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Hematoencephalic barrier. Ultrastructure and histophysiology of the endothelium capillary of the neuronal nuclei of the mesencephalon

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Summary. The ultrastucture of the dorsal periaqueductal nucleus capillaries of the mesencephalon in the cat was studied under the electron microscope in relation to the *hematoencephalic barrier*, and its four structural levels: 1. Endothelium; 2. Basal membrane; 3, Pericytes; and 4. Glial prolongations. An analysis was performed of what occurs in these four components (in a non-experimental histophysiological state, and without manipulation by markers) in the thinnest capillaries of the centre of the mesencephalic neuronal nucleus. Special attention was placed on the *first diffusion barrier* formed by the endothelium capillary as the intimate guardian of the Central Nervous System (C.N.S) neurons. The C.N.S. capillaries are formed from the continuous endothelium, with no fenestrations, and hermetic joining complexes, without pinocytosis vesicles on both sides of the plasmatic membrane (adluminal and external), and surrounded by a continuous basal membrane. The non-fenestrated capillaries of the C.N.S. are less permeable than those with similar characteristics located in other areas. In the C.N.S. these capillaries form a *selective physiological barrier* which determines the size of the molecules that are permitted to cross the capillary wall. It is suggested that the *electron-dense globules* found in the endothelium cytoplasm may be molecules assimilated from the blood, which might represent the *first level or step* to the selective diffusion entrusted to the *hematoencephalic barrier*. It is also suggested that the *elongated electron-dense particles* found in the endothelium cytoplasm and basal membrane may be macromolecules which are normally retained for an active defensive function. They would represent the *first and second level or steps* of the retention performed by the *hematoencephalic barrier* which blocks their passage to the confined space of the perivascular capillary.

Key words: Hematoencephalic barrier, Endothelium capillary, Histophysiologic capillary

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Introduction

The thin structure of the capillaries in the cerebral cortex has been subjected to various studies during different states of development (Donahue and Pappas, 1961), including their *perivascular space* in relation to the perineural and subarachnoid space (Wollam and Millen, 1955). The blood capillaries of the vertebrates have been classified both morphologically and structurally, based on the participation of three constituent elements within them: *fenestrations of the endothelium basal membrane*, and *pericapillary or pericyte cells* (Bennet et al., 1959). According to these authors, the capillaries can be classified into *three types*: With regard to pores or fenestrations: Type 1 = capillaries without fenestrations or perforation. Type 2 = capillaries with intracellular fenestrations or perforations. Type 3 = capillaries with intercellular fenestrations or gaps. With regard to basal membrane: Type A = capillaries with complete continuous basal membrane. Type B = capillaries without complete continuous basal membrane. With regard to pericytes: Type α = capillaries without pericytes. Type ß = capillaries with pericytes surrounding them completely, between the parenchymatous cells and the endothelium capillary. The importance of the reaction of exogenous proteins in the Central Nervous System (C.N.S.) capillaries has been indicated (Cancilla et al., 1972). The *hematoencephalic barrier* was suggested based on experimental and clinical data, on observing that when injected into the blood, certain vital colourants and some drugs did not stain or activate/act upon the C.N.S. (Wislocki and Leduc, 1952 for vital staining with silver nitrate and trypan blue). In ultrastructural terms, the thinnest and deepest capillaries of the C.N.S. are covered by a basal lamina and several prolongations of the glial cells (Peters, 1961).

The degree of retention of permeability of the *hematoencephalic barrier* has been studied experimentally by several authors. With the intravenous injection of *peroxidase, lanthanum* or *ferritin* it has been

observed that in the capillaries of the cardiac and skeletal muscle (Karnovsky, 1967), these markers appear 5 minutes afterwards in the endocytic vesicles of the adluminal wall of the endothelium, 10 minutes afterwards in the intractyoplasmic vesicles and 30 minutes afterwards in the basal membrane, but they never appear crossing the hermetic joint complexes of the endothelium. This information was enlarged upon by Mackenzie et al. (1987) for lanthanum in the nerve. Similar results were observed for ferritin in the diaphragmatic muscle, crossing the endothelium to the adventitia (Bruns and Palade, 1968). With the intravenous injection of peroxidase, it was observed that in the *capillaries of the cerebral cortex* (Reese and Karnovsky, 1962), this marker does not cross the endothelium capillary, since very few micropinocytosis vesicles containing peroxidase are seen in the adluminal side of the endothelium, and none in the perivascular side. These authors indicate the existence of an authentic endothelial barrier in the cerebral cortex, although ions and glucose are able to pass through.

