

STRUCTURAL ASPECTS OF THE "BLOOD-BRAIN BARRIER" AREA

IN

RAT CEREBRUM

by

Geoffrey Q. Fox

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SIGNED: Stephen Q Fox

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Wayne W. Ferris  
Wayne W. Ferris  
Associate Professor of Zoology

3 May 1963  
Date

## ABSTRACT

### STRUCTURAL ASPECTS OF THE "BLOOD-BRAIN BARRIER" AREA IN RAT CEREBRUM

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White laboratory rats were anesthetized with sodium pentobarbital. Their skulls were opened and the cerebral hemispheres exposed. Partial fixation of the cortex took place *in vivo* by dripping osmium tetroxide ( $OsO_4$ ) over the exposed surface. The hemisphere was then removed, the cortex diced, and then completely fixed in  $OsO_4$ . The tissue was embedded in methacrylate and Epon 812, then sectioned and viewed on an electron microscope. The Epon 812 sections were stained with lead hydroxide.

The study consisted of investigation of the major components of the blood-brain barrier: 1) capillary endothelium; 2) basement membrane; 3) engrossing sheath formed from membranes of astrocytic end-feet and neuronal processes; 4) neuroglial cells.

The capillary wall represents the first morphological component of the blood-brain barrier. Modes of nutrient transport across the endothelial cell were sought. A basement membrane completely engrosses the endothelial cell. This membrane was found to be lamellar in structure.

Three types of neuroglial cells were considered (oligodendroglia,

astrocytes, and microglia). Oligodendroglia were found to be quite similar to astrocytes but were distinguished by diffuse chromatin in the nucleus and clear mitochondria. Astrocytic end-feet membranes plus membranes from adjacent cellular processes were found to form a sheath which completely surrounds the basement membrane. The presence of astrocytic end-feet in conjunction with capillary endothelium represents a direct relationship of astrocytes with blood-brain barrier activities.

Characteristic features of neurons such as large circular mitochondria, lobated nucleus, and distinct endoplasmic reticulum make the neuron the most easily identifiable cell in the cortex.

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## INTRODUCTION

Perhaps the greatest value derived from the development of the electron microscope has been that it has enabled the scientist to re-investigate many disputed areas and from this, to add to or detract from the evidence supporting existing theories. A recent application of the electron microscope has been directed towards the study of the "barrier" areas within the central nervous system. Although "barrier" areas were known to exist even before the turn of the century, very little was understood about their morphology until rather recently.

One of the best known of the present day multitude of "barriers" is the blood-brain barrier. For this discussion, the term will be used to indicate the tissue area between the endothelial lining of the capillaries and the neuron within the central nervous system. The present investigation is concerned with the morphology of the blood-brain barrier in the cerebral cortex.

Ehrlich, in 1885, was the first to describe the existence of a barrier. After giving animals intravenous injections of coerulein-s dye, Ehrlich found that although most of the body tissues became heavily stained, the central nervous system remained relatively untouched by the dye. Following these initial findings, a number of German workers continued Ehrlich's studies by using a variety of dye stuffs. Goldmann, 1909-1913, clearly described the existence of a barrier between blood and brain. He gave intravenous injections of trypan blue and found

that the meninges and choroid plexuses were deeply stained while the central nervous system and cerebro-spinal fluid remained unstained. Goldmann (1913) injected the dye directly into the cerebro-spinal fluid and found that the entire nervous tissue was deeply stained. This established that, as far as trypan blue was concerned, an effective blood to brain barrier existed since the dye failed to leave the vascular bed except in the area of the choroid plexuses and meninges. Goldmann (1913) pointed out that this barrier was unidirectional and could be circumvented by direct injection into the cerebro-spinal fluid.

No new approaches to the problem developed for the next thirty years and investigators continued the use of dyes and other histological identifiable substances such as ferricyanide and silver (Stern and Gautier, 1922; Gärtner, 1927; King, 1939). In the early 1940's, a concentrated effort was initiated to determine the physiological, biochemical, and biophysical properties of this area. It was soon apparent that the brain did not contain one blood-brain barrier as the early investigators had thought, but several barriers, each of which was rather specific in its function. For instance, silver and acid aniline dyes injected intravenously will be retained on or within the capillary endothelium (Ehrlich, 1885; Lewandosky, 1900; Goldmann, 1909, 1913; Friedmann, 1942; Aird and Strait, 1944; Dempsey and Wislocki, 1955; Bakay, 1956). Most proteins will pass through the capillary endothelium but are then graded in their advancement towards a neuron (Friedmann, 1942; Tschirgi, 1952). The barrier is permeable to substances such as basic aniline dyes and many antitoxins (Friedmann, 1942). The list of substances continues to grow and each substance

appears to react in a slightly different manner (Friedmann, 1942; Dobbing, 1961).

The present investigation has two objectives: 1) a morphological examination of the ultrastructural aspects of various components of the blood-brain barrier in Epon embedded rat cerebral cortex; 2) a comparison of the above results with previous findings using methacrylate embedded material. Methacrylate embedding is one of the oldest and most standard procedures in electron microscopy today. It has been found that methacrylate can cause severe cytological distortion due to swelling during polymerization. The advantage of Epon over methacrylate is that polymerization swelling is reduced significantly, the result being higher quality preservation of cytological detail.

The following three blood-brain barrier components were given special attention: 1) capillary endothelium and adjacent membrane systems; 2) extracellular spaces and ground substance; 3) neuroglial cells. A general description of neurons was also deemed applicable.

## MATERIALS AND METHODS

Adult white laboratory rats were given intraperitoneal injections of sodium pentobarbital using the standard dosage of 25-35 mg/gm body weight. The right half of the skull was exposed and a hole was ground in the posterior medial portion of the parietal bone with a small burr dental drill. This opening avoided the superior sagittal and transverse sinuses. The burr was then replaced with a carborandum disk and the skull was cut anteriorly from the hole to the naso-frontal suture. The parietal, frontal and superior portion of the squamosal were chipped away to expose the right cerebrum. The dissection to this point was accomplished almost bloodlessly. The cerebral cortex was partially fixed in vivo by dripping 4% osmium tetroxide ( $\text{OsO}_4$ ) over the exposed area for 5-10 minutes. The right cerebrum was then freed from the cranial cavity and diced into millimeter cubes. These cubes were immediately placed in  $\text{OsO}_4$  for two hours, dehydrated through a chilled alcohol series and embedded in methacrylate. Both fixation and dehydration were carried out at 5°C. Additional material was prepared in a manner similar to that just described except that it was embedded in Epon 812 using DMP-30 as an accelerator. In both cases "00" gelatin capsules were used as an embedding receptacle and were placed in a 60°C oven overnight.

All sections were cut with glass knives on a Servall MT-1 ultramicrotome. The sections were mounted on 200 mesh grids coated

with a 0.25% Formvar solution. Grids for methacrylate embedded material were carbon coated according to Bradley (1954) using a Kinney SC-3 high vacuum evaporator. Epon 812 material was stained with lead hydroxide according to Lever (1960). Sections were viewed and photographed with a Philips EM-100B electron microscope.

## OBSERVATIONS

### Capillaries

The main component of all capillaries is a flattened squamous endothelial cell. The cell is ensheathed in a thin, darkly stained plasma membrane 8-10 millimicrons thick. The membrane surface facing the lumen of the vessel is surprisingly irregular (fig. 1). Bumps and ridges are quite common but of particular interest are numerous distributed finger-like processes projecting into the lumen. As may be seen in figures 1, 2, 3, these projections are of varying lengths and shapes and do not appear to orientate themselves with any regularity within the lumen.

The nucleus is distinct and somewhat more dense than the cytoplasm. The chromatin is distributed fairly homogenously. The nucleus generally protrudes well into the lumen of the vessel and many times is shaped to the contour of the vessel (Bloom and Fawcett, 1962). No noticeable nucleoli are present. The nuclear membrane stains slightly darker than the surrounding nucleoplasm and the cytoplasm and is thus easily identifiable.

The cytoplasm is characteristically less dense than the nucleus. The cytoplasm is very homogenous in spite of being broken up by mitochondria, vesicles, and other assorted cytoplasmic bodies (figs. 1, 3, and 4). The degree to which the cytoplasm is sandwiched between the plasma membranes varies considerably. In some instances the cyto-

plasmic wall is only about 40  $\mu$  thick while in other areas it reaches a thickness of over a micron. Mitochondria are found to be distributed at random, their sizes and shapes varying somewhat. They are morphologically similar to mitochondria of surrounding nerve processes but are much smaller. A circular or slightly ellipsoid shape seems to be most common. Cristae are sparse but extend the width of the mitochondria (figs. 4 and 5).

Pinocytotic vesicles are present throughout the endothelial cytoplasm and occasionally can be seen pinching off from the plasma membranes (fig. 4). They are generally circular, clear, and of uniform small size. They are distributed quite evenly between the plasma membranes. They tend to be slightly less osmophilic than the plasma.

The external plasma membrane presents a smooth outer surface in comparison to the luminal membrane and shows no unusual surface irregularities. Adjacent endothelial cells were found to be separated from one another by a space of 10  $\mu$ . Desmosomes were found to be associated with these junction areas (fig. 2) and, in a few cases, the finger-like processes are seen to be a continuance of these junctions (figs. 2 and 6).

The existence of fenestrations in the capillary wall of cortical vessels has long been questioned by investigators. No evidence was found to justify the existence of either intracellular or intercellular fenestrations. As can be seen in figures 1 and 3, the plasma membranes are continuous and unbroken around the cytoplasm.

The basement membrane is 100-200  $\mu$  thick and completely surrounds the external plasma membrane. The lamellar basement membrane

(fig. 7) is of a slightly higher density than the endothelial cytoplasm and represents a remarkably uniform layer around the capillary (fig. 5). Whether or not the basement membrane protrudes into the stomata could not be resolved.

Long fusiform cells, bounded by a distinct membrane, occur at irregular intervals within the basement membrane. The cytoplasm of these cells is distinctly less dense than that of the endothelial cell. Mitochondria are not abundant and do not assume any characteristic shape. Circular clear vesicles are present within the cytoplasm but not in great numbers. Occasionally there appear to be sparse cellular organelles present within the large areas of clear cytoplasm (figs. 2 and 8). No nuclei were found.

