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Glia-vascular complex and the neurohemal function of circumventricular organs

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The neurohemal zones of rat median eminence (ME) and subfornical organ were investigated electronmicroscopically and immunohistochemically (for glial fibrillary acidic protein - GFAP and for laminin) during pregnancy and lactation. The ultrastructural and immunohistochemical changes of the fenestrated capillaries and perivascular glia cells were described in relation to the diferent intensity of the neurohemal function.

Key words: neurohemal zone, circumventricular organs, electron microscopy, laminin.

It is well known that the brain vessels are not permeable for high molecular substances or it exists blood-brain barrier due to the specific structure of endothelial cells, basal membrane, pericytes [14]. Some hypothalamic zones and circumventricular organs, however, possess capillaries facilitating the delivery of neurotransmitters and hormones from neuronal endings into blood or neurohemal contact is available [13]. The neurohemal function is due to the characteristic structure of the neurogliohemal complex in these zones and its plasticity in relation with the neuroendocrine activity [9]. The capillaries are fenestrated with micropinocytotic - vesicles in endothelial cytoplasma. The pericapillary space is large - the s. c. "metabolitic lake" by Hartman [7]. Around the capillary wall glial sheath, neuronal and tanycytic terminals are situated. We are interested in the ultrastructural and immunohistochemical correlates of the different intensity of the neurohemal function.

We have chosen for our study the neurohemal zones in median eminence and subfornical organ of the rat. Median eminence is a component of the endocrine hypothalamus, especially of its hypophysiotrophic zone [6], which "drains" the neurosecretions (mainly different releasing hormones) into the hypophyseal portal circulation. The subfornical organ participates in mediation the central nervous response to osmotic stimuli [1]. There are many disputable problems about the plasticity of the glio-vascular association for instance the age-dependent fine structural changes, the role of the extracellular matrix component in ageing, changes of the number of fenestrated and non-fenestrated capillaries etc.

Material and Methods

Experimental animals. Wistar rats : midpregnancy, endpregnancy, lactation and ageing (20-month-old).

Electron microscopy. Intracardially perfusion with fixative solution, containing 2% paraformaldehyde and 2.5% glutaraldehyde. The tissue specimens from median eminence and subfornical organ were posfixed in 1% osmium tetraoxide in cacodylate buffer, dehydrated in alcohols and embedded in durcupan. The semithin sections were stained with toluidine blue and the thin sections were stained with uranyle acetate and lead citrate.

Immunohistochemistry — (procedure by SIGMA). Antigen-specific primary antibody (for Glial fibrillary acidic protein and for Laminin) was applied to deparaffinized tissue sections. Following a brief wash, the sections were incubated with a biotinylated secondary antibody - a stable extravidin-biotin complex was formed. Sites of antibody deposition were visualized by addition of freshly preparated substrate containing hydrogen peroxide and the electron donor chromogen, 3-amino-9-ethylcarba-zole (AEC). Bound peroxydase catalyses the oxidation of the AEC to form reddish-brown insoluble precipitate at antigen sites. The same procedure was performed without antigen as a control.

Results and Discussion

At the end of pregnancy the delivery of gonadotrophic releasing hormone from neuronal terminals to capillaries of median eminence is intense (so-called phasic secretion) [14]. The endothelial cells of the fenestrated capillaries in median eminence are characterized by cytoplasmic protrusions into lumen, micropinocytosis (Fig. 1), or fine structural signs of intense transport activity. The pericapillary space is enlarged in comparison with the pericapillary space of midpregnancy (Fig. 2, 3). Multivesicular bodies could be seen too. The pericytes are mainly fibrous pericytes after the classification of Mori and Nagano [8]. The neuronal and tanycytic endings are situated directly on the outer basal membrane and the glial sheath could not be seen. Rennels et al. [10] and Gregory et al. [5] supposed an other function of the perivascular space. They established transport of substances in this space in the length of the vessels from zones without blood-brain barrier to zones of the brain with blood-brain barrier.

