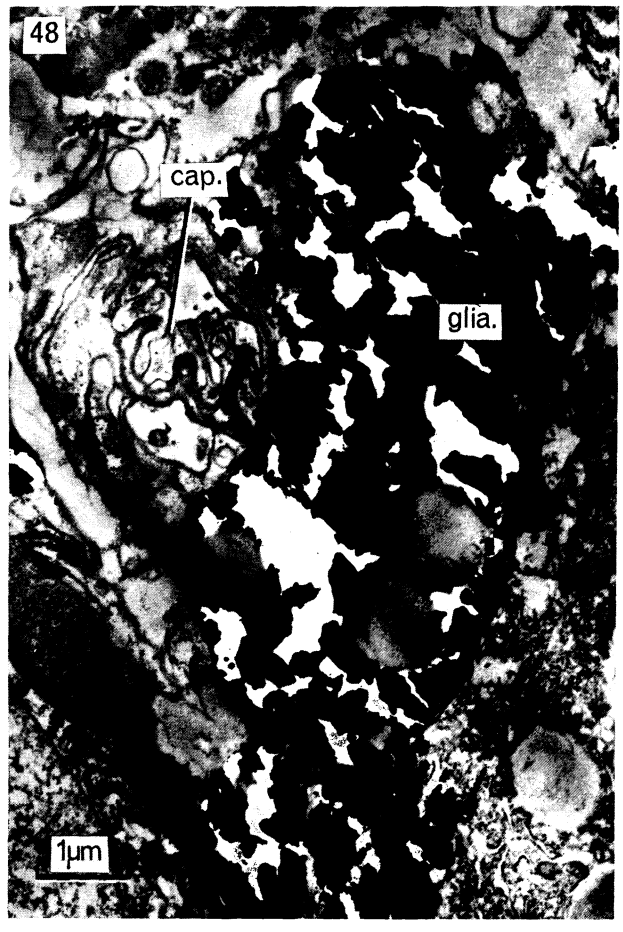


FIGURE 46. Electron micrograph of section of Golgi impregnated capillary showing labelled endothelium (optic lobe).

FIGURE 47. Electron micrograph of section of capillary showing labelled pericytes (optic lobe).

FIGURE 48. Electron micrograph of section of capillary showing labelled glia (optic lobe).

parts of the cells having flattened surfaces apposed to the basement membrane. However, the lining is discontinuous, especially in the smaller vessels, with bare areas of basement membrane often 1 μm or more across and so it differs from vertebrate endothelium, which is, in all but a few special zones, complete. The octopus endothelium is similar to that of vertebrates in being situated inside the basement membrane, however. The layer outside the basement membrane they described as complete (and this is confirmed here) and they termed it the pericyte layer. The terminology of Barber & Graziadei has been adopted in this present work, but it may have to be revised in the future. In the earthworm, Hama (1960) adopted the term 'endothelium' for the layer outside the basement membrane because it looked like the true limiting layer of the blood. He could find few flattened cells forming an inside lining to the basement membrane. Coggeshall (1965) working on a different earthworm also used 'endothelium' to describe the layer outside the basement membrane. It could be that the cephalopod pericyte layer and the oligochaete endothelium are the same thing and that in the cephalopod a proportion of the blood amoebocytes exists as fixed lining cells. Barber & Graziadei argue (perhaps rightly) that their morphology (i.e. no granules) indicates that they are not amoebocytes. However, it could be argued that fixed amoebocytes become freed into the blood stream on the appropriate signal and only then do they develop granules, perhaps as a result of phagocytosis. Clearly some sort of labelling experiment is required to see if, in the octopus, amoebocytes which are thought to originate in the white body (Necco & Martin 1963) become incorporated in the endothelium. Perhaps it is wrong to look for homologies in the cell types of blood vessel walls in such distinctly separated groups in evolution as the molluscs and annelids.

In this paper two main types of glial cell and dark cells of uncertain nature can be distinguished in the octopus brain. Additional cell types include the fibrocytes (so-called because of their close spatial relationship to collagen, which they presumably secrete) and the smooth muscle cells in the extracellular channels. (See also the subpedunculate tissue of the optic lobe mentioned on p. 20.) Other cells occasionally encountered are probably amoebocytes, since they contain vesicles with dense material inside and coincide in appearance with the amoebocytes seen in the blood (see Barber & Graziadei 1965). Experimental lesions (Gray & Young 1968) produced large numbers of these cells, judged to be actively phagocytic, in the vertical lobe. Presumably they migrate from the blood vessels or metamorphose from fixed non-vesicular cells, perhaps not readily distinguishable from small neurons. The two types of glia seen with the electron microscope were the protoplasmic and fibrous glia. The two types of glia were distinguished first by light microscopy by Stephens & Young (1969) in the preceding paper. The sheathing glia around the very large cells (described here in the suboesophageal ganglia) are classed above with protoplasmic glia. The two sorts have similar cytoplasm with bundles of fine fibres. However, the sheathing glia often possess large aggregations of glycogen granules. This together with the fact that their processes are often numerous and laminated perhaps warrants a distinct category for them. In the vertebrates, especially, the mammals it is usual to distinguish protoplasmic and fibrous astrocytes (with varieties in different parts of the nervous system) and oligodendroglia and microglia. Thus in the octopus the protoplasmic and fibrous glia can perhaps be considered analogous to astrocytes in

their trophic and supporting roles. Oligodendroglia are peculiar to the vertebrates and are concerned with the development and maintenance of myelin (which in its compact form is absent in *Octopus* and for that matter in all invertebrates—see Gray 1969). Large axons in the central nervous system of *Octopus* and other invertebrates have complex sheaths, however, but at present it is not clear whether the sheath cells should be regarded in a separate category or whether they are modified protoplasmic glia. The mesodermal microglia of the mammalian brain probably also have no counterpart in *Octopus*. In *Octopus* brain the only extravascular phagocytes are probably amoebocytes migrated from the blood vessels, but as mentioned above this needs further investigation. At present there is no reason to suspect an analogy between the dark cells of the octopus brain and the mammalian microglia (which have been described by some as dark cells—see Mugnaini & Walberg 1965). Although fibrous and protoplasmic glia can be distinguished on morphological grounds, there is no evidence for functional differences as yet. Stephens & Young (1969) found that fibrous glia tended to occur in tract regions of the octopus brain. Perhaps they function during development to guide nerve fibres along appropriate pathways by means of their very long running processes (Stephens & Young). Certainly in the adult such fibres can be found enclosed in groups within the folds of the fibrous glia, but the same can be said for protoplasmic glia, where they were described above encasing optic fibres entering the cortex of the optic lobe. Amoroso *et al.* (1964) described in a gastropod mollusc, glial cells containing fibres resembling those seen with the electron microscope in the octopus fibrous glia. In the gastropod the glia were related to fibre tracts.

Amoroso *et al.* (1964) also described sheathing and supporting glia similar in some respects to the octopus protoplasmic glia. However, much more work is needed on comparative studies using both light and electron microscopy before one can generalize about homologies between glia in molluscs in particular and invertebrates in general (see, for example, Merker & Harnak (1967) on Archiannelida, Zimmerman (1967) on earthworm, Gray & Guillery (1963) and Coggeshall & Fawcett (1964) on leech, Rosenbluth (1963) on Gastropoda, Scharrer (1964) and Heuser & Doggenweiler (1966) on Crustacea and Trujillo-Cenóz (1962) and Smith & Treherne, (1963) on Insecta and their references and other papers quoted here).

Since the blood-brain barrier and its underlying morphology still remain rather an obscure phenomenon in mammals, it is perhaps idle at present to speculate about the existence of such a mechanism in the octopus. However, it is useful to compare the morphologies of the two groups so that future physiological studies can proceed with more certainty (see Barber & Graziadei 1967*a*).

In the octopus there have apparently been no physiological experiments to attempt to demonstrate a blood–brain barrier. Morphological observations (p. 21) show that glia form linings around the glio-vascular channels and other extravascular zones that lead to the surface of the blood vessels. The evidence from fixed and sectioned material strongly suggests however, that the glial layer does not seal off the neuropil, which could often be observed separated only by extracellular space from the blood vessels with no intervening glia (as first reported by Barber & Graziadei 1967*a*). Thus the octopus glia may play a role in metabolic exchange, but the observations suggest that the glia

are probably not involved in any barrier phenomena in neurovascular metabolic exchange.

There is, however, a complete lining of pericytes separating the blood from the brain tissue. Barber & Graziadei (1965, 1967*a*) have pointed out that they resemble in some respects mammalian astrocytes. It is possible that the pericytes have a metabolic regulatory function. However, it must be remembered that pericytes are not exclusive to the brain blood vessels of the octopus but are a constant feature of octopus blood vessels in general (excepting possibly the arteries, p. 18), whether they are in or outside the nervous system. Thus they are clearly in this respect not analogous to the vertebrate astrocytes. Indeed, as argued above (p. 25), future work may decide that pericytes should be regarded as endothelium.

Little is known about the function of the glial lamellated sheaths found round the larger perikarya that were described above. From sections there is no evidence for seals (tight junctions) between the interlaced lamellae so presumably there are adequate extracellular channels for ions and the smaller molecules (see Kuffler 1967). The trophospongial specializations and the presence of glycogen in these and in the glial lamellae with which they are continuous are suggestive of some sort of nurse or nutritive function. However, experimental evidence is lacking in the octopus, and evidence from other animals on the role of glia in regulating the neuronal metabolism is controversial (see Wigglesworth 1960; Kuffler & Nicholls 1966).

The trophospongia described here in the octopus are common in the larger neurons of other cephalopods (Stephens & Young 1969; Young 1969) and indeed in the larger neurons of several other groups of invertebrates (see Bullock & Horridge 1965). The neurons may have cell bodies of 100 μm or much more in diameter. It is generally supposed that the trophospongial invaginations serve to increase the surface area of the neuron so that a certain surface area/volume relationship is maintained to ensure adequate diffusion of metabolites in and out of the cell (Bochenek 1901; van Gehucten quoted in Marinesco 1909). If this is so one wonders why the large neurons of vertebrates (the primary sensory dorsal root ganglion neurons and motor neurons of the cord for example may exceed 100 μm) have no trophospongia. There are, however, two interesting exceptions to this statement. Trophospongia were, in fact, first described by Holmgren (1899) in vertebrate neurons, the dorsal root ganglion cells of the fish *Lophius* and later in the supramedullary neurons of the fish *Spheriodes* (see Nakajima, Pappas & Bennett 1965; Scharf 1958). Now both these cell types are unipolar neurons, just as are the large invertebrate neurons that have trophospongia. So it seems that there is a correlation between the absence of dendrites and presence of trophospongium in neurons. It seems that in evolution the majority of vertebrate neurons have developed dendritic processes on their cell bodies sufficient to bring the surface/volume relationship above the critical level for metabolic transfer. The invertebrate neuron, however, has developed a unipolar morphology and the one relatively small limb extending from the cell body has not provided sufficient increase in surface area. So the larger cell bodies have developed trophospongia. But if this is so then it is pertinent to ask why the large dorsal root ganglion cells of the larger mammals, often with cell bodies well over 100 μm in diameter, have no trophospongia. The answer is that in fact these cell bodies often have numerous cytoplasmic

dendrite-like extensions, which obviously increase the surface area (see Holmgren 1899; Ramón y Cajal 1911; Scharf 1958).

It is perhaps being overspeculative to suggest that in vertebrates dendrites developed in evolution directly as outgrowths from the neuron cell bodies primarily as a method of increasing the surface area relative to volume. The vast dendritic receptive fields especially of neurons of the mammalian cerebral cortex are, of course, of importance in the synaptic organization and integration in the nervous system. Most invertebrate neurons, in contrast, have their dendritic and axonal ramifications arising as arborizations of the single and often slender arm of the unipolar cell; the synaptic fields are, as it were, out on a limb. The reasons for this curious dichotomy of neural organization are probably of fundamental importance and need closer attention.

The presence of extensive extracellular collagen-containing tunnels (the glio-vascular strands) and perivascular sleeves no doubt accounts for the comparatively large inulin space measured by Martin *et al.* (1958) (and see Barber & Graziadei 1967*a*). As described above, haemocyanin molecules can sometimes be detected in the extracellular spaces directly related to neuronal elements. Sometimes they occur in tightly packed arrays and give a paracrystalline appearance in sections (see Barber & Graziadei 1965; Gray & Young 1968). At present it is not possible to determine whether or not the molecules are artifactually liberated from the blood during tissue processing.

Stephens & Young (1969) describe branching lymph channels, detected after indian ink perfusion, which apparently feed into the blood vessels. These channels no doubt correspond to the extracellular haemocyanin-containing channels described above (see figure 34, plate 19). However, no connexions have yet been seen with the electron microscope between these and the lumina of the blood vessels. Possibly they have been missed because of sampling difficulties. On the other hand, it could be that although these channels may function as lymph ducts, since with the electron microscope they often appear 'empty' and lacking in collagen, they have no direct connexion with the veins, but fluid is transported from them into the veins across the extremely thin pericyte zones mentioned above (p. 18). The presence of indian ink in these channels could then be accounted for by assuming that it escaped out of the vessels into the channels by rupture of these extremely thin zones. This must be considered a possibility since free mitochondria and other cell debris were observed with the electron microscope in channels where extravascular haemocyanin occurred.

Extracellular channels were described (p. 19) running as tunnels 'through' the perikarya of the giant fibrous glia. Since the tunnels contain only fine granular material and lack collagen they may well act as ducts for metabolic exchange and probably form extensions of the lymph channels described by Stephens & Young (1969). Perhaps they could be described as acellular trophospongia, although in the past light microscopists have only recognized neurons and not glia as having trophospongia. Tunnels possibly of a similar nature occur in the giant glia of the leech central nervous system (type 1 spaces of Gray & Guillery 1963): Wigglesworth (1960) and Smith & Treherne (1963) have described extracellular lacunar channels in insect ganglia. In the present paper rather similar tunnels to those mentioned above in the octopus fibrous glia were seen between the smooth muscle cells (cf. Baxter & Nisbet 1963; Nisbet & Plummer 1966) of the artery

walls. Here also they presumably facilitate metabolic exchange and perhaps can be regarded as analogous to the vasa vasorum of the large arteries of vertebrates. Finally the possible functions of the smooth muscle fibres that lie in the cores of the collagen-containing extracellular channels need considering. The channels occur probably in all the lobes of the octopus brain and they form a complex and extensive system (compare Stephens & Young 1969). The 'thick' filaments presumed to be myofilaments because of their diameters and even spacing are in fact the diagnostic feature of these smooth muscle cells. The 'thin' filaments are often not detectable (see Hanson & Lowy 1961). As yet there is no evidence from electron microscopy that these muscle cells are innervated. Possibly their contractions play an important part in speeding up metabolic exchange in the extracellular compartments and perhaps also by massaging the glia that line these compartments and for that matter the tissues of the lobes in general. Such a mechanism may compensate for some sort of evolutionary deficiency in the vasculature. It is tempting to compare this system with that of the pulsating cardiac ganglia described in the octopus by Alexandrowicz (1963). Observations have yet to be made to determine whether in fact the octopus brain does pulsate *in vivo*. If the contractions are asynchronous then the pulsations may not be so readily observable as those detected by Alexandrowicz. Alexandrowicz (1963) discussed the possibility that the pulsation aided dispersion of neurosecretory material which may be present in the nerve endings in the cardiac ganglion. In the octopus brain, endings can frequently be observed with the electron microscope in the various lobes containing dense-cored vesicles and resembling neurosecretory particles described elsewhere, so Alexandrowicz's theory might well also apply to the octopus brain.

I am indebted to Professor J. Z. Young, F.R.S., for his encouragement and guidance throughout this work and for co-operating in the tissue preparation. Miss T. Charlton gave technical assistance. The photographic prints were prepared by Mr R. Boddy. Mrs J. Astafiev drew the text-figures.