A distinct outer membrane of 8  $\mu$  thickness completely engrosses the basement membrane. This outer membrane is composed of membranes from closely adjacent cellular elements surrounding the capillary. Two sources contribute to this sheath, neuroglial end-feet and neuronal processes (figs. 5 and 8).

#### Extracellular Space and Ground Substance

The main mass of nervous tissue is a complicated array of cellular elements. The problem of determining whether true extracellular spaces exist within this tangle is a difficult one. If spaces do exist, a new problem of whether a ground substance occurs within the spaces emerges. The only possible space that occurs regularly is in the area located between cellular membranes. The separation between membranes ranges from 14  $\mu$  to about 30  $\mu$  with occasional larger

triangular and irregular areas formed by the junction of membranes (figs. 9 and 10). Whether this separation is a true extracellular space or not is still problematical, but indications are that spaces are present. In figures 9, 10 and 11, a substance of distinct density is present. This eosinophilia is believed to represent the ground substance of the cortex.

### Neuroglia

Three types of neuroglial cells were examined, oligodendroglia, astrocytes, and microglia.

Oligodendroglial cells contain a large, circular nucleus with a distinct nuclear membrane. Dense nucleoli of moderate size are sometimes present. The chromatin material is finely granular and aggregated into small clumps which are evenly distributed throughout (figs. 12 and 13). No unusual accumulation takes place along the nuclear membrane.

The cytoplasm forms a scant boundary layer around the nucleus. Its density and consistency appear to be almost identical to that of the nucleus. Cuboidal mitochondria are most frequent although elongate types are not uncommon, especially in the hillock region (fig. 12). The mitochondria appear distinctively clear against the cytoplasmic background. The cristae are few with distinct membranes. Their length does not exceed the radius of the mitochondria thus leaving large clear areas. Small clumps of dark aggregated material, remarkably similar to nuclear chromatin in appearance are found in the cytoplasm. Clear vesicles are present throughout but tend to be

more numerous in the region of processes. Luse (1956), Schultz et al. (1957) and DeRobertis et al. (1959) all report that oligodendroglial processes are short and do not branch extensively. The density of the cytoplasm appears to remain fairly constant towards the periphery of the cell.

Astrocytes are usually about the same size as oligodendroglia. Their nuclei are usually ovoid but in some cases they are almost circular. Nucleoli are not recognizable but a distinct nuclear membrane is. The chromatin is dense and is distributed in distinct aggregates throughout the nucleus (fig. 15). Without fail, this chromatin distinctly lines the nuclear membrane and this, it is thought, may be used as a distinguishing characteristic between this cell and the oligodendroglia for which it is often confused. Large osmophobic areas exist between the chromatin.

The cytoplasm is less dense than that of the oligodendroglia. It also tends to occupy a wider space about the nucleus. Mitochondria are numerous and generally spherical or cuboidal in shape. They are also very dense and stand out like dark spots against the cytoplasmic background (figs. 14 and 15). Short, dark, beaded, filamentous structures intertwine amongst the mitochondria and throughout the cytoplasm (fig. 15). In methacrylate sections the cytoplasm is distinctly clear.

Processes from the astrocytes are long and elaborate and their end-feet rest upon the basement membrane of cerebral capillaries (fig. 16). The difficulty in identifying all end-feet processes makes the determination of the quantitative relationship between astrocytes and

capillaries a difficult one.

Microglial cells are readily identifiable. They are about half the size of an astrocyte and appear to be dark, roughly ovoid cells. The nucleus is large and stains heavily. A distinct nuclear membrane is present with occasional nuclear "pores" (fig. 17). Chromatin material is extremely dense and homogenous throughout. Several nucleoli are sometimes seen. The cytoplasm is very scant but is as dense as the nucleus. Mitochondria are present but not numerous. The cell in its entirety is the most electron dense structure present in the cortex with the exception of the myelinated nerve sheaths. Microglia appear to act as satellite cells to neurons and capillaries. Their specific function is still being sought.

#### Neuron

The neuron is the most easily identifiable structure in the cortex. Its appearance is very distinct and in only rare cases is it confused with other cells such as microglia. It is the largest cell type to be found. The nucleus takes up a good share of the main visible mass of the cell. A large, dense, nucleolus is almost always present. The main mass of cortical tissue is composed of neuronal processes.

The nuclear area is very evident in the neuron, however the nuclear membrane is not as distinct as it is in neuroglia. The nuclear area is slightly less dense than the cytoplasm and the chromatin is evenly distributed. The nucleolus is the darkest element in the cell and is very large. Multinucleolated neurons have been reported in

rat (Palay and Palade, 1955).

The cytoplasmic mass surrounding the nucleus is very sparse, due mainly to the great number of large mitochondria present. These mitochondria are clear and distinctively circular, sometimes attaining a diameter of one micron (fig. 18). The nuclear membrane tends to surround a portion of the mitochondria. A thin slip of cytoplasm remains to separate the two membranes. The entire nucleus thus appears lobated (fig. 19). A large number of mitochondria of all sizes are always found in the hillock regions. The cytoplasm contains randomly arranged irregular open areas; clear vesicles are present in these areas and often seem to be associated with the spaces. These cavities are bounded by a membrane and represent either an unusually swollen, or a large endoplasmic reticulum (Palay and Palade, 1955) (fig. 18). Ribosomes are distributed throughout the cytoplasm but are most concentrated along the membranes lining the endoplasmic cavities.

The nerve processes vary considerably in size. Some are myelinated while others are not. Elongate mitochondria and neurofibrillae are always present.

## DISCUSSION

As was stated earlier, one of the purposes of this paper is to compare work using methacrylate embedded material with similar material embedded in Epon. Therefore, all references cited on the following pages will refer to work done with methacrylate.

Several questions arise as to the morphological role the cerebral capillaries play in blood-brain barrier activities. The first of these to be considered concerns the finger like processes of the endothelium projecting into the lumen of the vessel. Only the presence of these pseudopodia (Palade, 1961) has been mentioned to date. It was found in this study that their sizes extend over a great range and their orientation is anything but orderly. This irregularity may have an effect on blood flow and presents an interesting biophysical problem.

Pinocytotic vesicles have long been known to exist in the endothelium (Lewis, 1931). Rhodin (1963) states that they exist only in areas of greater than 100  $\mu$  thickness and this study concurs with those findings. As was stated earlier, the capillary endothelium was found to reach a minimum thickness of 40  $\mu$ . This is far from the average thickness which is calculated to be around 500  $\mu$ . Areas of below 100  $\mu$  are quite rare in cortical capillaries and thus it is believed that other transport mechanisms are involved in these areas. At any rate, Palade (1961) argues that the pinocytotic vesicles present in capillary endothelial cells are the main transporters for nutrient

material. If this is so, the question arises as to whether pinocytotic mechanisms in the central nervous system are selectively different from those in other parts of the body, and if so, how specific are they?

The absence of fenestrations in the capillary wall of cortical vessels has long been recognized by investigators. Farquhar and Hartmann (1956) stated that the luminal plasma membrane may have a few perforations but nothing that could be classified as an endothelial fenestration. Maynard et al. (1957) found nothing to suggest fenestrations similar to those found in other areas of the body i.e. glomerular capillaries (Pease, 1955). No evidence was found in this study to contradict previous findings.

The blood-brain barrier was initially believed to be located in the membranes surrounding the cerebral capillaries since this was the area where a great number of the dyes used by the early investigators were retained (Lewandowsky, 1900). Gärtner (1927) suggested that the surrounding membranes were pial investments and that because of this, permeability characteristics were uniform. Since that time, the basement membrane has been recognized as being the external investment of the capillary endothelium. Dempsey and Wislocki (1955) watered rats on a 1.5% silver nitrate solution for six months and found that silver deposition takes place at the basement membrane. In capillaries in other parts of the body, the basement membrane may or may not be present. When it is present, it usually presents a very diffuse irregular appearance (Bennett, et al., 1959). In the cortex, as was previously stated, it is believed to be a distinct layer because of the fact that it is

neatly sandwiched between the external plasma membrane of the endothelial cell and the various membranes of cellular elements in the nervous tissue. Disputes still reign over the structure or structures to be termed basement membrane. Farquhar and Hartmann (1956) define the basement membrane as a laminated layer directly adjacent to the external plasma membrane of the endothelium. This concurs with more recent work on basement membranes by Bennett, et al. (1959). On the other hand, Dempsey and Wislocki (1955) describe two distinct layers. The first is a lamina of variable thickness with moderate homogenous density in contact with the endothelial plasma membrane. The second layer, surrounding the first, is made up of collagen. This study found no evidence to support the idea that two distinct layers occur between the membrane systems. In figure 7 it is evident that a lamellar structure exists. However, it should be noted that the laminated area appears to be of equal density throughout with no suggestion of an additional collagen layer present.

Long fusiform cells are present in the basement membrane (Farquhar and Hartmann, 1956). Since 1956 other investigators have confirmed their presence (Bennett et al., 1959; Palade, 1961; Pierce, 1963). So little has been mentioned about these cells, however, that their function can only be generally adduced. They are fairly numerous but do not seem to be associated with any specific adjacent cellular element. Two possible functions have been suggested by Farquhar and Hartmann (1956): 1) they may play a part in capillary contraction; 2) they may be responsible for microglial formation.

## Extracellular Space and Ground Substance

The presence or absence of ground or fundamental substance in nervous tissue has been discussed for a half century. Observations of the cortex by light microscopists led histologists to believe that large extracellular spaces existed. Their confusion arose from misinterpreting minute cellular elements for spaces.

In 1955, Hess (1955b) using the periodic acid-Schiff (PAS) reaction on brain material concluded that whereas glial and nerve cells gave a negative reaction, some unknown substance gave a positive reaction. This substance was interposed between the cellular elements and accordingly thought of as the ground substance. Dempsey and Wislocki (1955) reached a similar conclusion. Davson and Spaziani (1959) using cytochemical methods have calculated that 15% of the brain is extracellular space.

Considering only the morphology of the area, it has become evident in recent years that no extracellular spaces exist. Wyckoff and Young (1956) found no evidence of extracellular spaces to account for the ground substance, and Gardner (1960) has confirmed these findings.