Selective experiments for locating the effectivity of the *hematoencephalic barrier*, conducted on rats, rabbits and cats using *fluorescent hydrocholrhydric proflavine* administered by three different methods (intravenous, subarachnoid and intraventricular) showed the following results: *Intravenous method*: It did not cross the endothelium capillary of the brain, in contrast to what occurs in other tissues. It penetrated the choroid plexus, but did not pass through to the cephalorrachidian fluid. In the brain the barrier was formed by the endothelium capillaries. *Subarachnoid method*: The C.N.S. nuclei were stained. The endothelium capillary was permeable towards the nervous tissue. The glia did not form a barrier. *Intraventricular method*: It penetrated freely towards the C.N.S. The choroid plexus was permeable. The ependymal epithelium did not establish a barrier (Rodríguez, 1955).

The *hematoencephalic barrier* has four structural levels: 1.- *Endothelium*; 2.- *Basal membrane*; 3.- *Pericytes*; 4.- *Glial prolongations*. An extensive revision of the *microvascular pericytes* was performed recently by Díaz-Flores et al. (1991) with regard to their morphological and functional characteristics.

In the analysis of what occurs in these four components or levels, in a normal histophysiological, non-experimental state without manipulation by markers, we observed the thinnest capillaries in the central area of a neuronal nucleus of the mesencephalon under the electron microscope, placing special emphasis on the *first diffusion barrier*, formed by the endothelium capillary, which is the closest guardian of the C.N.S. neurons.

Materials and methods

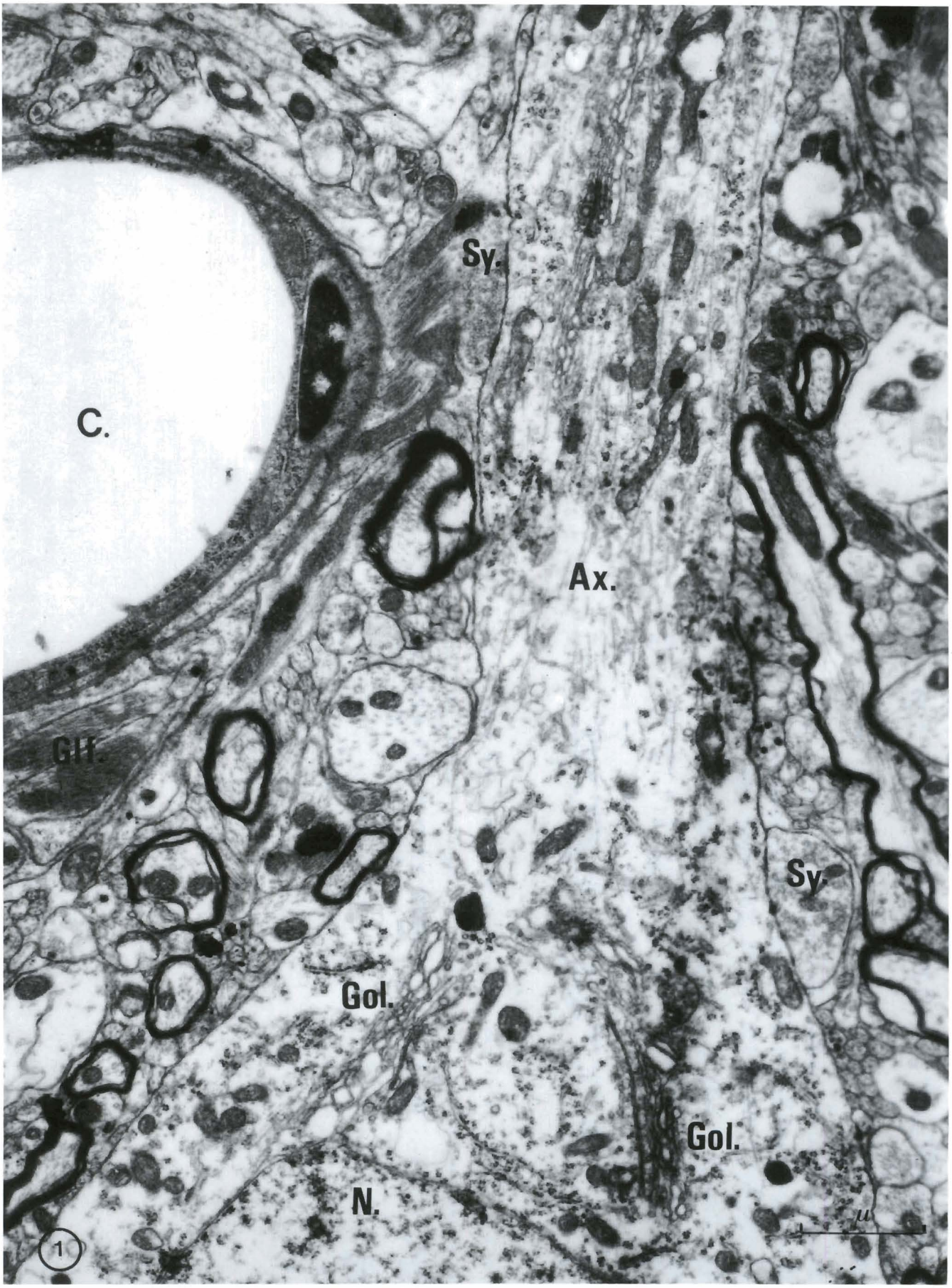
Household cats were subjected to anaesthesia through the peritoneum. They were placed on artificial mechanical respiration using a *respirator* connected to a tracheal tube. A vascular perfusion was then performed with 2.5% glutaraldehyde and a sodium cacodylate buffer (45 mM), perfusing before and afterwards with Ringer washing solution. After fixing, depending on the degree of stiffness of the animal, the cranial cavity was opened, the encephalon removed *in toto* and submerged completely in a buffer-sucrose bath. It was dissected until the mesencephalon was isolated. This was cut transversely into sections of about 3 millimetres thick. Pieces of the nervous tissue surrounding the Silvian acqueduct ependyma were obtained, and then postfixed in 3% osmic acidic for three hours. They were then washed with 2% uranyl acetate, and added to araldite. The dorsal periaqueductal nucleus of the mesencephalon was located topograpically by the observation of semithin sections stained with 1% toluidine blue. After locating the neuronal nucleus, this was again cut into ultrathin sections, which were observed under the transmission electron microscope, stained with lead citrate as per the Reynolds method (1963).

