

# Ultrastructural study of a special type of ependymal cell at paraventricular level of the golden hamster third ventricle

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**Summary.** The present paper describes the presence of a special cell located in the ependymal wall at the level of the paraventricular nucleus. At this level, ultrastructural observation of these ependymal cells, unlike most other mammalian species, shows the presence of nucleolus-like bodies in their cytoplasm and occasionally basal processes. These processes appear perpendicular to the ependymal surface and end in contact with the basal membrane of hypothalamic capillaries. Mitochondria, endoplasmic reticulum and numerous filaments are present in the basal processes. Nucleolus-like bodies or nematosomes consist of round or ovoid unbound masses of granular appearing material of variable density located in the apical cytoplasm of the cells. Some of their ultrastructural characteristics are similar to other ependymal specialized cells which are classically termed tanycytes. These findings point out the possibility that those special cells may also be implicated in a ventricle-blood vessel communication.

**Key words:** Tanycyte, Nucleolus like body, Nematosome, Electron microscopy, Nervous system

## Introduction

The ependymal layer of the cerebral ventricles has been the subject of many different studies using light and electron microscopy (SEM or TEM). The conventional ependymal cells (EC) are squamous, cuboidal or columnar in shape and possess numerous cilia (Leonhardt, 1980). Usually, they are arranged in a single or double layer and their basal portion does not have projections into the subependymal zone. In these layers it has been possible to prove the presence of several cell types depending on the species and the areas of the ventricle studied. These variations suggested that different ependymal cells have different functions and

that the morphology of the hypothalamic ependymal layer reflects varying metabolic states of the animal (Bleier, 1977). Different types of cells have been studied in birds (Kuenzel and van Tienhoven, 1982), rodents (Tennyson and Papas, 1962; Mitro and Palkovits, 1981; Jordan et al., 1987), rabbits (Brio et al., 1991) and in other mammals, including man (Leonhardt, 1980). Some studies suggest that the transport of neuropeptides and neurohormones present in the cerebrospinal fluid (CSF) would be carried out with active or passive participation of the ependymal cells (Knigge and Joseph, 1974; Scott et al., 1975).

One type of specialized ependymal cell, classically termed tanycyte (Horstman, 1954), has a long basal process related to neurons and/or capillaries and has apical protrusions in contact with the ventricular lumen. It was proposed that such ependymal cells may be involved in absorption, hormonal secretion and transport between hypothalamic neurons and CSF of the third ventricle (Paul, 1968; Bleier, 1971; Akmayev and Popov, 1977). Thus, it is possible that the ependyma in various parts of the ventricular wall, especially the tanycyte ependyma, allows communication between the CSF and subependymal neuropil through its processes (Uemura et al., 1975). Some data suggest that the tanycytes could be implicated in the hypothalamic control of hypophyseal adrenocorticotrophic function by means of a feedback mechanism (Akmayev and Popov, 1977).

To our knowledge the ultrastructural organization of the hamster third ventricle at paraventricular level has not been studied in detail. There is only one previous study on the development of ependymal cells (Hannah and Geber, 1977). In other rodents, this zone of the ventricle has been described as a monolayer of light cells without tanycytes (Mitro and Palkovits, 1981). Extrapolations used in the literature, may well be unjustified because the organization of the ependyma might deviate somewhat from one mammal to the other well-known species like rat (Millhouse, 1975), rabbit (Brio et al., 1991) or cat (Leonhardt, 1980).

The aim of our study is to describe the ultrastructure of a special type of ependymal cell situated at the top of

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the hamster third ventricle at the level of the paraventricular nucleus. This type of cell has some similarities with tanycytes, but its spatial location and other ultrastructural characteristics belong to conventional ependymal cells. Its possible functional role, in the communication between CSF and blood stream, is discussed. The present paper is part of a wider ultrastructural study that will supply new data about the ultrastructural changes of aging hamster brain.

### Materials and methods

Ten young adult male golden hamsters (*Mesocricetus auratus*), from our laboratory stock, were employed in the present study. They were housed in a temperature controlled room ( $20 \pm 2$  °C) with photoperiod of 14:10 h light/dark cycles and with free access to laboratory chow and water.

Before sacrifice, animals were anaesthetized with sodium pentobarbital (10.5 mg/10 g body weight) and transcardially perfused with 4% glutaraldehyde and 1mM  $\text{CaCl}_2$  in 0.05M cacodylate buffer at pH 7.3. After perfusion, brains were removed and fixed in the same fixative for 18 hours at 4 °C. The hypothalamic area at paraventricular nucleus level was dissected out and the tissue blocks were postfixed in a solution of 1% osmium tetroxide and 2% potassium ferrocyanide in 0.05M cacodylate buffer (pH 7.3) for 1 hour, dehydrated in a graded series of acetone and embedded in Durcupan-ACM (Fluka). Semithin sections, 0.5  $\mu\text{m}$  in thickness, were obtained and stained with the first step of the polychromatic method described by Tolivia et al. (1994) to locate the ependymal wall level. Ultrathin sections were obtained on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 109 microscope.