Hess (1955a) did show that extracellular spaces do exist in new born animals and that there is a substance present in these spaces. However he thought these spaces disappeared in the adult animal. It is thought that there is justification in assuming that spaces exist between cellular membranes and that this area is filled with some amorphous material. Distinct areas, bounded by membranes of closely adjacent cellular elements, are present in both methacrylate and Epon

embedded material. A distinct separation between parallel cellular membranes is also common. These spaces have a distinct osmophilia. Whether these spaces represent swollen separations due to the embedding medium could not be positively determined. It is believed that these spaces do not represent artifacts. All components of a system must be capable of a limited amount of independent movement and this movement would be severely restricted if all cellular elements were joined.

If these spaces are present in the central nervous system and are occupied by the ground substance, then new biochemical techniques will have to be developed to bring them to light.

### Neuroglia

The relationship of neuroglial cells to cerebral capillaries was recognized very early. Lewandowsky (1900) first suggested that they may be related to the blood-brain barrier. Gartner (1927) hypothesized that the glial membranes and investing pia membranes formed an effective permeability shield around the blood vessels. More recently there has been an increasing amount of interest directed towards the identification and function of the neuroglia.

Work has been conducted on the physiologic importance of the neuroglia by Dempsey and Wislocki (1955), Bakay (1956), Bairati (1958), DeRobertis et al. (1959, 1961), and Hyden (1962), who generally agree that neuroglial cells play an important part in effecting an exchange of nutrients from blood vessels to nerve tissue. It is very possible that neuroglia are the moderators of the blood-brain barrier. Maynard et al. (1957) demonstrated that astrocytic processes contact the base-

ment membrane surrounding the capillary wall. Since then a number of investigators have concurred with this work (Farquhar and Hartmann, 1957; DeRobertis et al., 1959).

The difficulty in identifying all astrocytic end-feet processes makes the determination of the quantitative relationship between astrocytes and capillaries a difficult one. The density of the cytoplasmic end-feet appears to be essentially identical to that of nerve fibers. It is near impossible to separate unmyelinated nerve fiber from astrocytic end-feet and processes. Two criteria, which serve in only a fundamental capacity, are available at present: 1) comparison of the relative area that the structure in question occupies in relation to an adjacent unmyelinated and easily identifiable cross sectioned nerve fiber; 2) the presence or absence of neurofibrillae. Even with this limited approach to identification, it is evident that astrocytic end-feet engulf great portions of the cerebral vessels. With this close morphological relationship, they are undoubtedly an extremely important element in the barrier mechanism.

Another problem is identification of oligodendroglia and astrocytes. These two neuroglial cells are often confused with one another. Photographic evidence presented by various workers appears highly contradictory and correlation between photographs and the written description is questionable.

Oligodendroglia of the cerebral cortex are closely adjacent to neurons. DeRobertis et al. (1959) believe that they "attach" themselves to the perikaryon of the neuron. Whether this is true in most instances is questionable. They are also found in the white matter of

the spinal cord where they are arranged in rows between fasciculi (DeRobertis and Gerschenfeld, 1961). These two types are somewhat different in structure and the present discussion is confined to the cortical oligodendroglia. Both types are believed to have had the same function, that is, the synthesis and maintenance of myelin sheaths. Functionally then, they are a type of Schwann cell. This relationship to myelin formation is lost in the cortical area where the distance between an oligodendroglia and a myelinated nerve is sometimes very great. For this reason and also because of its close relationship to neurons, the function of this cell in the cortical areas is still being closely examined (Tschirgi, 1952; Mannery, 1954; Bakay, 1956; Dobbing, 1961).

Three identifying criteria for separation of oligodendroglia from astrocytes has emerged from this study.

1. Chromatin material is finely distributed throughout oligodendroglia with only minute clumping present. In astrocytes the chromatin tends to form large aggregates which invariably concentrate heavily on the nuclear membrane.
2. Mitochondria are distinctively clear in oligodendroglia whereas in astrocytes they tend to be osmophilic and thus set themselves off sharply from the cytoplasmic background.
3. The cytoplasmic mass in oligodendroglia is moderately dense with no unusual characteristics to point out. Cytoplasm of astrocytes is very scant and contains beaded fibers which are quite characteristic of these cells.

## SUMMARY

The blood-brain barrier as defined by this study is composed of the following elements: 1) capillary endothelial cells; 2) basement membrane; 3) engrossing sheath formed from membranes of astrocytic end-feet and neuronal processes; 4) neuroglial cells, specifically astrocytes.

Capillary endothelium varies tremendously in thickness and has a very irregular luminal surface. Pinocytosis is believed to be the main nutrient transport system but other active transport systems must be assumed to exist. No fenestrations occur in the cortical endothelium.

A basement membrane completely surrounds the endothelium. This membrane has a lamellar structure and is evenly sandwiched between the external endothelial plasma membrane and the sheath formed by the surrounding cellular elements.

A sheath made up of membranes from astrocytic end-feet and other cellular elements completely surrounds the basement membrane.

Identifying characteristics were established for oligodendroglia, astrocytes, and microglia. Astrocytes play a direct role in blood-brain barrier activities. End-feet from astrocytic processes make direct contact with the basement membrane. The extent of the basement membrane surface area contacted by these end-feet is still disputed since the absolute identification of end-feet is difficult.

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Figure 1. Cross section of a rat cerebral capillary showing a number of short pseudopodia (P) projecting into the lumen (L) and pinocytotic vesicles (lines). A basement membrane (B) containing a satellite cell (S) completely surrounds the endothelium. Several astrocytic processes (A) are in contact with the basement membrane. X23,000.

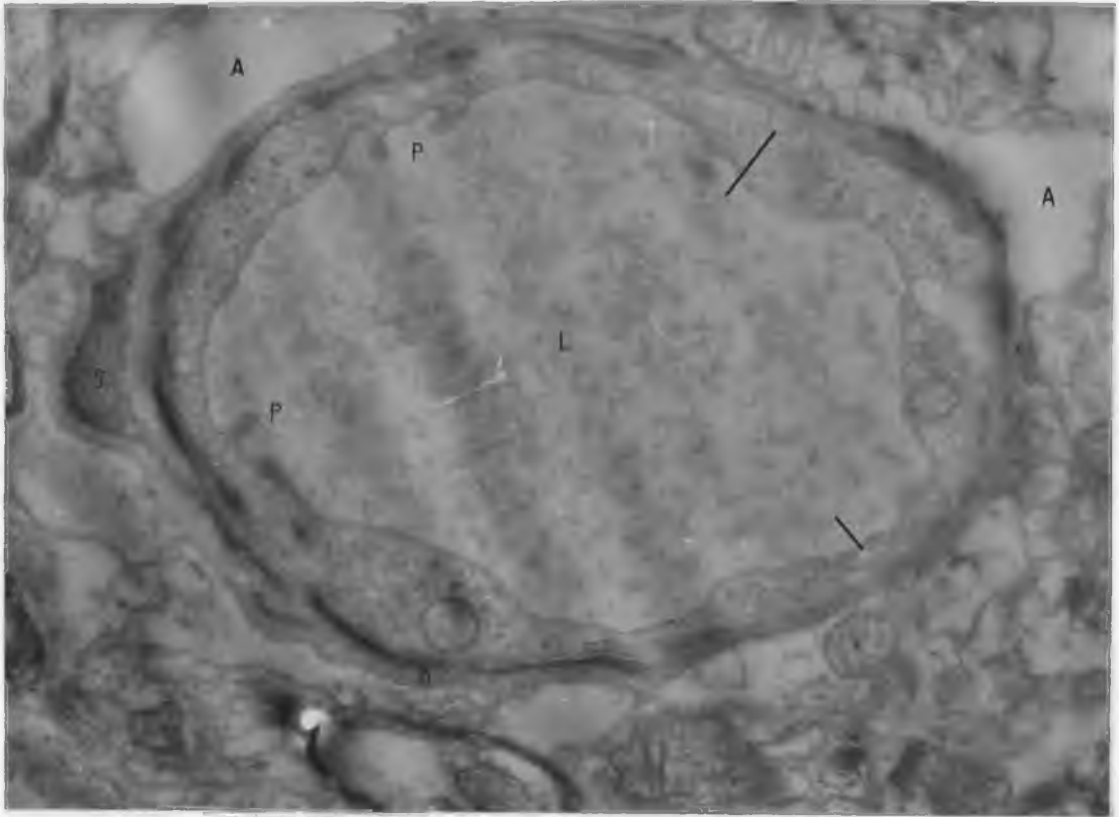


Figure 2. Section of the wall of a rat cerebral capillary showing desmosomes (arrows) at the junction of two endothelial cells. Associated with the junction is a prominent pseudopodia (P). A large satellite cell (S) with a circular mitochondria (M) is present within the basement membrane (B). Note that the cytoplasm of the satellite cell is less osmophilic than that of the endothelium. X26,000.

