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Ultrastructure of the *area postrema* of the monkey, *Macaca fascicularis*

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Summary. The *area postrema* of the monkey, *Macaca fascicularis,* were a pair of oval organs at the caudal end of the floor of fourth ventricle. Their ependymal lining was covered by well-developed microvilli with occasional overlying supraependymal cells. Two types of lining cells were present: pyramidad- and flattened cells. The pyramidal cell showed a long extending basal process resting on the underlying blood vessels.

In transmission electron microscopy, the organ showed numerous fenestrated sinusoids characterized by a distinct perivascular space containing mast cells, macrophages and collagen fibrils. The parenchyma of the organ was composed of neurons and glial elements. Only one type of neuron ranging from 9.5 to 15μm could be distinguished. The neurons contained an indented nucleus surrounded by organelle rich cytoplasm. The soma of the neuron was enclosed by glial element resembling astrocyte. The glial processes terminated on the blood vessel where they were "tunnelled" by a variable number of nerve fibres some of which gained a direct access to the external basal lamina of the perivascular space.

Synapses in the neuropil predominantly of the axodendritic variety were observed. Axon terminals containing round agranular vesicles were seen to make synaptic contacts with the neuronal soma. No structural changes were observed in the area postrema following bilateral cervical vagotomy. However, degenerating axon terminals were observed in the subpostremal zone 7, 14 and 21 days after vagotomy suggesting a direct afferent projection into this region.

Key words: Monkey-*Area postrema*-Ultrastructure-Vagotomy

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Introduction

The *area postrema,* an area lacking a typical blood-brain-barrier, has been considered as one of the circumventricular organs located at the floor of fourth ventricle (Klara and Brizzee, 1977). It has been shown to be a chemoreceptor zone involved in vomiting (Borison and Wang, 1949; Brizzee and Borison, 1952; Borison, 1977), osmoreception (Clemente et al., 1957) and cardiovascular regulation (Ferrario et al., 1979). Most of the anatomical studies on *area postrema* have been at the light microscopical level (Wislocki and Putman, 1924; Cammermeyer, 1947; Borison and Brizzee, 1951; Brizzee and Neal, 1954) and those at the ultrastructural level were mainly on the non-primate species (Shimizu and Tshii, 1964; Spacek and Parizek, 1969; Rohscheneider et al., 1972; Dempsey, 1973; Torack et al., 1973; Klara and Brizzee, 1977; Manni et al., 1982). The only available information on the ultrastructure of *area postrema* of primate was on the squirrel monkey, *Saimiri sciureus* (Klara and Brizzee, 1975).

The present study aimed, primarily, to study the ultrastructure of the *area postrema* of the monkey, *Macaca fascicularis* which, as far as is known, has not been fully described. Furthermore, this study also considered the possibility of a primary afferent projection of the vagus nerve to the area postrema which has been demonstrated under light microscope by using autoradiographic examination (Beckstead and Norgren, 1979) and horseradish peroxidase labelling method (Kalia and Mesulam, 1980).

Materials and methods

A total of 4 normal and 7 experimental monkeys *(Macaca fascicularis)* of either sex weighing 2.5 - 4.2 kg were used in this study. One of the normal monkeys was used for scanning electron microscopy. The normal monkeys

were anaesthetized by an intraperitoneal injection of sodium pentobarbital (0.5 ml per kg body weight). After tracheostomy and thoracotomy, the animals were perfused with 200 ml Ringer's solution followed by a mixed aldehyde solution in 0.1M cacodylate buffer. The aldehyde solution was infused in two steps: first with a dilute (500 ml 1% paraformaldehyde and 1.25% glutaraldehyde) and then a concentrated fixative (1000 ml 4% paraformaldehyde and 5% glutaraldehyde). After the perfusion which lasted for about 1 hour, the brain was removed. The roof of the fourth ventricle was carefully dissected away and the *area postrema* exposed. For scanning electron microscopy, the required area was trimmed into the desired size. The tissue blocks were postfixed in 2% osmium tetroxide in 0.1M cacodylate buffer at pH 7.4. They were dehydrated in a graded series of ethanol and critical point dried in liquid carbon dioxide. They were then mounted on a specimen stub, sputter coated with gold for 3 minutes and viewed in a Philips 505 scanning electron microscope operated at 20 kV.

For transmission electron microscopy, the brainstem including the *area postrema* was removed from the perfused brain. 100µm Vibratome sections were cut from the blocks and postfixed in 2% osmium tetroxide. The sections were dehydrated in alcohol and embedded in araldite mixture. Ultrathin sections were doubly stained in uranyl acetate and lead citrate and examined in a 400T electron microscope.

For the experimental monkeys, bilateral vagotomy at the level of the omohyoid muscle was performed under sodium pentobarbital anaesthesia. A segment from 5 to 10 mm of the nerve was removed. The animals were allowed to survive for 1, 3, 5, 7, 10, 21 and 28 days. They were perfused according to the procedures described above and the *area postrema* was processed for transmission electron microscopy.

Results

1. General

Under the scanning electron microscope, the *area postrema* appeared as paired oval swellings at the floor of the caudal end of the fourth ventricle (Fig. 1). The organ measured 0.2 x 1 mm. The boundary between the *area postrema* and the rest of the ependymal lining was demarcated by a groove containing an accumuation of pleomorphic supraependymal cells (Fig. 2) which had all the features of the phagocytic supraependymal cells described in other parts of the brain (Walsh et al., 1978; Ling et al., 1986). Sometimes, the supraependymal cells extended over the ependymal lining of the *area postrema* (Figs. 2, 3). The ependyma of the fourth ventricle was lined by an admixture of microvilli and tufts of long cilia, while the lining of the *area postrema* was generally devoid of cilia (Fig. 2). In the latter, the lining was composed of long and closely packed microvilli lying flat on the luminal surface of the ependymal cells (Fig. 3).

