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Ultrastructure of the mouse spinal cord ependyma

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INTRODUCTION

Extensive studies on the ultrastructure of the brain ventricular ependyma have revealed substantial regional morphological differences (see Leonhardt, 1980 and Low, 1982 for reviews). However, no conclusive answer regarding the function(s) of the ependyma in unspecialized areas has been gained. Diffusion barrier, secretion, mechanical support, transcellular and ciliary transport are among functions proposed. The ependyma of the central canal has only been investigated to a minor degree and, to our knowledge, only Seitz, Lohler & Schwendemann (1981) and Sturrock (1981) have reported on the particular ultrastructure in mice. Recent findings in our laboratory made us believe some of the reported observations by Sturrock to be due to inadequate preservation of the ependyma (Bjugn, Haugland & Flood, 1988). It is the purpose of the present communication to provide a morphological description of well-preserved spinal canal ependyma of the mouse along with a discussion of functional aspects.

MATERIAL AND METHODS

Eight female, albino mice (Bom: NMRI, obtained through SIFF, Oslo, Norway) weighing from 30 to 40 g, with free access to water and standard pelleted food, were used. These were selected for their excellent tissue preservation from a total of 72 mice used in a study on the preservation of the mouse spinal canal ependyma (Bjugn *et al.* 1988). All animals were anaesthetised with an intraperitoneal injection of Equithesin® (1 ml/100 g body weight). They were then subjected to vascular perfusion fixation and postfixation using a modified Tyrode's solution (Romeis, 1948), containing 50% of normal NaCl amount, 0·06 M sucrose, 2% dextran T-40 and 3% glutaraldehyde (pH 7·2, 37 °C, 550–560 m-osmol). After fixation the spinal cord was isolated. Transverse sections from the mid-cervical, mid-thoracic and mid-lumbosacral level were cut and prepared for transmission electron microscopy (TEM) as described elsewhere (Bjugn *et al.* 1988).

Specimens for scanning electron microscopy (SEM) were dehydrated in increasing concentrations of acetone and critical point dried using liquid CO_2 as a transitional medium. The specimens were cracked open, exposing the central canal, with the aid of a razor blade. They were then mounted on specimen supports, coated by approximately 20 nm thermally-evaporated gold–palladium alloy (60/40) and examined in a Jeol T 200 scanning electron microscope.

RESULTS

In well-preserved specimens the ependyma lined a cylindrical central canal at all levels of the spinal cord (Figs. 1, 6). Within the central canal and along its entire length

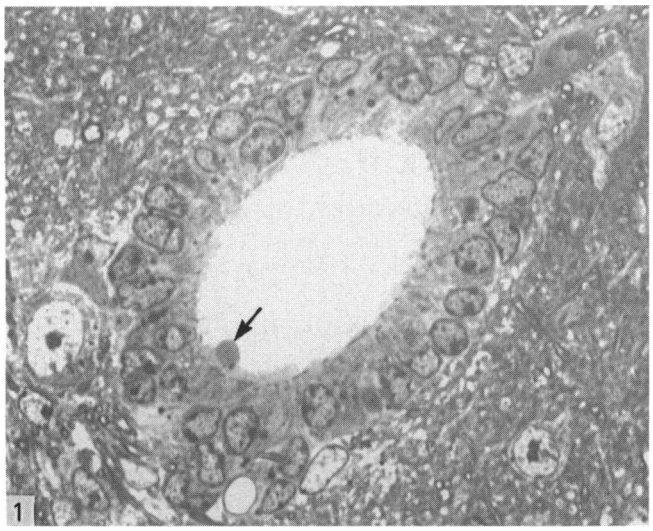

Fig. 1. Light micrograph of well-preserved mouse central canal ependyma from the cervical level. Arrow points to a supraependymal neuronal bulb. × 1000.

