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# Combined TEM and SEM analysis of the rostral wall of the human III ventricle

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**Summary.** A combined morphological study using transmission and scanning electron microscopy was carried out on the central zone of the rostral wall of the III ventricle from a sample taken during the operation of a patient with a retrochiasmatic craniopharyngioma. Under the scanning electron microscope, the apical cell wall of the ependymocytes generally appeared folded and lack in ciliae, but with numerous microvilli developed to different extents. The flat surface cells were scarce and were polygonal in shape and limited by 3-7 prominent edges. Ultrastructural analysis revealed the existence of several types of ependymocytes and subependymal epithelial cells arranged in different layers; in both layers the presence of abundant neurofilaments and intercellular junctions was striking.

**Key words:** Ependyma, III Ventricle, Humans, SEM, TEM

## Introduction

In recent years, the ependymal cells lining the lumen of the III ventricle have acquired great functional importance since groups of cells such either form specialized areas such as the circumventricular organs or otherwise contain tanycytes that participate in processes of secretion and/or transport between the ventricular wall and the cerebrospinal fluid (CSF) (Weindl and Joynt, 1972; Scott and Krobisch-Dudley, 1975; Kozlowski and Coates, 1985).

The epithelium of the middle ventricle has been analyzed in many animal species using different techniques and under different experimental conditions (Brightman and Palay, 1963; Kobayashi et al., 1970; Vigh-Teichmann et al., 1971; Weindl and Joynt, 1972; Scott et al., 1972, 1974; Coates, 1977, 1978; Akmayev and Popov, 1977; González-Santander, 1979; Kotrschal et al., 1985; Riesco et al., 1988).

In humans, such studies are difficult to carry out and hence few works have been published; some of them were done on autopsy material with the consequent phenomenon of cell autolysis (Bruni et al., 1972; Flament-Durand et al., 1978). We therefore believe that the description offered here, using the combined techniques of scanning and transmission electron microscopy (SEM and TEM), of a sample obtained intraoperatively may be of interest for gaining further insight into the epithelium lining the rostral portion of the III ventricle.

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## Materials and methods

The material employed was from a 10-year-old boy operated on a retrochiasmatic supraselar craneopharyngioma removed through the ventricular pathway via the right interventricular foramen. During the operation a sample was taken from the central portion of the rostral wall of the III ventricle, as far as possible from the tumor; under the surgical microscope, it had normal morphological characteristics.

Immediately after removal, the piece was washed in isotonic saline and cut for TEM and SEM studies. The first steps in processing were the same for both techniques: fixing by immersion in 5% glutaraldehyde in phosphate buffer (0.1M, pH=7.4) postfixing in 1% osmium tetroxide in the same buffer, and dehydration in a progressive acetone series. Later, treatment varied according to the fate of the samples.

In the case of SEM, the dehydrated pieces were dried by the critical point method on a Polaron E-3000 apparatus and mounted on a slide holder with colloidal silver. After sputtering on a coat of gold (20 nm), the pieces were observed under a Philips SEM-500 scanning electron microscope.

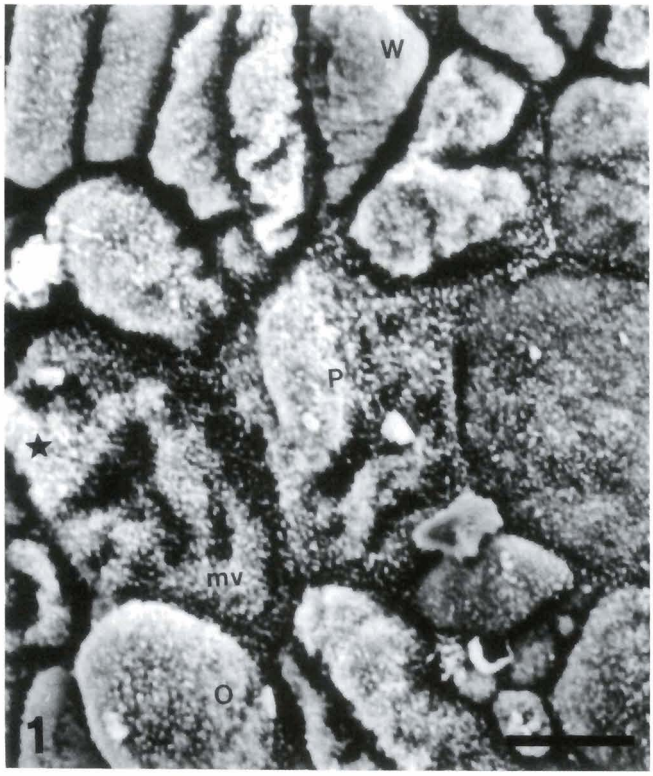
For the ultrastructural analysis, the dehydrated samples were embedded in araldite. They were then cut into ultrathin sections (45 nm) on an LKB ultramicrotome. These pieces were mounted in grids and contrasted with the Reynolds' method (1963).