In 1µm semithin sections stained with methylene blue, the *area postrema* is a highly vascularized organ composed of neuronal and glial elements (Fig. 4) The ramified blood vessels were characterized by a wide perivascular space. In transmission electron microscopy, the blood vessels were typical sinusoids of fenestrated type (Fig. 5). The cytoplasm of the endothelial cells contained numerous pinocytic vesicles especially on their outer walls resting on the inner basal lamina (Fig. 5). A variable number of membrane bound electron dense granules were present (Fig. 5). Lying external to the inner basal lamina was the perivascular space containing macrophages, a variable number of mast cells, fibroblasts and collagen fibrils (Fig. 5). An external basal lamina delimited the perivascular space which was ensheathed by glial processes.

The lining epithelium of *area postrema* consisted of flattened and pyramidal shaped ependymal cells (Figs. 6, 7). Both were devoid of cilia. The pyramidal ependymal cells were endowed with more microvilli than flattened ones. In some sections, the flattened ependymal cells which were attenuated rested directly on the somata of neurons (Fig. 6). The pyramidal cells often showed a long basal process penetrating the deeeper zone of *area postrema* where it often terminated as an end-foot on the external basal lamina of the perivascular space (Fig. 7). The basal process gave rise to a variable number of secondary processes which contained bundles of filaments. These secondary processes ramified between the nerve fibres separating them into fasciculi in the neuropil (Fig. 7).

2. Neurons and glial elements

The neurons were round and pyriform often forming a cluster of two to five cells adjacent to the blood vessel (Fig. 8). The soma is almost completely enveloped by the glial processes which appeared to surround more than one cell and to cover at the same time the perivascular space (Fig. 8). The size of the neurons ranged from 9.5 to 15µm. The nucleus was deeply infolded (Fig. 8). The cytoplasm showed cisternae of rough endoplasmic reticulum which may be stacked. A variable number of lipofuscin granules were located on the indented side of the nucleus (Fig. 8). A well-developed Golgi complex was present.

On the cell body, axo-somatic synapses were present. The axon terminal contained round agranular vesicles with occasional dense cored vesicles measuring the size of about 70 nm (Fig. 9). Similar axon terminals also made synaptic contacts with the main dendrites of the cell (Fig. 10).

In the neuropil, there was a wide occurrence of axodendritic synapses. The commonest form of axon terminals were those containing round evenly dispersed agranular vesicles with some larger dense-cored vesicles (Fig. 11). They made synaptic contacts with dendrites of different sizes. The second common axon terminals were smaller. Their agranular synaptic vesicles appeared to form a cluster (Fig. 12). Furthermore they contained more large dense cored vesicles. In a few axon terminals, profiles resembling smooth endoplasmic reticulum were present (Fig. 13). The round agranular vesicles which were sparse formed a small clump adjacent to the presynaptic membrane thickening (Fig. 13).

In the neuropil among the nerve fibres were present randomly distributed myelinated axons. They were particularly common in the subpostremal region.

The commonest glial cells in the *area postrema* were the astrocyte-like cells with long extending processes (Fig. 8). They surrounded the somata of the neurons especially those located adjacent to the blood vessels (Fig. 8). The terminal processes of the glial cells formed a mesh-like structure tunnelled by bundles of nerve fibres (Fig. 14). Some of the nerve fibres had a direct contact with the external basal lamina of the perivascular space (Fig. 15).

Other glial cells present in the *area postema* were the microglial cells. Oligodendrocytes were extremely rare.

A striking change of the *area postrema* following vagotomy was the presence of numerous mast cells (Fig. 16) which invaded the parenchyma, reaching as far as the subependymal zone. Some of them were intercalated between the ependymal cells. Often, a cluster of 4 - 7 mast cells resided in a widened interstitial space (Fig. 16). Occasional plasma cells were present in the neuropil.

The search for degenerating axon terminals in the neuropil of *area postrema* of the vagotomized animals has not been successful. Darkened degenerating axon terminals, however, were commonly seen in the subpostremal region in the 7, 14 and 21 day survival monkeys. The terminals showed closely packed synaptic vesicles some of which were swollen (Fig. 17). The darkened axon terminal was presynaptic to one or more dentrites (Fig. 18). In some terminals, dense myelinated bodies were present (Fig. 19).

Fig. 1. Scanning electron micrograph showing the paired oval *area postrema* (arrows) at the caudal end of the floor of fourth ventricle. X 50

Fig. 2. Scanning electron micrograph showing the borderline zone between the covering epithelium of *area postrema* and the rest of the ependyma of fourth ventricle covered by well-developed cilia (C). Supraependymal cells (SC) are accumulated at the junctional zone but sometimes distributed over the ependyma of the *area postrema.* X 146

Fig. 3. Scanning electron micrograph showing the parallel arrays of microvilli lying flat on the luminal surface of the ependymal cells of area postrema. SC, supraependymala cell. X 5,700

Fig. 4. A portion of *area postrema* with its covering ependymal lining. The parenchyma shows numerous profiles of blood capillaries (C) which are characterized by a wide perivascular space. The rest of the tissue is filled by pale staining neurons (N) and smaller and denser glial elements. 1μm Araldite section stained in methylene blue. X 740

Fig. 5. Perivascular space (PVS) containing a mast cell (MC) and a macrophage (MP). The external basal lamina (EBL) forms a continuous layer separating the perivascular space from the glial elements. The inner basal lamina (IBL) covers the endothelium which is fenestrated. The endothelial cell is characterized by numerous pinocytotic vesicles and the presence of a variable number of electron dense granules (Circle, Inset). X 11,500; Inset, X 14,000

Fig. 6. A flattened ependymal cell with its slender microvilli projecting into the lumen of the fourth ventricle (V). Immediately beneath to it is a pale neuron. X 8,050

Fig. 7. A pyriform ependymal cell which appears to have more microvilli than the flattened cell depicted in Fig. 6. The cell presents a long tapering basal process abutting on the external basal lamina (EBL) of the perivascular space. Extending from the main basal processes are secondary processes which divide the nerve fibres into small fasciculi. X 4,900

Fig. 8. Two neurons of different sizes lying next to a blood vessel. The nuclei of the cells are typically infolded. The Golgi apparatus (G) lies preferably at the indentation of the nucleus. The cytoplasm forms a thin zone around the nucleus. The somata of the neurons are espoused by the cytoplasmic processes of the astrocyte-like glial cell (AS). Similar glial processes rest on the surface of the perivascular space (PVS) separated by the external basal lamina (EBL). X 9,000