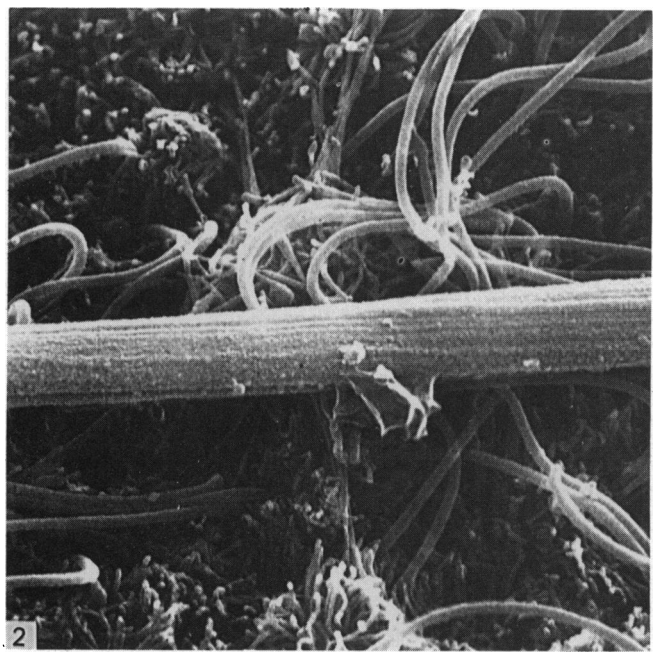

Fig. 2. Scanning electron micrograph of Reissner's fibre in the mouse central canal. × 10000.

Reissner's fibre was present. The diameter of the fibre ranged between 0·4–1·1 μm and the surface showed fine longitudinal stripes in SEM (Fig. 2). No cells were observed in the lumen.

The ependyma surrounding the central canal had the features of a simple, cuboidal epithelium. The nuclei of neighbouring cells were situated at various distances from the lumen. This was especially evident along the dorsoventral axis, where the

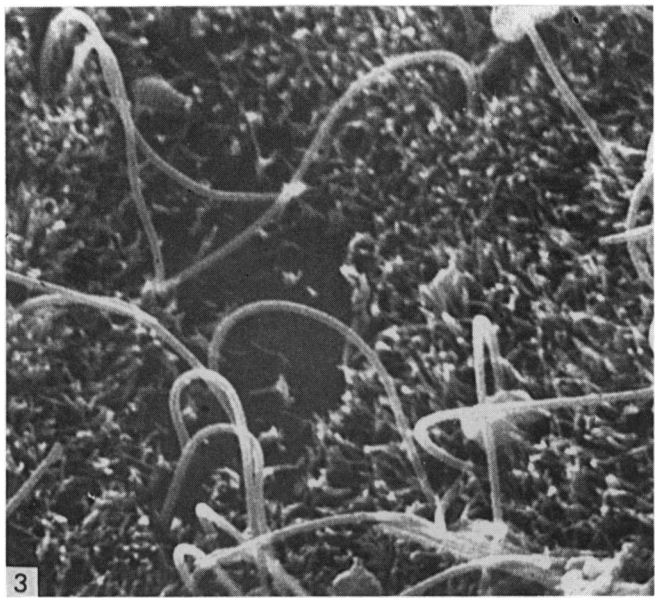

Fig. 3. Scanning electron micrograph of the mouse central canal showing microvillar-poor area devoid of kinocilia. ×8000.

ependyma had a pseudostratified appearance (Figs. 1, 6). In a few instances blood capillaries were located within the ependymal epithelium.

When studying the cerebrospinal fluid-contacting surface of the ependyma, several microvilli-covered cytoplasmic protrusions gave the impression of small microvillar 'bushes' bulging into the lumen. Individual cells were seen to have a highly variable number of kinocilia. Of the kinociliary-poor cells, a few were, in addition, almost free of microvilli (Fig. 3). There was no regular pattern of distribution or intracellular differences between cells containing various numbers of kinocilia. The kinocilia were about 0·35 μm in diameter and rarely longer than 10 μm.