When the neuroendocrine activity is lower for instance during ageing the cytoplasmic endothelial protrusions are scarce and the micropinocytosis is weak (Fig. 4). The pericytes are mainly granular (phagocytosis). In tanycytic endings lipid droplets could be seen and in neuronal endings exist disk bodies. In the neuropil near the capillaries secondary lysosomes are seen.

The functional plasticity of the glia-vascular complex in neurohemal zones we have studied also applying immunohistochemical reactions for glial fibrillary acidic protein and for laminin.

The glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed in normal, reactive and neoplastic glial cells. The GFAP immunoreactivity of rats in midpregnancy is strong around the capillaries of median eminence and in subfornical organ (Fig. 5). The GFAP immunoreactivity is weak around the vessels of median eminence at the end of pregnancy and in subfornical organ during lactation (Fig. 6). These changes of the GFAP immunoreactivity around the vessels

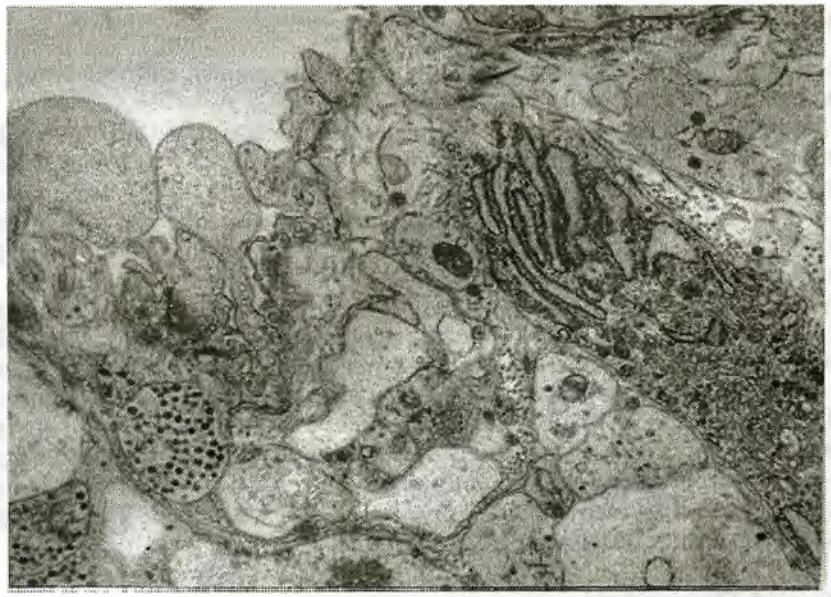

Fig. 1. Median eminence of the rat at endpregnancy. Endothelial cytoplasmic protrusions into the lumen of the fenestrated capillary (× 6000)

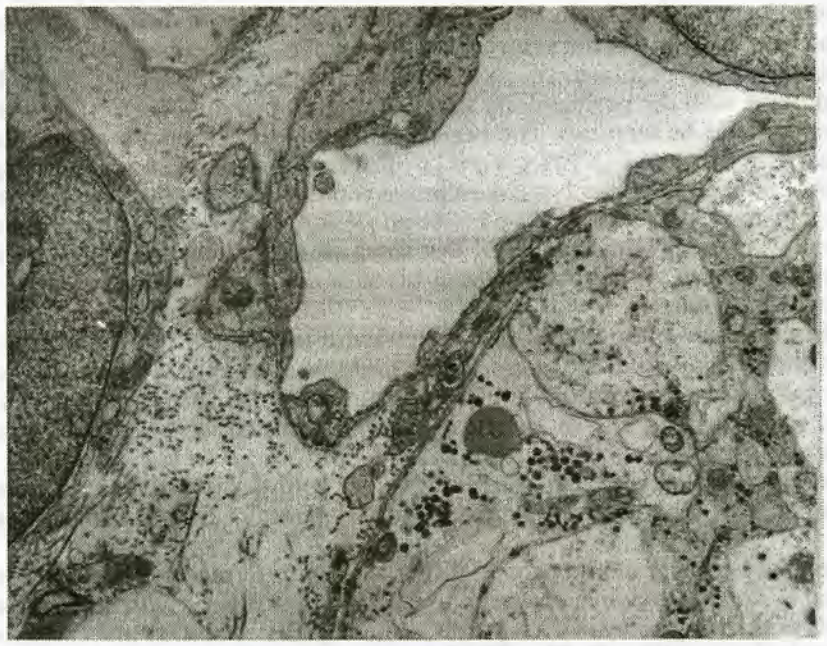

Fig. 2. Median eminence of the rat at endpregnancy. Enlarged pericapillary space (× 6000)