List of abbreviations

art.	artery	*h.*	haemocyanin molecules
b.	extracellular channels between muscle fibres	*is.*	trunk initial segment
bm.	basement membrane of blood vessel	*lu.*	lumen of blood vessel
bmx.	basement membrane of extracellular zones	*m.*	mitochondrion
c.	centrioles	*mus.*	muscle cell or fibre
cap.	capillary	*myf.*	myofilaments
col.	collagen	*n.*	neuron
dgc.	dark glial cell (or its cytoplasm)	*ncb.*	neuron cell body (perikaryon)
end.	blood vessel endothelium	*neur.*	neuropil
e.	extracellular channel	*np.*	neural process
ex.	extracellular zone	*nuc.*	nucleus
f.	fibrils of protoplasmic glia	*o.ax.*	optic nerve axon
ff.	fibrils of fibrous glia	*peri.*	blood vessel pericyte
fib.	fibrocyte or its process	*pgl.*	protoplasmic glia
fgl.	fibrous glia or process(es)	*pp.*	pericyte plaque
G.	Golgi membranes	*r.*	centriole rootlet
ger.	granular endoplasmic reticulum	*sg.*	sheathing glia
gl.	glycogen	*sgf.*	sheathing glial fibrils
glia.	glial cell	*syn.*	synapse
gvt.	glio-vascular tunnel	*t.*	trophospongium
		ve.	vein
		x.	gaps in endothelium

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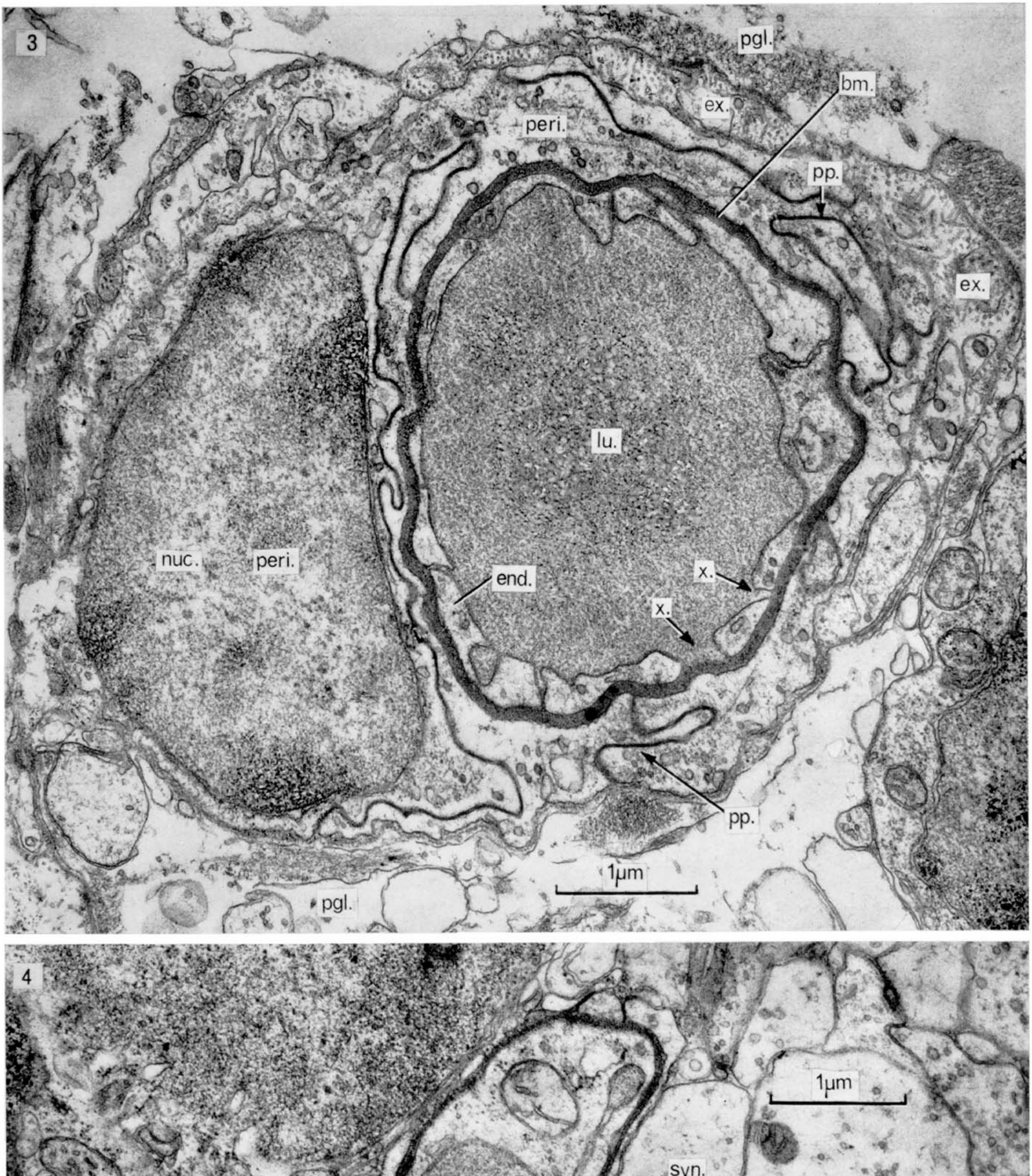
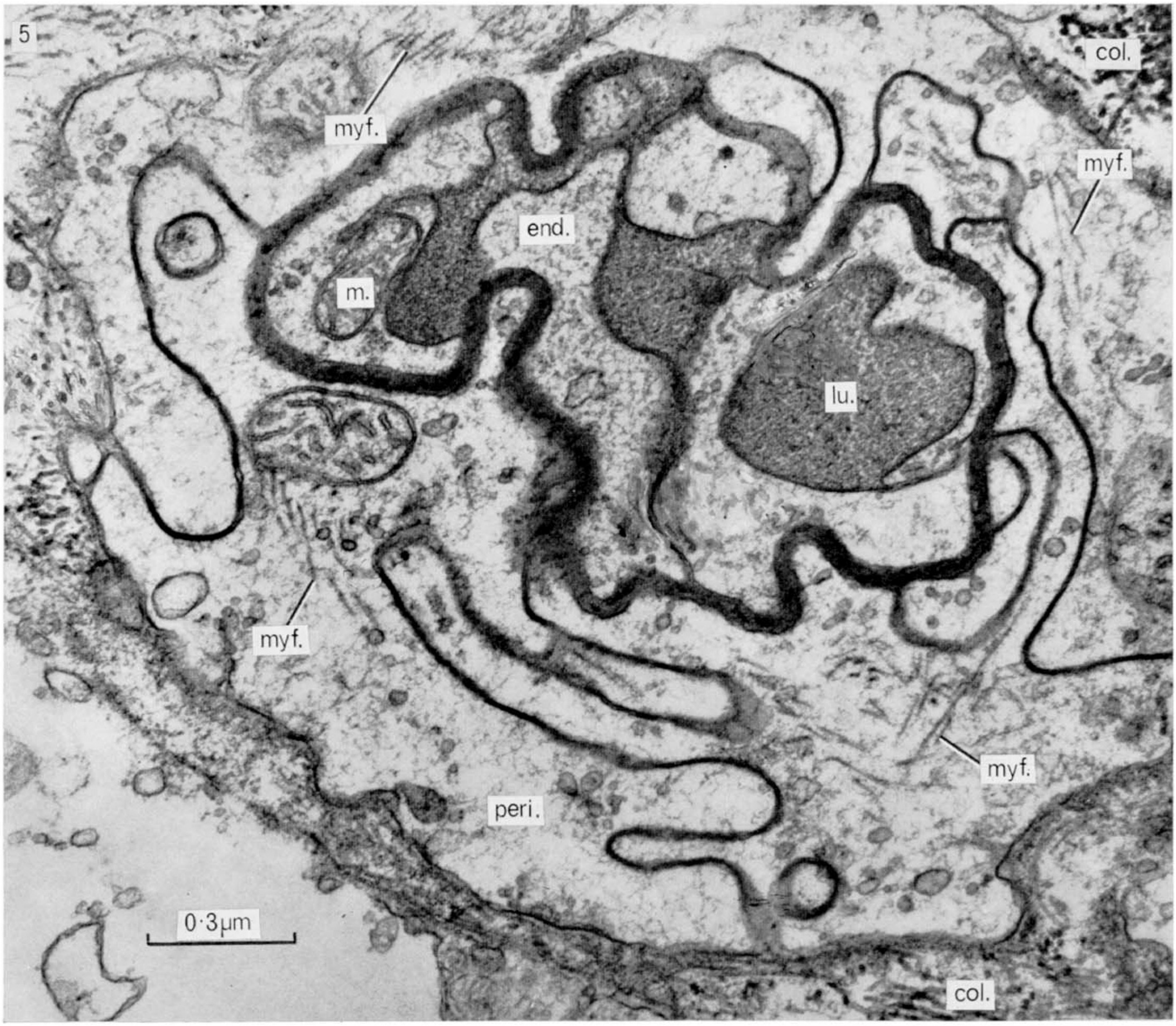


FIGURE 3. Cross-section of a capillary in the optic lobe.

FIGURE 4. Cross-section of a capillary and neighbouring synaptic zones in the optic lobe.



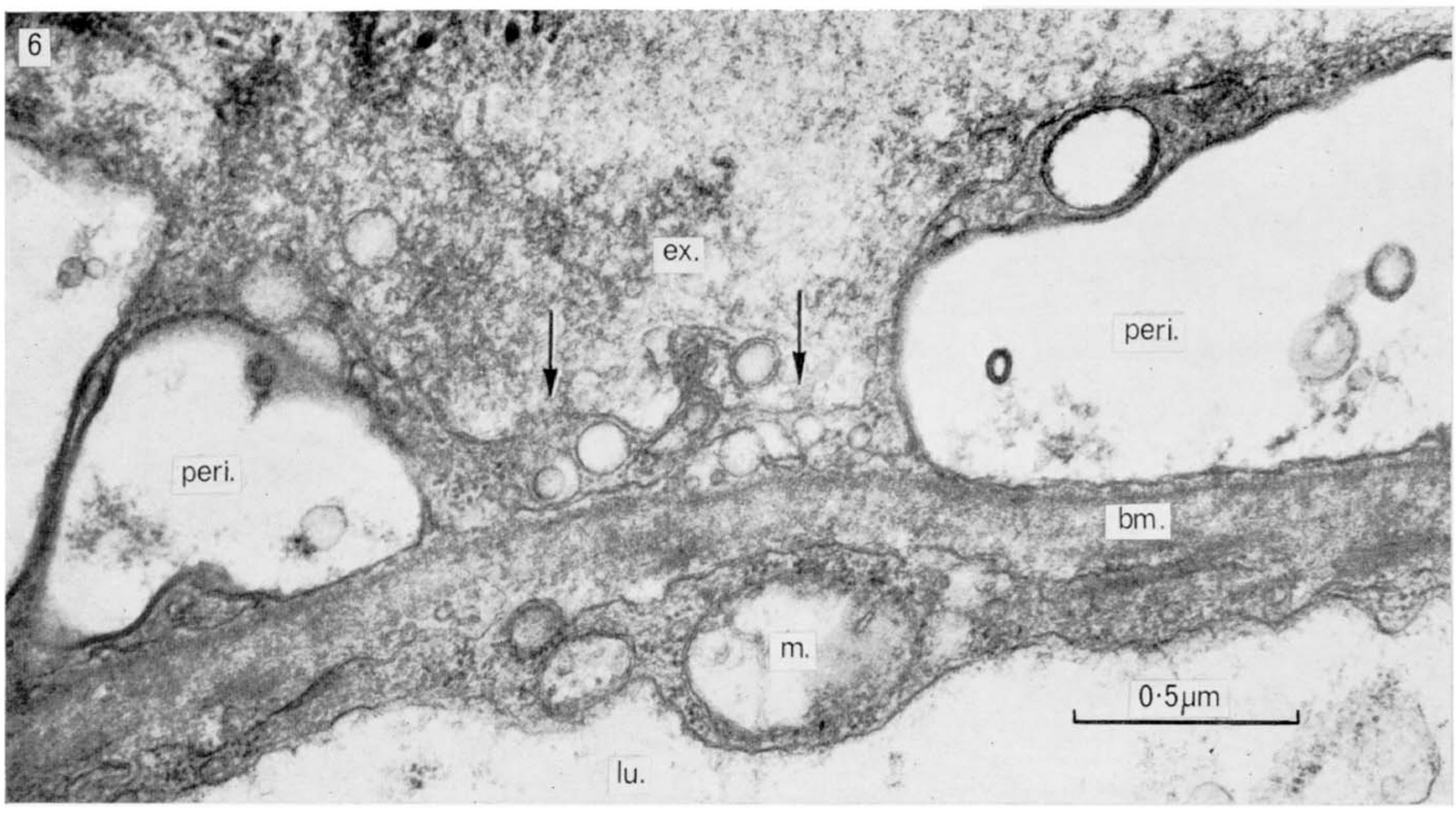
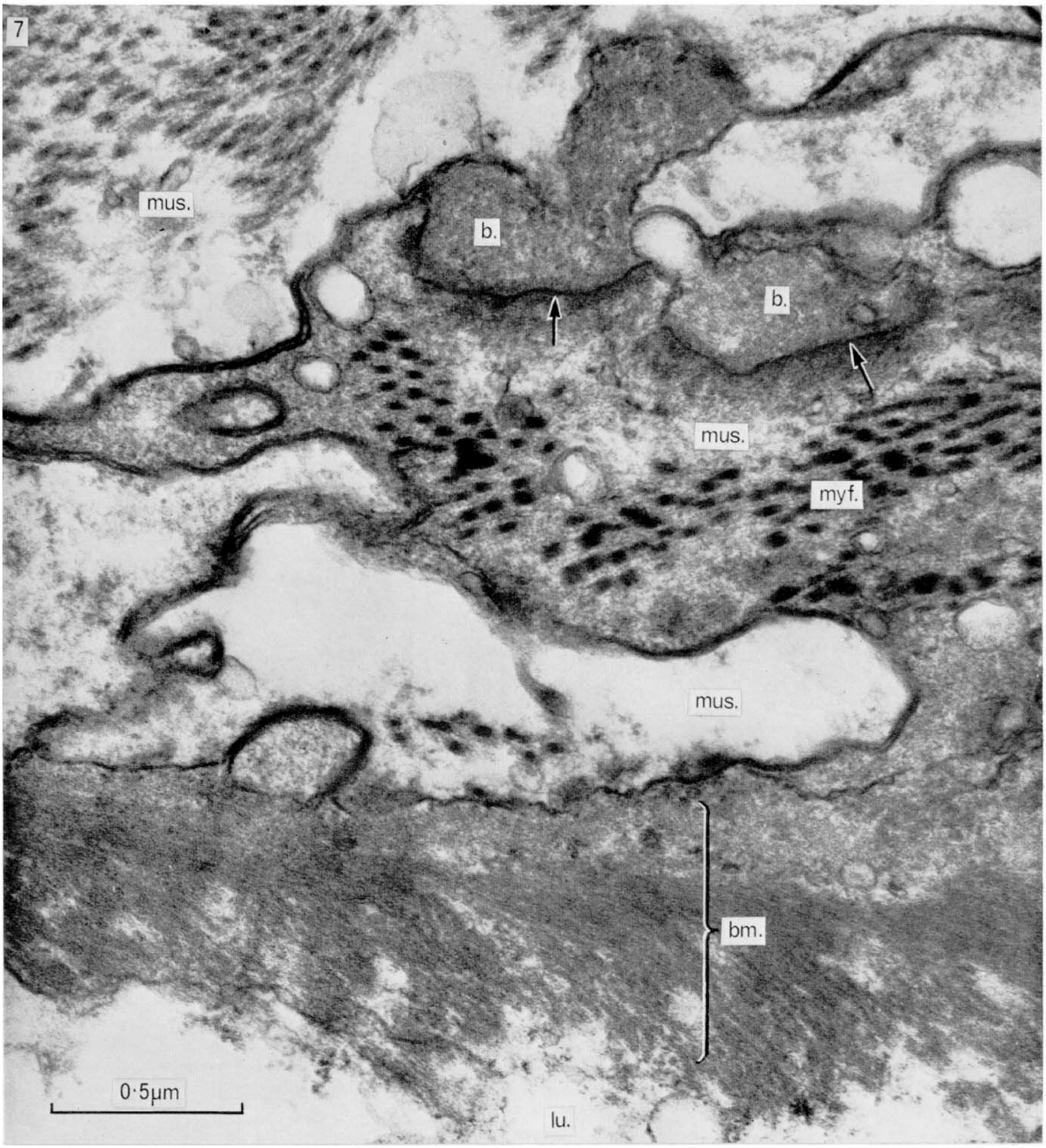


FIGURE 5. Cross-section of a capillary, the pericytes of which contain myofilaments (optic lobe).

FIGURE 6. This pericyte zone in the wall of a vein (medulla, optic lobe).



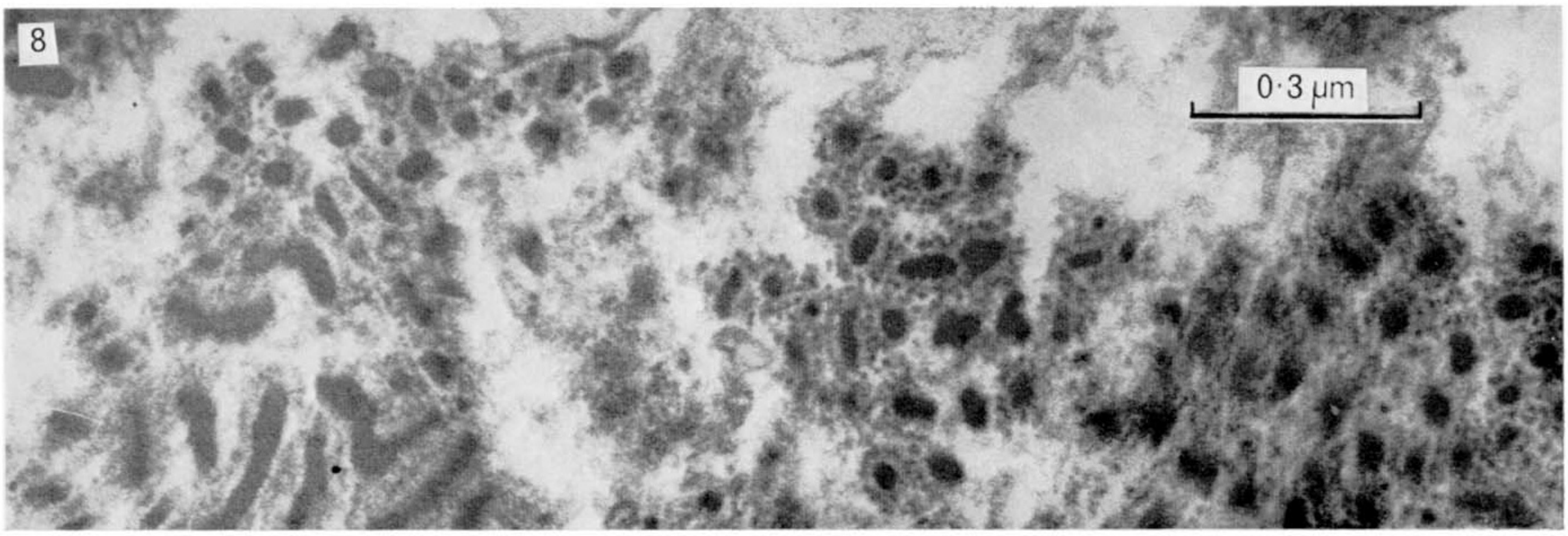


FIGURE 7. The muscular wall and basement membrane of an artery in the optic lobe.

FIGURE 8. High magnification micrograph of cross-section of myofilaments (see figure 7).