### Results

At the level of the paraventricular nucleus (PVN), the ependymal wall of the third ventricle is formed by a monolayer of cells. Most of them have a cuboidal shape and exhibit no considerable individual variation, but some ependymal cells show a special morphology which partly resembles that of classical tanycytes. These cells were characterized by the presence of one basal process which projected into the neuropil, perpendicular to the ependymal wall (Figs. 1, 3). Occasionally, two or more processes of the same cell were observed ending on the same capillary (Fig. 5).

These cells sent their processes towards the hypothalamic capillaries, ending close to the basal membrane of the continuous endothelium (Figs. 4, 6). These processes contained some longitudinally oriented mitochondria, scarce free ribosomes, some cisterns of

the endoplasmic reticulum and numerous filaments (Fig. 6).

The subependymal neuropil was composed of a feltwork of intermingled neuronal and glial cell processes. Basal processes of these cells, as has previously been mentioned, protruded perpendicularly to the ependyma surface in contrast to the most neuronal and glial processes that ran parallel to it (Figs 1, 3, 5). The basal part of the ependymal cells was bordered by the neuropil and close to it astrocytes cell bodies could be frequently found (Fig. 2).

The ultrastructural characteristics of the nucleus and cytoplasm of these cells were in all similar to the rest of the ependymal cells at this level. The nuclei were central, round and euchromatic with a small crown of dense chromatin beneath the nucleolema (Figs. 1-3, 7, 8). The nucleolus was quite conspicuous and some nuclear inclusions could be observed occasionally (Fig. 8).

The cytoplasm was electron lucent and contained abundant free polyribosomes, a poor rough endoplasmic reticulum, mitochondria, vesicles, Golgi complex, dense bodies and round bundles of filaments in perinuclear position (Fig. 7). Golgi complex constituted a very prominent feature of the supranuclear cytoplasm (Fig. 9). Mitochondria were large and mostly located in the apical part of these cells. Multivesicular and dense bodies may be seen close to the Golgi complex. The basal cytoplasm was poor in organelles, and only a few mitochondria and little endoplasmic reticulum could be observed (Figs. 1-3, 5).

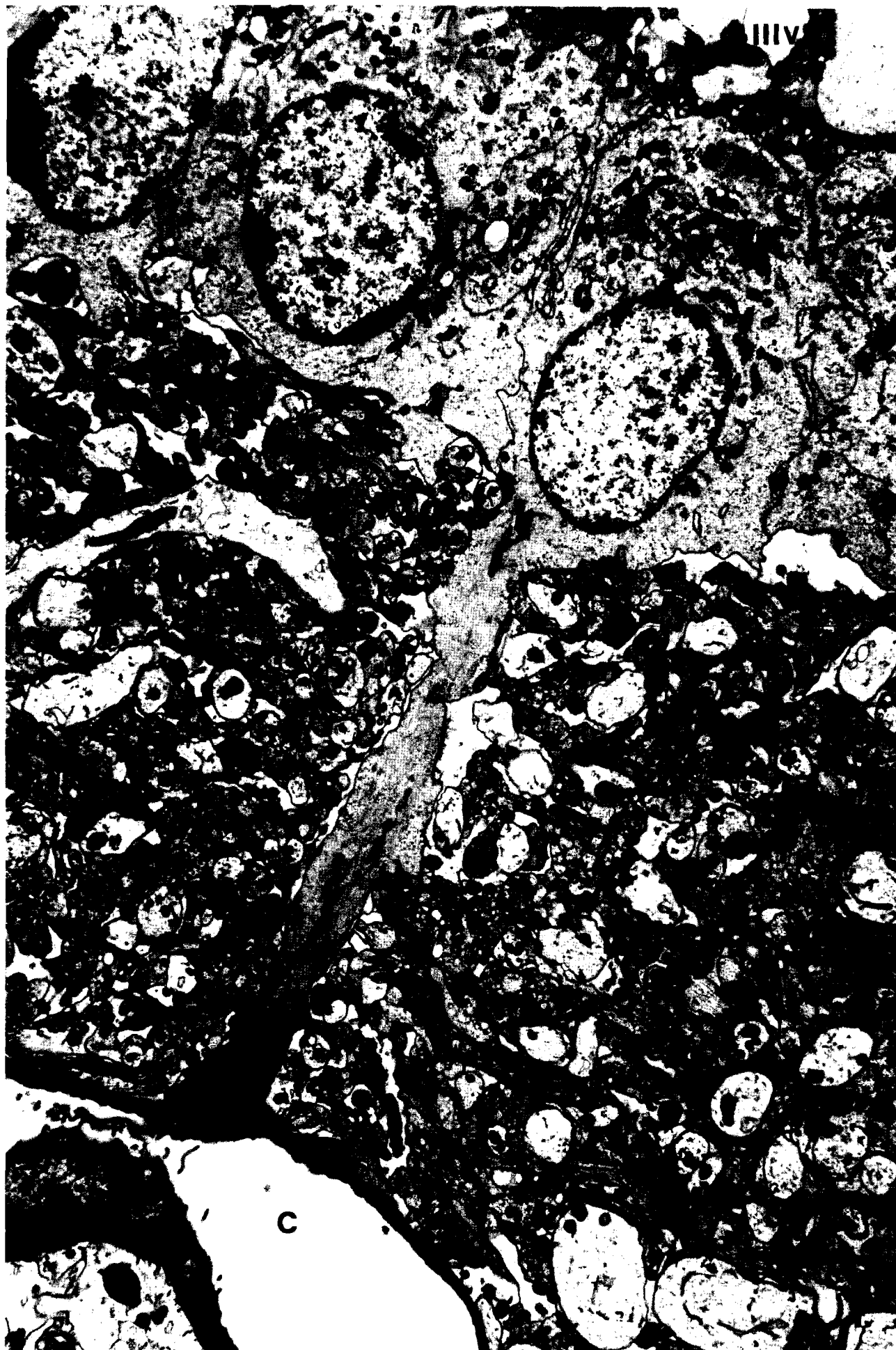
The apical surface had abundant microvilli and cilia. As usual, the cilia contained 9+2 microtubules (Fig. 11) and the basal bodies were found in the apical cytoplasm of the cells (Fig. 10). Lateral walls of the ependymal cells had membrane interdigitations. Zonula occludens and zonula adherens could be distinguished in the apical part (Fig. 12).