Fig. 9. Two axon terminals (A1, A2) forming axosomatic contacts with the soma of the neurons. The terminals are filled with round agranular vesicles with occasional larger dense-cored vesicles (arrows). X 22,750

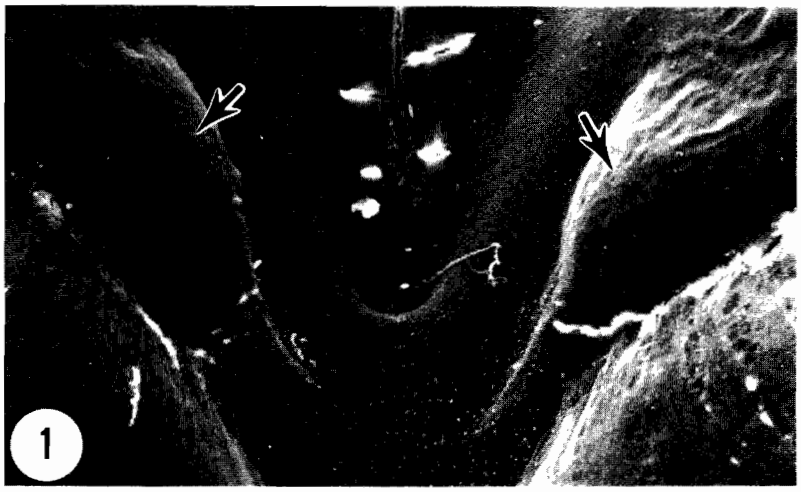
Fig. 10. An axon terminal (A) containing round agranular vesicles is making synaptic contact with a large dendrite (D). X 22, 750

Figs. 11-13. Three different types of axodendritic synapses in the neuropil of *area postrema.* In the first type (Fig. 11), the axon terminal (A) is filled with numerous evenly dispersed round agranular vesicles. Interspersed among these are some dense-cored vesicles which are slightly bigger in size. In the second type of synapse (Fig. 12), the round agranular vesicles are clustered in the centre of the axon terminal, while the dense-cored vesicles are located at the periphery. In the third type (Fig. 13), the axon terminal is filled with profiles of smooth endoplasmic reticulum. The round agranular sypnaptic vesicles form a small cluster subjacent to the presynaptic membrane thickening. Fig. 11, X 22,750; Fig. 12, X 29,750; Fig. 13, X 29,750

Figs. 14, 15. Ramified astrocytic glial processes (gp) abut on the external basal lamina (EBL) of the perivascular space. Nerve fibres (nf) in bundles penetrate between the glial processes to reach near to (Fig. 14) or in contact with (Fig,. 15) the external basal lamina. Fig. 14, X 14,000; Fig. 15, X 17,500

Fig. 16. A cluster of three mast cells located in the wide interstitial space of neuropil in *area postrema.* 7 days after bilateral vagotomy. X 6,300

Figs. 17-19. Degenerating axon terminals (A) in the subpsotremal zone 21 days after bilateral vagotomy. The density of the axon terminals is greatly enhanced. Some of the clumped synaptic vesicles are swollen (Fig. 17). The terminal is presynaptic to one (Fig. 17) or more dendrites (Fig. 18). In some terminals dense lamellated bodies (1b) are present (Fig. 19). X 29,750



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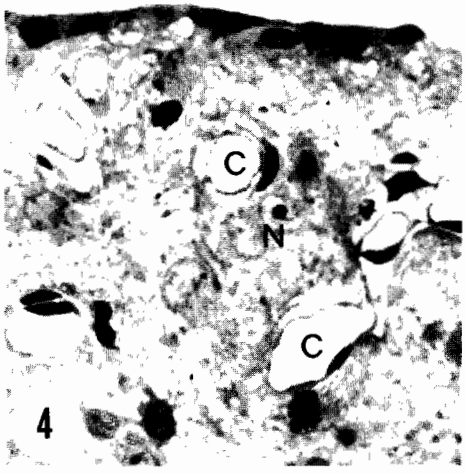

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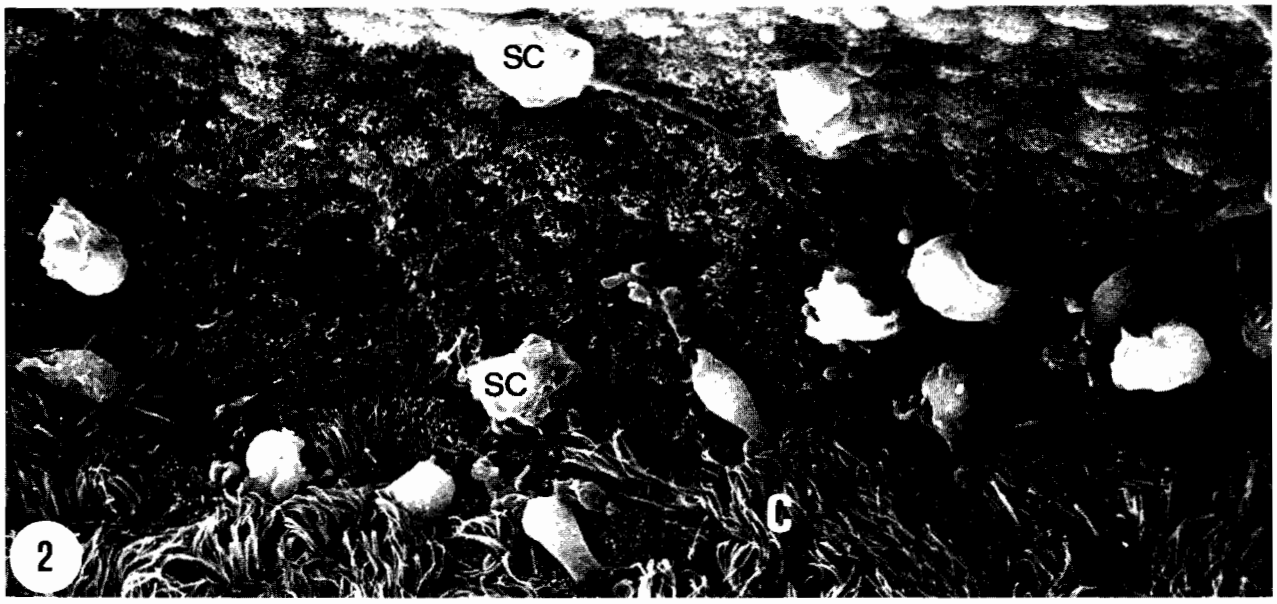