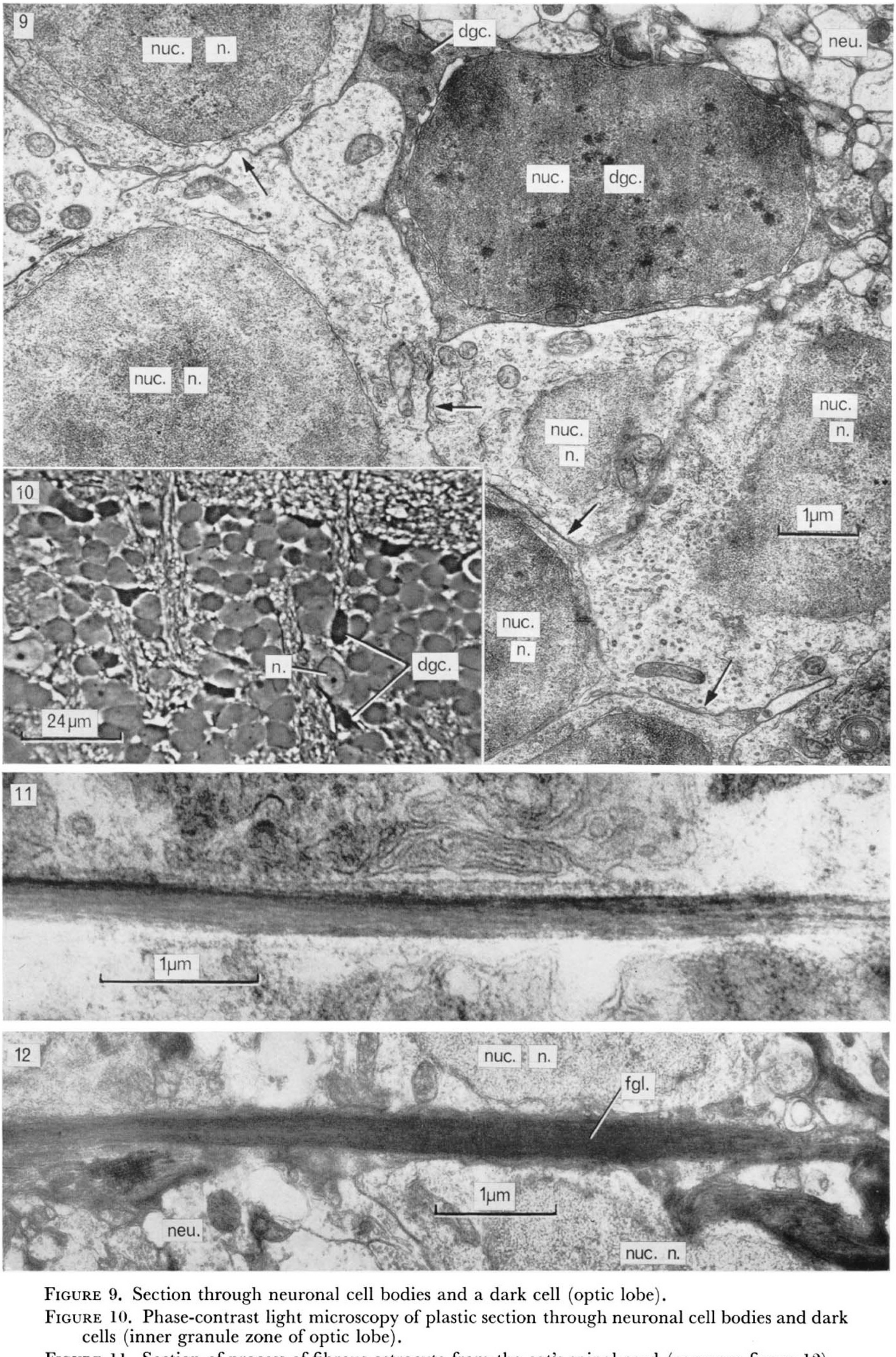


FIGURE 9. Section through neuronal cell bodies and a dark cell (optic lobe).

FIGURE 10. Phase-contrast light microscopy of plastic section through neuronal cell bodies and dark cells (inner granule zone of optic lobe).

FIGURE 11. Section of process of fibrous astrocyte from the cat's spinal cord (compare figure 12).

FIGURE 12. Section through process of fibrous glia in the optic lobe of the octopus (compare figure 11).

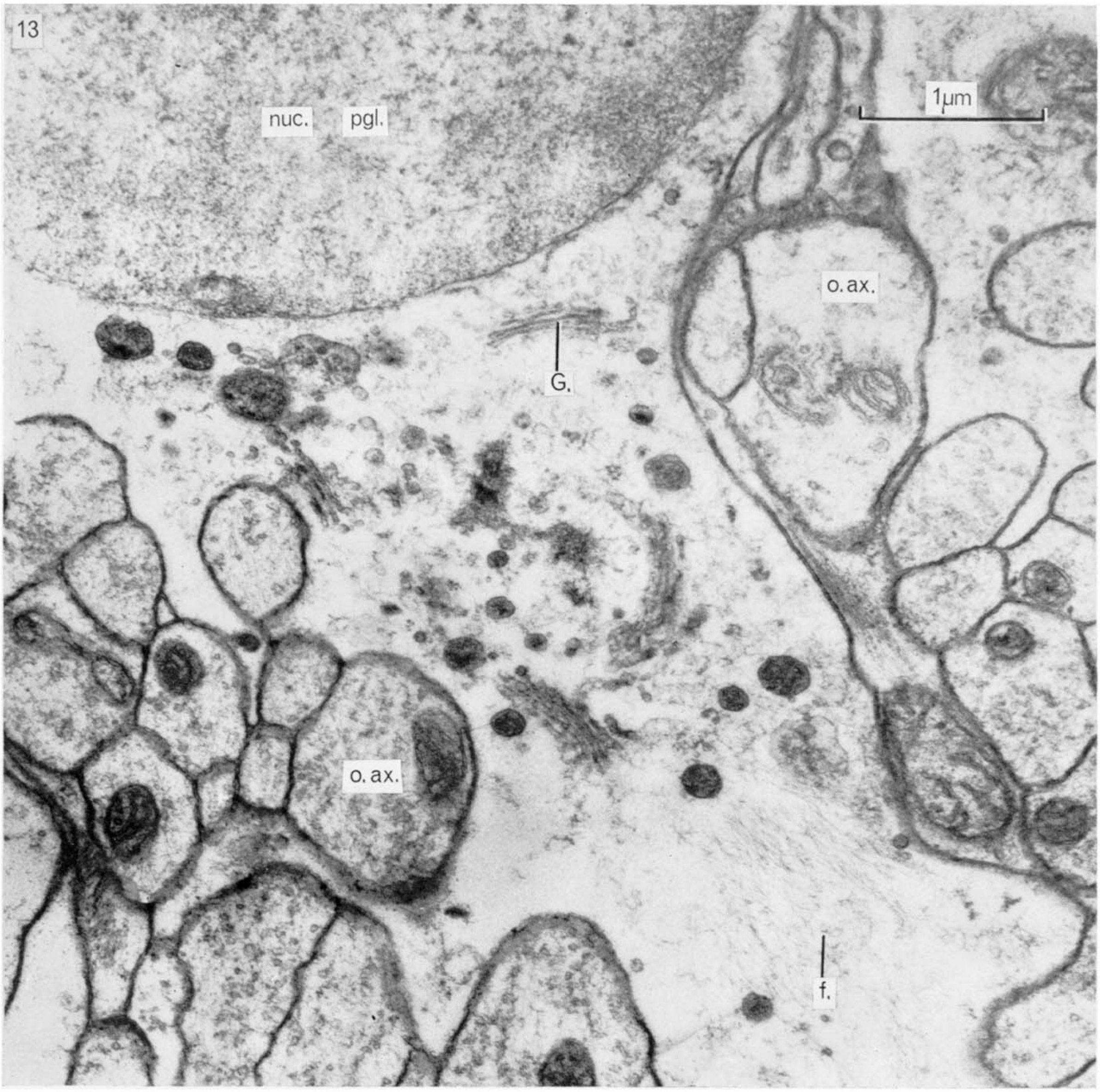


FIGURE 13. Part of a protoplasmic glial cell with adjacent optic nerve axons (external granule zone of optic lobe).

FIGURE 14. Cytoplasmic zone adjacent to the nucleus of a protoplasmic glial cell.

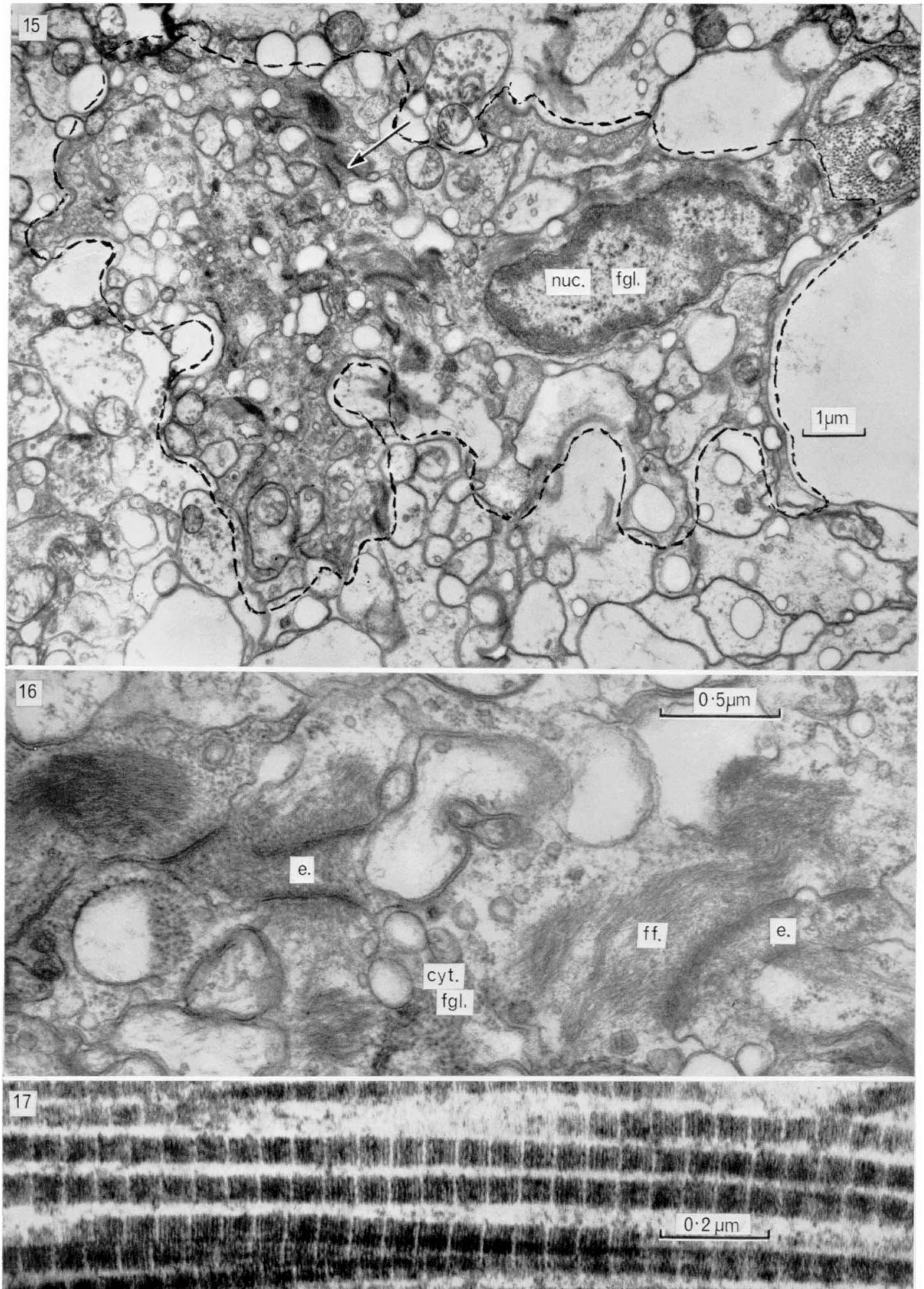
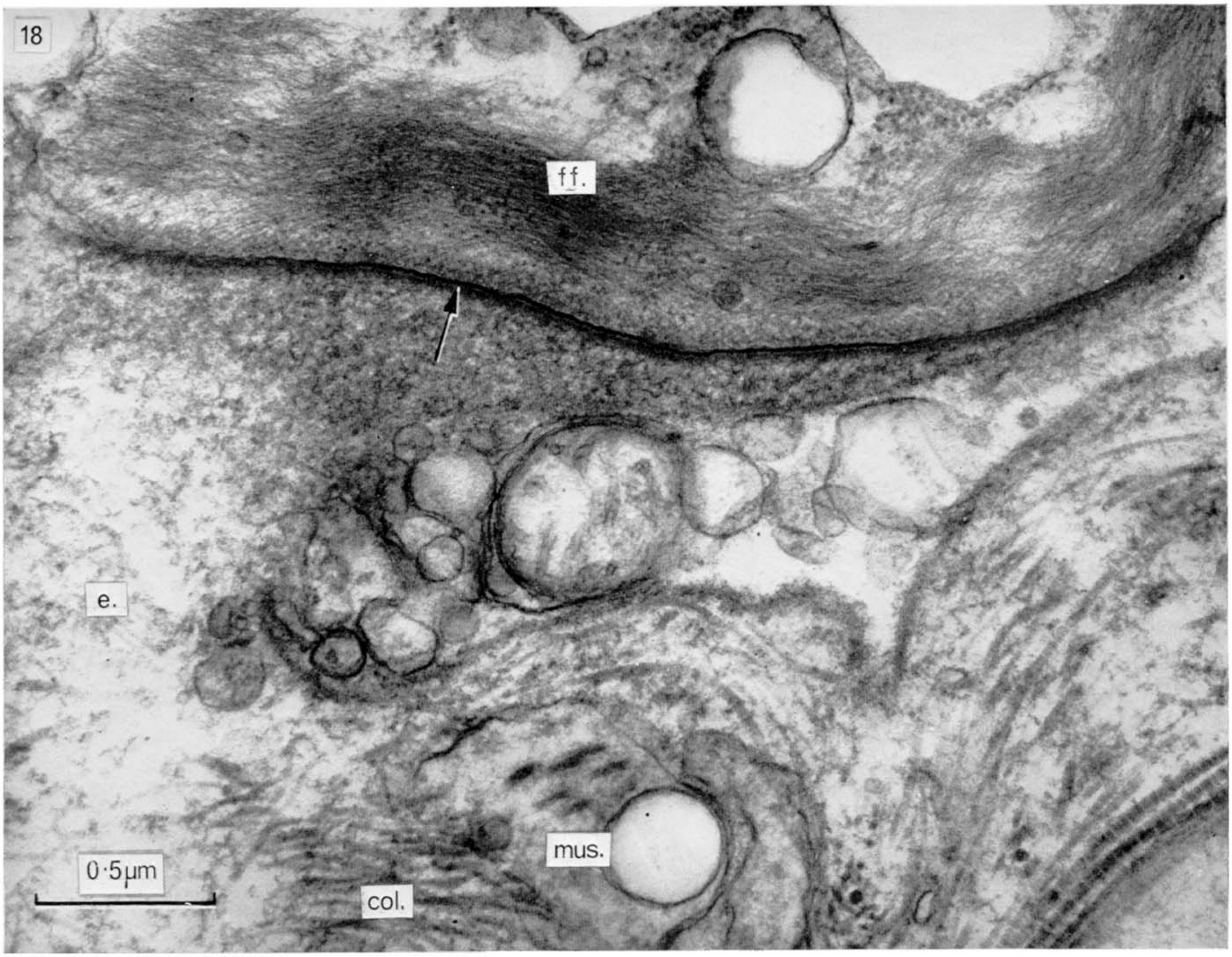


FIGURE 15. Section through a fibrous glial cell seen at low magnification. Region indicated by arrow is enlarged in figure 16 (optic lobe medulla).

FIGURE 16. Enlarged portion of the fibrous glial cell shown in figure 15.

FIGURE 17. Octopus 'collagen'.