Supraependymal neuronal bulbs, containing mitochondria, empty and dense-core vesicles and neurofilaments were regularly seen in sections from all segments. These bulbs were globular structures, 3·2–5·2 μm in diameter. About one third of the bulbs were seen to be in continuity with subependymal somata (cerebrospinal fluid-contacting neurons), rich in granular endoplasmic reticulum (Fig. 4). One third of the bulbs were in contact with ependymal cells by means of zonula adhaerens-like junctions (Fig. 5) and one third appeared free in the lumen next to the ependymal surface. Zonula occludens-like junctions were observed between the bulbs and the ependymocytes (Fig. 5).

Apical plasmalemma covered protrusions/luminal vesicles, devoid of microvilli and with a cytoplasmic-like matrix containing ribosomes and/or empty-looking vesicles were observed at all levels of the spinal cord examined (Fig. 6).

The apical part of the lateral surface of the ependymocytes was usually, but not always, connected by a junction (zonula occludens or gap junction, see below) followed by a number of zonulae adhaerentes further down (Fig. 7). The junction appeared either as a 0·2–0·7 μm long section of closely apposed parallel cell membranes, presumably representing a gap junction, or as a single very short segment of partial membrane fusion, probably forming a zonula occludens. More basally,

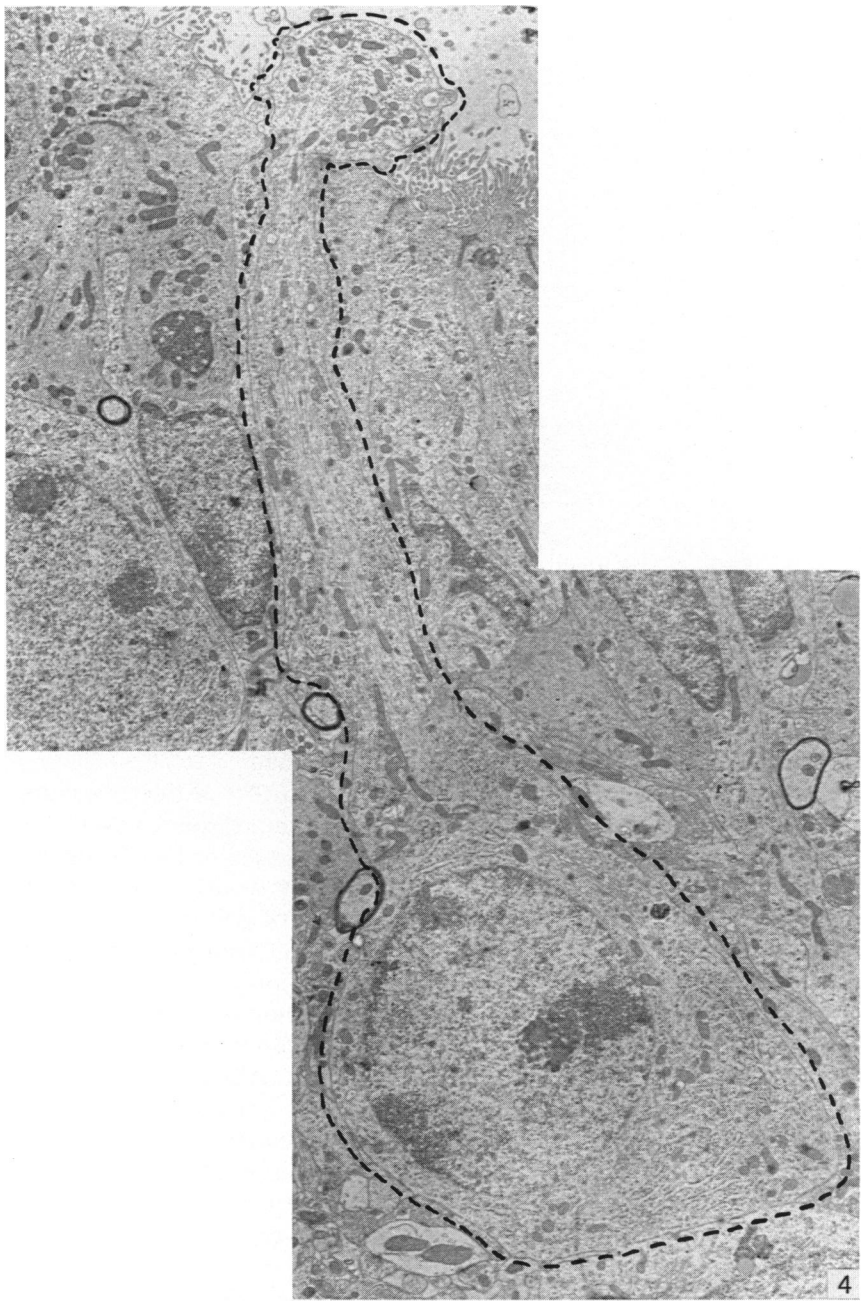

Fig. 4. Transmission electron micrograph of a cerebrospinal fluid-contacting neuron in the mouse spinal cord. The cellular outline is indicated. ×4500.