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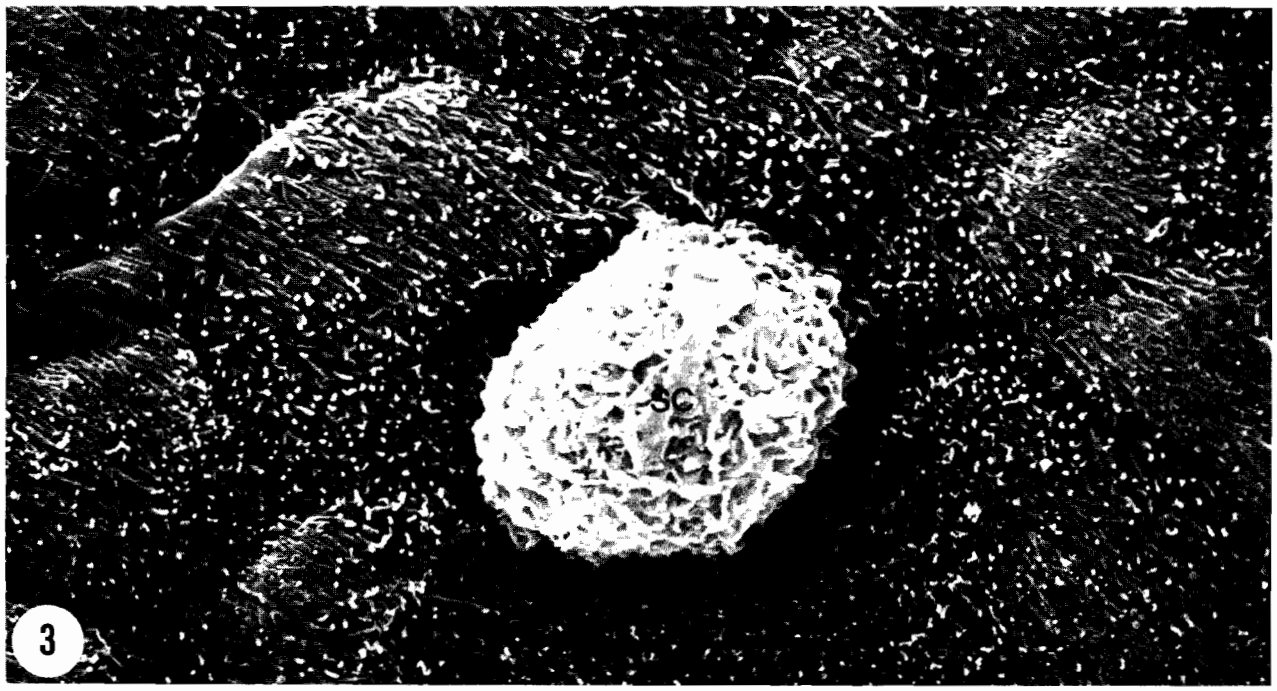