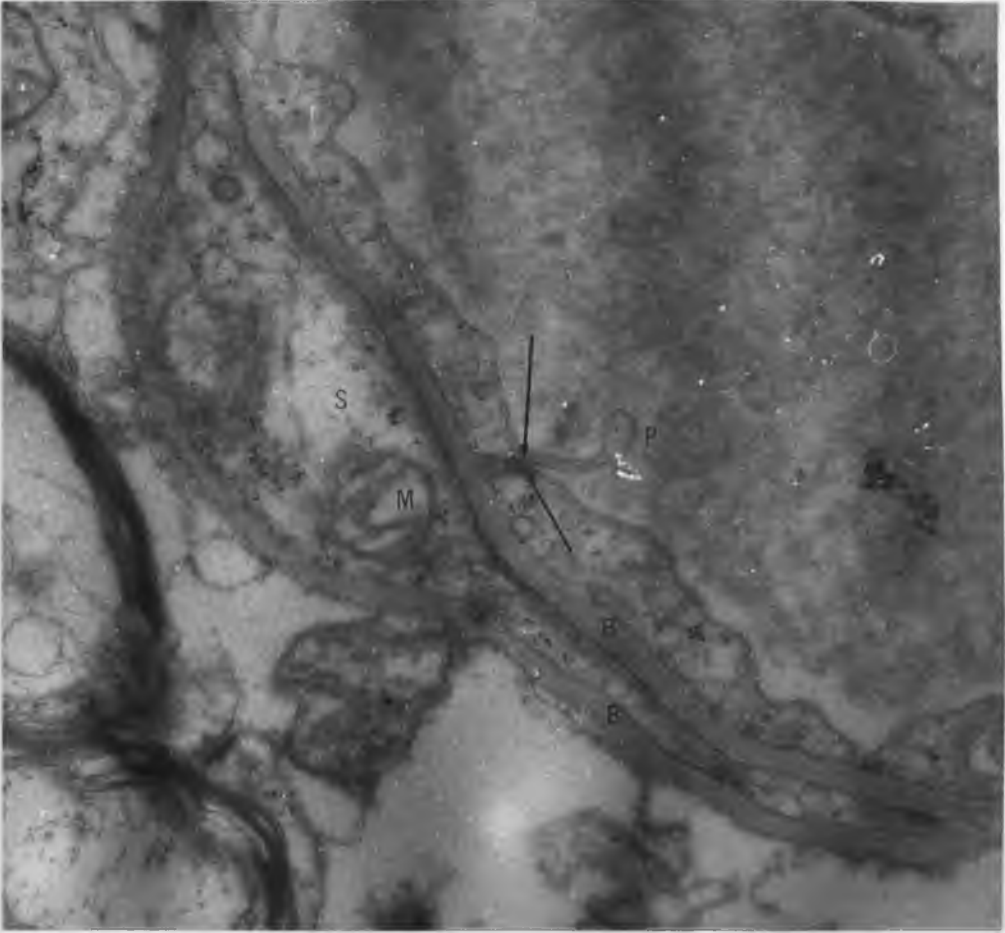


Figure 3. Longitudinal section of a rat cerebral capillary with numerous pseudopodia (P) and mitochondria (M). X30,500.

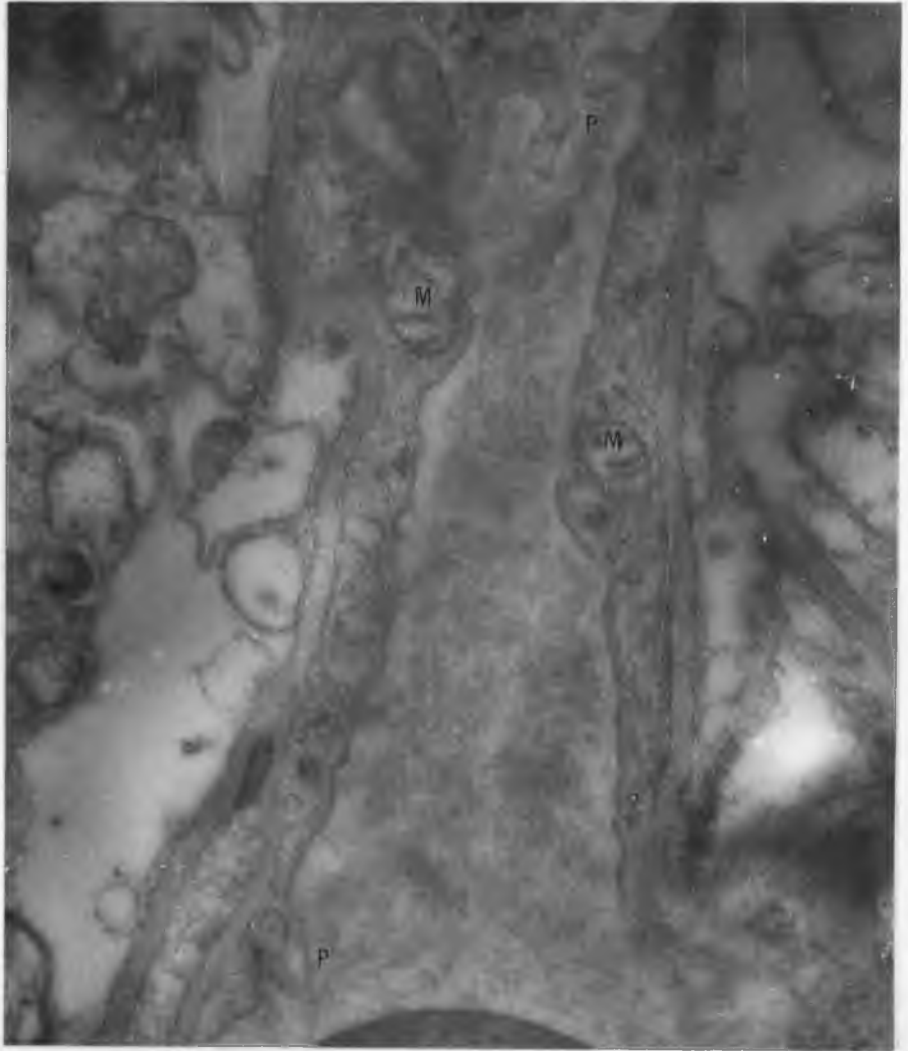


Figure 4. Endothelial wall showing pinocytotic vesicles (arrows) pinching off the luminal plasma membrane. Numerous vesicles (V) are present throughout and a characteristic mitochondria (M). A leucocyte (W) occupies the lumen. X19,000.

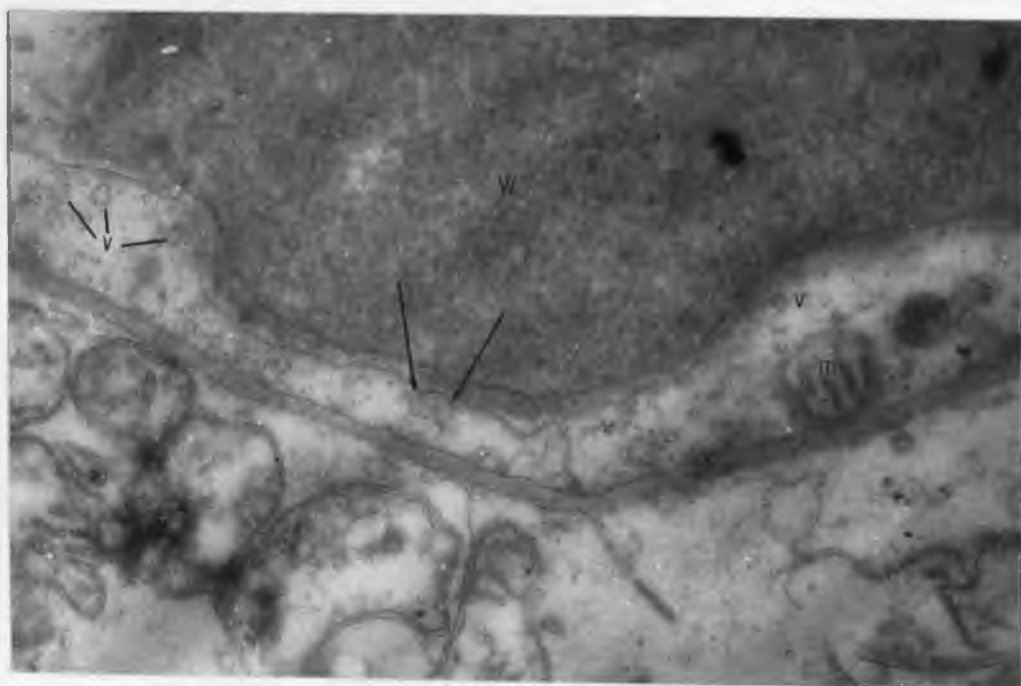


Figure 5. Cross section of a rat cerebral capillary.

Numerous mitochondria (M) are present in the endothelium. The basement membrane (B) forms an even layer around the capillary. A large astrocytic process (A) makes contact with the basement membrane. Present within the lumen is a leucocyte (W). X18,000.

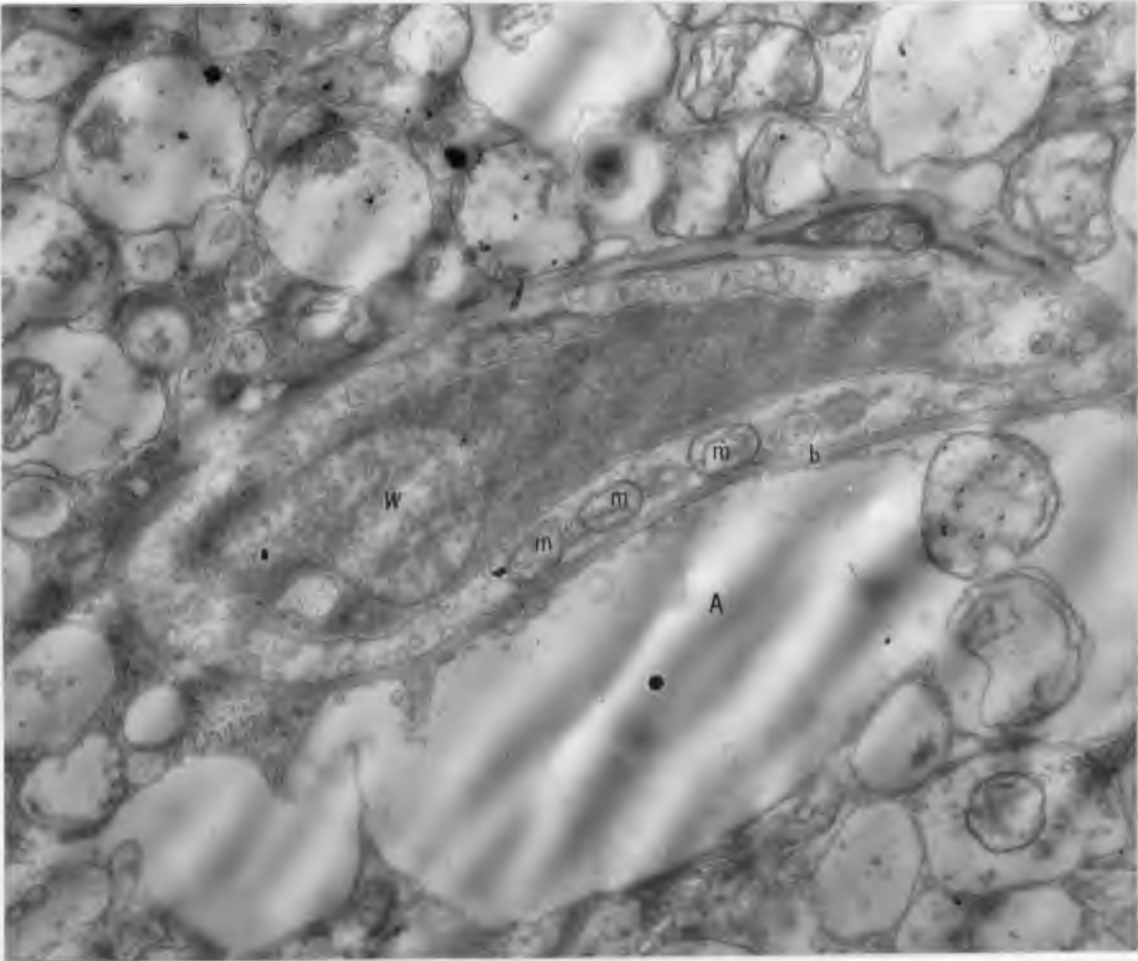


Figure 6. Junction of two cerebral capillaries with an erythrocyte (E) and leucocyte (W) present within the lumen. Junction of two endothelial cells (arrow) is flanked by two distinct pseudopodia (P). Basement membrane (B); Satellite cell (S). X26,000.