extensive gap junctions were the predominant junctional structure (Fig. 7), but fasciae adhaerentes were also observed.

The basal surface of the ependymocytes was rather irregular and in contact with the neuropil (Fig. 6). Basal processes indicative of tanycytes were infrequently observed. No synapse-like contacts were observed between elements of the neuropil and the ependymocytes.

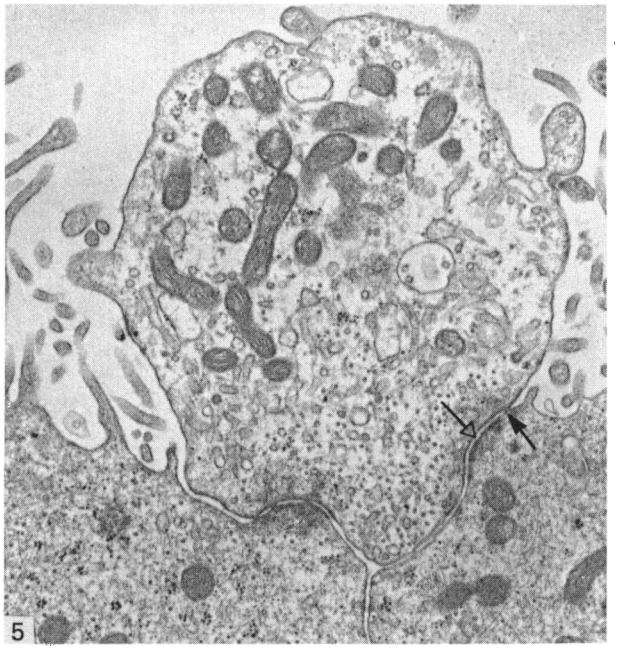

Fig. 5. Transmission electron micrograph of the mouse central canal showing different contacts between a supraependymal neuronal bulb and the ependyma. Open arrow points to a zonula adhaerens. Closed arrow points to a zonula occludens-like junction. ×20000.

Except for the kinocilia, the ependymocytes revealed no dominant organelle features pointing to a specific function. Mitochondria of the crista type were small and ovoid-looking and were the most conspicuous organelle. They were mostly dispersed throughout the apical part of the cytoplasm (Fig. 6). The endoplasmic reticulum was inconspicuous, the rough type being seen most frequently. The Golgi apparatus was rather elaborate and located in the middle and apical part of the cell. Its longitudinal axis was parallel to the apical-basal axis of the ependymocyte. Each Golgi apparatus consisted of five to ten cisterns. Lipid droplets were observed in the middle and basal part of some ependymocytes. The nucleus was located in the mediobasal part of the cell and contained predominantly euchromatin with heterochromatin along the nuclear envelope.