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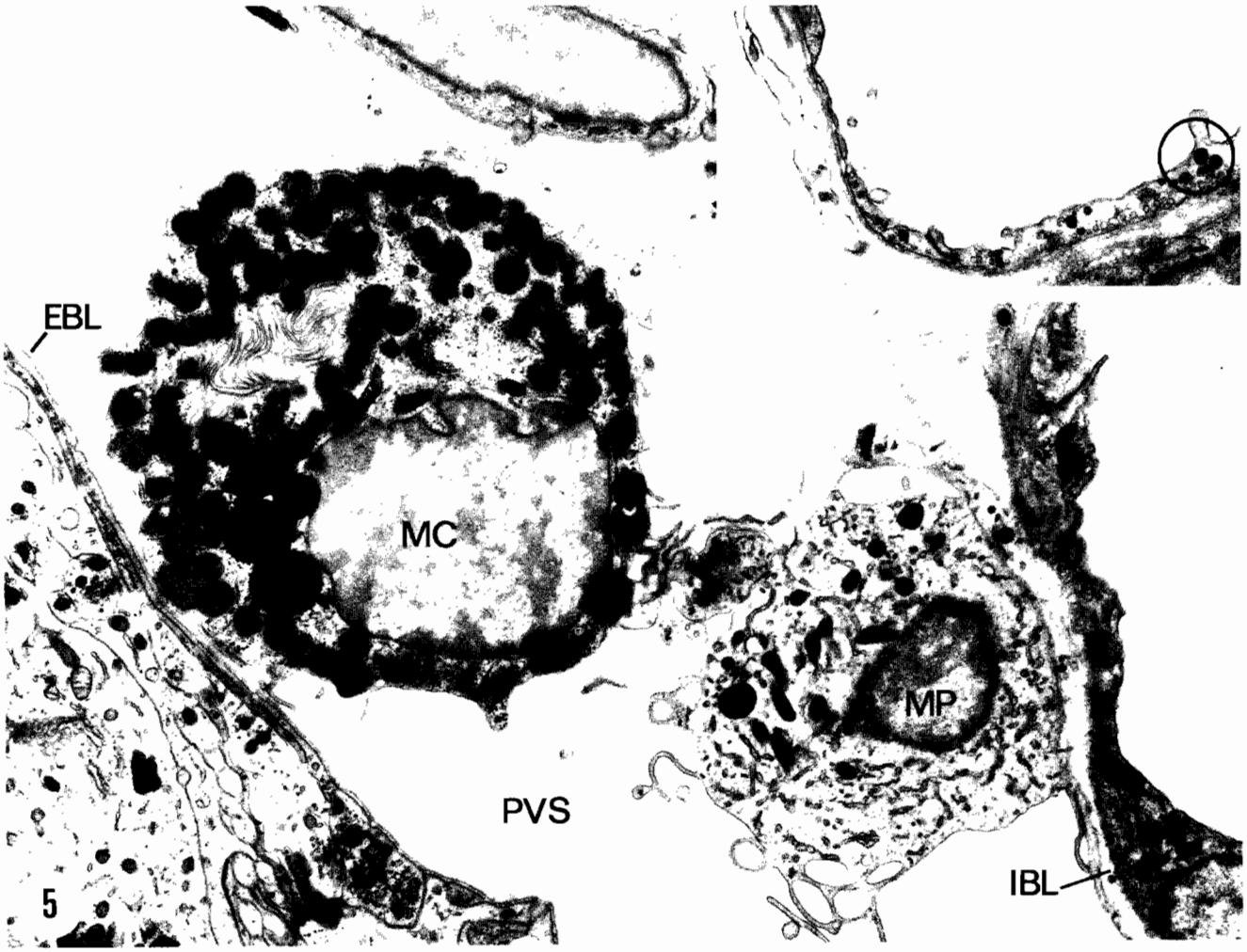
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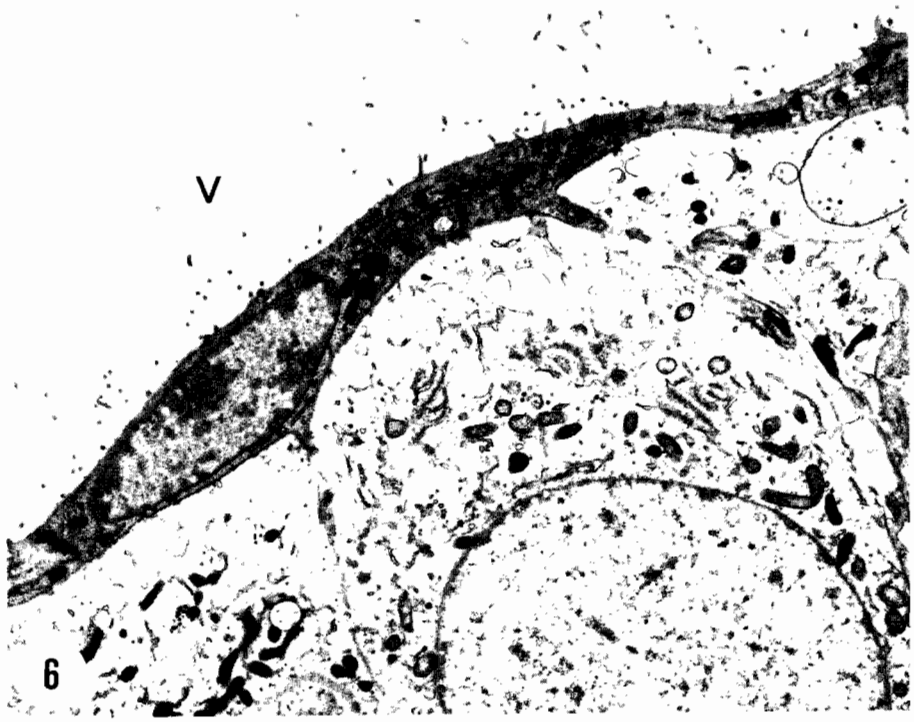
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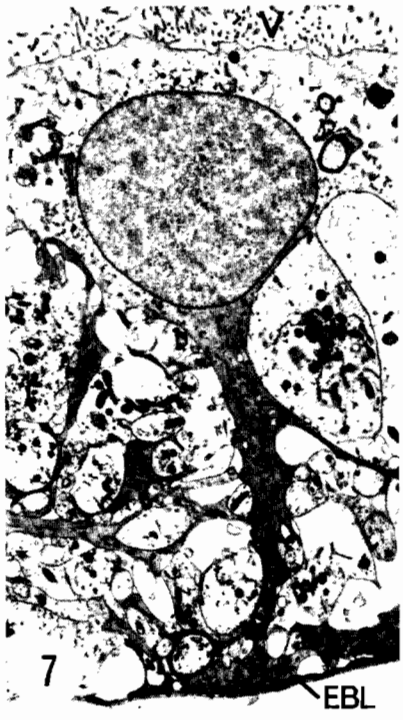
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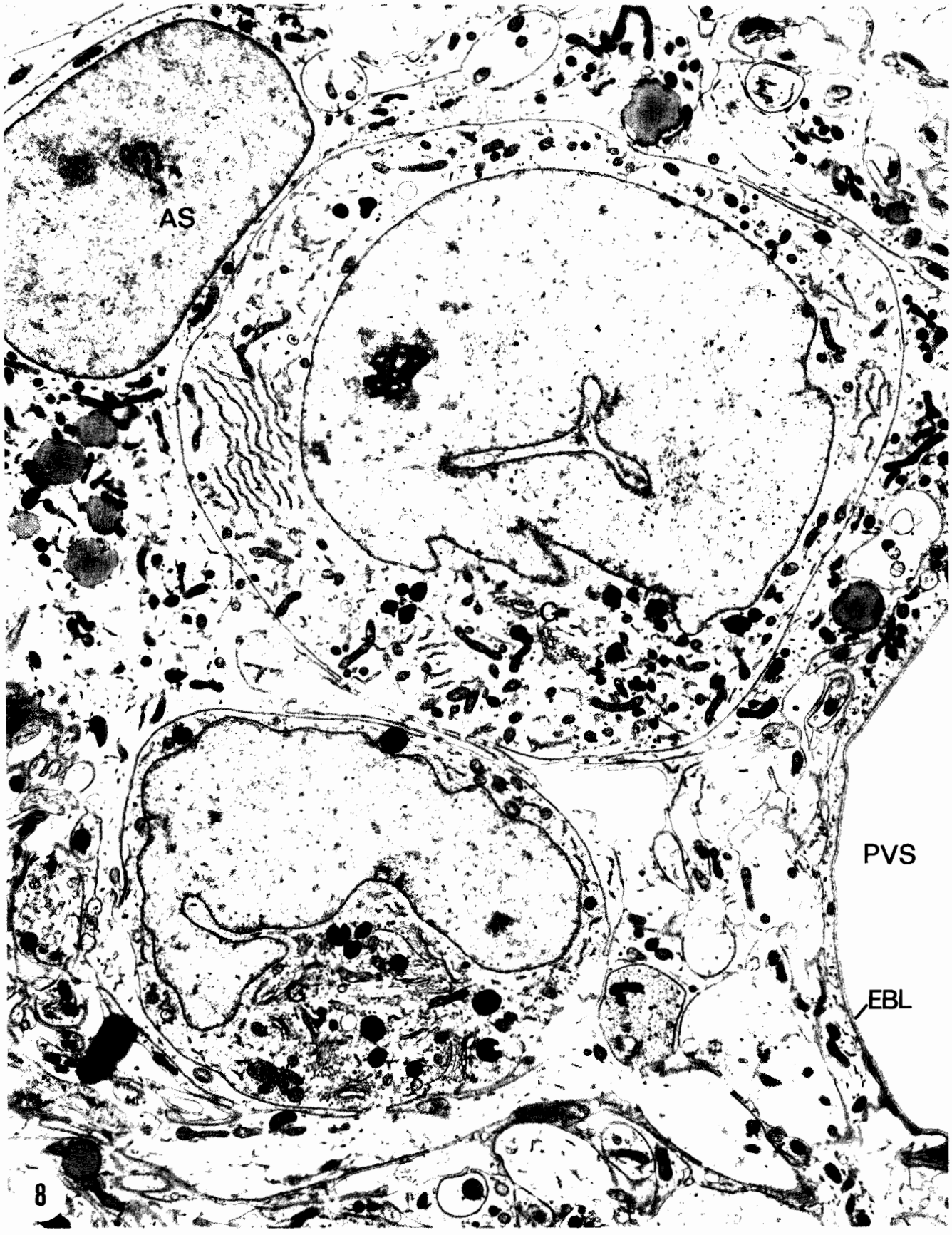
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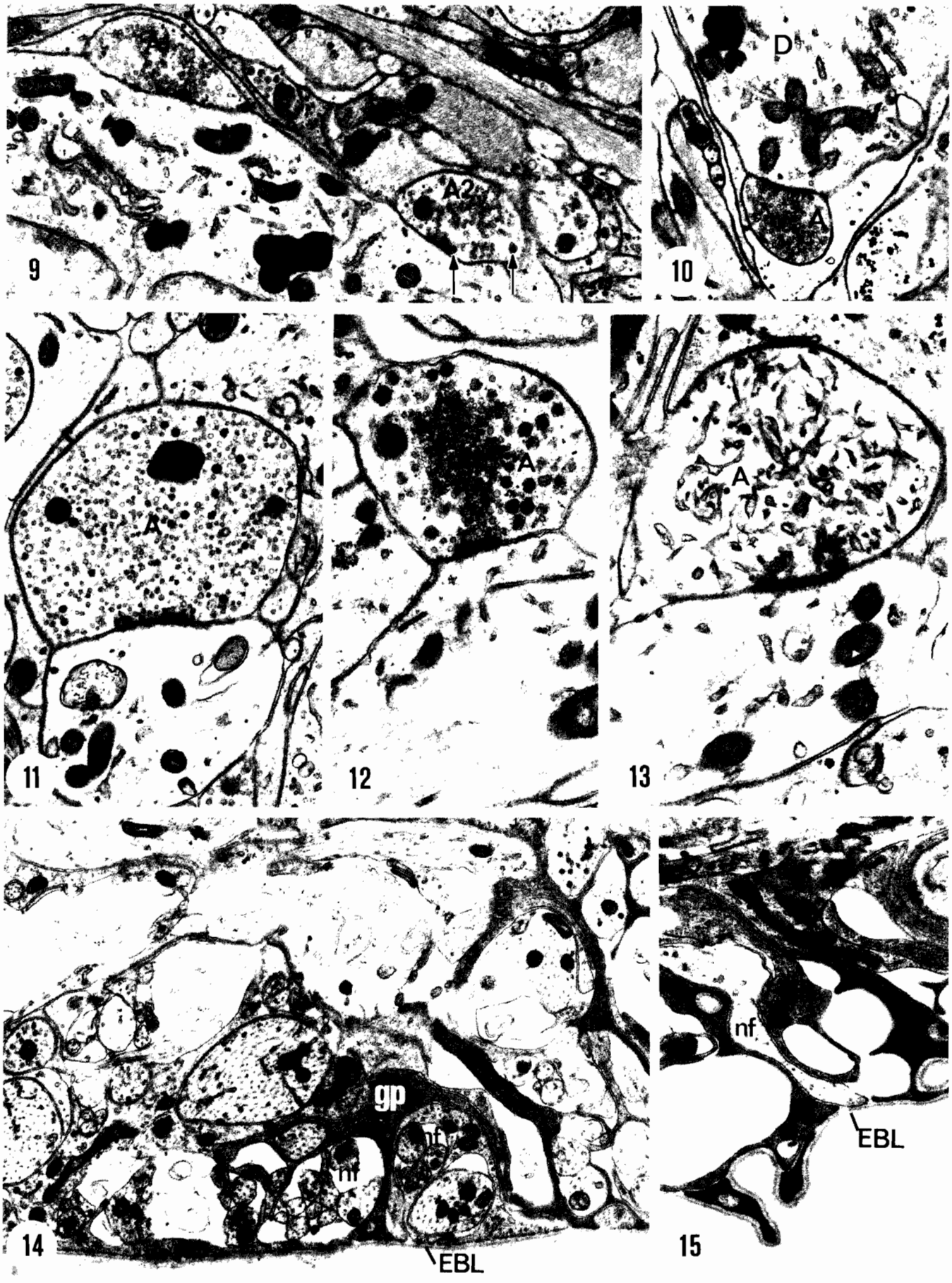
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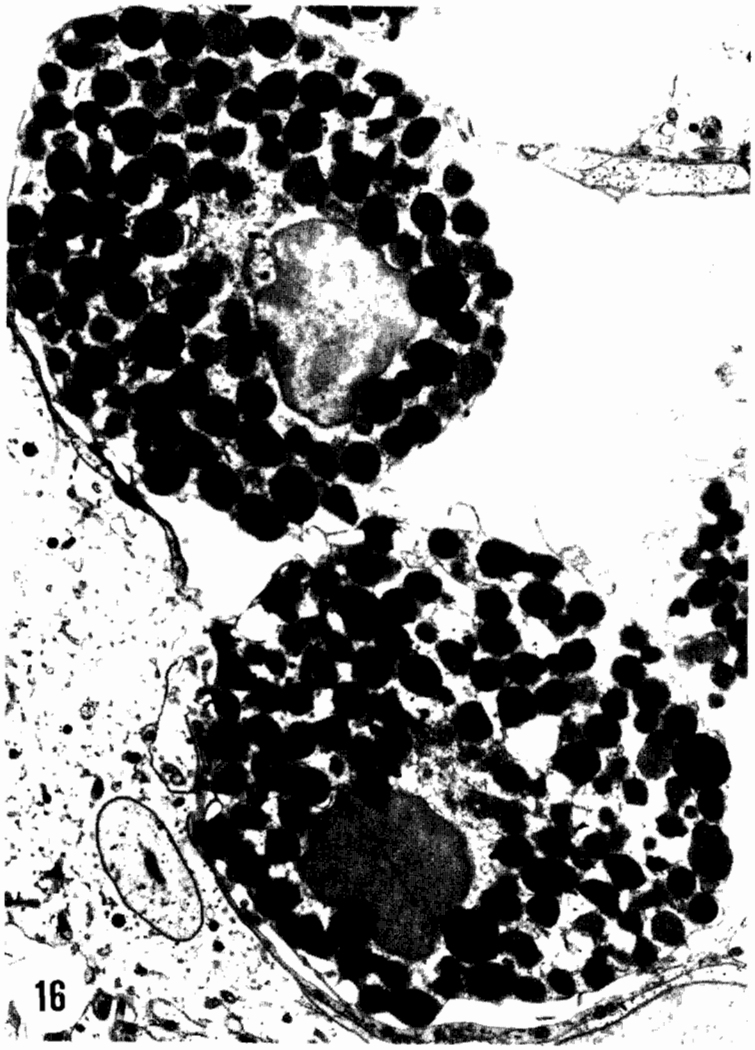