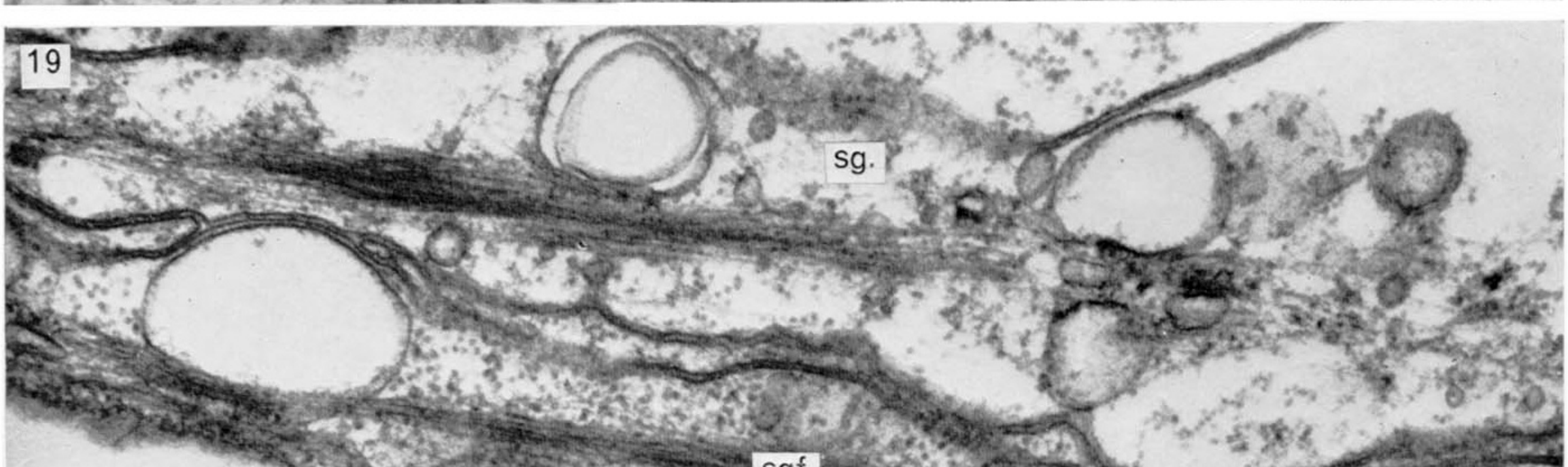
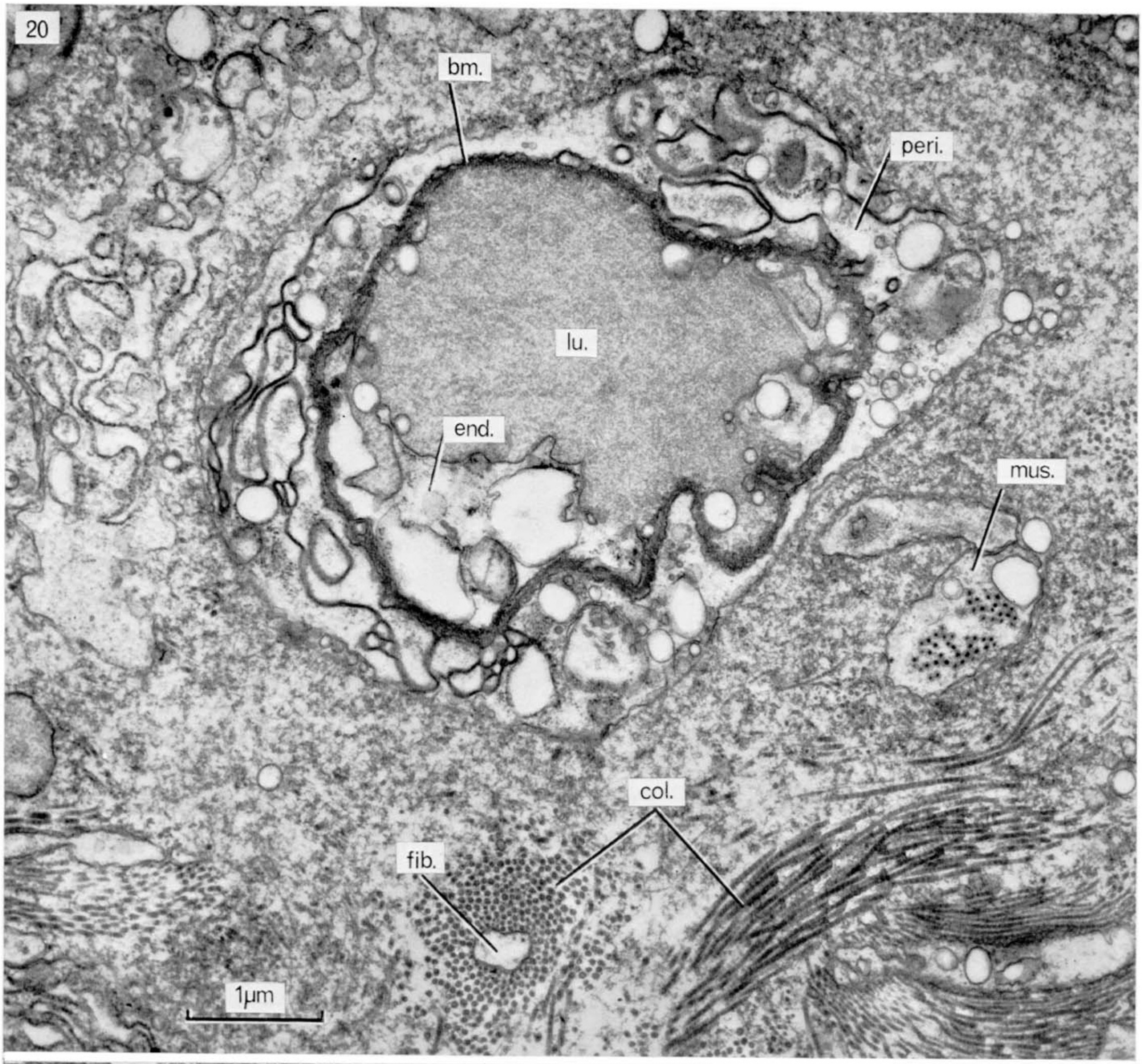


FIGURE 18. Part of fibrous glial cell (above) where it is apposed to an extracellular zone. The surface membrane shows specializations (medulla, optic lobe).

FIGURE 19. Glial folds forming sheath round large neuron cell body (cortex of suboesophageal lobe).



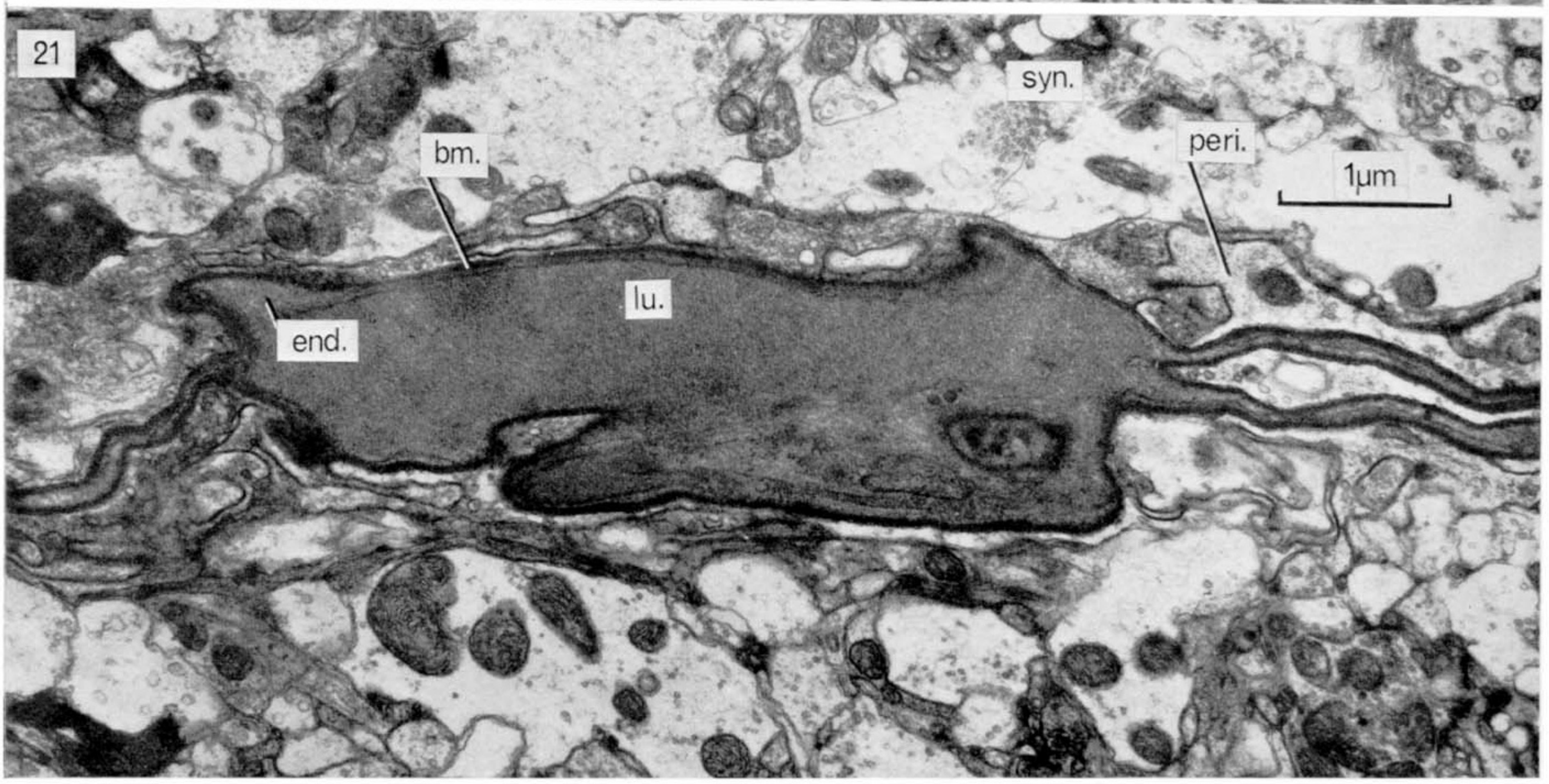


FIGURE 20. Cross-section of capillary lying in an extensive extracellular zone. The zone contains collagen, fibrocyte and muscle processes (medulla optic lobe).

FIGURE 21. Longitudinal section of capillary in plexiform zone of cortex of optic lobe.

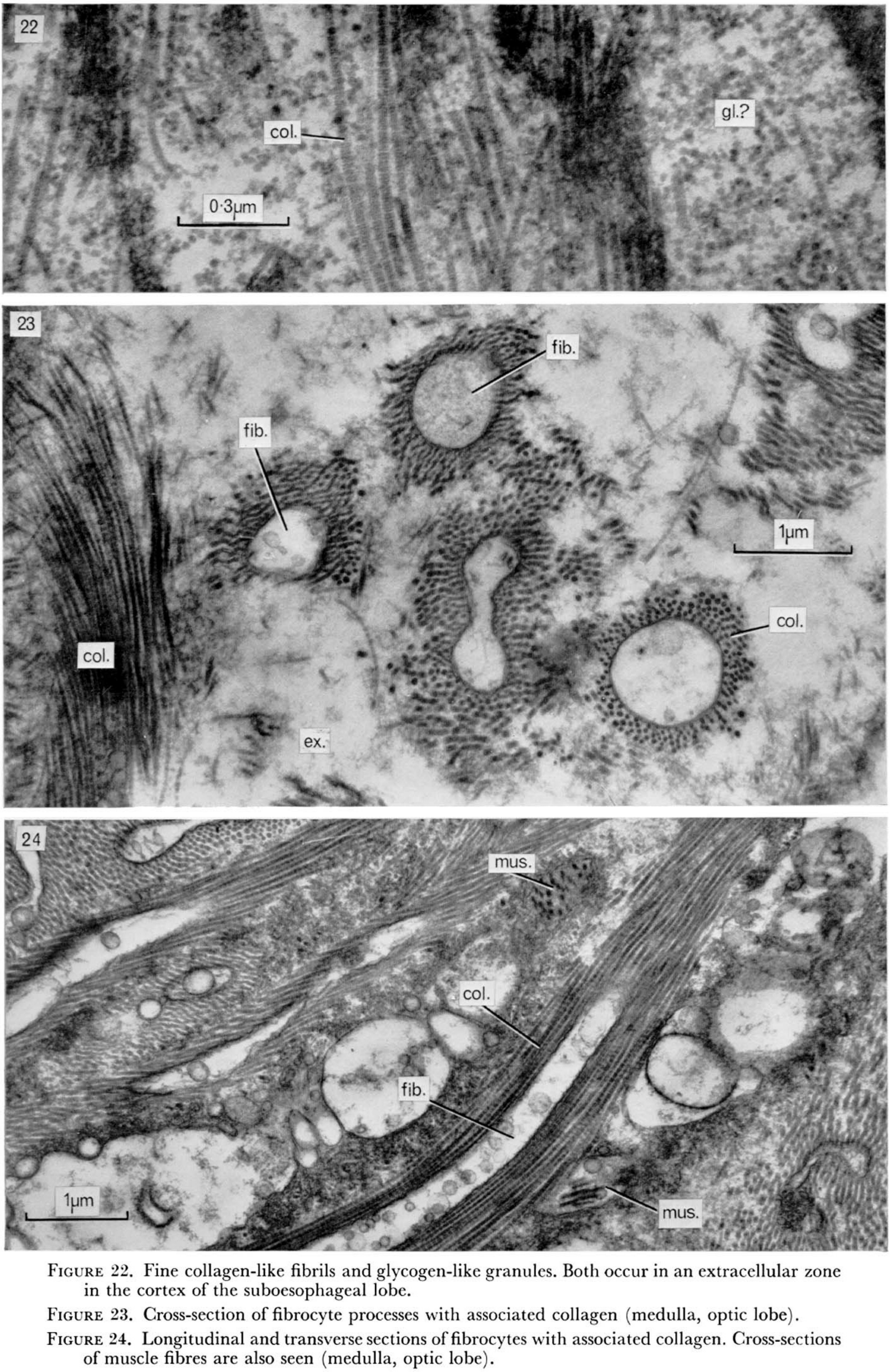


FIGURE 22. Fine collagen-like fibrils and glycogen-like granules. Both occur in an extracellular zone in the cortex of the suboesophageal lobe.

FIGURE 23. Cross-section of fibrocyte processes with associated collagen (medulla, optic lobe).

FIGURE 24. Longitudinal and transverse sections of fibrocytes with associated collagen. Cross-sections of muscle fibres are also seen (medulla, optic lobe).

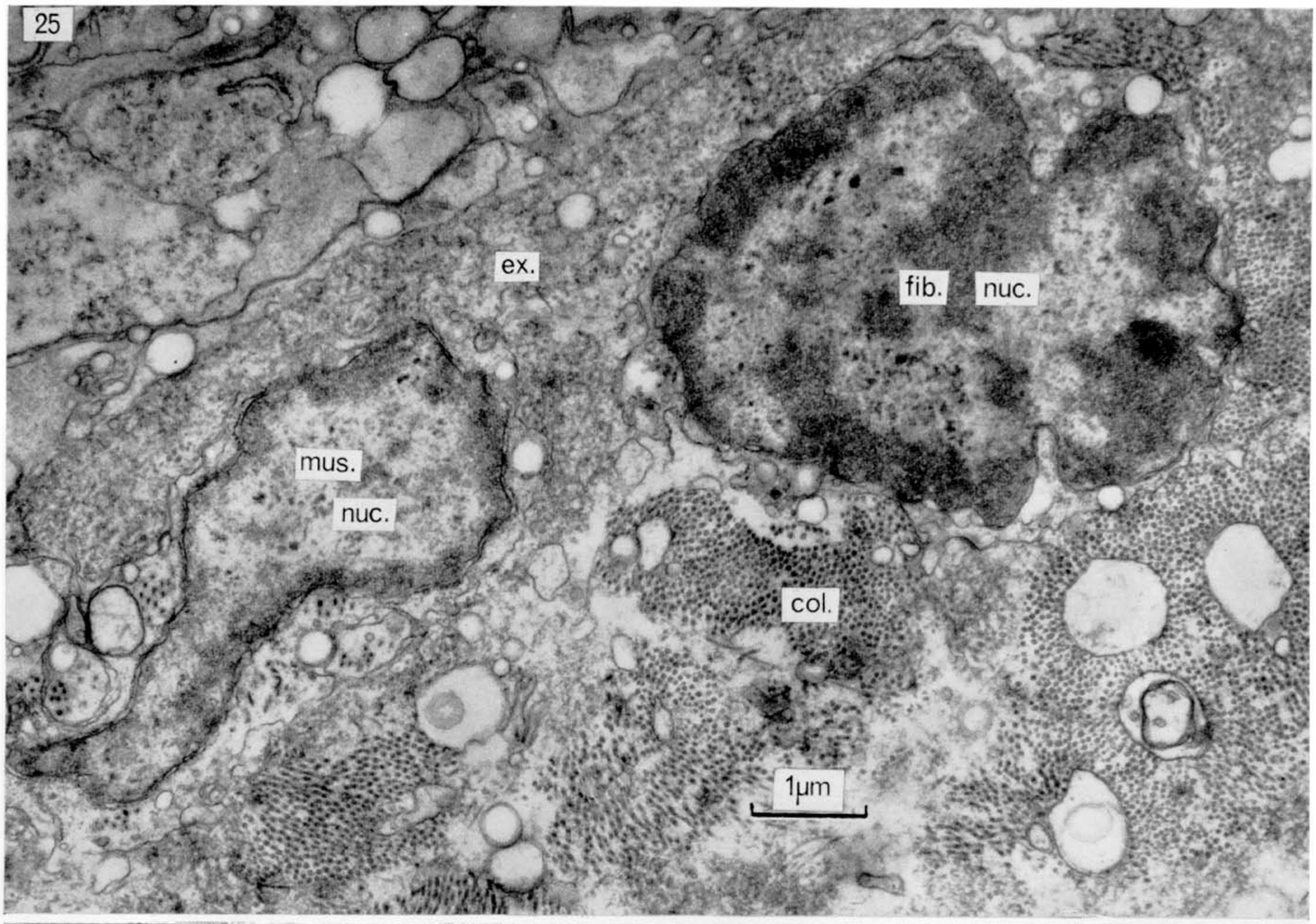
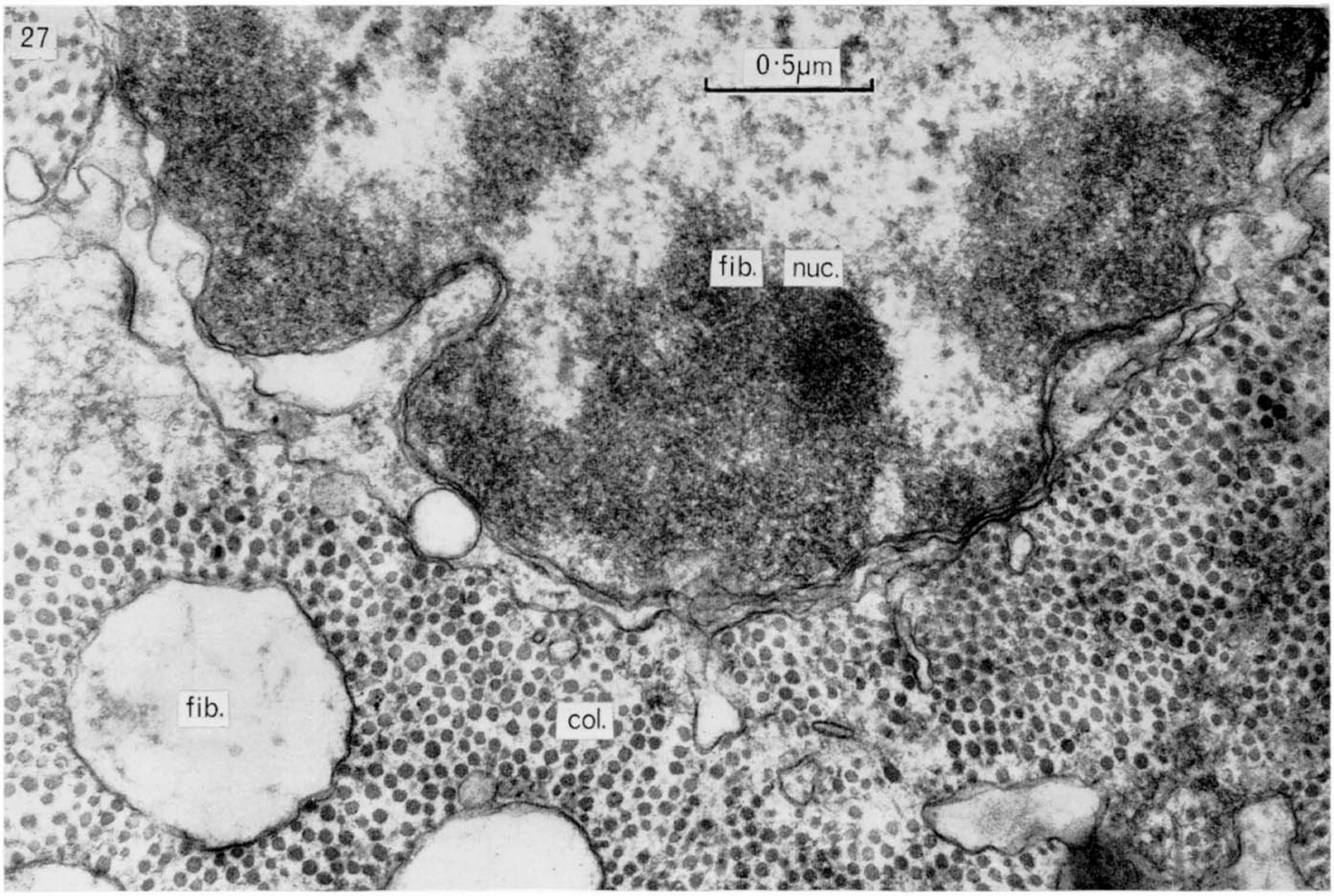