DISCUSSION

In the present study, the ultrastructure of the ependymocytes in the central canal was found to be essentially identical to that described earlier in the spinal cord of the mouse (Seitz *et al.* 1981) and rat (Bruni & Reddy, 1987). Furthermore, the ultrastructure was nearly identical to the cuboidal ependymocytes in the unspecialised areas of the ventricles (see Leonhardt, 1980; Low, 1982). No regional variations in ependymal structure were found along the length or circumference of the central canal. This is in contrast to the especially rich distribution of kinociliary-poor cells in the ventromedial part of the central canal in the rabbit and rat as described by Leonhardt (1980) and in contrast to the ependyma of the brain ventricular system in mice (see Leonhardt, 1980; Low, 1982).

In all segments examined the central canal was found to be round to ovoid in cross section and, apart from the luminal vesicles/apical protrusions, free of amorphous

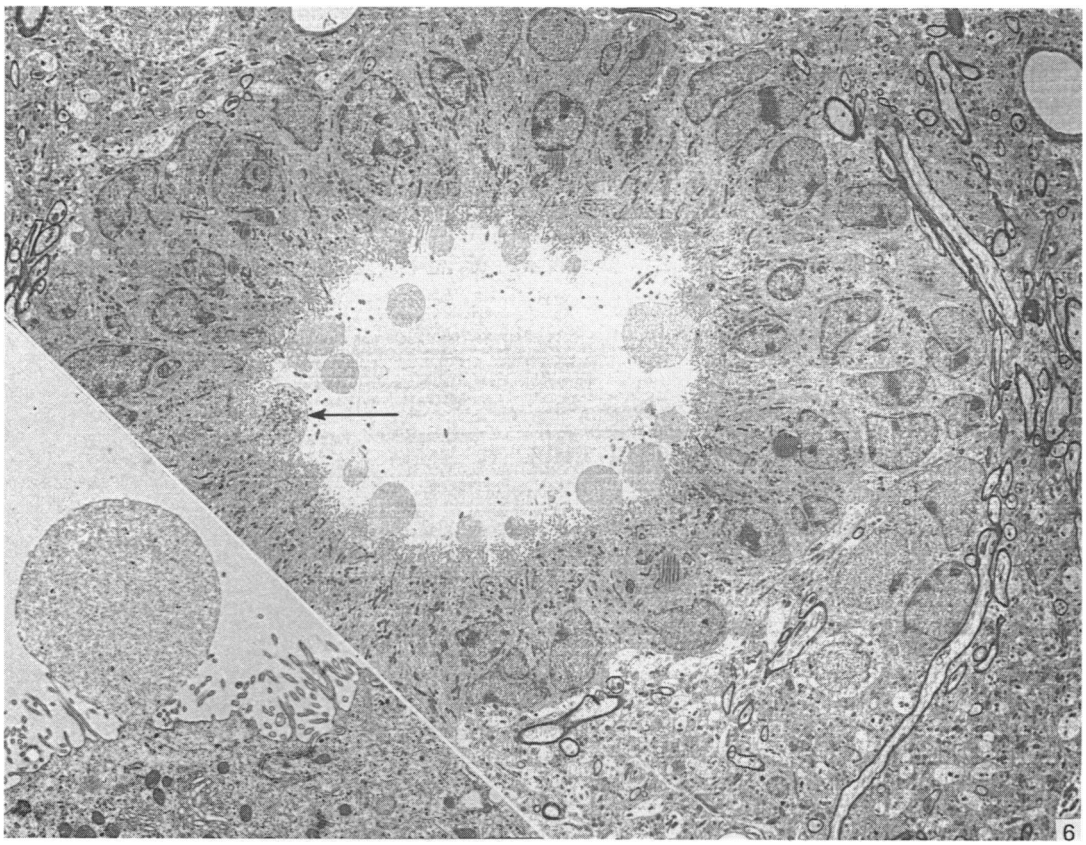

Fig. 6. Transmission electron micrograph of mouse spinal canal showing luminal vesicles/apical protrusions at the lumbosacral level. Arrow points to a supraependymal neuronal bulb. × 1600. Inset shows detail of ependyma and an apical protrusion. × 5400.