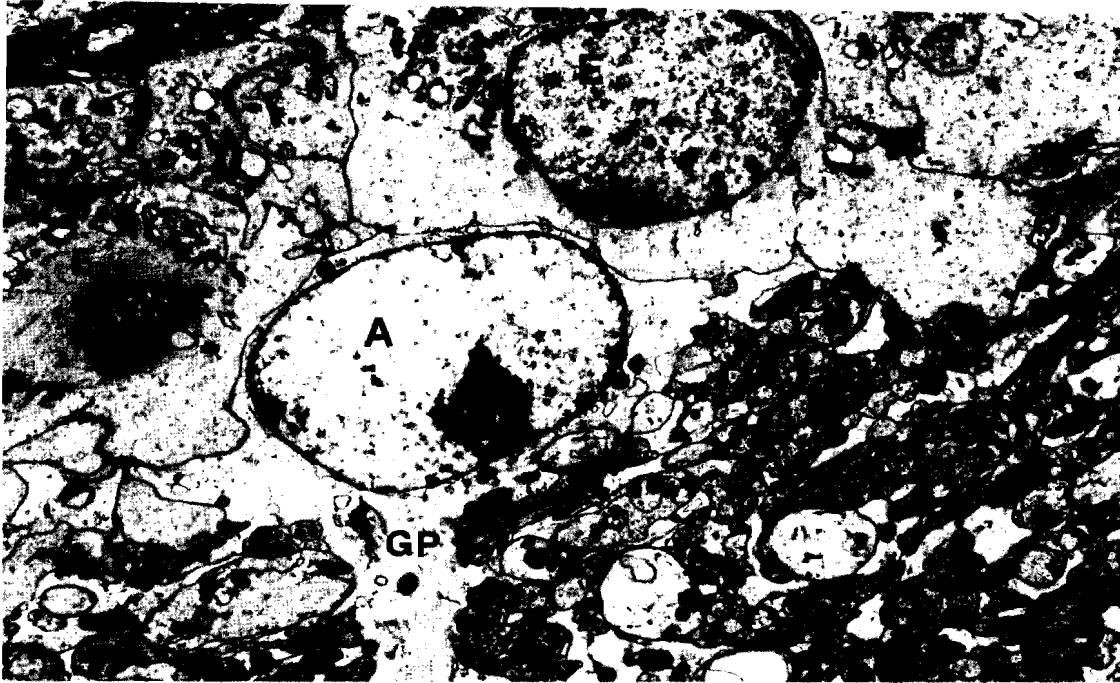
Nucleolus-like bodies (NLBs) or nematosomes were frequently observed over the apical pole of the nucleus (Fig. 8). These NLBs showed a spherical or ovoid shape of unbound electron-dense material with a granular texture and clear cores (one or more per NLBs) (Figs. 8-10). Most cells showed only one NLB but some cells had two or more NLBs (Fig. 10). Basal corpuscles of cilia and mitochondria (Figs. 8-10) were observed in the vicinity of NLBs. The granular texture and electron density of these cytoplasmic bodies resembled that of the nucleolus.