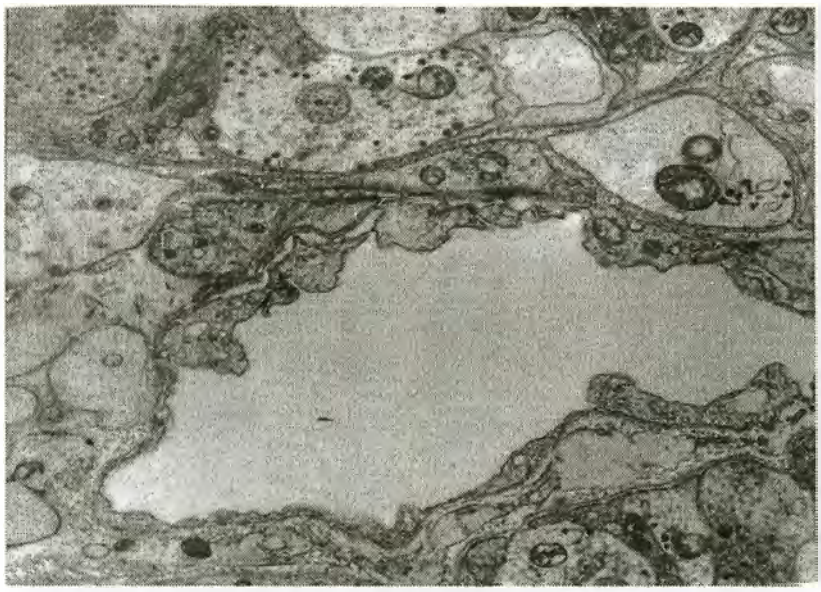

Fig. 3. Median eminence of the rat at midpregnancy. Fenestrated capillary (× 6000)

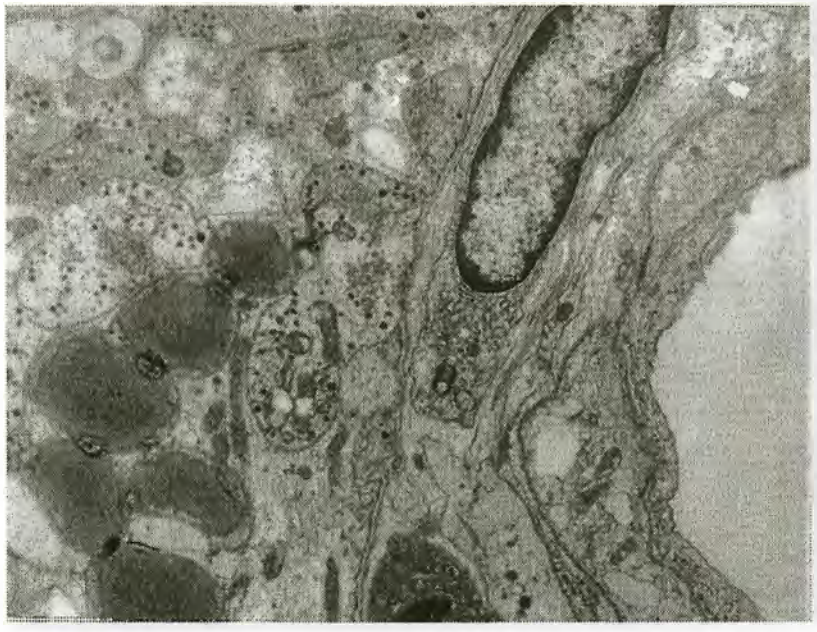

Fig. 4. Median eminence of the 20-month-old rat. Secondary lysosomes in the neuropil near the capillary (× 6000)