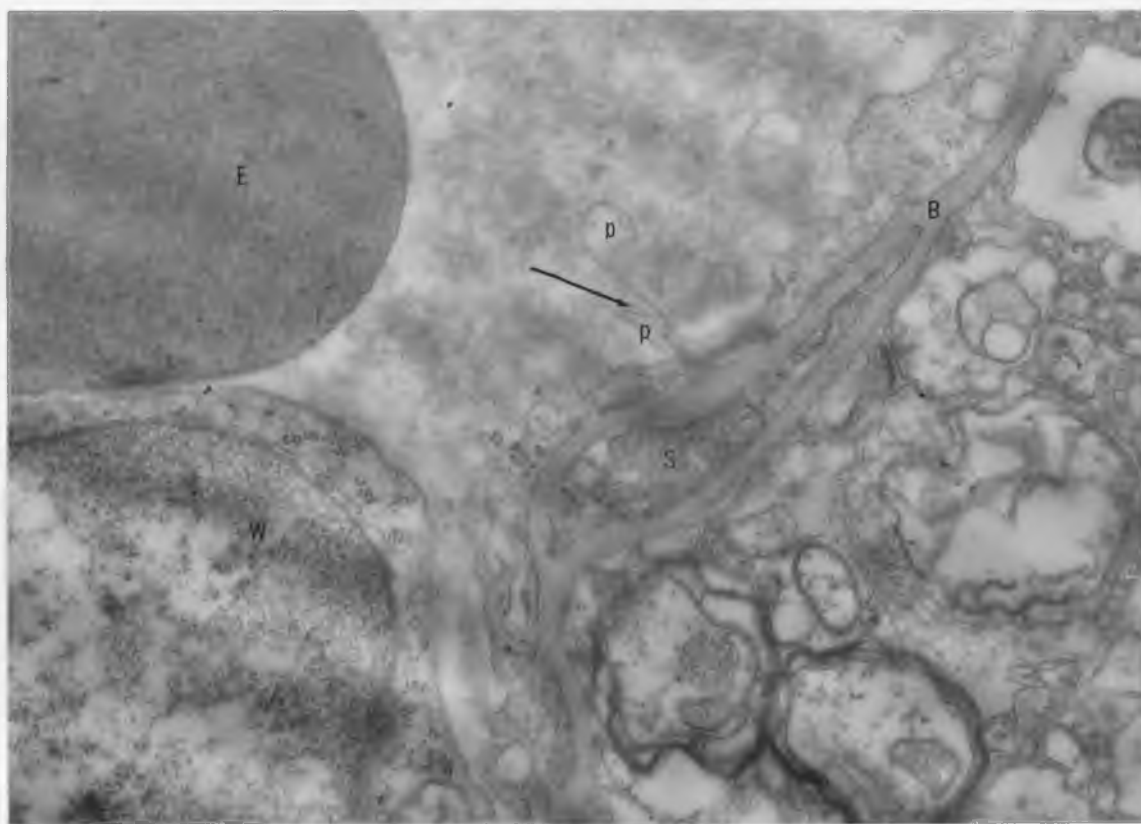


Figure 7. Section of endothelium (En) with adjacent basement membrane (B). The basement membrane has a distinct lamellar structure. X108,000.

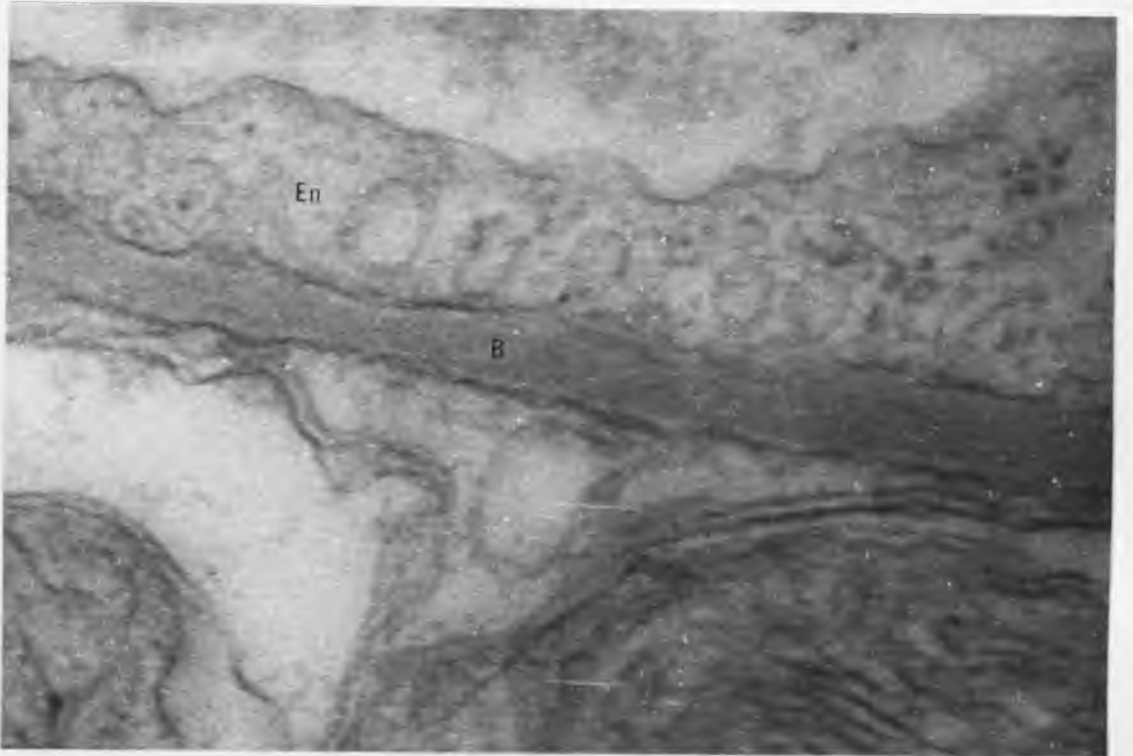


Figure 8. Cross section of a rat cerebral capillary with a leucocyte (W) occupying the lumen. Numerous satellite cells (S) with their characteristic osmophobic cytoplasm are present. An astrocytic process (A) surrounds half of the capillary. X16,000.

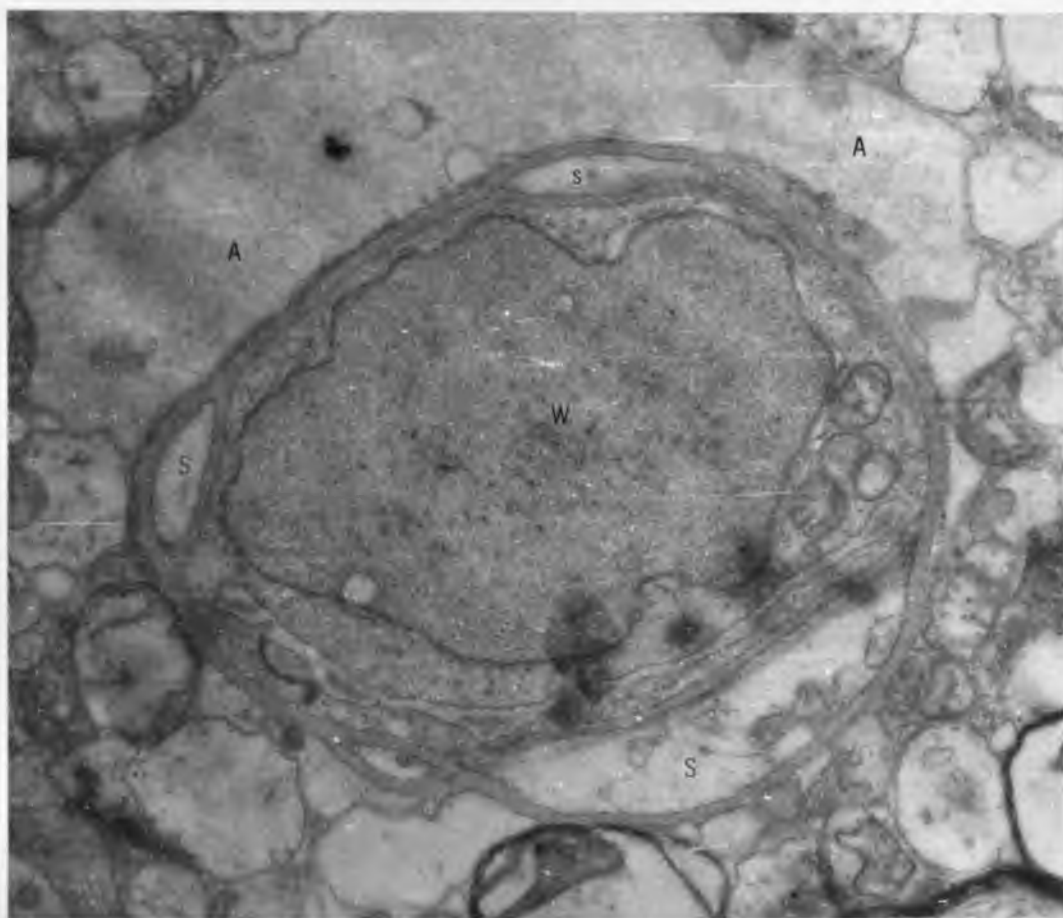


Figure 9. Extracellular space (arrow) bounded by membranes of nerve processes. X20,000.