### Discussion

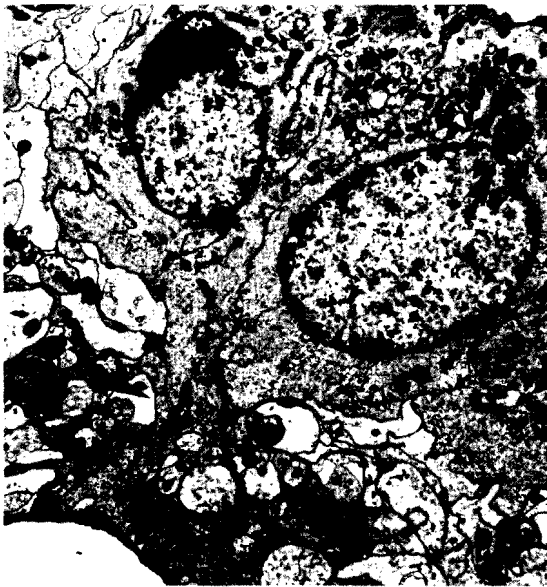
The ependymal cells, as previously commented, show marked morphological variations along the ventricle and among species. At level of the

**Fig. 1.** Electron micrograph of the ventricular wall at paraventricular level with an ependymal cell sending one basal process through the underlying neuropil to a continuous capillary. IIIv: third ventricle; C: capillary.  $\times 8,000$





**Fig. 2.** Ultrastructure of the ependymal layer showing an astrocyte (A) located under the ependymal cells (E). GP: glial, process. x 5,580



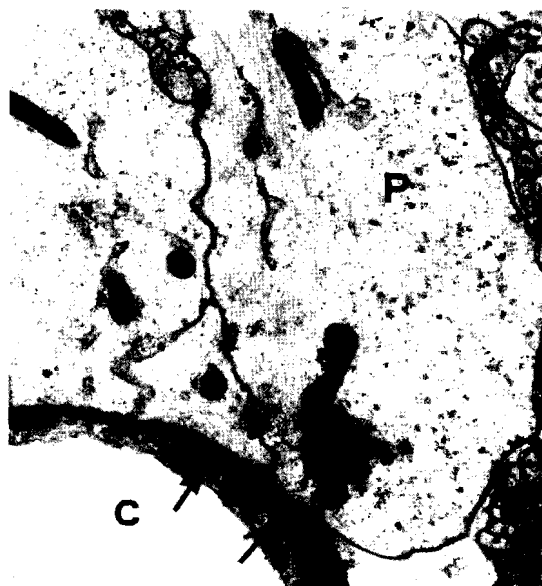
**Fig. 3.** Ependymal cell with one basal process (arrowheads). C: capillary. arrow: NLB. x 4,185



**Fig. 4.** Distal segment of the basal process (P) shown in Fig. 3 terminating on the basal membrane (arrowhead) of a capillary (C). x 15,500