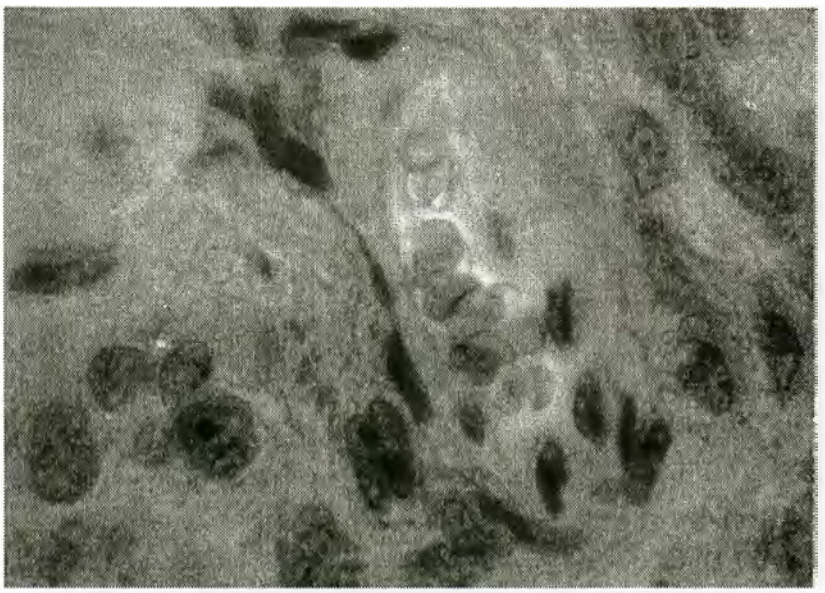

Fig. 5. GFAP reaction in the subfornical organ of the rat at midpregnancy. Glial positive sheath around the capillary (× 1000)

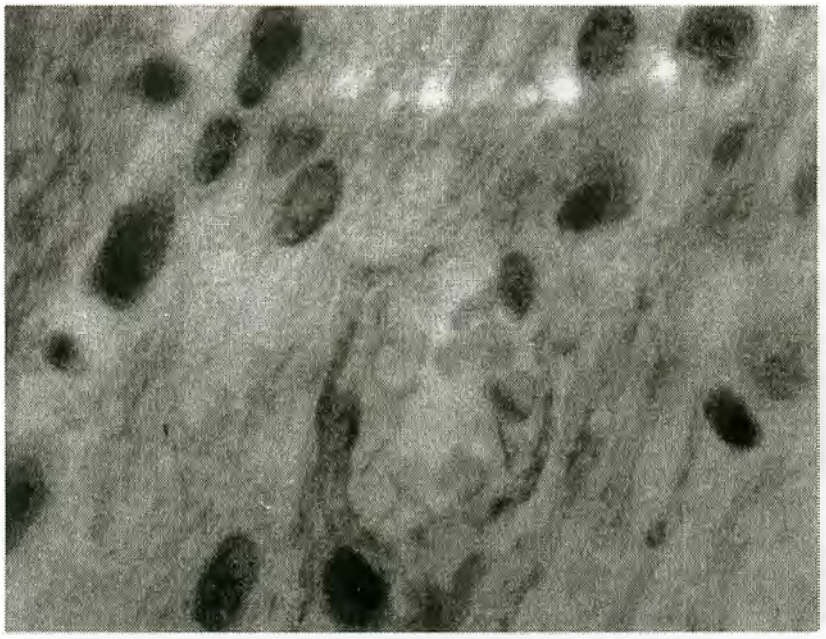

Fig. 6. GFAP reaction in the subfornical organ of the rat in lactation. Weak reaction around the capillary (× 1000)