Results

We have studied the ultrastructure of the C.N.S. capillaries at the level of the parenchyma of the dorsal periaqueductal nucleus of the mesencephalon, observing the ultrastructural details of: the *endothelium capillary*, the *basal membrane*, the *pericytes* and the juxta-capillary *glial prolongations*.

The capillaries of the C.N.S. could be seen, perfectly rounded, in the transverse section, corresponding to the pressure of the vascular perfusion pression performed, which guarantees positive fixing (Fig. 1). The capillaries were very small (4.63 µm. in external diameter and 3.58 µm. internal diameter), without fenestrations (they were continuous) and only one endothelium cell was observed in the transverse section, with a chromatin-dense elongated nucleus under its karyotheca. Its cytoplasm was a narrow lamina (0.122 µm. in thickness) with an electron-dense appearance containing some crested mitochondria, a small portion of the rough endoplasmic reticulum, and *osmiophilic electron-dense globules* of 15.2 to 16.8 nm, in diameter, which were free and disseminated or grouped throughout the cytoplasm of the endothelium cells. (Figs. 2, 3). The endothelium cytoplasm also contained several *extremely electron-dense elongated particles*, of variable size, between 14 and 35 nm, in length and 5.6 to 7.7 nm. in thickness (Figs 4, 5), distributed irregularly throughout the whole thickness of the cytoplasm flap. On the internal (adluminal) surface of the endothelium cell, the plasmatic membrane showed several short microvilli (Fig. 3). The endothelium cells were joined to each other by *hermetic joints* where the external lamina of the plasmatic membrane unit were joined together.