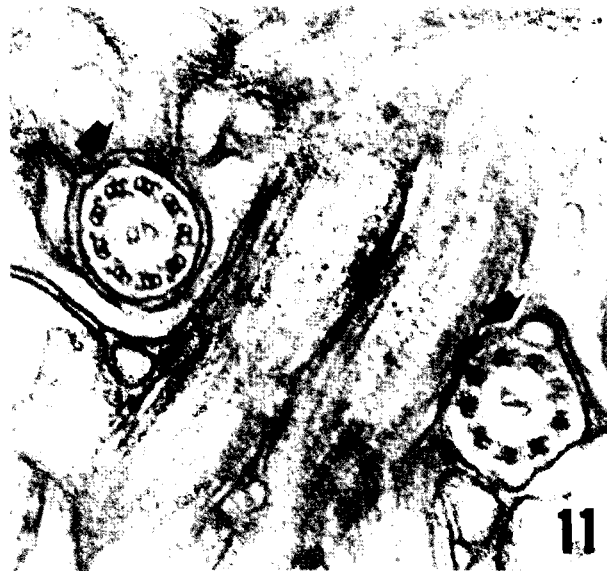
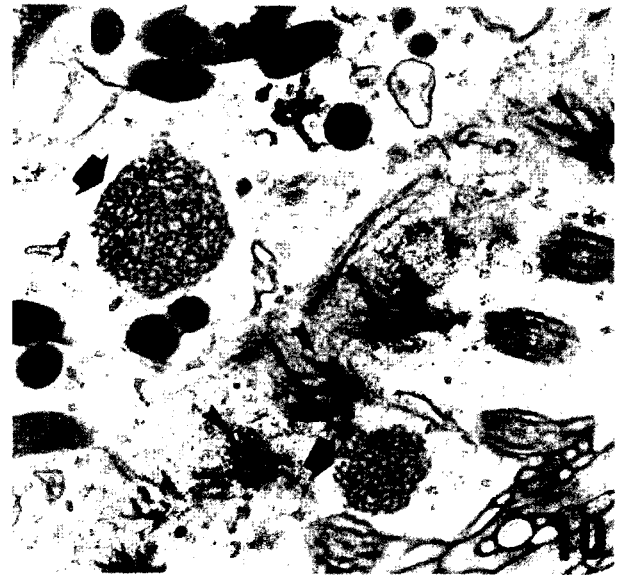
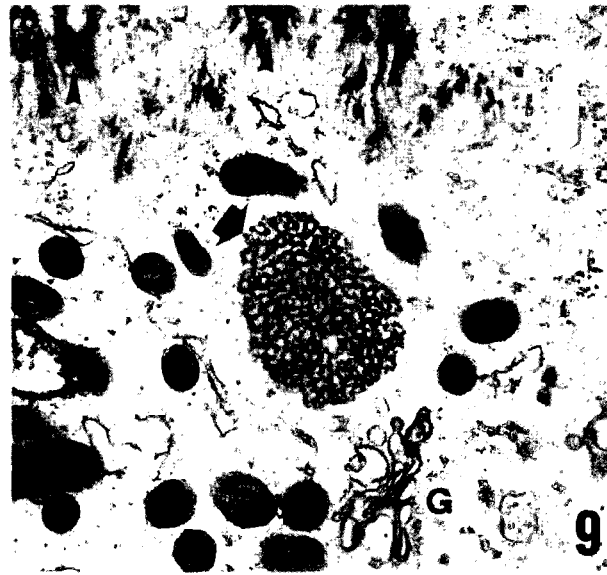
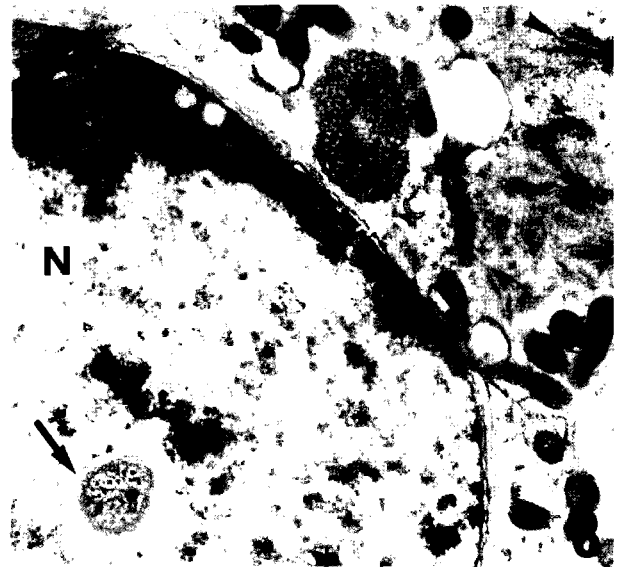
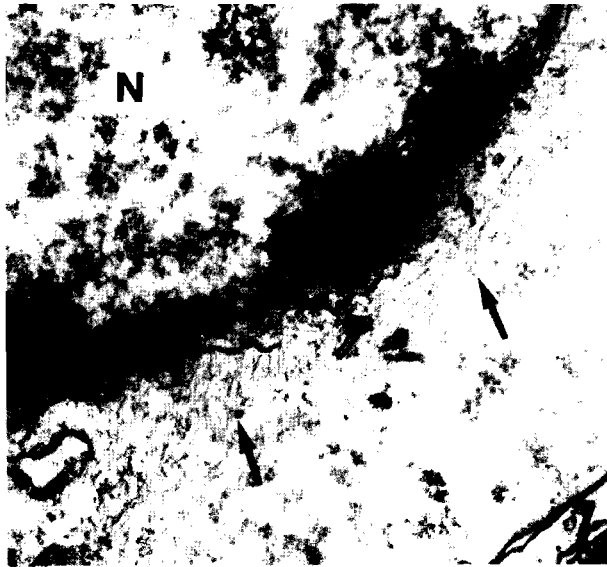


**Fig. 5.** Low power micrograph of one ependymal cell sending two basal processes (P) to pericapillary space. C: capillary. x 4,650



**Fig. 6.** Detail of Fig. 5 showing one terminal swelling of a basal process (P) containing mitochondria, filaments and rough endoplasmic reticulum. Basal membrane (arrows). C: capillary. x 27,500

*Hamster special type of ependymal cell*



### *Hamster special type of ependymal cell*

**Fig. 7.** High power micrograph of ependymal cell nucleus (N). A great number of filaments can be seen (arrows). x 40,500