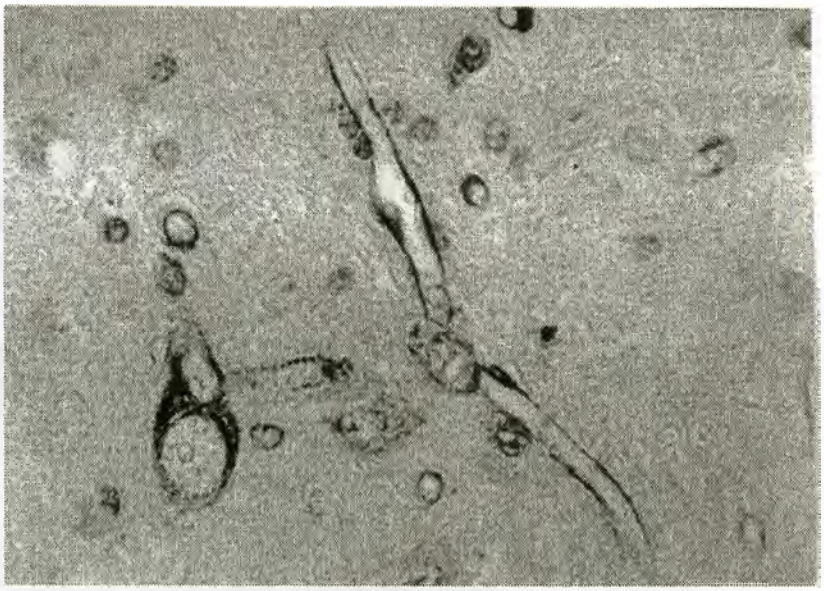

Fig. 7. Laminin immunoreactivity in median eminence of young rat. Positive reaction at the capillary walls (× 250)

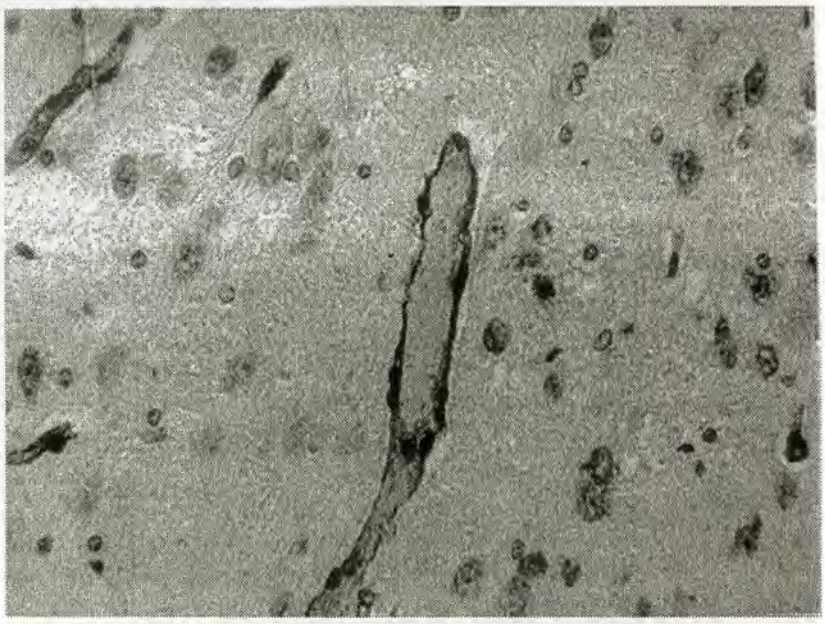

Fig. 8. Laminin immunoreactivity in subfornical organ of young rat. Positive reaction in the capillary wall (× 250)