The *basal membrane* surrounding the endothelium formed a continuous whole with a regular thickness of 0.022 µm. In its amorphous content we found no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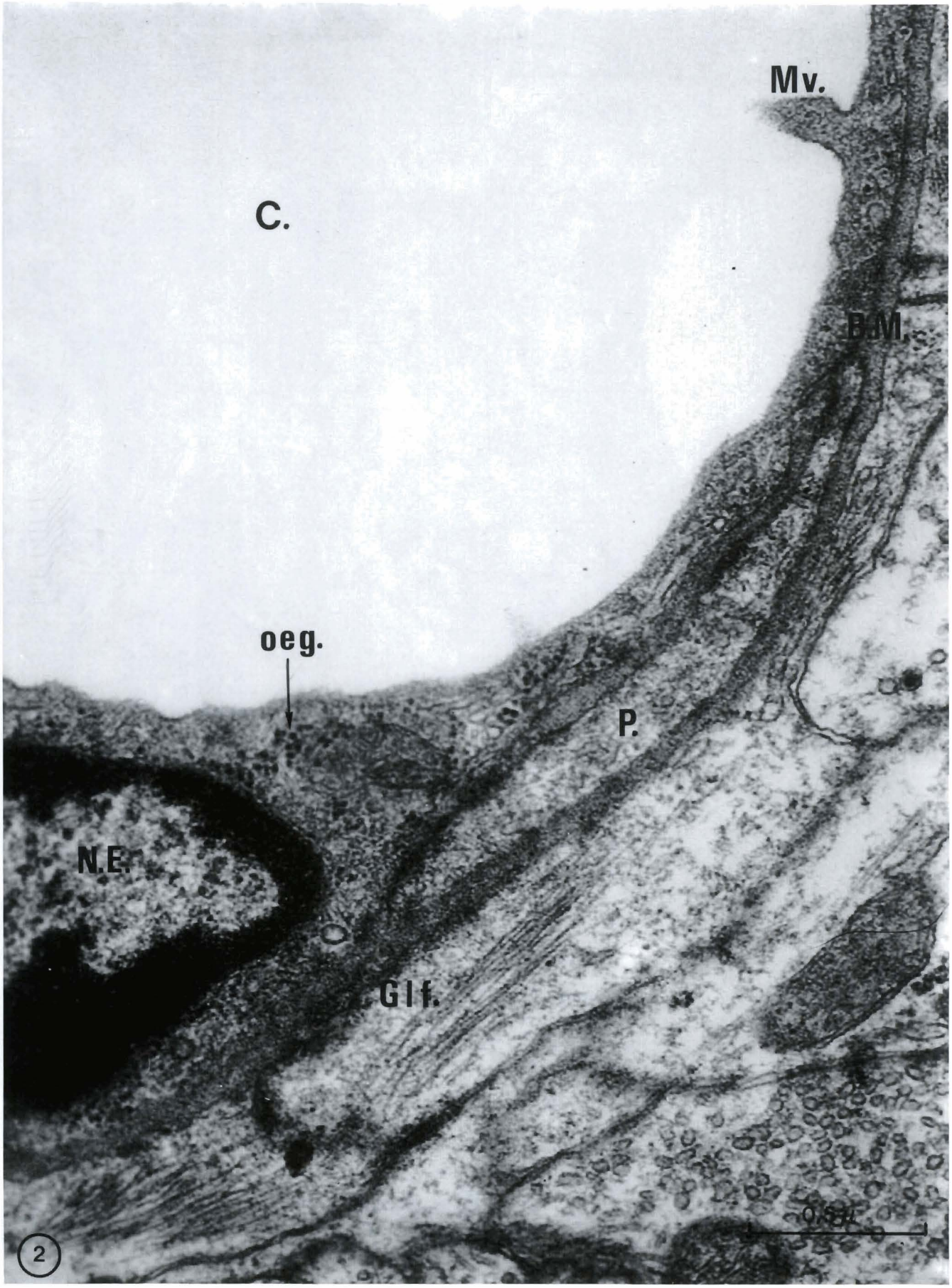
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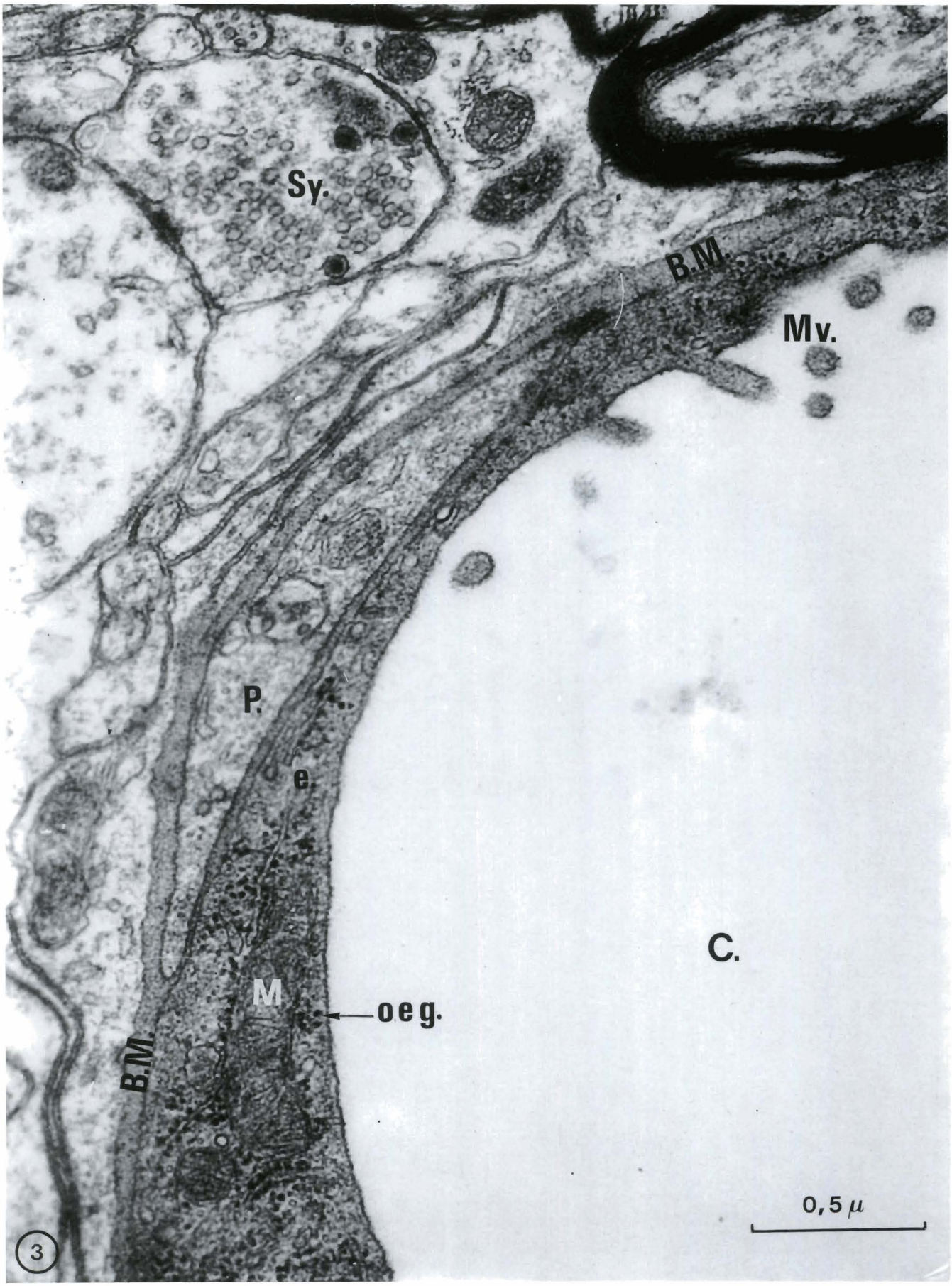
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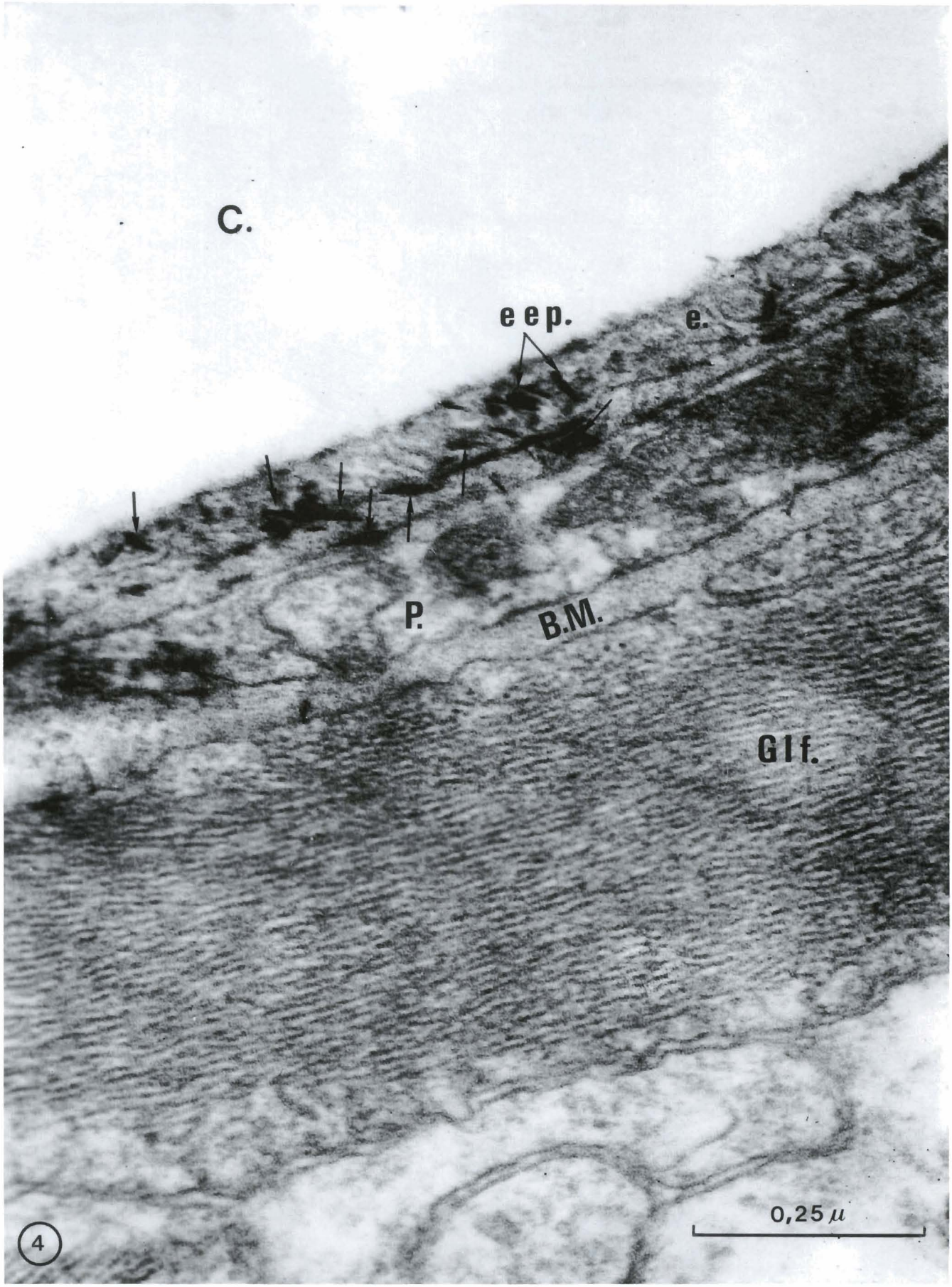