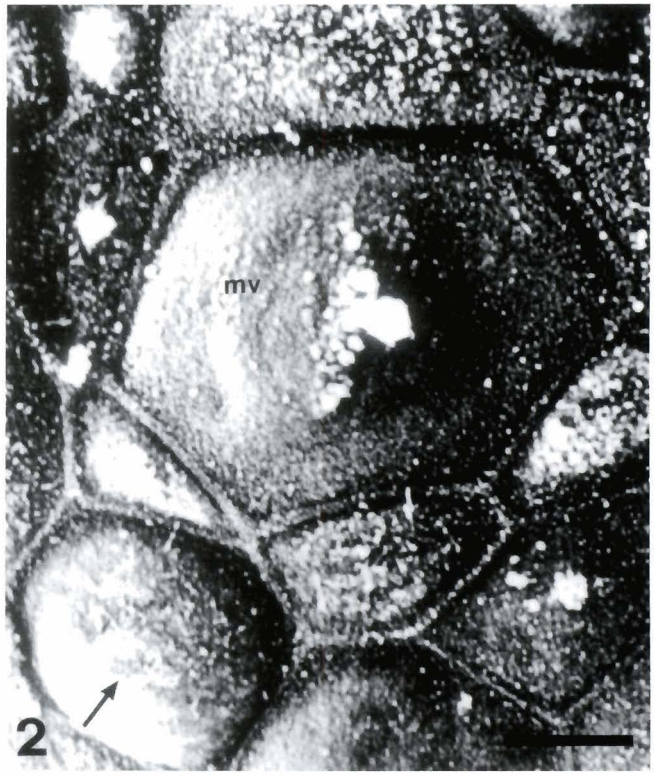
Observation was carried out under a Philips EM-201 transmission electron microscope.

## Results

*Scanning electron microscopy.* The rostral ependyma of the III ventricle showed an aciliate epithelium, with a predominance of folded apical cellular surfaces, covered with microvilli and limited by depressed edges.

The irregular undulated contours of the cell surfaces could be seen, some of these arranged like a wedge among neighbouring cells (Fig. 1). The microvilli



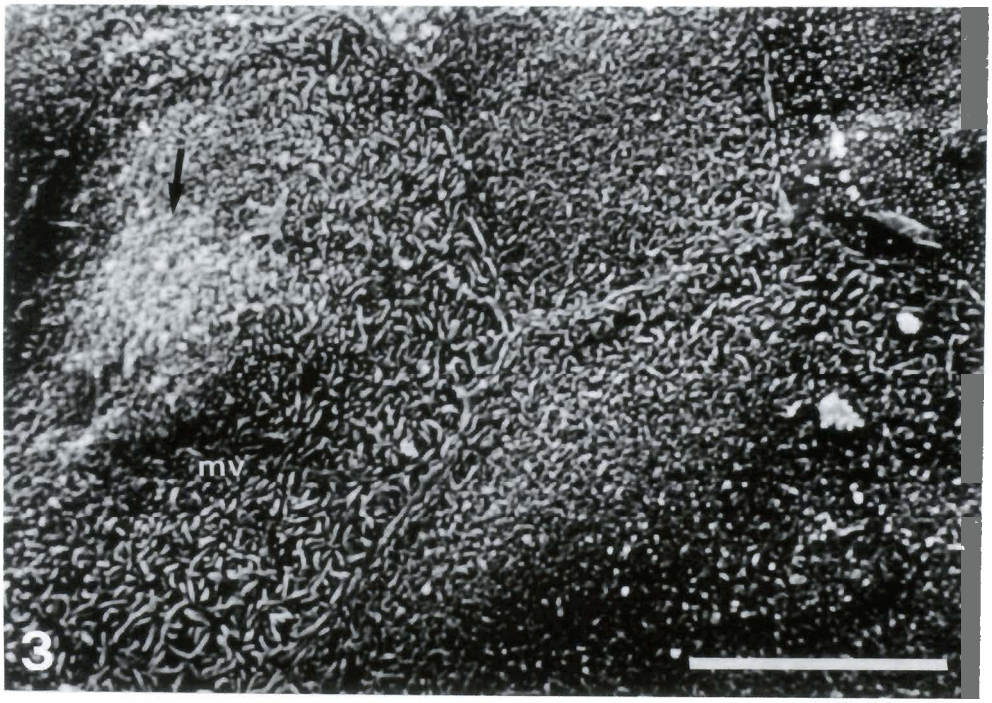
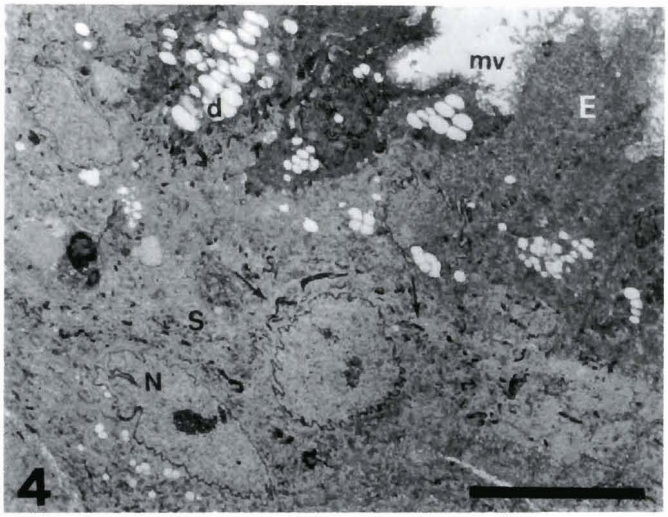