**Fig. 8.** An NLB (asterisk), mitochondria and basal corpuscles (arrowhead) in the apical part of an ependymal cell. N: nucleus; arrow: nuclear inclusion. x 16,500

**Fig. 9.** An NLB (arrow) in the supranuclear cytoplasm of an ependymal cell. No limiting membrane is observed. Around the body there are aggregates of free ribosomes, mitochondria and basal corpuscles of cilia (arrowheads). G: Golgi dictiosomes. x 28,800

**Fig. 10.** Two NLBs (arrows) separated by a linear group of basal corpuscles (arrowheads). x 28,800

**Fig. 11.** Transversal section of a typical cilia feature of the ependymal cells (arrows). x 57,800

**Fig. 12.** Lateral junctions between ependymal cells. x 34,500

paraventricular nucleus (PVN) of the hamster, the ependymal wall is thin and consists of a single layer of columnar cells. As we previously comment, in other rodents, it has been described as the «light cell zone» without tanocytes (Mitro and Palkovits, 1981).

The existence in the hamster of basal processes in some of those cells approach them to other ependymal specialized cells which are termed tanocytes (Horstmann, 1954). The process of these type of ependymal cells is rather short as compared to the long processes of true tanocytes and unlike these (Rodriguez et al., 1979), no synaptoid contacts were seen between processes and subependymal fibres or cells.

Moreover, tanocytes appear mostly in the central part of the third ventricle of the rat. At this level, their processes connect the third ventricle with the capillary vessels of the median eminence and basal hypothalamus. In relationship with their morphology and topographic situation a possible function of transport, between the ventricle and the brain vessels, has been suggested (Horstmann, 1954; Knigge and Scott, 1970; Millhouse, 1971; Wagner and Pilgrim, 1974; Akmayev and Popov, 1977).

The term «tanocyte» has been fairly confusing. Horstmann (1954) did not include the existence of cilia as a criterion for tanocytes. However, Leonhardt (1980) recommended the term «tanocyte» only for the scarcely ciliated ependymal cell (*kinocilienarme Ependymzelle*) having a basal process. Uryu et al. (1988) studying the ependyma in the posterior mesencephalon of the chicken, concluded that Leonhardt's definition of «tanocytes» is the most appropriate. On the other hand, conventional ependymal cells do not have a basal extension. Therefore, some ependymal cells located at level of the PVN in the hamster third ventricle have both: basal process, with capillary endings and apical cilia. These cells have the structure of both, the tanocyte and the unspecialized cuboidal ependyma; for this reason we could term them «tanocyte-like cells». In this sense, Blinzinger (1962) described, in the lateral ventricles of the hamster, richly ciliated ependymal cells that project basal processes into the subependymal layer. Hirano and Zimmerman (1967) report, in the lateral ventricle of the rat, the presence of a ciliated cell with basal processes that ends on the wall of capillaries. Redecker (1990) reports some tanocyte-like cells in the

median eminence of the gerbil which were labelled with glial fibrillary acidic protein- (GFAP) and vimentin-antibodies, as occurs with classical tanocytes. But the existence of this type of cell at the level of the PVN has never been reported before in the hamster.

The functional significance of the basal processes in these hamster cells is unknown. According to Horstmann (1954) the basal processes could provide mechanical support for the effect of movement or an increase in load on the brain wall. However, the findings of processes that project into subependymal neuropil and contact blood vessels suggests that these cells may act not only as possible supporting element but also as communication pathway between the ventricle and vascular elements.

Some authors give a radically different interpretation to the function of tanocytes, considering them undifferentiated cells which frequently decrease gradually and finally disappear (Tennyson and Papas, 1962). This theory has not been accepted by the majority of the authors, but Gould et al. (1990) consider that tanocytes could mature into ependymal cells developing cilia and losing the basal process. Millhouse (1972) also interpreted tanocytes as undifferentiated cells. For these authors this type of cell aways a signal, on the ependyma, to differentiate into glial or ependymal cells.

The presence of NLBs in the apical cytoplasm of ependymal cells has not been previously reported. The nematosomes or NLBs appear as typical cytoplasmic inclusions in many cell types including hypothalamic neurons (Le Beux, 1972; Van den Pol., 1982; Menéndez and Alvarez-Uría, 1987), neurons of the medulla oblongata (Kishi, 1972), adenohipophysial cells (Hindelang-Gertner et al., 1974), tipulid spermatocytes (Fuge, 1976), embryonic ectodermal cells (Takeuchi and Takeuchi, 1982; Takeuchi and Sonta, 1983) and oocytes (Azevedo, 1984). NLBs observed in other cell types are similar to those described in the present paper. In the literature these inclusions are described as spherical or ovoid masses of granular or filamentous material, mainly associated with cisternae of RER and free ribosomes. Likewise, the close association to mitochondria cannot be considered as fortuitous (Hindelang-Gertner et al., 1974). It has been reported that NLBs are mainly composed of proteinaceous material (Kishi, 1972) but the prevailing idea is that they consist of ribonucleo-