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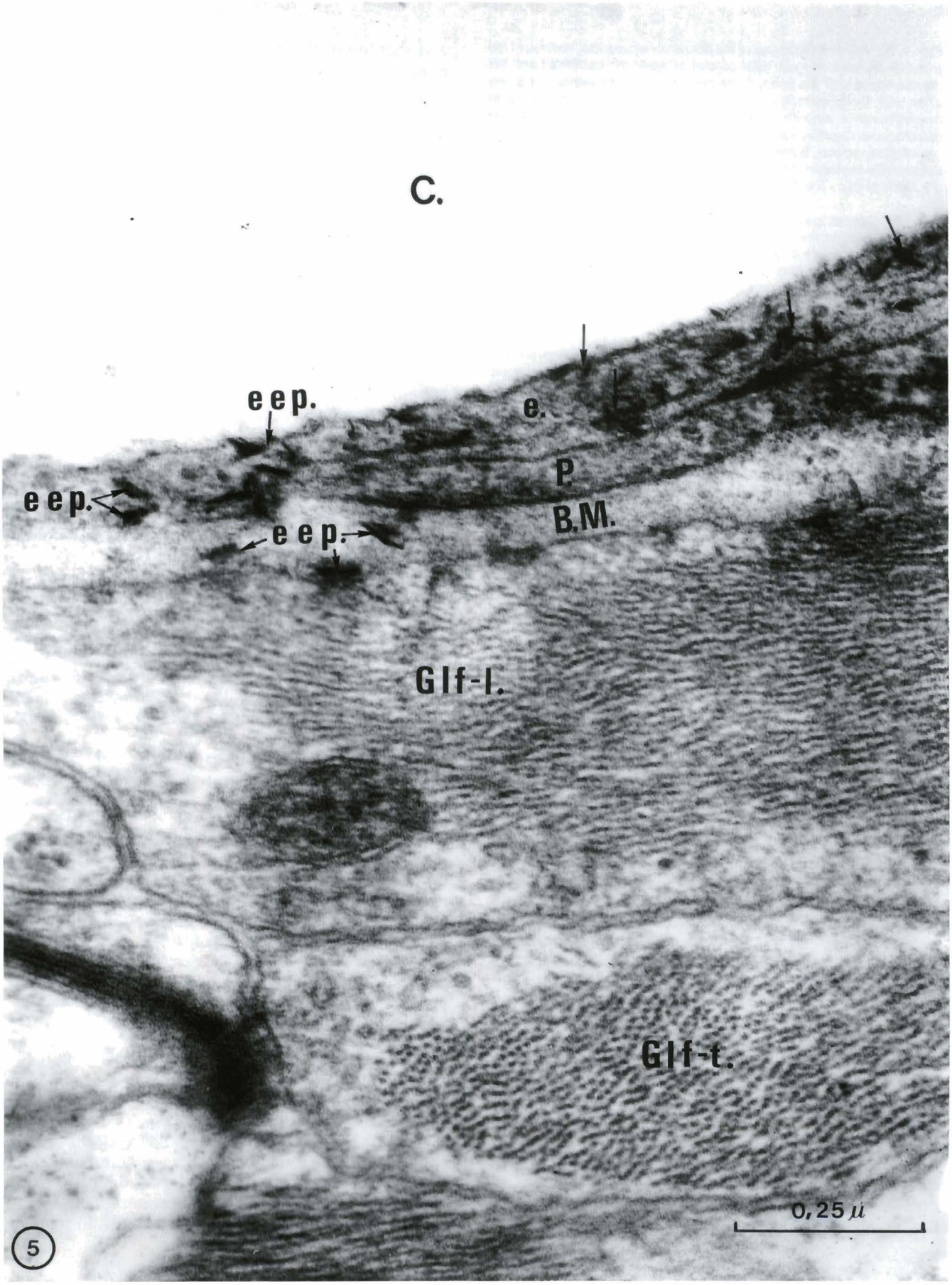
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Fig. 1. Neuron and capillaries of the dorsal periaqueductal nucleus of the mesencephalon in the cat. Glial contact between the capillaries and the commencement of the axon. Cone showing the commencement of the axon with Golgi complex. Commencement of the axon (Ax), with neurofilaments, smooth endoplasmic reticulum, polyribosomes and mitochondria. Capillary wall (c), with continuous endothelium and lack of pericapillary space. Synaptic bouton (Sy) at the point of glial contact between the capillaries and axon. Golgi (Gol). Gliofilaments (Glf). Pericyte (P). Nucleus (N). x 6,800