FIGURE 25. Section through a smooth muscle cell (left) and a fibrocyte (optic lobe medulla).
FIGURE 26. Enlarged portion of the muscle cell shown in figure 25.



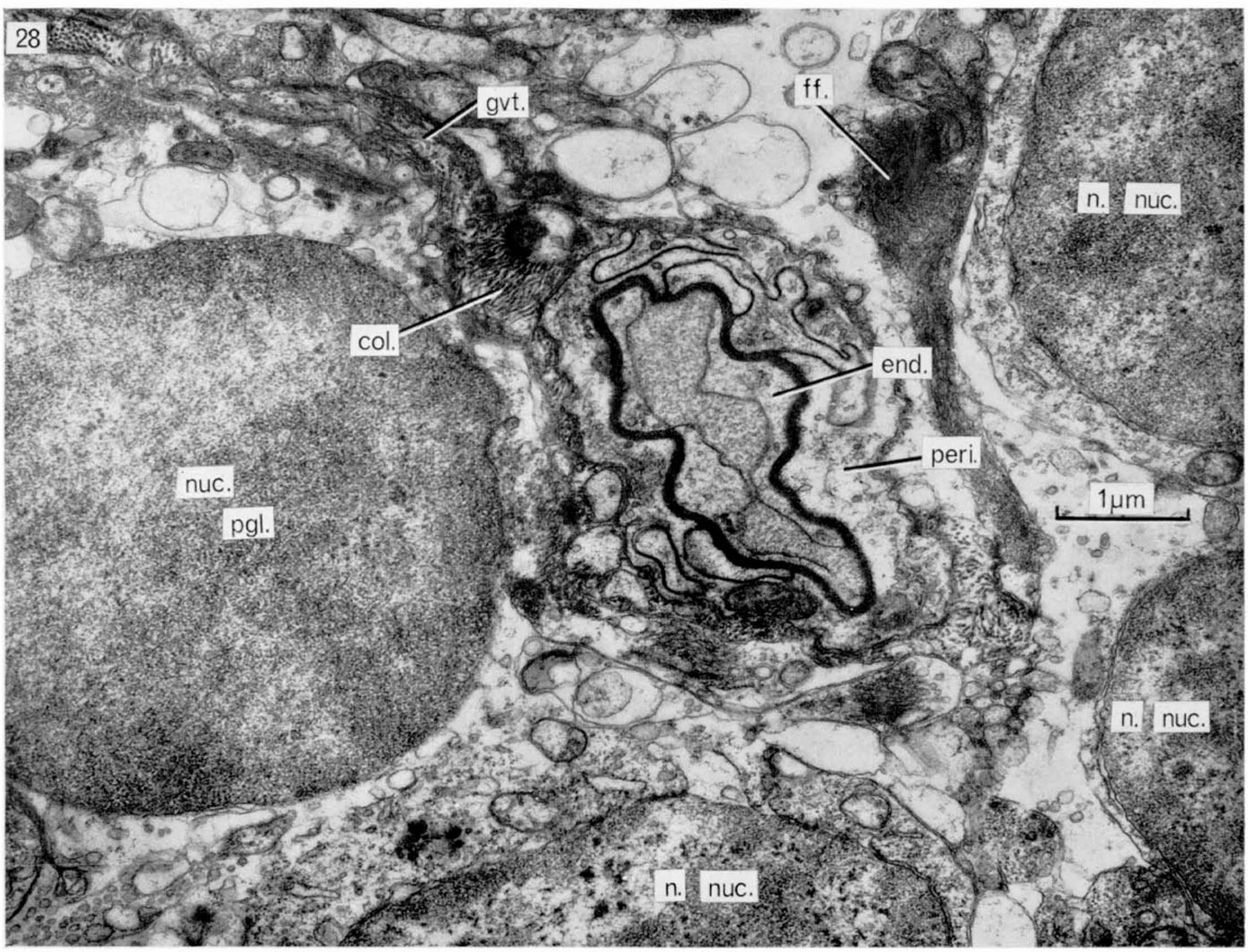
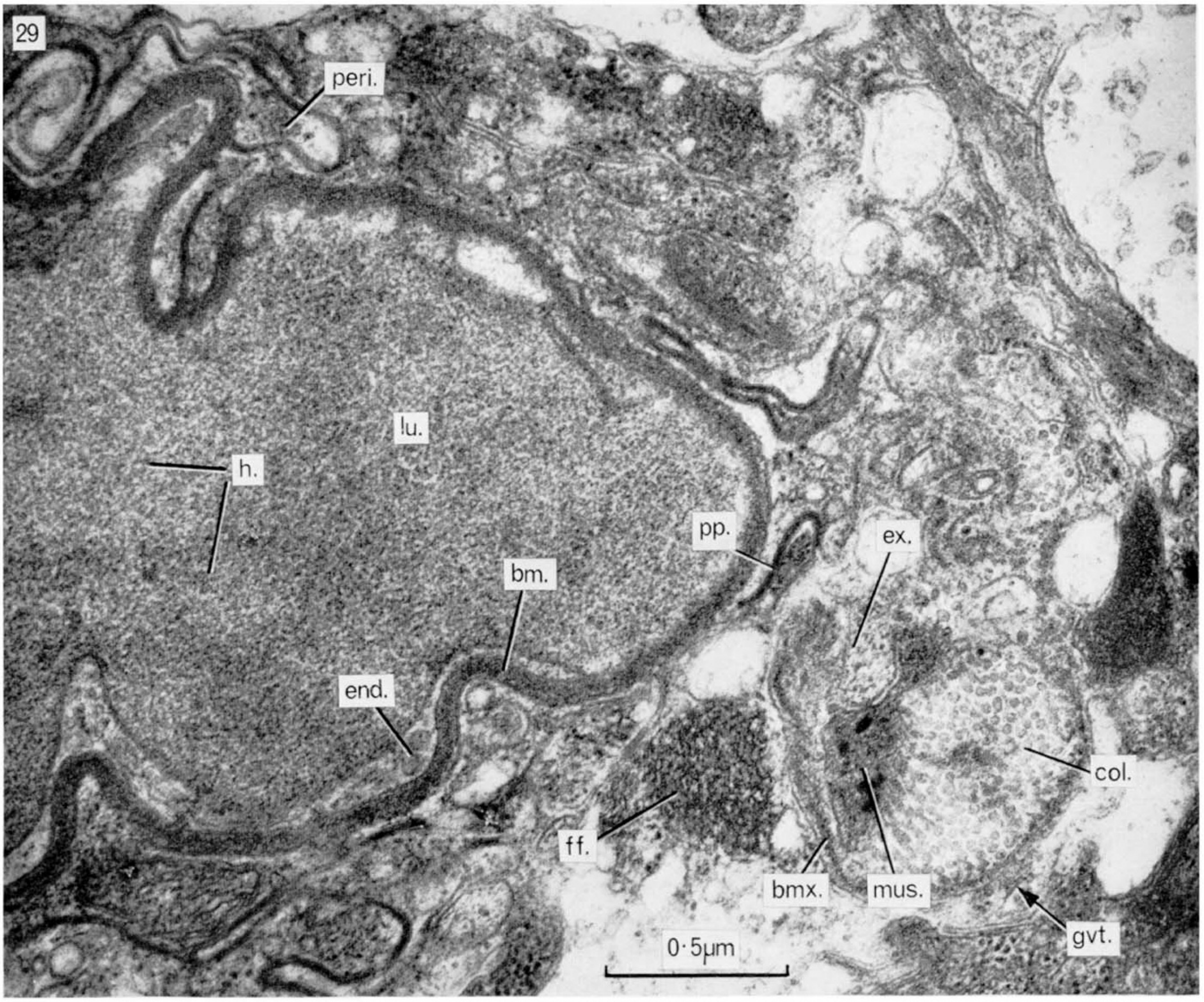


FIGURE 27. Enlarged portion of the fibrocyte shown in figure 25.

FIGURE 28. Cross-section of a capillary with a glio-vascular tunnel extending away above and to the left (medulla optic lobe).



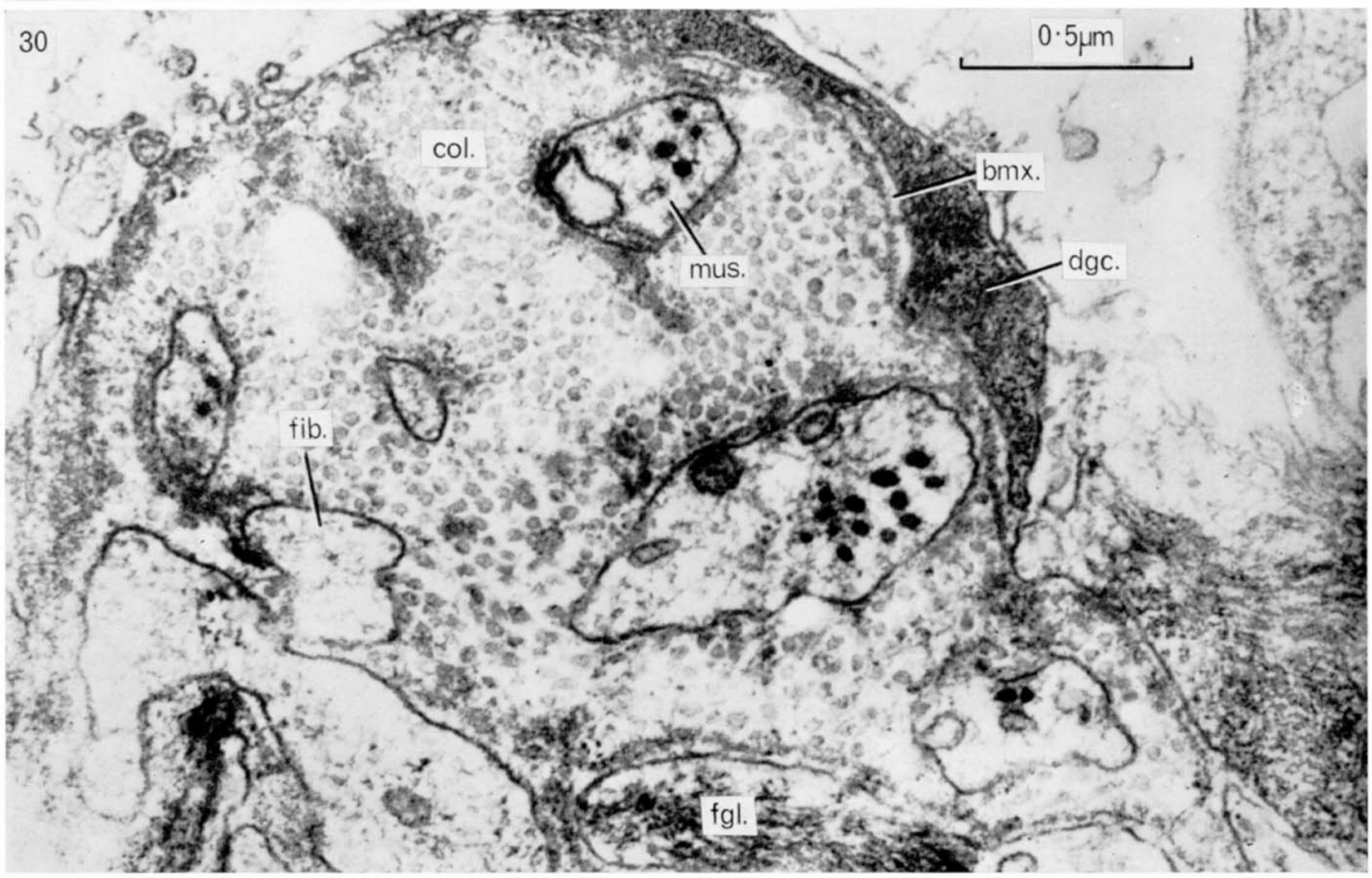
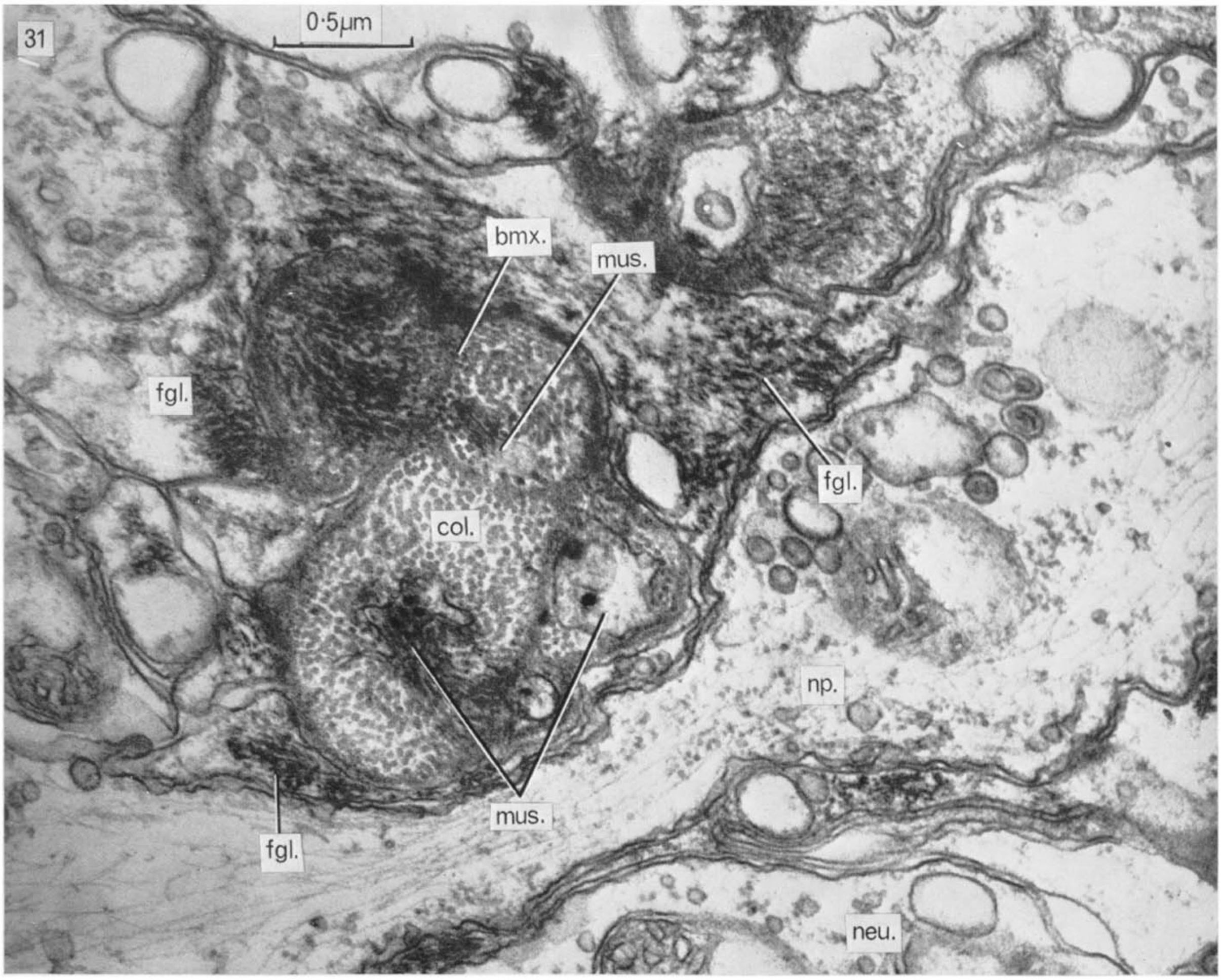
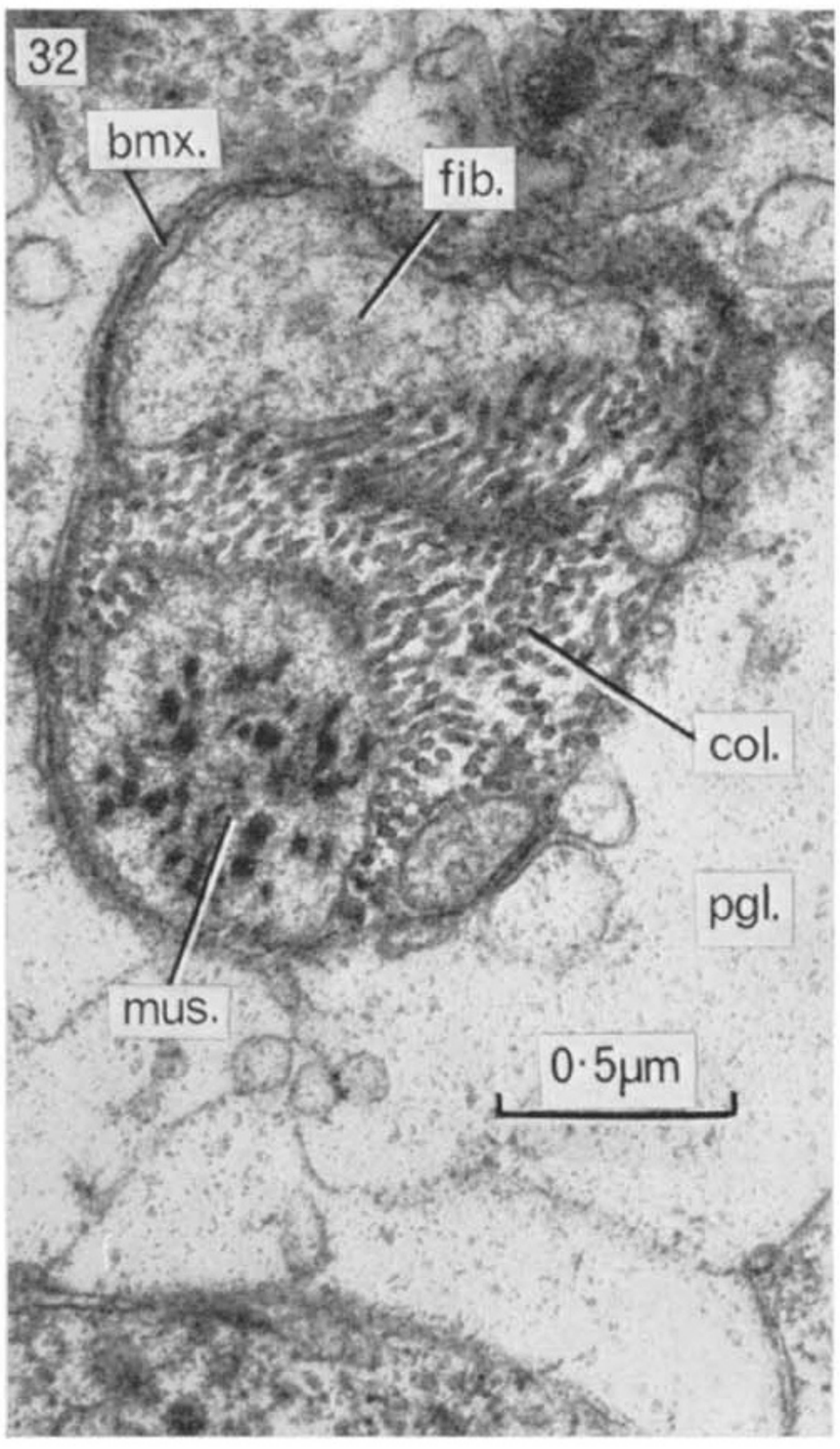