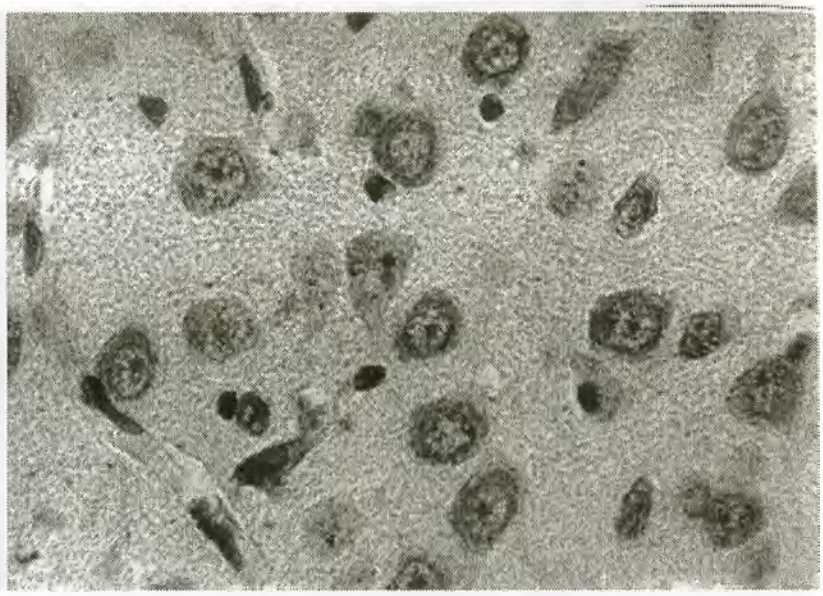

Fig. 9. Laminin immunoreactivity in cells outside the basal membrane (× 500)

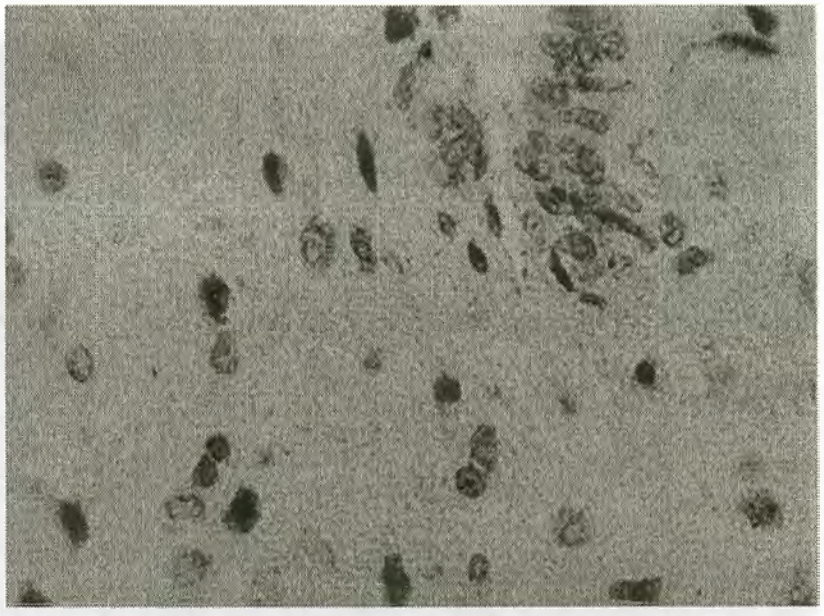

Fig. 10. Laminin immunoreactivity in median eminence of 20-month-old rat. Weak reaction in capillary walls (× 250)

of median eminence and subfornical organ in terms when the neurohemal function is intense are probably due to the withdrawal of the glial sheath, observed electronmicrosopically, facilitating the neurohemal contact. In ageing rats the reaction for GFAP in median eminence and subfornical organ is intense, many positive cells could be seen in the neuropil, probably due to the increased number of glial cells in these animals.

In order to receive more information about the role of the microenvironment for the plasticity of the neurohemal contacts we have studied the laminin immunoreactivity. Laminin is the major non-collagenous glycoprotein of the basement membrane. Several components of the extracellular matrix and basal membrane are known to influence the neuronal growth during development and regeneration [11]. Moreover, after S i i r o n e n et al. [12] the laminin synthesis increases during nerve regeneration. After G a v a z z i et al. [4] the extracellular matrix is involved in regulation of neuronal ageing and the role of laminin durung degeneration and in reduced plasticity of axons in old age is still unknown.

We have seen some differences of the laminin immunoreactivity in neurohemal zones of median eminence and subfornical organ of young and ageing rats. In young animals the reaction is strong in vessels wall (Fig. 7, 8). Characteristic is the laminin immunoreactivity in cells outside basement membrane (Fig. 9). S o i n i et al. [13] have seen in histiocytoma laminin positive histiocytes, but after these authors it is uncertain whether the positivity for laminin in cells results from de novo synthesis of laminin by these cells or their capacity to absorb exogenous laminin on their surface.