Figure 10. Extracellular space (arrow) bounded by membranes of adjacent nerve processes. An osmophilia similar to that present in the nerve fibers is present within the space. X20,000.

Figure 11. Extracellular spaces (arrows) bounded by membranes of adjacent nerve processes. A distinct osmophilia seems to be present. X45,000.

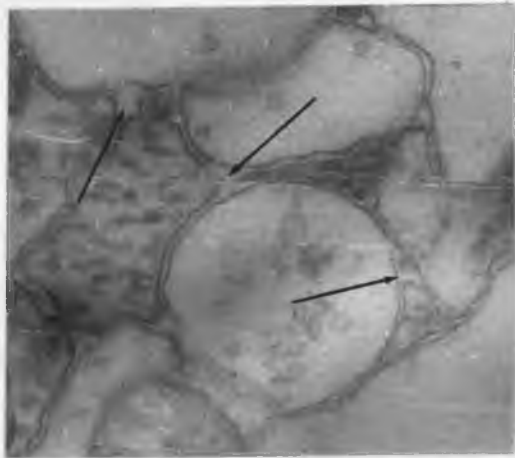
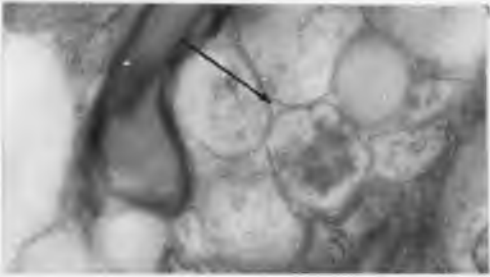
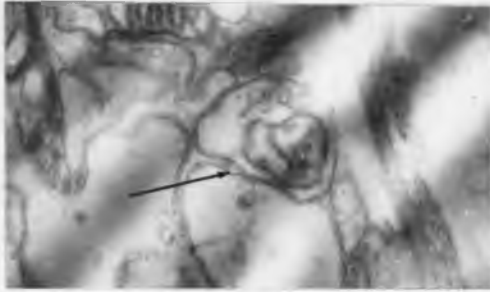


Figure 12. Oligodendroglia cell. Nucleus (Nu) contains evenly dispersed chromatin granules and is bounded by a distinct membrane (Nm). Mitochondria (M) are large and clear. Cytoplasm forms only a thin layer about the nucleus. X10,000.

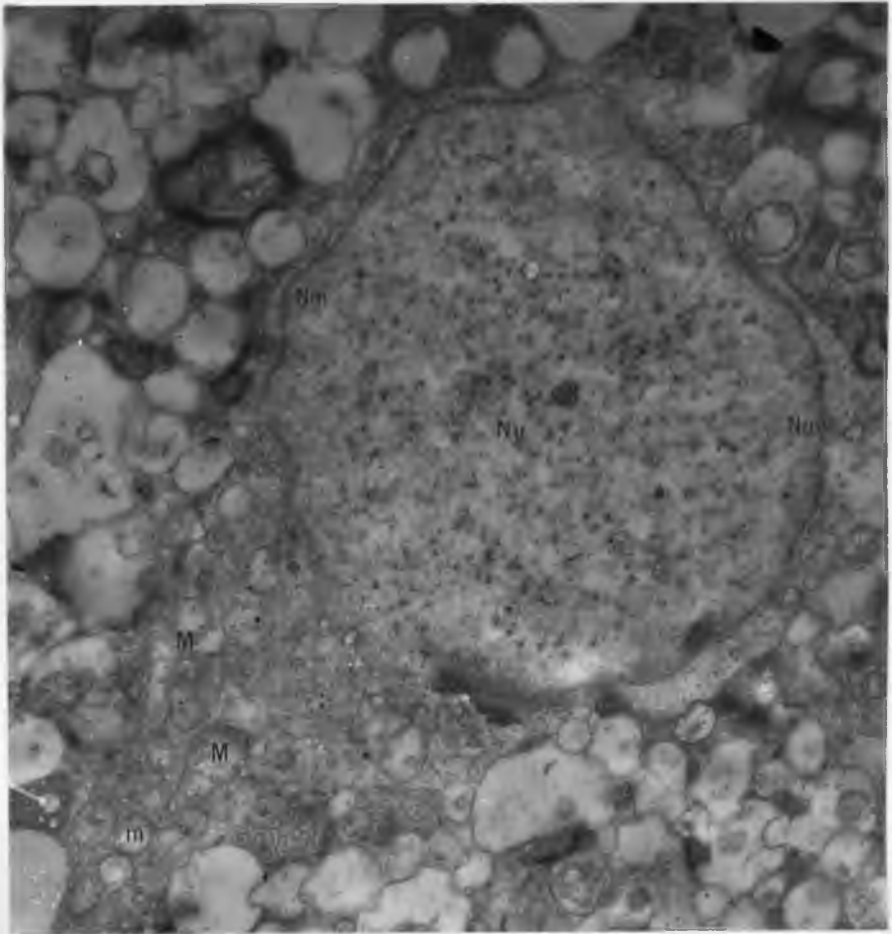


Figure 13. Oligodendroglia cell. Distinct nucleolus (Nc) present within the nucleus (Nu). Clear mitochondria (M) abound in the thin layer of cytoplasm about the nucleus. X16,000.

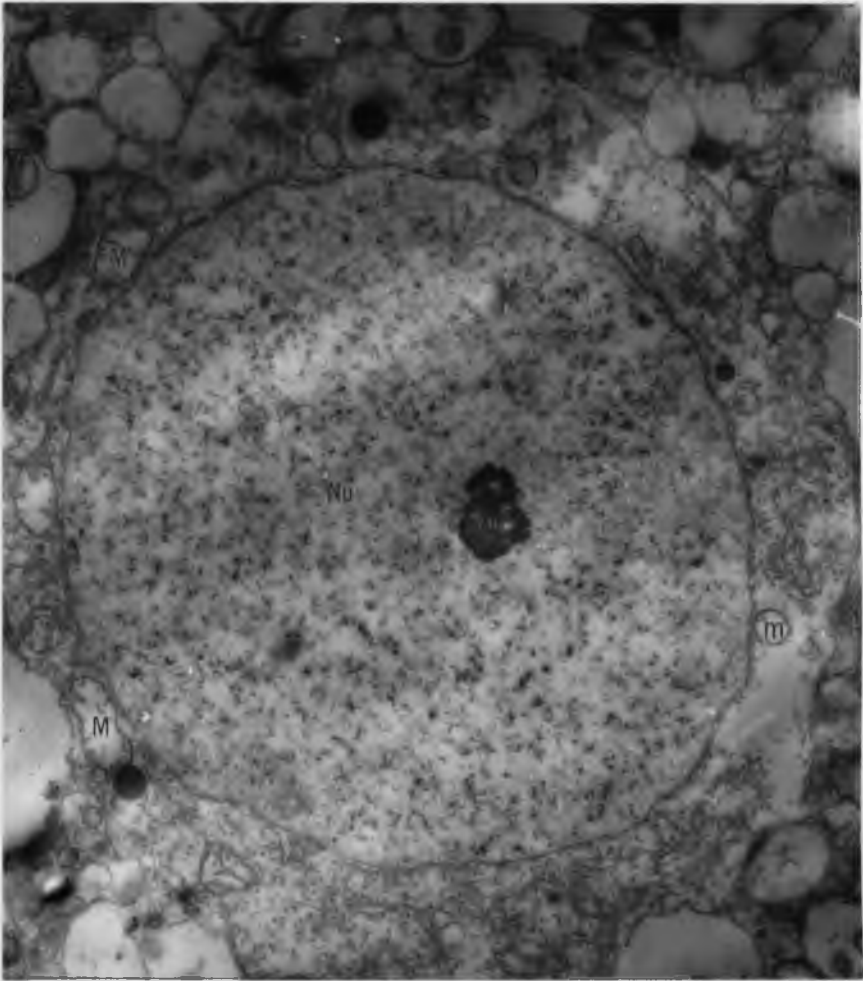


Figure 14. Astrocyte with nucleus (Nu) containing irregularly distributed aggregations of chromatin (C) which forms a heavy border (arrows) along the nuclear membrane. Osmophilic mitochondria (M) present within the osmophobic cytoplasm. X14,000.