FIGURE 29. Cross-section of part of a capillary with adjacent glial and muscular processes (optic lobe).

FIGURE 30. Cross-section of a glio-vascular tunnel. Protoplasmic and fibrous glia lie below and a dark process is seen on the right (optic lobe).





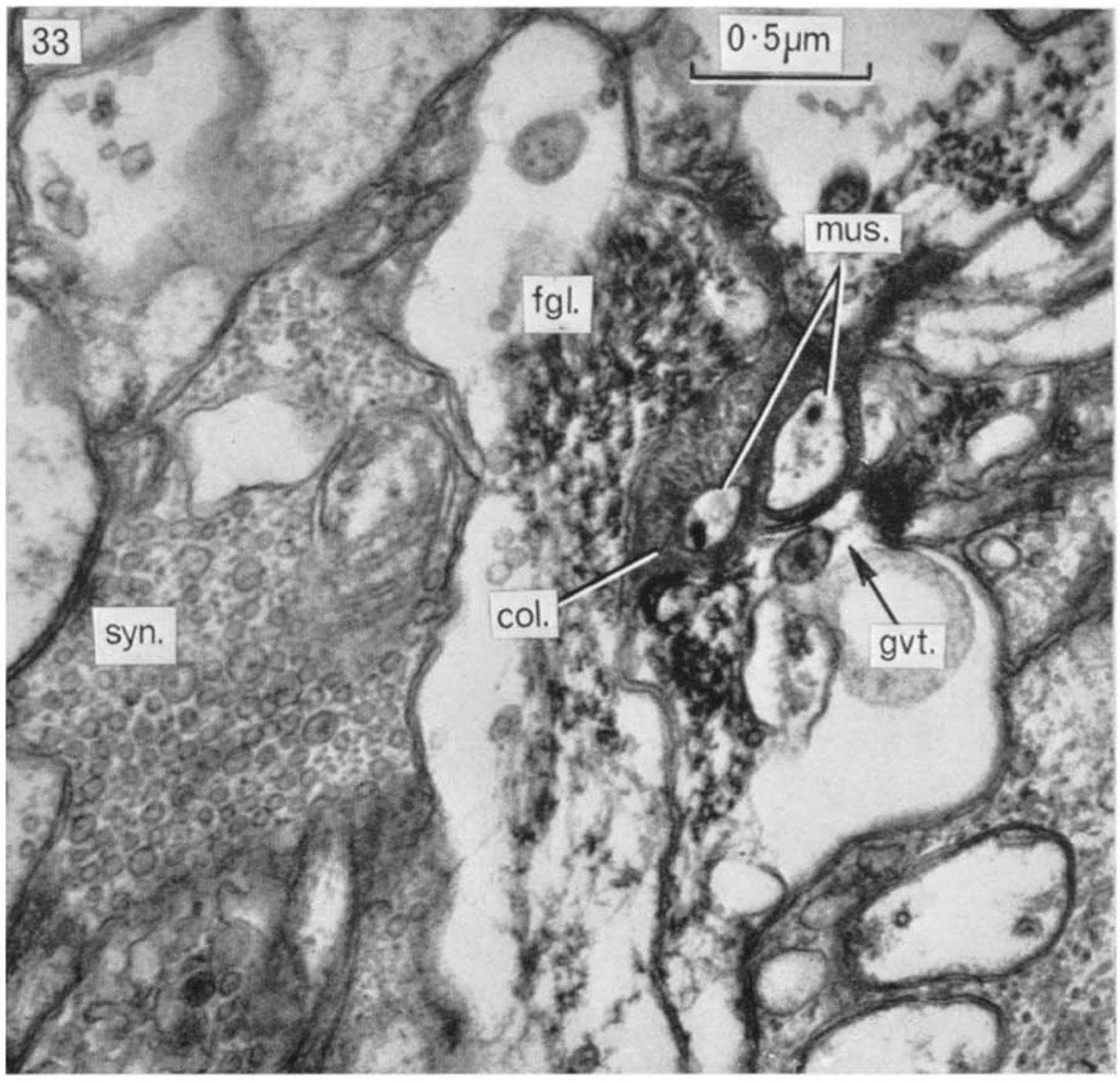
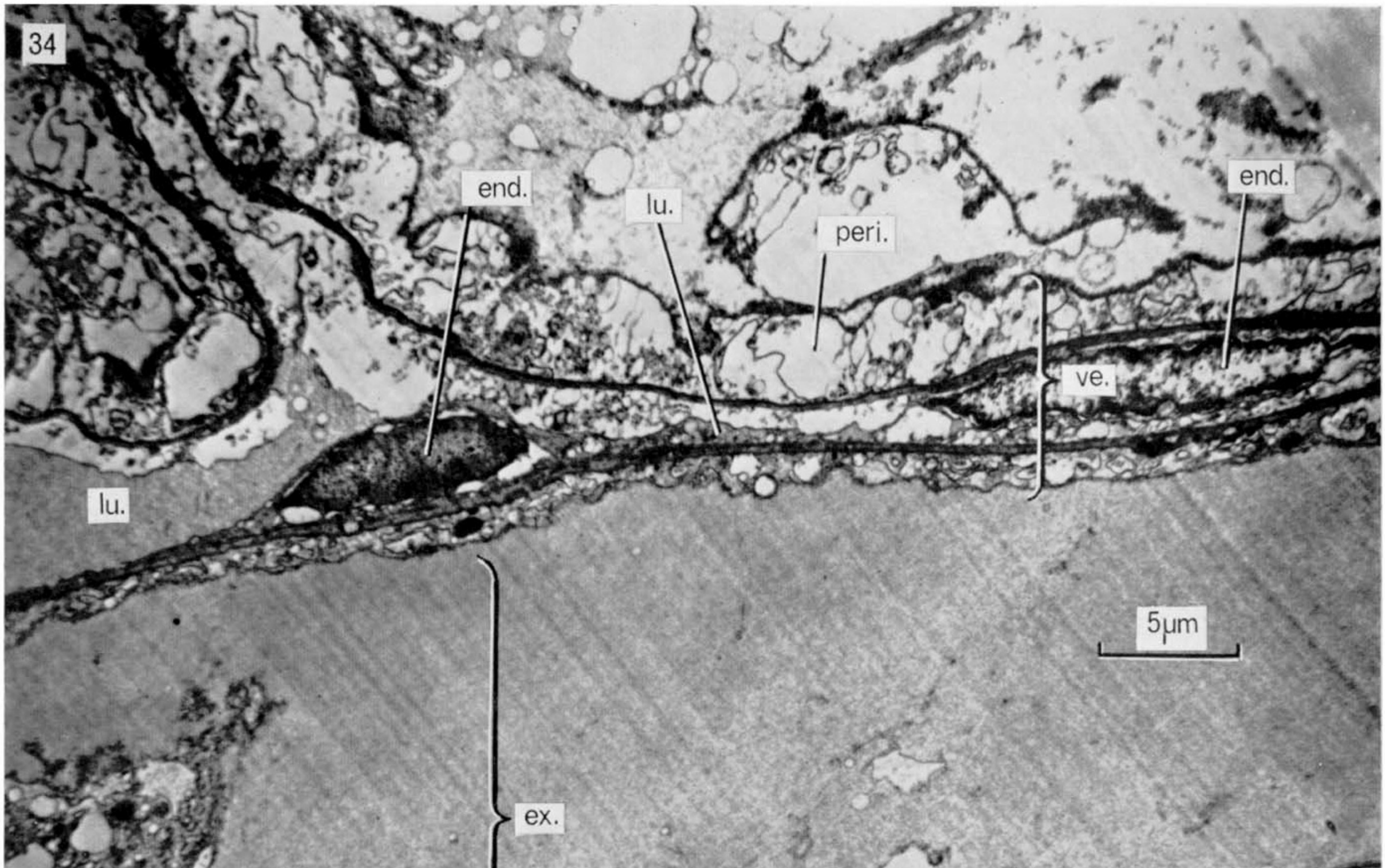


FIGURE 31. Cross-section through small glio-vascular tunnel. It is divided into compartments by a basement membrane (medulla suboesophageal lobe).

FIGURE 32. Cross-section through a small glio-vascular tunnel (optic lobe).

FIGURE 33. Cross-section through a very small glio-vascular tunnel. It contains two muscle fibres each with only one thick myofilament.



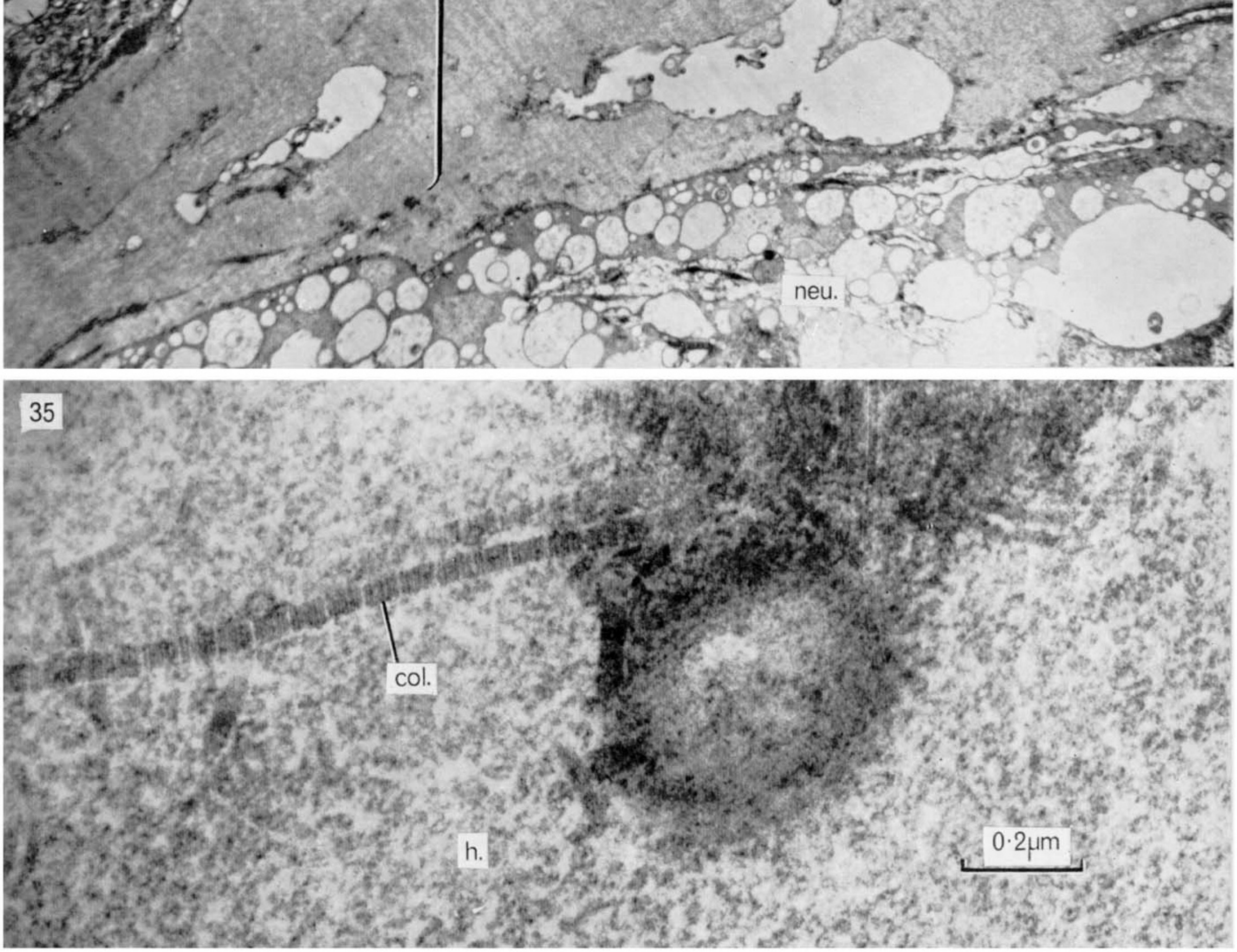


FIGURE 34. Longitudinal section through a vein situated adjacent to a large extracellular channel which contains haemocyanin (low magnification, medulla of optic lobe).

FIGURE 35. Extracellular zone containing a collagen fibre and haemocyanin molecules.