## Hamster special type of ependymal cell

proteins of preribosomal material (Hindelang-Gertner et al., 1974; Takeuchi and Takeuchi, 1982). Several authors consider that NLBs may be a transient storehouse of the necessary ribonucleoproteins for an active and rapid metabolism differentiation processes (Takeuchi and Sonta, 1983; Menéndez and Alvarez-Uría, 1987; López et al., 1990). The presence of NLBs in the apical cytoplasm of some ependymal cells suggests an increased cellular activity and somehow supports the theory of its undifferentiated state.

Some ultrastructural features of the cells described in the present paper: irregular microvilli, surface vesicles, supranuclear prominent Golgi complex, NLBs, etc, suggest a dynamic interaction between these ependymal cells and the cerebrospinal fluid. These morphological characteristics, as was pointed out by Pollay and Curl (1967) and Booz (1975) in true tanycytes, regard the releases of secretory products to the CSF by the ependymal cells.

These findings also point out that other types of ependymal cells, not only true tanycytes, can be implicated in a possible ventricle-blood vessel communication. In spite of this, more morphological and physiological investigations are needed in order to clarify the functional role of these cells.

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### References

- Akmayer I.G. and Popov A.P. (1977). Morphological aspects of the hypothalamic-hypophyseal system. VII. The tanycytes: their relation to the hypophyseal adrenocorticotrophic function. An ultrastructural study. *Cell Tissue Res* 180, 263-282.
- Azevedo C. (1984). Development and ultrastructural autoradiographic studies of nucleolus-like bodies in oocytes of viviparous teleost (*Xyphophorus helleri*). *Cell Tissue Res* 238, 121-128.
- Bleier R. (1971). The relations of ependyma to neurons and capillaries in the hypothalamus: a Golgi-Cox study. *J. Comp. Neurol.* 142, 439-464.
- Bleier R. (1977). Ultrastructure of supraependymal cells and ependyma of hypothalamic third ventricle of mouse. *J. Comp. Neurol.* 174, 359-376.
- Blinzinger K. (1962). Elektronenmikroskopische untersuchungen am ependym der hirventrikel des goldhamsters (*Mesocricetus auratus*). *Acta Neuropathol.* 1, 527-532.
- Brio M.A. del, Riera P., García J.M. and Alvarez-Uría M. (1991). Cell types of the third ventricle wall of the rabbit (*Oryctolagus cuniculus*). *J. Submicrosc. Cytol. Pathol.* 23, 147-157.
- Booz K.H. (1975). Secretory phenomena at the ependyma of the third ventricle of the embryonic rat. *Anat. Embryol.* 147, 143-159.
- Fuge H. (1976). Ultrastructure of cytoplasmic nucleolus-like bodies and nuclear RNP particles in the late prophase of tipulid spermatocytes. *Chromosoma* 56, 363-379.
- Gould S.J., Howard S. and Papadaki L. (1990). The development of ependyma in the human fetal brain: an immunohistological and electron microscopic study. *Dev. Brain Res* 55, 255-267.
- Hannah R.S. and Geber W. (1977). Specializations of the ependyma in the third ventricle of the developing hamster. *Am. J. Anat* 149, 597-603.
- Hindelang-Gertner C., Stoeckel M.E., Porte A., Dellmann H.D. and Madarasz B. (1974). Nematosomes or nucleolus-like bodies in hypothalamic neurons, the subfornical organ and adenohypophysial cells of the rat. *Cell Tissue Res.* 155, 211-219.
- Hirano A. and Zimmerman H.M. (1967). Some new cytological observations of the normal rat ependymal cell. *Anat. Rec.* 158, 293-302.
- Horstmann E. (1954). Die Faserglia des Selachiergehirns. *Z. Zellforsch.* 39, 588-617.
- Jordan F., Rieke G. and Thomas W. (1987). Presence and development of ependymal cells in primary tissue cultures derived from embryonic rat cerebral cortex. *Dev. Brain Res.* 35, 97-110.
- Kishi K. (1972). Fine structural and cytochemical observations and cytoplasmic nucleolus-like bodies in nerve cells of the medulla oblongata of the rat. *Z. Zellforsch.* 132, 523-532.
- Knigge K.M. and Joseph S.A. (1974). Thyrotropin releasing factor (TRF) in cerebrospinal fluid of the third ventricle of the rat. *Acta Endocrinol.* 76, 209-213.
- Knigge K.M. and Scott D.E. (1970). Structure and function of the median eminence. *Am. J. Anat.* 129, 223-244.
- Kuenzel W.J. and Van Tienhoven A. (1982). Nomenclature and location of avian hypothalamic nuclei and associated circumventricular organs. *J. Comp. Neurol.* 206, 293-313.
- Le Beux Y.J. (1972). An ultrastructural study of a cytoplasmic filamentous body, termed nematosomes, in the neurons of the rat and cat substantia nigra. The association of nematosomes with other cytoplasmic organelles in the neuron. *Z. Zellforsch.* 133, 289-325.
- Leonhardt H. (1980). Neuroglia. In: *Handbuch der mikroskopischen Anatomie des Menschen.* Oksche A. and Vollrath L. (eds). Springer Verlag, Berlin, Heidelberg. pp 177-666.
- López J., Tolivia J., Diaz C. and Alvarez-Uría M. (1990). Ultrastructural study of lamellar and nucleolus-like bodies in the Harderian gland during postnatal development of the master (*Mesocricetus auratus*). *Anat. Rec.* 228, 247-254.
- Menéndez A. and Alvarez-Uría M. (1987). Nucleolus-like bodies in the posterior subnucleus of the rat paraventricular nucleus during postnatal development. An ultrastructural cytochemical and morphometric study. *J. Submicrosc. Cytol.* 19, 101-105.
- Millhouse O.E. (1971). A Golgi study of the third ventricle tanycytes in the adult rodent brain. *Z. Zellforsch.* 121, 1-13.
- Millhouse O.E. (1972). Light and electron microscopic studies of the ventricular wall. *Z. Zellforsch.* 127, 149-174.
- Millhouse O.E. (1975). Lining of the third ventricle in the rat. In: *Brain endocrine interaction. II. The ventricular system.* 2nd. Int. Symp. 1974. Karger, Basel. pp 3-18.
- Mitro A. and Palkovits M. (1981). The third ventricle. In: *Morphology of the rat brain ventricles, ependyma and periventricular structures.* Bibliotheca anatomica n.21. S. Karger (ed). Basel. New York. pp 48-84.
- Paul E. (1968). Histochemische studien an dem Plexus choroidei, an der Parphyse und am Ependym von *Rana temporaria* L. *Z. Zellforsch.* 91, 519-546.
- Pollay M. and Curl F. (1967). Secretion of cerebrospinal fluid by the