Fig. 2. Dorsal periaqueductal nucleus capillaries (c) of the mesencephalon in the cat. Without fenestrations. Without pericapillary space. Formed by one single endothelium cell. Cellular nucleus (N.E) with dense chromatin adhering to the karioteca. Microvilli (Mv). Gliofilaments (Glf). Endothelium closely surrounded by thin basal membrane and glial cell prolongations. Cytoplasm of endothelium with osmiophilic electron-dense globules (o.e.g.). Basal membrane (B.M.). Pericyte (P). x 21,800

Fig. 3. Thin basal membrane (BM) wrapped around endothelium and thin pericytic flap (P). Internal surface of endothelium (e) with several short, isolated microvilli (Mv). Cytoplasm of endothelium with osmiophilic electron-dense globules (o.e.g.) and mitochondria (M). At the side of the capillaries (c) is an axo-dendritic synapse (Sy). x 21,800

Fig. 4. Capillary wall (c) of the dorsal periaqueductal nucleus of the mesencephalon in the cat. Cytoplasm of the endothelium (e) with extremeley electron-dense elongated particles (e.e.p.) which can also be seen in the surrounding basal membrane (BM). Thick bunch of gliofilaments (GLF) closely surrounding the capillaries. Pericyte (P). x 50,000

Fig. 5. Capillary wall (c) of the dorsal periaqueductal nucleus of the mesencephalon in the cat. Endothelium (e) and basal membrane (B.M.) with extremely electron-dense elongated particles (e.e.p.). Pericyte (P). Two thick bunches of gliofilaments surrounding the endothelium capilary transversely (Glf - t) and longitudinally (Glf - l). x 50,000

collagen fibres, but we did observe several *extremely electron-dense elongated particles* in some capillaries, disseminated throughout the thickness of the basal membrane, which were identical (with the same morphology and dimensions), (Fig. 5) to those described above in the cytoplasm of the endothelium cells.

In the thickness of the basal membrane some short, small pieces of cytoplasmic flaps from some pericytes could be seen. These flaps only occupied a small part of its circumference and were located throughout the thickness of the basal membrane (Figs. 2 - 5).

Outside the basal membrane, in close contact with the *juxta-endothelium area*, we observed several *gliofilament bunches* closely surrounding the capillaries, and adopting a transverse (Fig. 5) or longitudinal (Figs. 2, 4 and 5) position. Very close to the capillary wall we observed a few axo-dendritic synapses, some with collinergic synaptic vesicles and others with adrenergical ones. (Fig. 3).

Discussion

The *hematoencephalic barrier* was conceptually established based on clinical and experimental data, on observing that the introduction of *vital colourants* into the bloodstream did not stain the C.N.S. parenchyma, and that some therapeutical substances administered intravenously did not reach the grey matter in the brain, despite the fact that these areas were well-vascularized. Thus, a barrier was established between the cerebral parenchyma itself and the blood. This capacity of isolation of the C.N.S., or impermeability to macromolecules carried in the blood, seems to be a gradual process in the course of pre and post natal development, since it has been observed that *certain colourants*, when injected into the blood of young animals, produce slight accumulations of the substance in some cells of the cerebral trunk (Wislocki and Leduc, 1952; Rodríguez, 1955).