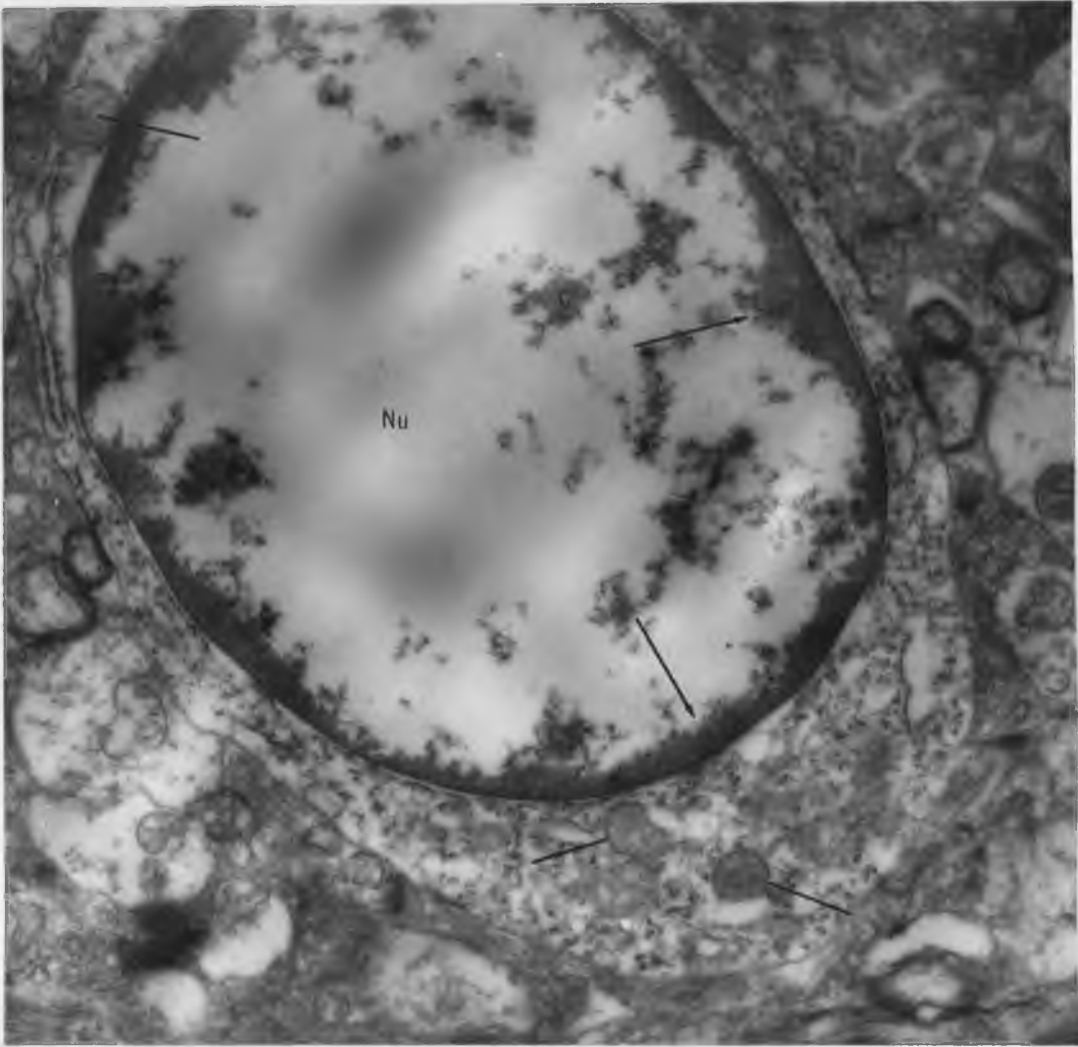


Figure 15. Astrocyte with characteristic chromatin aggregations (C) within the nucleus. Numerous osmophilic mitochondria (lines) are present within the cytoplasm. Beaded filaments (arrows) are also distributed throughout the cytoplasm. X14,000.

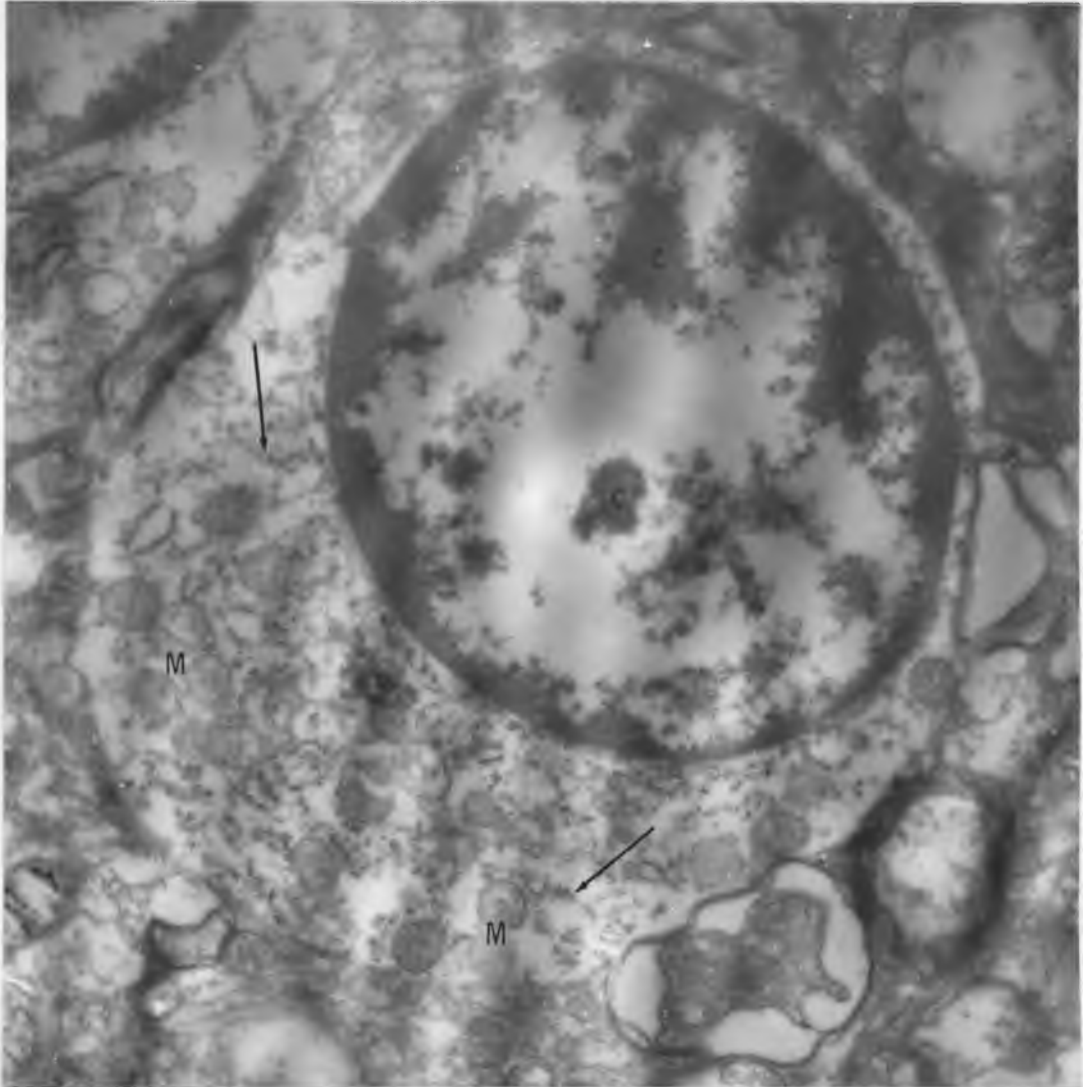


Figure 16. Cross section of a rat cerebral capillary surrounded by astrocytic end-feet (A). A large satellite cell (S) is present within the basement membrane. X11,000.

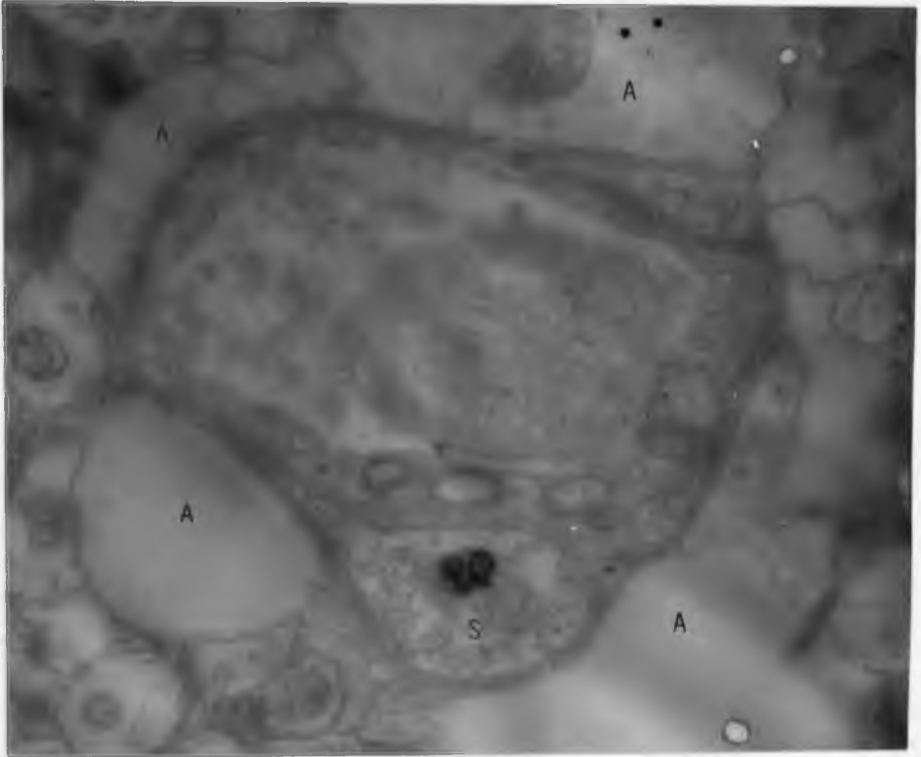


Figure 17. Microglia cell with large osmophilic nucleus (Nu). Scant cytoplasm surrounds the nucleus. A "pore" can be seen on the nuclear membrane (arrow). X20,000.

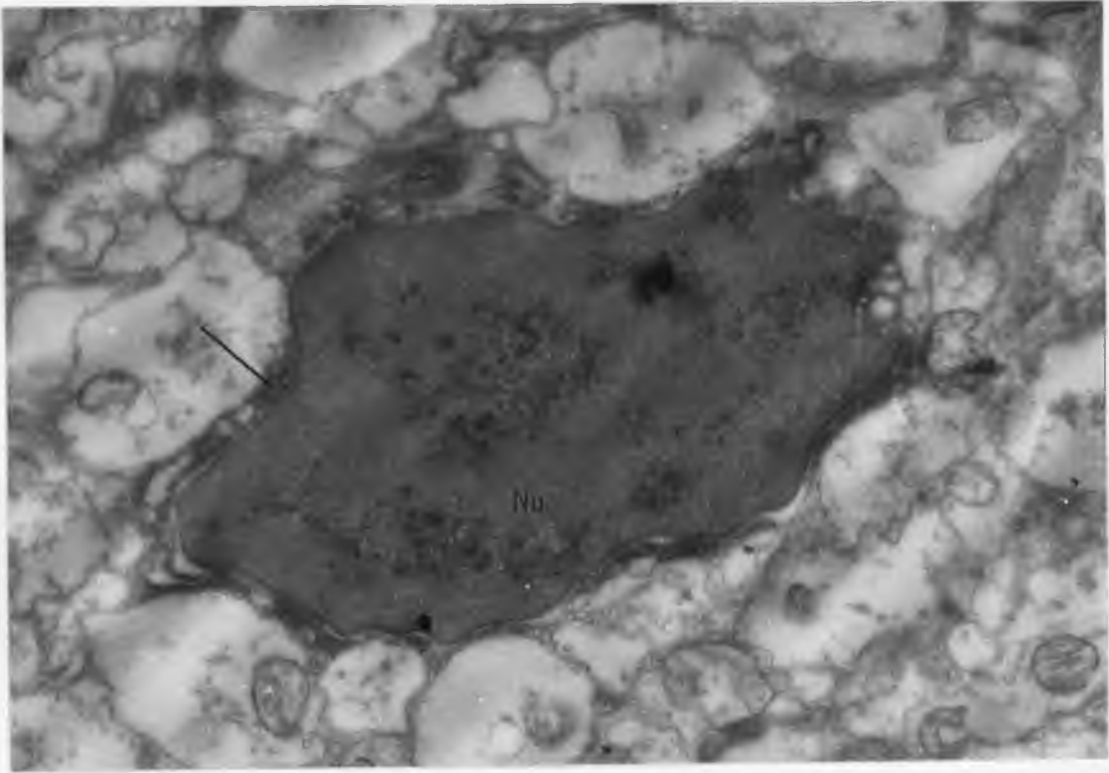


Figure 18. Neuron with abundant large mitochondria (M). A distinct endoplasmic reticulum (arrow) is present. Nucleus (Nu).  
X12,000.