*Hamster special type of ependymal cell*

- ventricular ependyma of the rabbit. *Am. J. Physiol.* 213, 1013-1038.
- Redecker P. (1990). The glial architecture of the median eminence of the Mongolian gerbil (*Meriones unguiculatus*); a study of glial fibrillary acidic protein (GFAP) immunoreactivity in semithin sections. *Acta Histochem.* 88, 139-147.
- Rodríguez E.M., González C.B. and Delannoy L. (1979). Cellular organization of the lateral and postinfundibular regions of the median eminence in the rat. *Cell Tissue Res.* 201, 377-408.
- Scott D., Krobisch-Dudley G., Paull W.K., Kozlowski G.P. and Ribas J. (1975). The primate median eminence. I. Correlative scanning-transmission electron microscopy. *Cell Tissue Res.* 162, 61-73.
- Takeuchi I.K. and Sonta S.I. (1983). Silver staining of the nucleolus-like bodies in postimplantation rat embryos and the nudge in chinese hamster oocytes. *J. Electron Microsc.* 32, 176-179.
- Takeuchi I.K. and Takeuchi Y.K. (1982). Ultrastructural and cytochemical studies on nucleolus-like bodies in early post-implantation rat embryos. *Cell Tissue Res.* 226, 257-266.
- Tennyson V.M. and Papas G.D. (1962). An electron microscope study of ependymal cells of the fetal, early postnatal and adult rabbit. *Z. Zellforsch. Mikrosk. Anat.* 56, 595-618.
- Tolivia J., Navarro A. and Tolivia D. (1994). Polychromatic stain of epoxy semithin sections: a new and simple method. *Histochemistry* 101, 51-55.
- Uemura H., Asai T., Nozaki N. and Kobayashi H. (1975). Ependymal absorption of luteinizing hormone releasing hormone injected into the third ventricle of the rat. *Cell Tissue Res.* 160, 443-452.
- Uryu K., Hirunagi K. and Fujioka T. (1988). Specialized ependyma in the posterior mesencephalon of the chicken: the fine structure of the subsubtrochlear organ. *Cell Tissue Res.* 254, 531-538.
- Van Den Pol A.N. (1982). The magnocellular and parvocellular paraventricular nucleus of the rat: intrinsic organization. *J. Comp. Neurol.* 206, 317-345.
- Wagner H.J. and Pilgim C.H. (1974). Extracellular and transcellular transport of horseradish peroxidase (HRP) through the hypothalamic tanycyte ependyma. *Cell Tissue Res.* 152, 477-491.

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