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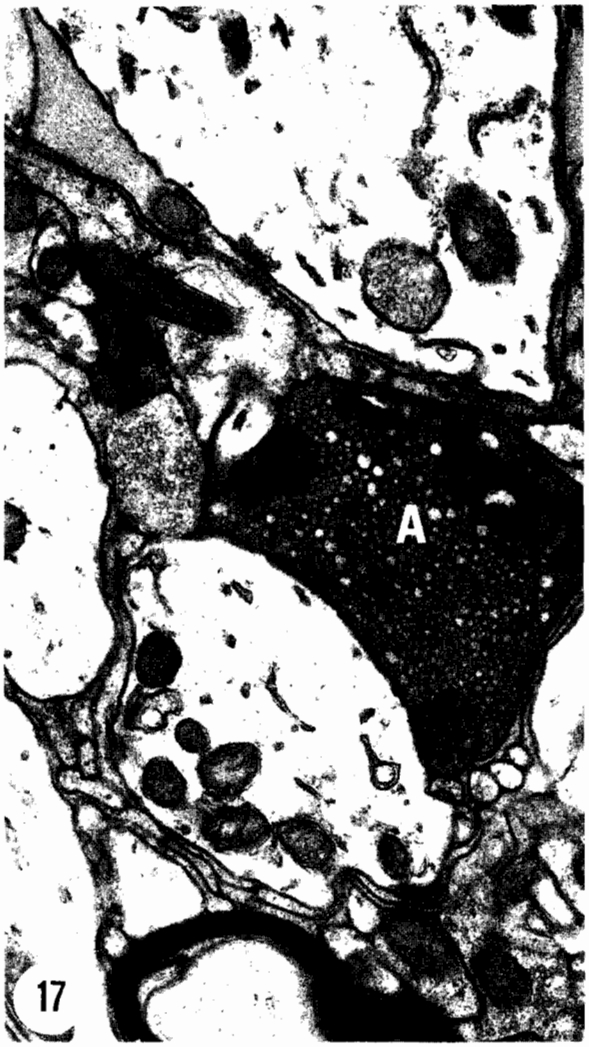