The capillaries that penetrate into the neuronal nuclei of the C.N.S. become thinner and thinner. They are only constituted by the two first structural levels: 1. *The endothelium*; 2. *The basal membrane* of the four possible structural levels that separate it from the parenchyma of the C.N.S.

At first it was thought that the prolongations of the protoplasmic glias, both fibrous and restrictive, intervened in the *hematoencephalic barrier.* It was later found that this was not necessary, upon observing that the perivascular space was a continuation of the subarachnoid space surrounding the encephalon and spinal medulla, (Wollam and Millen, 1955).

Contact between vessels and the C.N.S. as they progress deeper into the neuronal nuclei (Fig. 1) becomes closer and closer, and it can be seen that the encephalic vessels gradually lose their muscular sheath, and that their perivascular space is reduced. The deepest capillaries are formed by an endothelium lamina covered by the continous basal membrane surrounded by some prolongations of the glial cells, (Peters, 1961) (Fig. 1).

The thinnest juxta-neuronal capillaries (3.58 μm. in diameter) only have an endothelium and basal membrane. They belong to the ones classified by Bennett et al. (1958) as type 1A α.

The cytoplasm of the thin endothelium flap is electron-dense, and contains several crested mitochondria, a small portion of rough endoplasmic reticulum and *osmiophilic electron-dense globules* of 15.2 to 16.8 μm, in diameter, freely disseminated or grouped (Figs. 2, 3). It also contains *extremely electron-dense elongated particles* of variable size, between 14 and 35 nm. in length and from 5.6 to 7.7 nm. in thickness (Figs. 4, 5). It does not contain cytoplasm, filaments or microtubules. The adluminal plasmatic membrane has few short microvilli and no micropinocytosic vesicles (Figs. 3, 5). The endothelium cells are joined by *hermetic joint complexes* and the external lamina are joined to the plasmatic membrane unit. There are no fenestrations or pores.

The nature of the *osmiophilic electron-dense globules* contained in the cytoplasm of the endothelium cell is unknown, and apparently, due to their morphology and dimensions, they are not ribosomes, nor glucogen, with the particles of which we have established, in principle,

a differential diagnosis (Fig. 2,3).

The basal membrane is continuous in a circular form, with a regular thickness of 0.022 μm., with an amorphous content, inside which there are several extremely electron-dense elongated particles with identical morphology and dimensions to those described in the endothelium cytoplasm. We do not know the nature of these *electron-dense elongated particles*, which are observed in the cytoplasm of the endothelium cells, and to a lesser extent, in the adjacent basal membrane (Figs. 4, 5).

Occasionally there are thin flaps of pericytes wrapped in the basal membrane itself (Figs. 2, 3).

The extracellular perivascular space is practically non-existent, since it is so narrow or small, that it may measure between 20 and 30 nm. In some places, between the capillaries and the neurons, some incomplete laminas are interposed, like astrocytic glial processing plates that surround the capillaries peripherically (Fig. 1).

In conclusion; the thinnest capillaries of the C.N.S. are formed the continous endothelium, without fenestrations, with hermetic joint complexes, without pinocytic vesicles at both sides of the plasmatic membrane (adluminally and externally) and with a continous surrounding basal membrane.

These ultrastructural characteristics mean that the non-fenestrated capillaries of the C.N.S. are less permeable than those with similar characteristics in other areas. In the C.N.S. these capillaries establish a *selective physiological barrier* which determines the size of the molecules that are allowed to pass through the capillary wall.

A possible function is suggested for the C.N.S. capillaries studied, as a normal activity of the *hemato neuronal* C.N.S., since the capillaries analyzed in the mesencephalon nucleus have not been treated with any substance, in order not to introduce any distortion factor into the interpretation of images.

It is suggested that the *osmiophilic electron-dense globules* may be macromolecules assimilated from the blood, during a normal- functional process of the endothelium cell, which could possibly be the *first level, or step*, to the selective diffusion task entrusted to the *hemato encephalic barrier.*

It is suggested that the *extremely electron-dense elongated particles* may be macromolecules trapped by the endothelium and its basal membrane, in an actively defensive function, possibly representing the *first and second layer, or step*, of the retention carried out by the *hematoencephalic barrier*, which prevents them from passing through to the small perivascular capillary space.

It is suggested that the C.N.S. capillaries form an authentic *hematoencephalic barrier* in the *neuronal nuclei* through the existence of the following in the thin capillaries: integrity of the endothelium plasmatic membrane; hermetic joint complexes; and molecule-retaining mechanisms, both at cytoplasmic level and in the basal membrane. For this reason we were unable to see micropinocytic vesicles on the adluminal or basal membrane sides, but we did observe molecules retained in the cytoplasm of the endothelium and in the basal membrane.

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