**Fig. 1.** Detail of the ventricular ependymal surface, folded and covered with microvilli that are irregular in size and density. The surface morphology, bordered by the depressed intercellular edges, varies from circular (O) to polygonal cells (P), though with a predominance of wedge-shaped cells (W). The asterisk shows the velvet-like aspect of some ependymocytes due to the presence of microvilli (mv). Bar = 10 μm. x 7,500

**Fig. 2.** Polygonal ependymocytes with 3-7 clearly visible edges outstanding and a smooth apical membrane whose centre harbours microvilli (mv) or which exhibits cupola-like protrusions (arrow). Bar = 10 μm, x 7,500.

**Fig. 3.** Detail at greater magnification of the microvilli (mv) and of a protrusion (arrow) in the centre of the smooth ventricular membrane of a polygonal ependymocyte. Bar = 1 μm. x 15,000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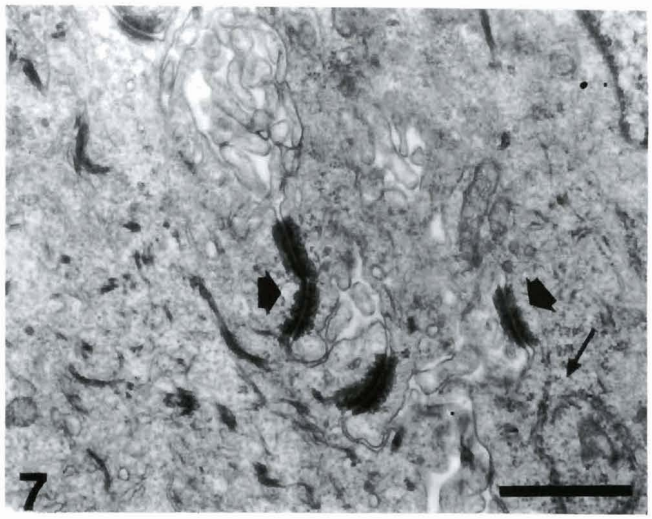
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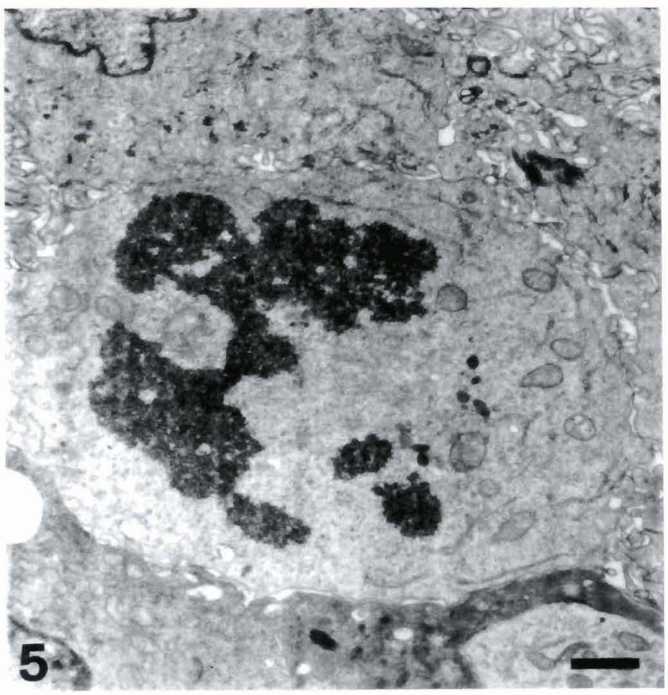