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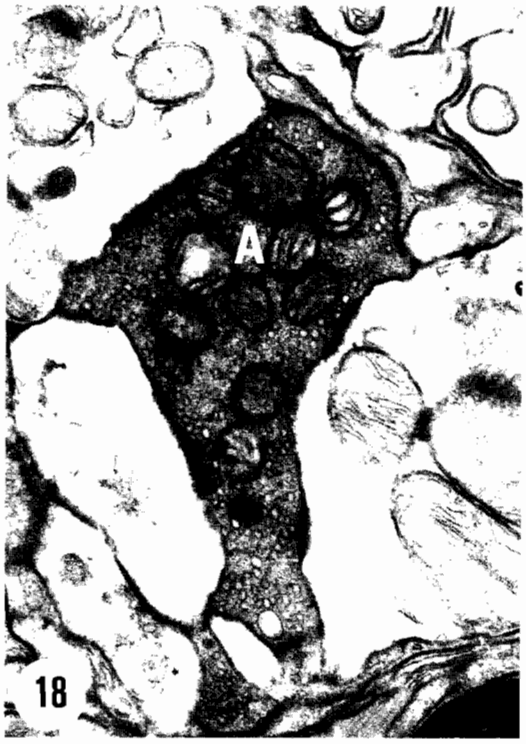