In aged rats the laminin immunoreactivity in neurohemal zones of median eminence and subfornical organ is very weak (Fig. 10). There are many speculations about this finding, seen in other tissues of ageing animals. For instance G a v a z z i et al. [4] have shown reduced laminin immunoreactivity in the blood vessel wall of ageing rats correlating with a reduced innervation, or it has been supposed that the changes of the laminin content affect the neuronal plasticity. The further studies of the morphological substrate of the neurohemal function in the brain could give new data about the local factors, influencing the intensity of the neurohemal contact.

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References

1. B e l o v e j d o v, V. T., V. G. B o u r n e v a. Lactation-associated redistribution of the Glial fibrillary acidic protein within rat subfornical organ. — C. R. Acad. Bulg. Sci, 48, 1995, No 3, 111-113.
2. B e l o v e j d o v, V. T., V. G. B o u r n e v a. Morphometric study of the different types of vessels in subfornical organ of lactating rats. — C. R. Acad. Bulg. Sci. (in press).
3. C o l l i n s, F., D. H. W o o l m a n.The circumventricular organs. — In: Progress in anatomy (Ed. R. J. Harrison, R. L. Honnes). Cambridge Univ. Press, 1981,123-141.
4. G a v a z z i, I., K. S. B o y l e, D. E d g a r, T. C o h e n. Reduced laminin immunoreactivity in blood vessel wall of ageing rats correlates with reduced innervation in vivo and following transplantation. — Cell Tiss. Res., 281, 1995, 23-32.
5. G r e g o r y, T. F., M. L. R e n n e l s, O. R. B l a u m a n i s, K. F u j i m o t o. A method for microscopic studies of cerebral angioarchitecture and vascular-parenchimal relationship, based on the demonstration of "paravascular" fluid pathways in the mammalian central nervous system. — J. Neurosc. Methods, 14, 2985, 5-14.
6. H a l a s z, B. The trophic dependece of the anterior pituitary on the diencephalon; the hypophisiotrophic area of the hypothalamus — In: Hypothalamic control of the anterior pituitary (Eds. J. Szentagotai, B. Flerco, B. Mess, B. Halasz). Budapest, Acad. Kiado, 1968, 110-155.

7. H a r t m a n, J. F. Electron microscopic of the neurohypophisis in normal and histamine-treated rats. — Z. Zellforsch., **48**, 1958, 291-308.
8. M o r i, S., M. N a g a n o. Electron microscopic investigation on three types of pericytes in the rat brain. — In: Int. Cong. on Electron Microscopy (Kyoto), 1986, 3175-3188.
9. P a l a c i o s-P r ü, E. Z a m b r a n o, R. V. M e n d o z a. Development of the neurogliohemal complex in the mouse neurohypophisis. — J. Subnicr. Cytol. Pathol., **27**, 1995, No 2, 217-225.
10. R e n n e l s, M. L., T. F. G r e g o r y, O. R. B l a u m n i s, K. F u g i m o t o. Evidence for a "paravascular" fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. — Brain Res., **326**, 1985, 47-63.
11. S a n e s, J. R. Extracellular matrix molecules that influence neural development. — Ann. Rev. Neurosci., 12, 1989, 491-516.
12. S i i r o n e n, J., M. S a n d b e r g, V. V u o r i n e n, M. R o y t t a. Laminin-bl and collagen type IV gene expression in transected peripheral nerve-reinnervation compared to denervation. — J. Neurochem., **59**, 1992, 2184-2192.
13. S o i n i, Y., H. A u t i o-H a r m a i n e n, M. M i e t t i n e n. Immunoreactivity for laminin and type IV collagen in malignant and benign fibrous histocytoma. — J. Pathol., **158**, 1989, 223-228.
14. Г и ц о в, Л., В. Б у р н е в а. Функционална морфология на гонадотропния хипоталамус. С., БАН, 1990, 1 — 87.