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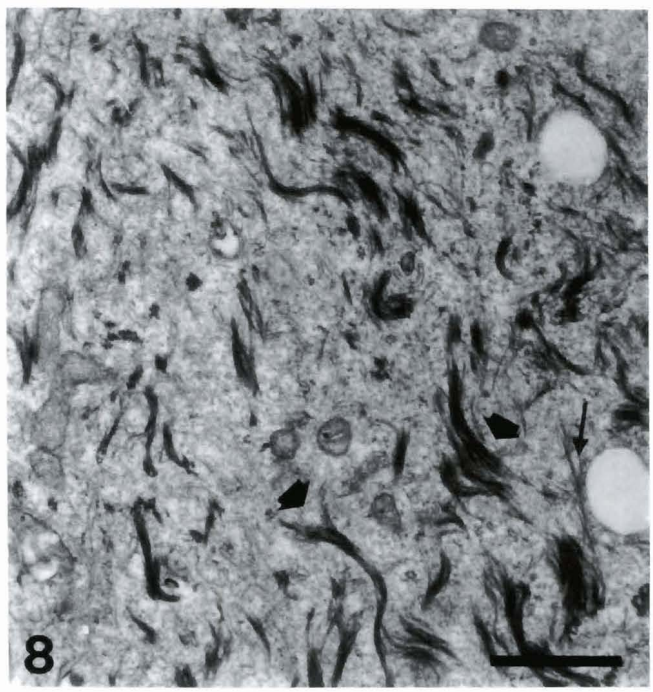
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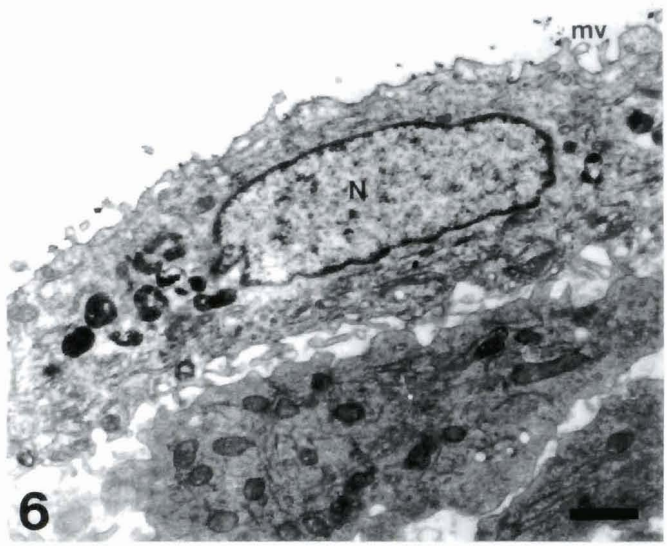
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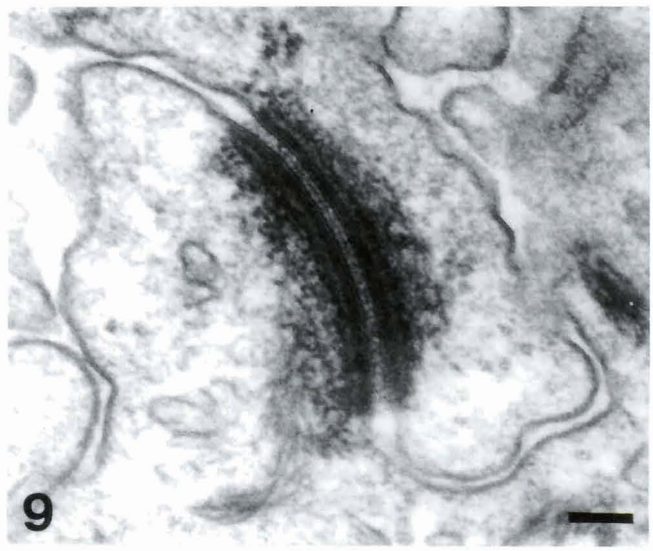
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covered the whole of the apical surface and in some cells the greater size and density of the microvilli conferred a velvet-like aspect on the epithelial surface. Only in isolated areas were polygonal cell surfaces found; these were flat, aciliate, with short microvilli and were limited by 3-7 clear edges standing out from the ventricular cavity (Fig. 2). Occasionally, the cells with a flat lumen surface exhibited a cupola-like protrusion with pointed microvilli (Fig. 3).