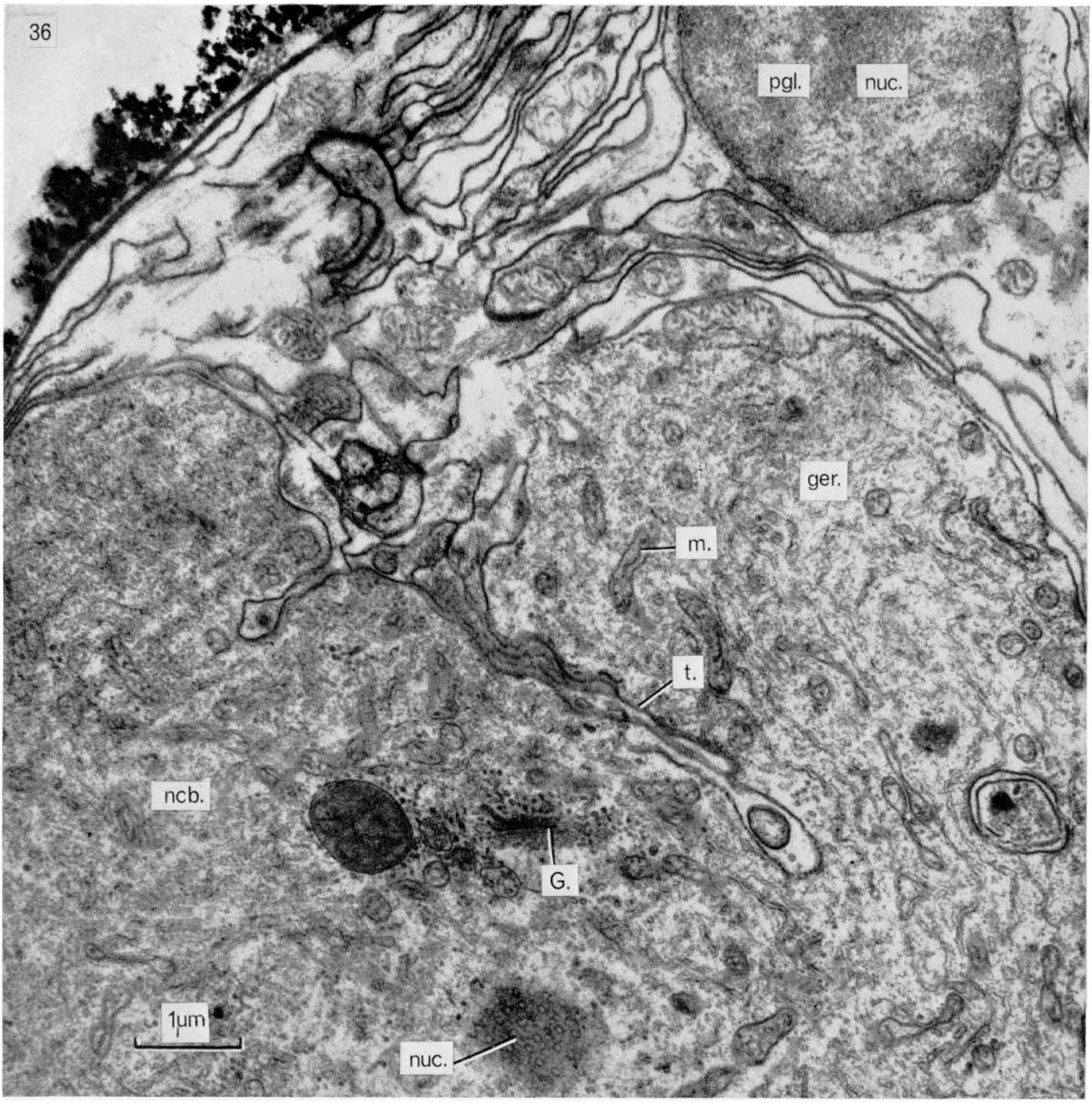


FIGURE 36. Small trophospongial invagination in medium-sized neuron in outermost zone of optic lobe.

FIGURE 37. Surface region of medium-sized neuron with glial lamellae and glial indentation (medulla, optic lobe).

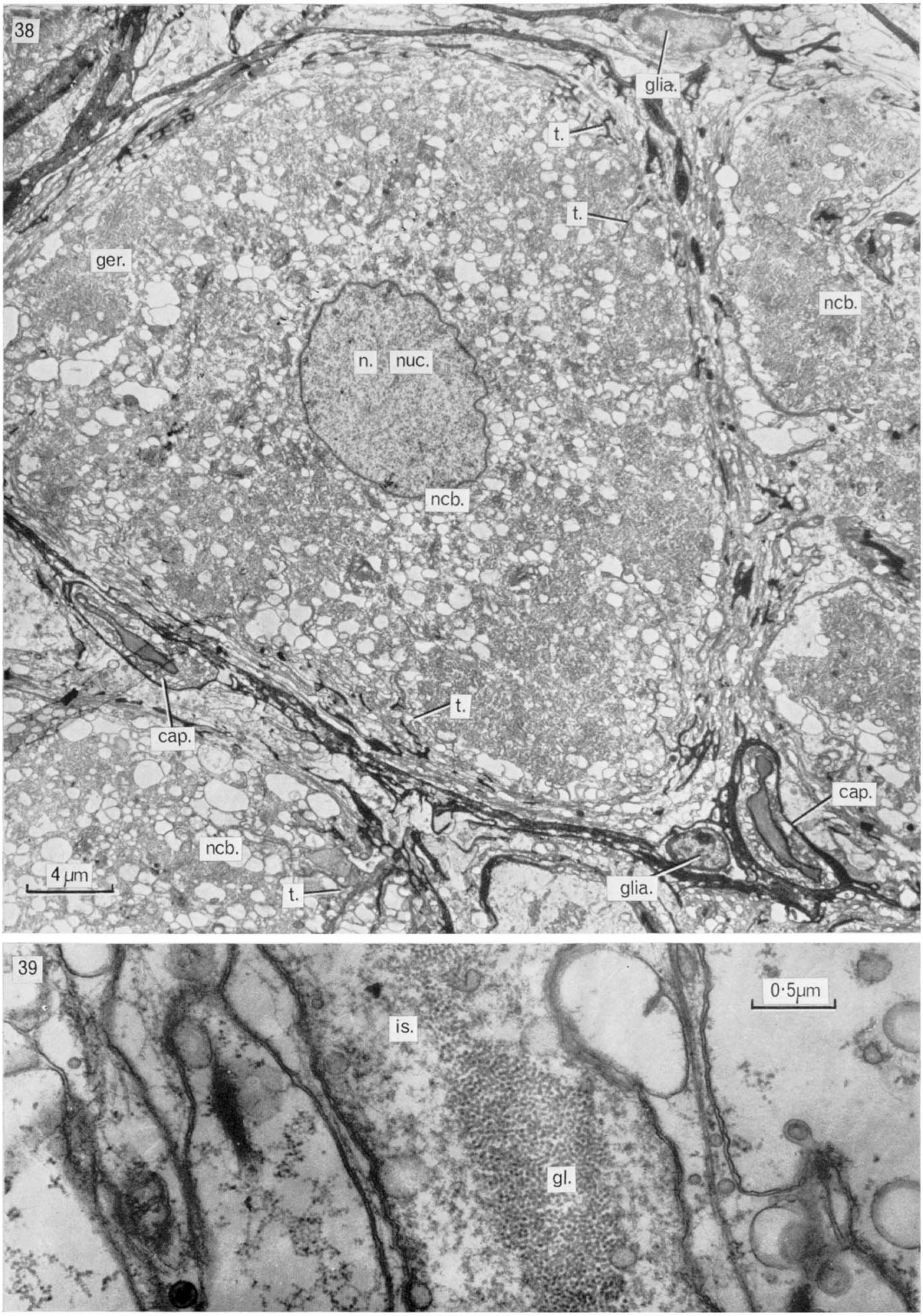
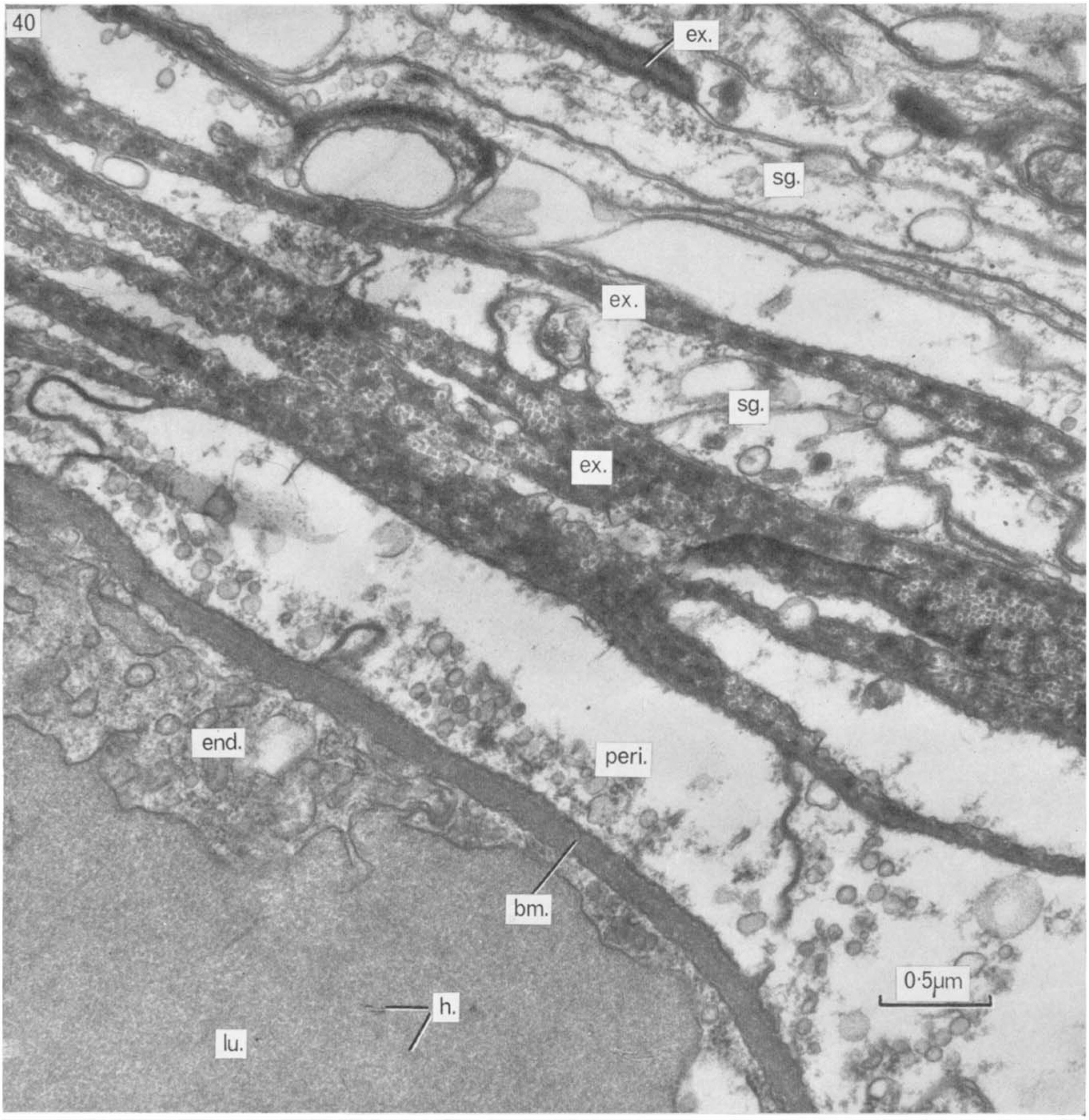


FIGURE 38. Section taken at low magnification through the large neuron cell bodies in the cortex of the suboesophageal lobe.

FIGURE 39. An axonal initial segment of a large neuron of the suboesophageal lobe. The axon contains glycogen.



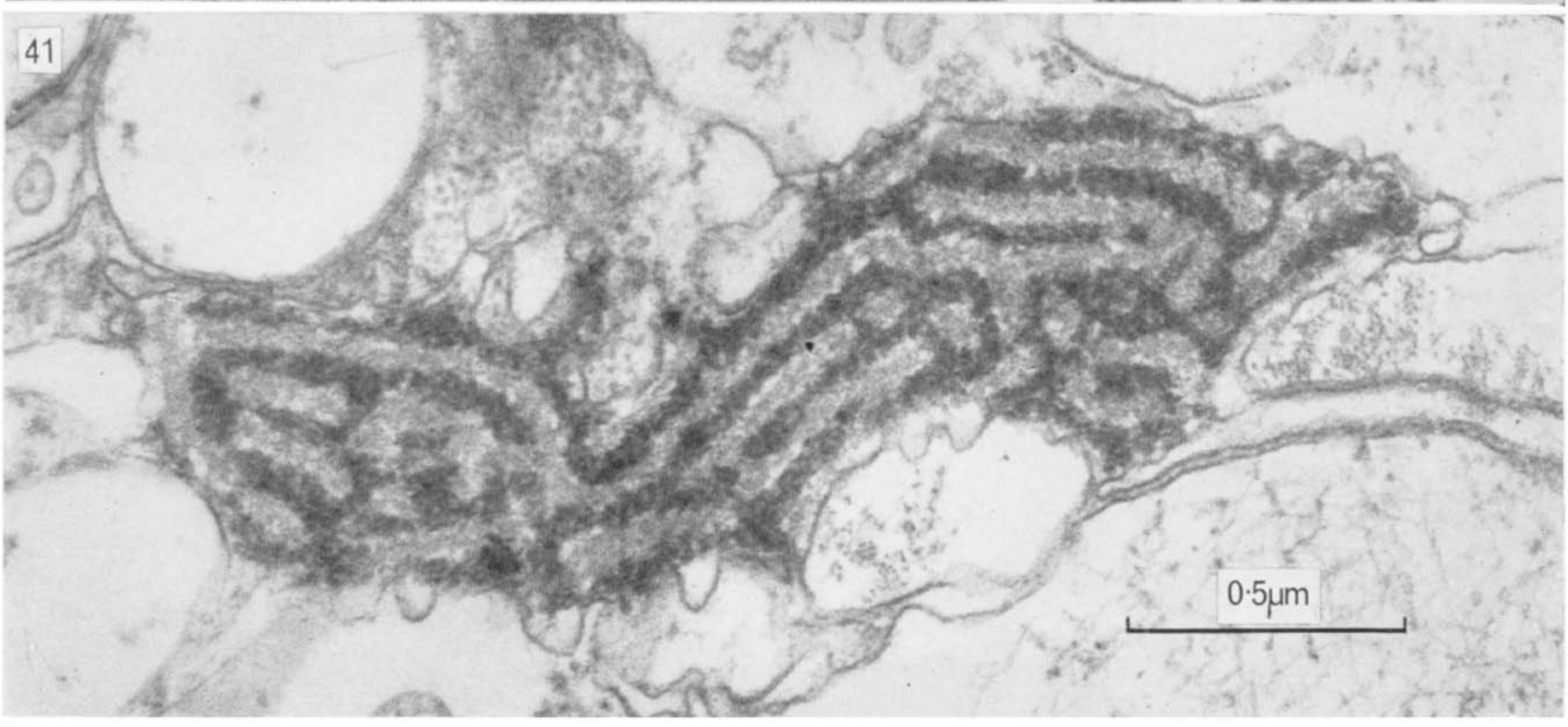
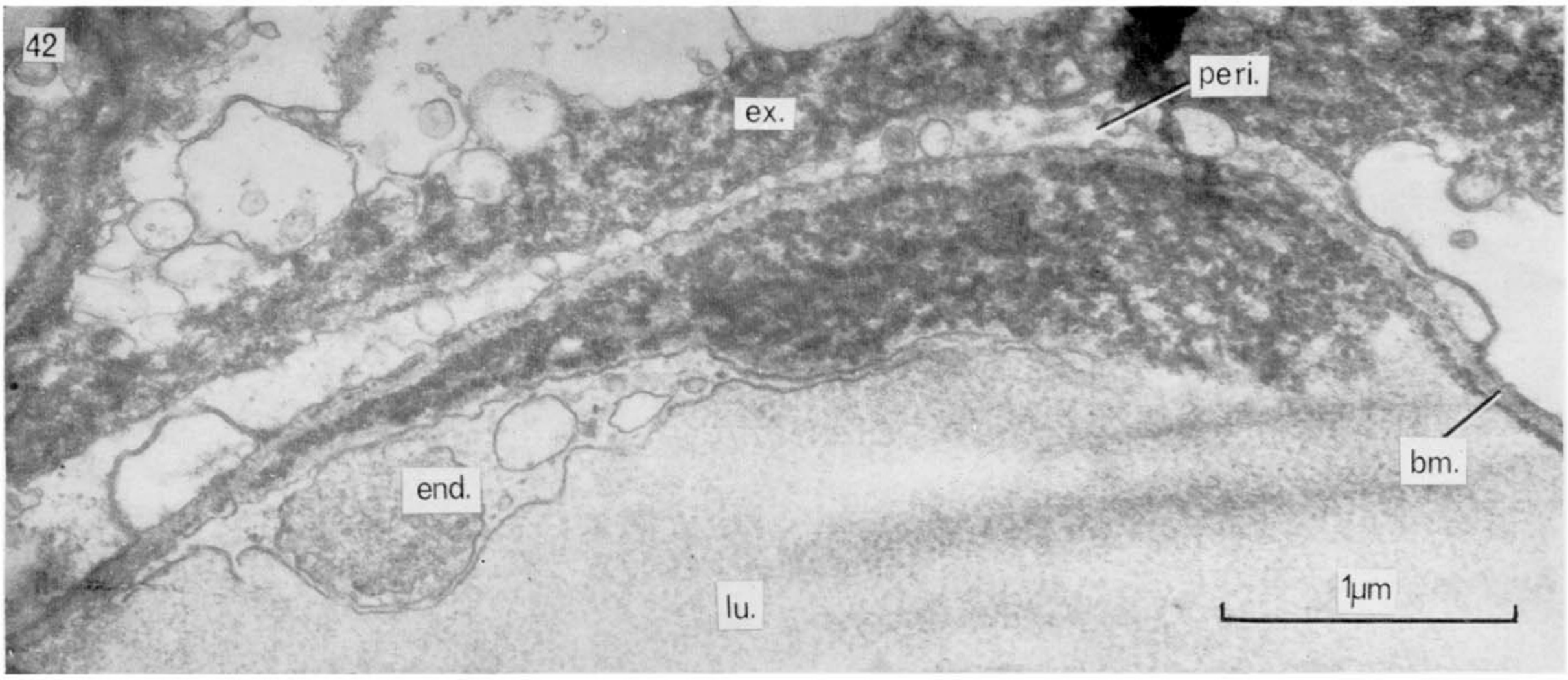


FIGURE 40. Portion of blood vessel (cross-section) and glial folds and extracellular zones forming part of the environment of a large cell body of a neuron of the suboesophageal lobe.

FIGURE 41. Section through the peculiar extracellular material encountered in the cortex of the suboesophageal lobe.