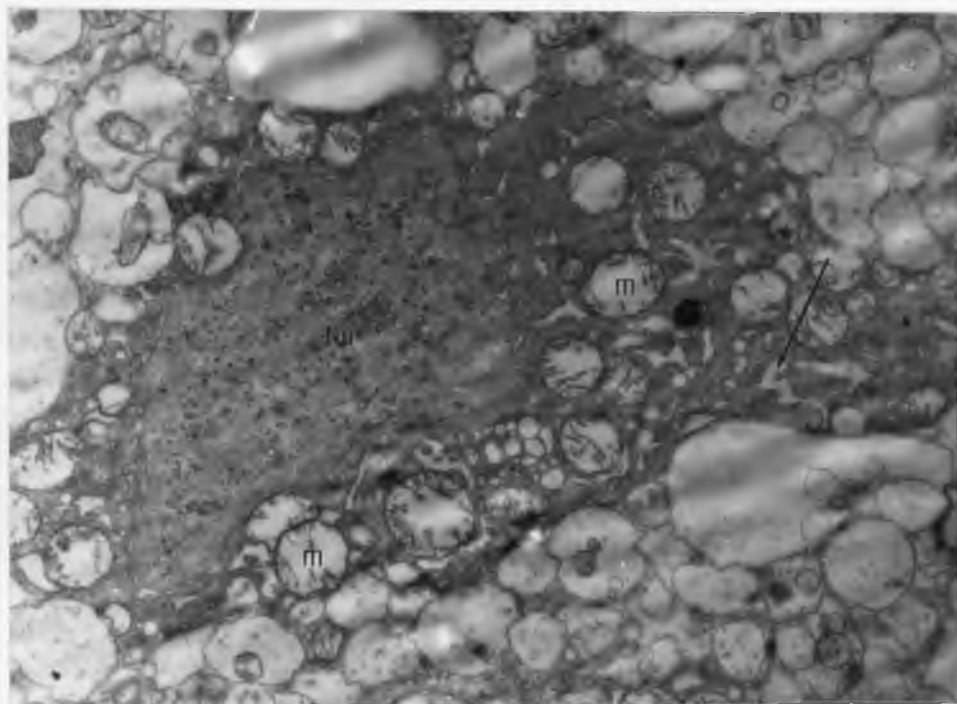


Figure 19. Neuron with distinct nucleus (Nu) containing a nucleolus (Nc). Mitochondria (M) in close proximity to nuclear membrane (arrow) gives the nucleus a lobated effect. X20,000.

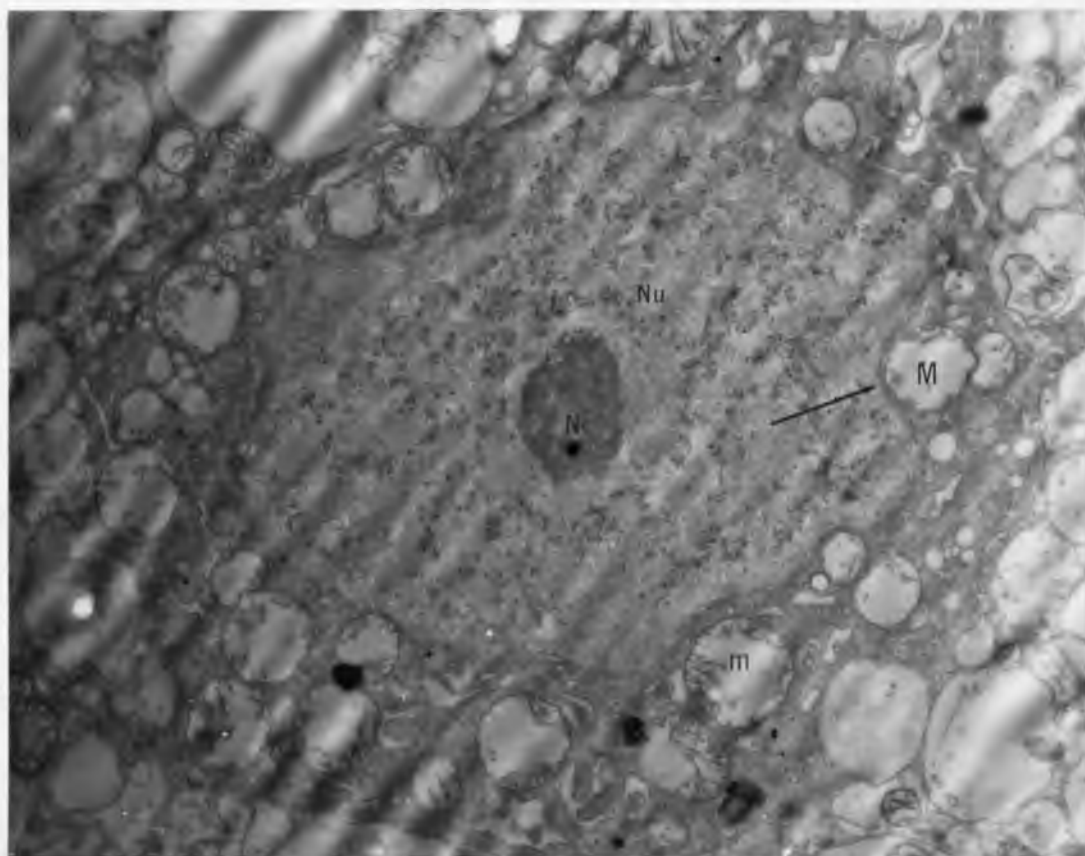


Figure 20. Methacrylate embedded section showing three cell types: 1) neuron; 2) oligodendroglia; 3) microglia. X5400.

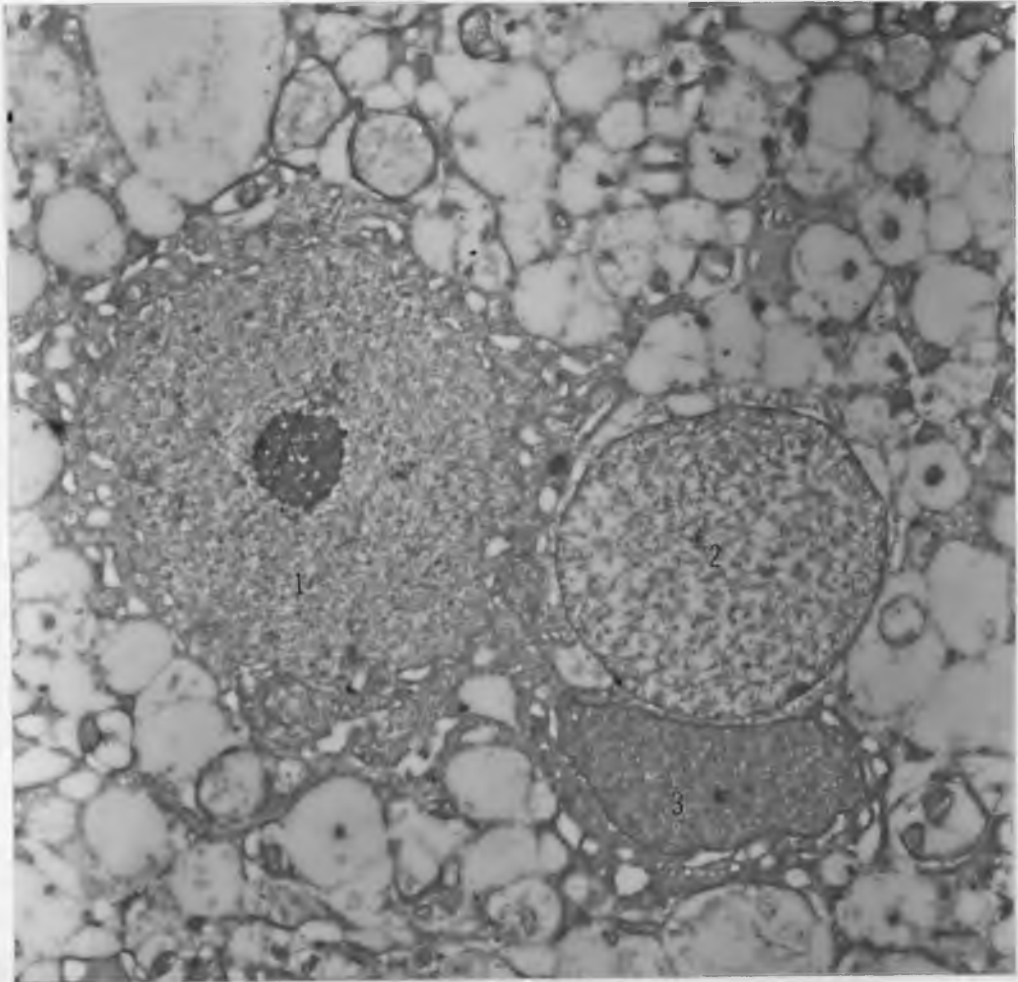


Figure 21. Methacrylate embedded astrocyte with nucleus (Nu) surrounded by clear cytoplasm (Cy). X5400.