*Transmission electron microscopy* evidenced an epithelium composed of an aciliate ependymal layer and several strata of subependymal cells (Fig. 4) with a few mitoses (Fig. 5).

The ependymal layer had different cell types in contact with the ventricular lumen. Some ependymocytes had large, irregular nuclei, poor in chromatin and with a clearly visible nucleolus (Fig. 4); the apical membrane had small microvilli and the cytoplasm was poor in organelles. Figure 4 shows two varieties of this cell type: a) ependymocytes, similar to the former kind, but with abundant lipid droplets on the basal pole; b) ependymocytes with the apical membrane projected towards the ventricular lumen and arborized by large numbers of microvilli, under which some small apical vesicles could be observed.

The scarce flat areas observed under the SEM corresponded to ultrastructural images in which the ependymocytes were extremely flat, with a hypochromatic nucleus arranged horizontally (Fig. 6). The apical membrane exhibited very short microvilli and the cytoplasm contained numerous dense bodies and cisternae of rough endoplasmic reticulum.

The subependymal cells, which resembled one another, featured a large nucleus; this was poor in chromatin and had a clearly visible nucleolus (Fig. 4). Among the cytoplasmic organelles the mitochondria were outstanding, with a somewhat electron-translucent matrix and few cristae and the neurofilaments (Figs. 7, 8); they also had some cisternae of rough endoplasmic reticulum and free ribosomes (Fig. 7).

Both in the ependymal and in the subependymal layers abundant junction complexes were observed; these were of the *macula adherens* type and were intimately related with sheaves of neurofilaments (Figs. 7, 9).

## Discussion

The ependyma of mammals had numerous and different types of cell surfaces. However, the SEM studies carried out by Clementi and Marini (1972) and Scott et al. (1974) allowed these authors to reduce the types of cellular surfaces present in the ventricular cavities to four: a) ciliate ependymocytes: b) monociliate ependymocytes, with numerous microvilli; c) ependymocytes with vesicular formations and with a peripheral border of microvilli; and d) smooth ependymocytes, with some microvilli arranged peripherally.

In its caudal third, the ependymal lining of the III ventricle of humans belongs to the ciliate type, while in the middle and -above all the rostral- thirds there is a predominance of extensive ciliate zones; the separation between both is signalled by a broad oligociliate band (Bruni et al., 1972; Allen et al., 1978; Flament-Durand et al., 1978); in this sense the transition is not as pronounced as in rodents (Brightman and Palay, 1963; Scott et al., 1972; Allen et al., 1978; Flament-Durand et al., 1978; Bleier and Siggelkow, 1986; Riesco et al., 1988).

Among all mammals, humans exhibit the greatest extension of aciliate areas at the rostral level of the III ventricle (Bruni et al., 1972; Allen et al., 1978; Flament-Durand et al., 1978). In our study the whole piece analysed had an aciliate ependyma and we believe that the lack of oligociliate zones of transition might be due to the limited extension of the sample owing to the prevailing surgical possibilities.