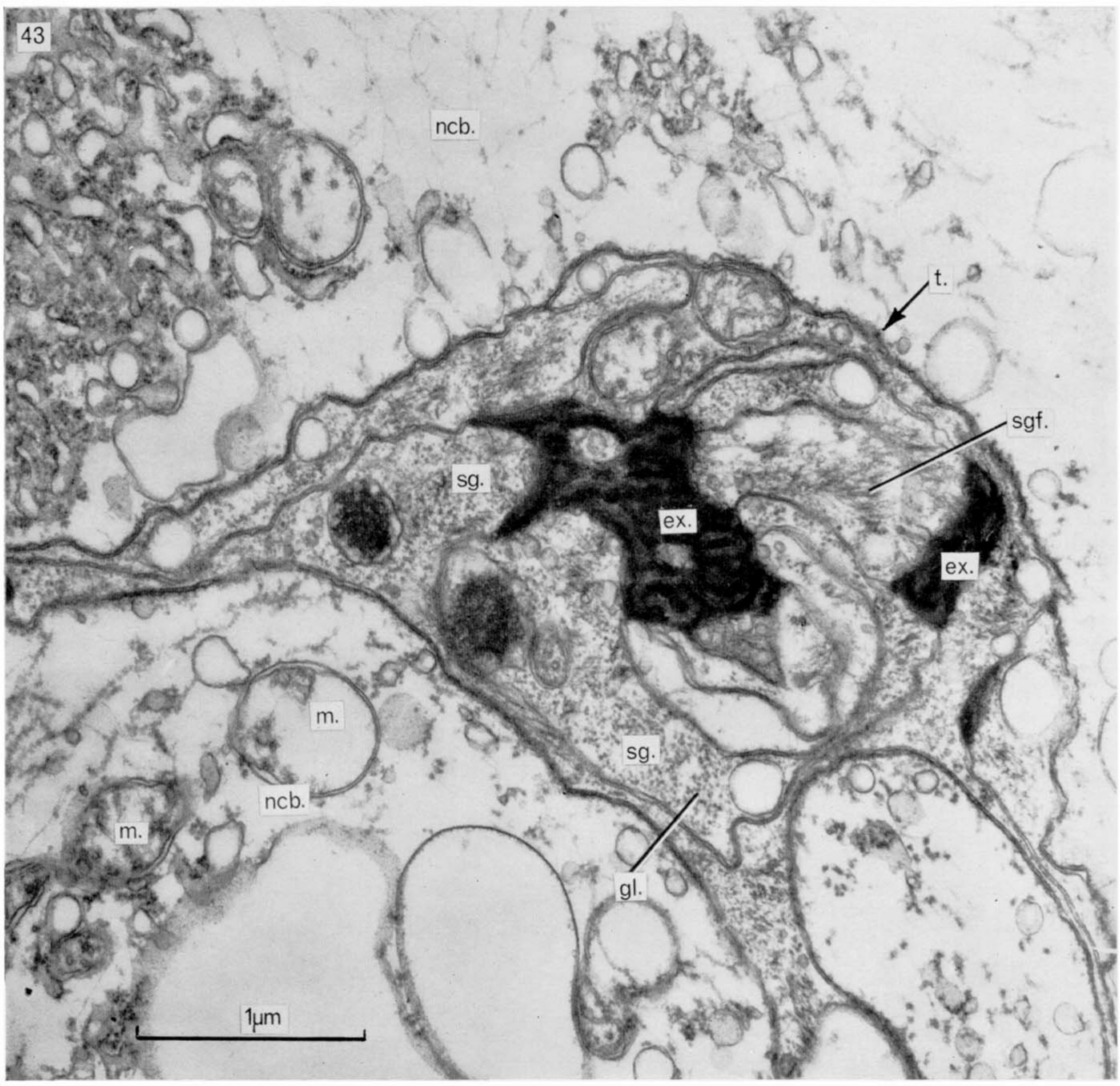


FIGURE 42. Part of the perimeter of a small vein. The basement membrane contains additional extracellular material (see figure 41).

FIGURE 43. Cross-section through a trophospongial invagination into a large neuron cell body of the suboesophageal lobe.

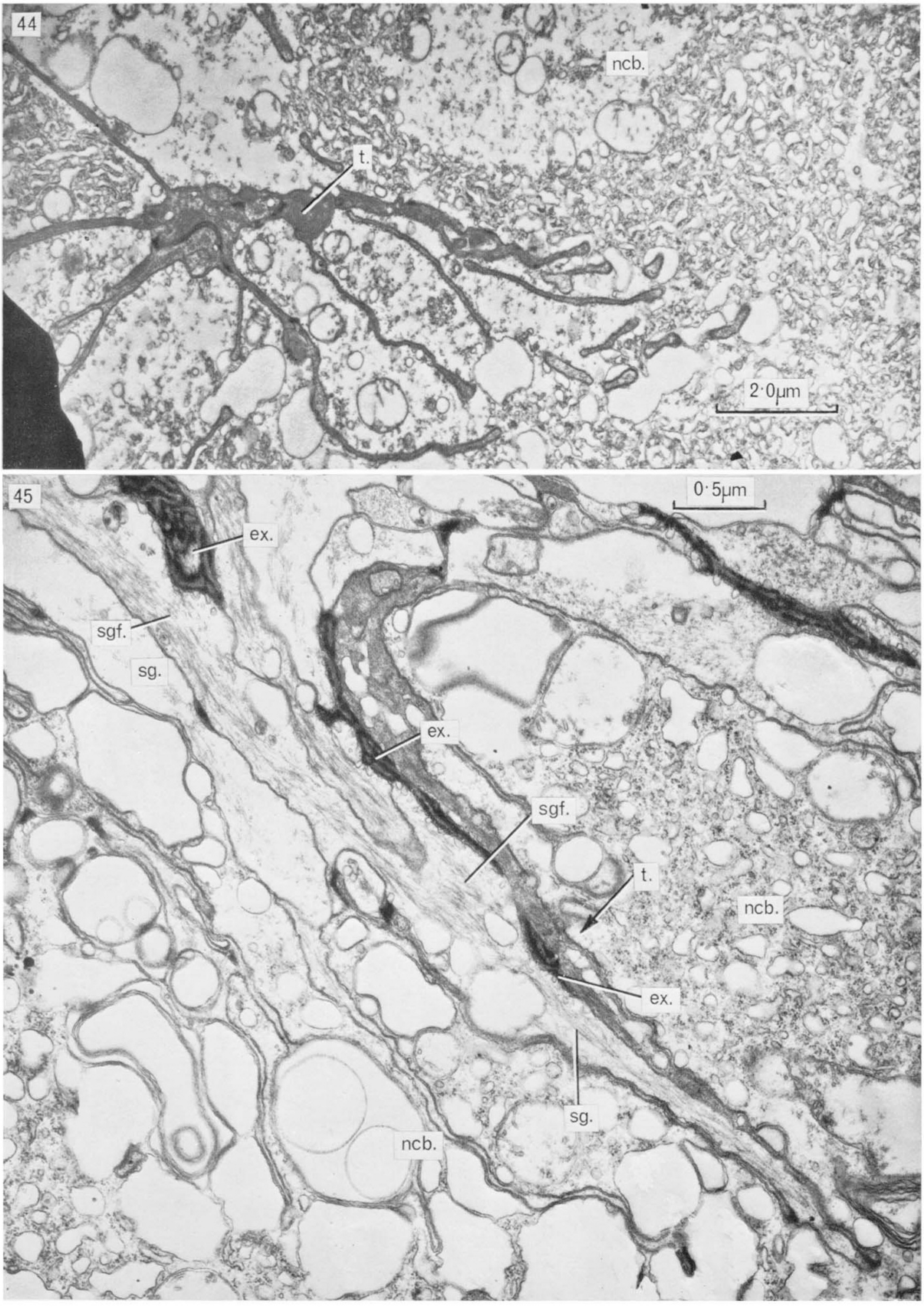
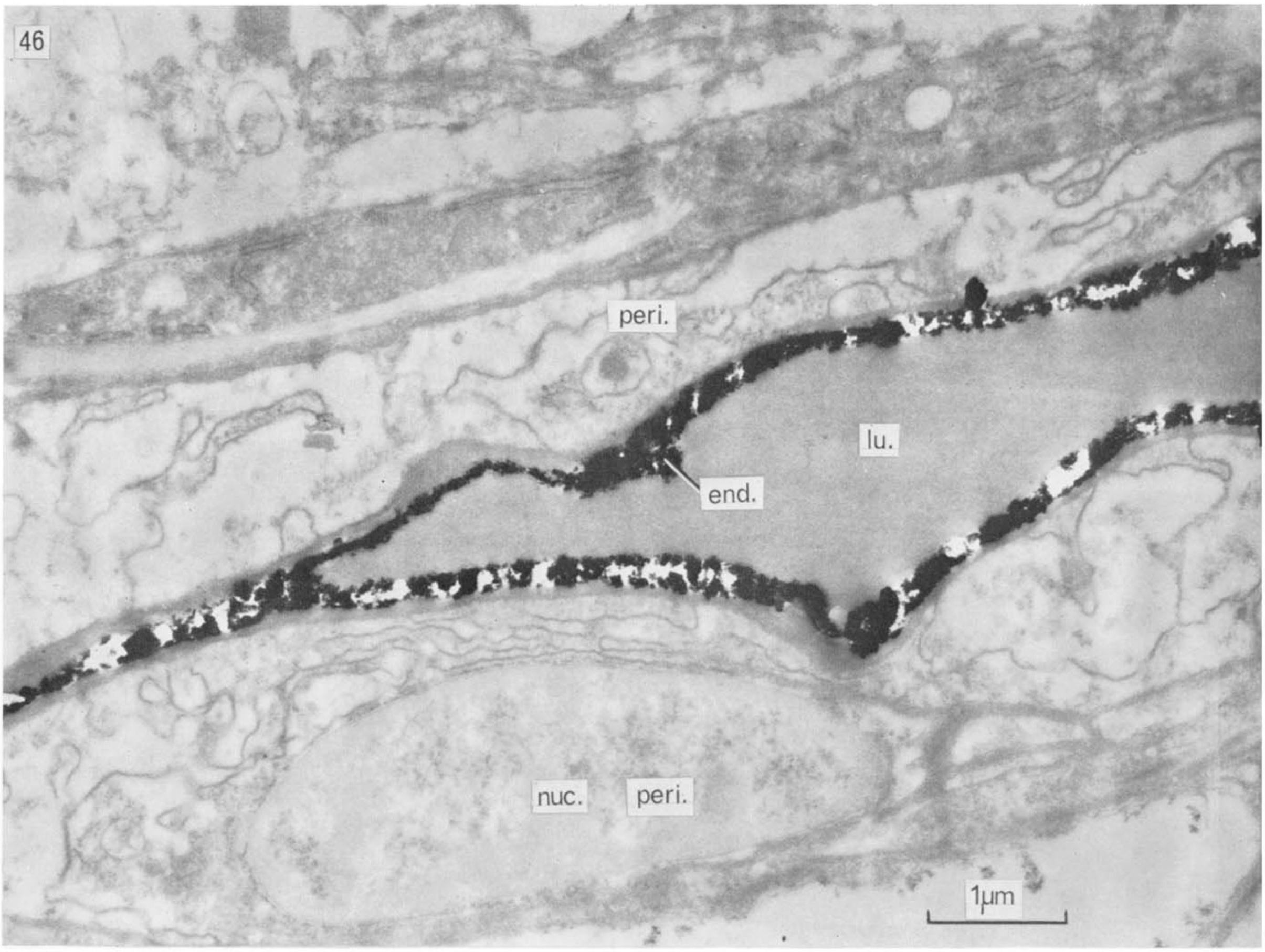
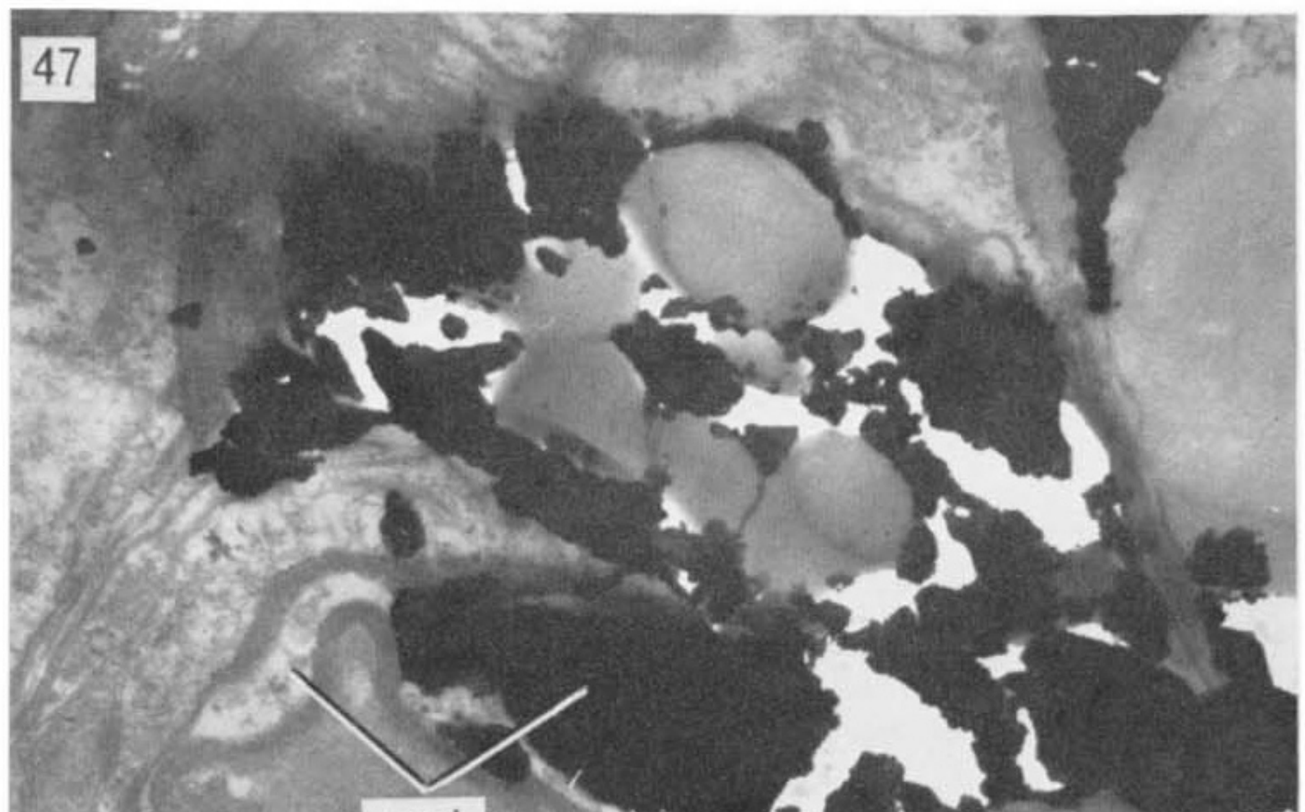


FIGURE 44. Low-power cross-section through a trophospongium which has numerous side channels (neuron of suboesophageal lobe).

FIGURE 45. Longitudinal section of a trophospongium of a large neuron (suboesophageal lobe).





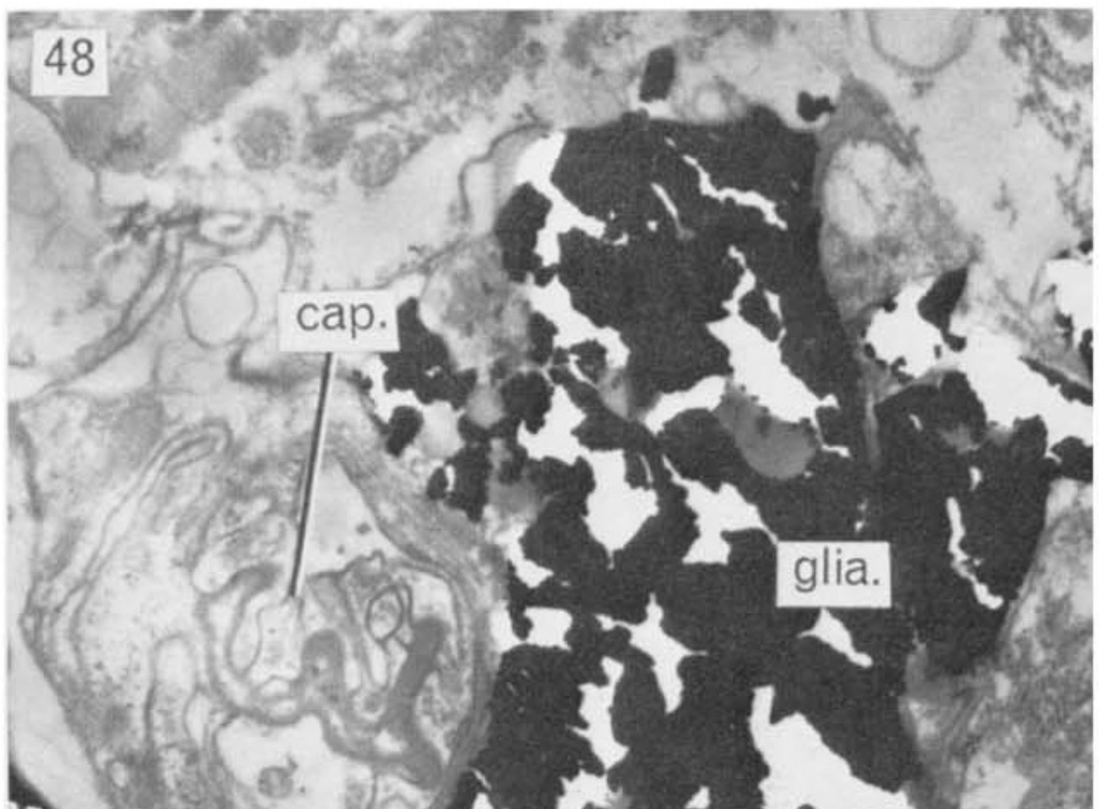


FIGURE 46. Electron micrograph of section of Golgi impregnated capillary showing labelled endothelium (optic lobe).

FIGURE 47. Electron micrograph of section of capillary showing labelled pericytes (optic lobe).

FIGURE 48. Electron micrograph of section of capillary showing labelled glia (optic lobe).