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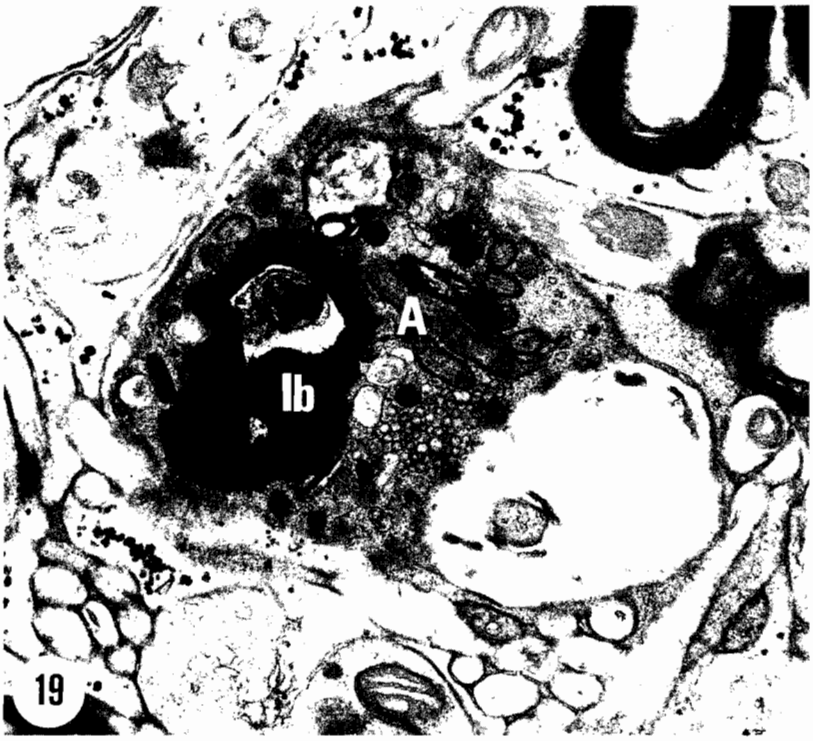

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Discussion

The ultrastructure of the *area postrema* of the monkey, *Macaca fascicularis,* is in general agreement with that reported in other species including rat (Dempsey, 1973), mouse (Rohrsneider et al., 1972), rabbit (Shimizu and Ishii, 1964), squirrel monkey (Klara and Brizzee, 1975), cat (Klara and Brizzee, 1977) and guinea pig (Manni et al., 1982). As in the cat (Klara and Brizzee, 1975), the ependymal suface of the *area postrema* in the monkey is modified and endowed with numerous microvilli. The latter appeared to lie flat on the surface of the ependymal cells. There is no evidence of secretion into the cerebral spinal fluid as described by Klara and Brizzee (1975) in the cat's *area postrema.* The functional significance of such arrangement of the microvilli is obscure. In the transmission electron microscopy, some neurons were located immediately beneath the attenuated ependymal cells. It is possible that the neurons may detect the changes of the cerebral spinal fluid in the fourth ventricle. Whether or not the abundant microvilli on the ependymal cells could serve as a mechano- or chemoreceptor is uncertain.

Some of the ependymal cells had their long basal processes extending onto the external basal lamina of the perivascular space. These long processes had secondary processes which penetrated the neuropil. They probably would serve more of a supportive role for the nerve fibres which were grouped into fasciculi, than of a neuroendocrine function as suggested by Klara and Brizzee (1975).

The neurons of the *area postrema* are peculiar in that except form some small areas of axo-somatic contacts, the cell bodies are enveloped by the astrocytic glial processes which also surround the external basal lamina of perivascular space. The close structural relation would suggest a nutritive as well as a supportive role for the neurons. With such arrangements, it is conceivable that nutrients would have to leave the perivascular space to reach the neurons via the glial processes. The perivascular space with its external basal lamina is surrounded by the glial processes separating the neuropil from the blood vessels. However, in certain areas some glial terminal processes were tunnelled by nerve fibres gaining a direct contact with the external basal lamina. Such arrangement would provide a sensory receptor to detect the changes of the contents in the perivascular space. This is deemed to be of vital importance in an organ where blood-brain-barrier is known to be inefficient (Dempsey, 1973). Substances which may have entered the perivascular space through the fenestrated capillaries could not only be monitored by the macrophages present but also could be detected by the nerve fibres which terminate on the blood vessel. Therefore, even though the present study could not confirm the existence of nerve endings within the perivascular space as described by Dempsey (1973) and Klara and Brizzee (1975) in the *area postrema* of rat and squirrel monkey respectively, it would still support the view of a chemoreceptive function for the *area postrema* as postulated by Brizzee and his co-wosrkers (Borison and Brizzee, 1951; Brizzee and Borison, 1952; Brizzee and Neal, 1954).

The commonest synaptic contacts in the *area postrema* of the monkey was of the axo-dentritic variety. Axon terminals containing different types of synaptic vesicles of different configurations were observed. They might represent afferent terminals from different sources. The afferents into the *area postrema* have been shown to be derived from the vagus nerve and aortic nerve (Kalia and Mesulam, 1980; Kalia and Welles, 1980) and hypothalamus (Hosoya and Matsushita, 1981). The present study could not confirm a direct sensory vagal projection in the *area postrema* as described by Kalia and Mesulam (1980). On the other hand, axon terminals which showed signs of degenerative changes, were common in the subpostremal zone following bilateral vagotomy. These changes probably resulted from transganglionic degeneration following the transection of the peripheral process of the vagal sensory neurons. Similar phenomenon had also been described in the central processes of the sensory ganglion cells of the vagus, glossopharyngeal and carotid nerves projecting to the nucleus of the tractus solitarius after the nerves are cut peripheral to the respective sensory ganglia (Majumdar et al., 1983) and in the dorsal motor nucleus after bilateral vagotomy (Ling et al., 1986). The present observations therefore strongly indicate that the vagal afferent input is primarily to the neuropil of the subpostremal zone rather than to the *area postrema* proper.

A remarkable feature of the *area postrema* of the vagotomized monkeys is the presence of numerous mast-cells in the neuropil. It is possible that their contents which would include serotonin may be released into the neuropil which would then affect the neurons. It is interesting to note that *area postrema* may monitor serotonin secreted by the supra-and subependymal 5HT plexus (Klara and Brizzee, 1977). In the present study, it would appear that vagotomy elicits the increase in number of parenchymal cells. It is likely that these mast cells are derived from the perivascular space where these cells are normally located. The mast cells probably respond to the degenerating axon terminals following vagotomy. It has been noted that the number of mast cells was dramatically increased in the distal portions of sectioned peripheral nerves (Olsson, 1968). In the present study, the close structural relationship between the mast cells and the ventricular lumen suggests also a possible release of substance into the cerebral spinal fluid. However, how it would affect the ventricular system is unclear.

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