The rostral wall morphology of the human III ventricle, obtained by the intraoperative biopsy has modifications with regard to the one existing in the pieces coming from autopsies, probably due to the preparative techniques of tissue several hours after death

**Fig. 4.** Transmission electron micrograph of the ependymal layer (E) and subependymal strata (S) of the rostral wall of the human III ventricle. The ependymocytes display different shapes, electron densities and cytoplasmic organelles. Outstanding among the subependymal cells are the cellular nuclei (N), with clearly visible nucleoli and abundant neurofilaments. d: lipid droplets; mv: microvilli; arrow: neurofilaments. Bar = 10 μm. x 2,350

**Fig. 5.** Cell located in the subependymal stratum in the process of mitosis. Bar = 1 μm. x 9,000

**Fig. 6.** Flat ependymocyte, with nucleus (N) arranged horizontally. The apical membrane is fairly smooth and exhibits a scanty development of microvilli (mv). The morphology of the apical membrane and the flattened aspect of the cell is in agreement with the images represented in Figs. 2 and 3 obtained with the SEM. Bar = 1 μm. x 9,000

**Fig. 7.** Subpendymal cells, with abundant ribosomes either free or associated with cisternae of the endoplasmic reticulum (thin arrow). Note the existence of several *maculae adherentia* on the intercytoplasmic membrane (thick arrow). Bar = 1 μm. x 17,500

**Fig. 8.** Note abundance of isolated neurofilaments (thin arrow) or in sheaves (thick arrow) in subependymal cells and the presence of mitochondria with a somewhat electron-lucent matrix and few cristae. Bar = 1 μm. x 17,500

**Fig. 9.** Ultrastructural image of a *macula adherens*, common both in the junctions among ependymocytes and in the intercytoplasmatic junctions of the subependymal cells. Bar = 0,1 μm. x 82,400

with the consequent distortion of tissue surfaces and autolysis. Although the folded aspect of the ependymal cellular surface, covered with microvilli together with the sunken aspect of the cell edges are in agreement with the SEM descriptions made in human ventricles by Bruni et al. (1972), Allen et al. (1978), Flament-Durand et al. (1978) and Polak et al. (1988), the cells with a stretched surface have not been described in the rostral wall and only Flament-Durand et al. (1978) described them in very exiguous areas of the ventricular surface.

Two observations were of interest in the morphology analyzed by us: the lack of supraependymal cells and/or fibres and the presence of cupola-like protrusions on the apical membrane of some of the polygonal and flat ependymocytes. Bruni et al. (1972) and Flament-Durand et al. (1978) did not observe supraependymal cells in the III ventricle of humans either; by contrast, Allen et al. (1978) reported their presence both in humans and in other animals. In previous works we have observed their existence in the rostral wall of the middle ventricle of the dog, cat and rat (Riesco et al., 1988).

The presence of vesicles on the apical membrane of the aciliate ependymocytes is well documented in different species: man; monkeys; sheep; cats; rodents (Leonhardt and Lindner, 1967; Knowles and Kumar, 1969; Bruni et al., 1972; Flament-Durand et al., 1978). The functional meaning of these structures remains to be elucidated. Currently, they are not thought to be artefacts but rather the morphological expression of particular functional situations (Millhouse, 1975) or as substances existing between the ependymocyte and the CSF (Knigge and Scott, 1970; Phillips et al., 1977; Kiktenko, 1986; Ratjova and Odehnal, 1989).

The morphological variations in the ependymocytes observed in the ultrastructural study have also been reported by other authors in different animal species (Rodríguez, 1969; Bruni et al., 1972; Mikami, 1975; Millhouse, 1975; González Santander, 1979). However, in the present study we did not observe the presence of either tanycytes or neuronal ependymocytes, and therefore believe that the differences observed must be due to different functional cellular states.

The abundance of neurofilaments and also of intercellular junctions of the *macula adherens* type, both in the ependymal layer and in the subependymal strata are in agreement with the data reported for the ependymal epithelia of other mammal species (Wagner and Pilgrim, 1974; Nakai and Naito, 1975).

Mitoses were only observed -and then in small numbers- at the level of the subependymal layers. In this sense, Aikawa and Suzuki (1986) have suggested that mitoses would only appear in the ependymal layer under pathological conditions, while they do appear -and with a certain degree of frequency- in the subependymal strata, specially in young animals; with age their numbers gradually decrease (Sturrock, 1985). In our own case, the age of the patient would have favoured the appearance of mitoses and although we are unaware of the fate of these, their location immediately below the ependymocytes seems to point to a substitutional role for these cells.

According to the results obtained in the TEM and SEM studies, it may be concluded that the rostral wall of the III ventricle in humans is mostly formed of aciliate ependymocytes, with irregular surfaces covered with microvilli and variable ultrastructural characteristics depending on their functional state. The absence of specialized cells, tanycytes or neuronal ependymocytes, and/or supraependymal structures and also of oligociliate zones may be due to the limitations imposed in the collection of the sample during the operation.

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