material. This is in accordance with earlier findings in mice (Kuwamura, McLone & Raimondi, 1978; Seitz *et al.* 1981) and other mammalian species (Tulsi, 1982; Rodriguez *et al.* 1985). We contend that the material-filled lumen described below the cervical level by Sturrock (1981) is caused by inadequate fixation (Bjugn *et al.* 1988). In the mouse (Sturrock, 1981) and rat (Bruni & Reddy, 1987) the central canal has been described as being collapsed in the lower segments of the spinal cord. In animals showing inadequate tissue preservation we have earlier found the central canal to be partially collapsed. However, in animals showing good tissue preservation, the central canal has always been round to ovoid in cross section at all levels examined (Bjugn *et al.* 1988). Reissner's fibre was seen to be continuous down to the lumbosacral level, as described in other mammalian species (Tulsi, 1982; Castenholz, 1984; Rodriguez *et al.* 1985). The proposal by Sturrock (1984) that Reissner's fibre contributes to the amorphous material observed by him in the mouse central canal cannot be supported by our findings.

In the present study, zonula occludens-like junctions represented 40–50% of the most apical junctions in the specimens. No zonulae occludentes were observed in the mouse (Seitz *et al.* 1981) or rat (Bruni & Reddy, 1987) spinal cord ependyma. Brightman & Reese (1969) and Mack, Neuhaus & Wolburg (1987) contend that the ordinary rat ventricular ependymal cells are devoid of zonulae occludentes. The

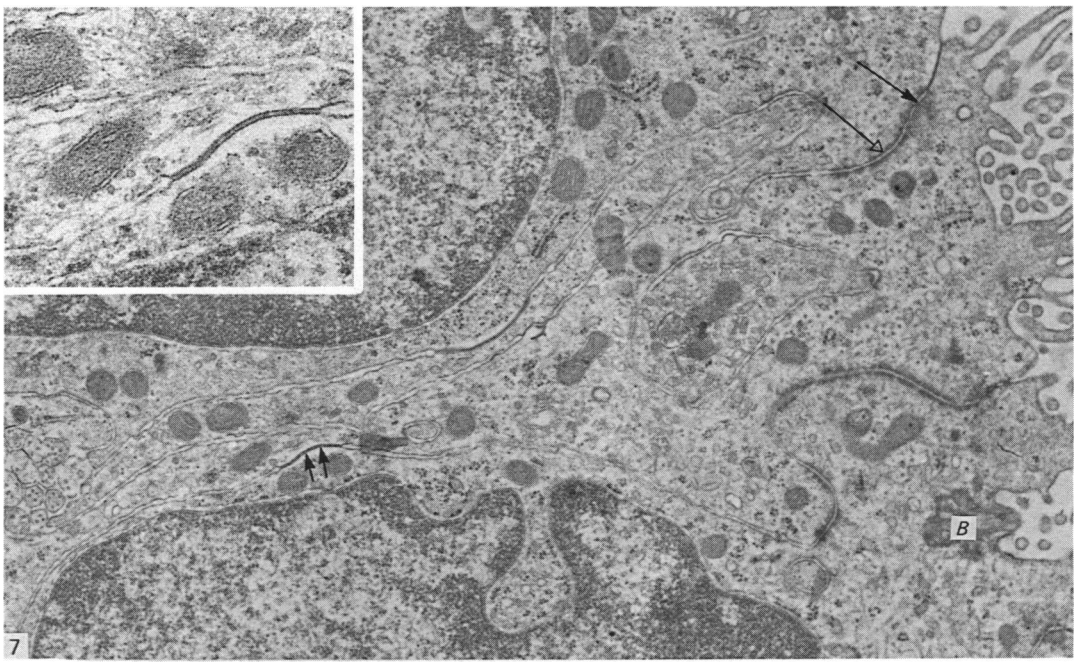

Fig. 7. Transmission electron micrograph of mouse spinal cord ependyma showing various junctional types along the lateral border of the ependymocytes. Arrow points to a zonula occludens-like junction. Open arrow points to a zonula adherens. Double arrow points to a gap junction. *B* indicates basal foot of cilia. ×16000. Inset shows larger magnification of the indicated gap junction. ×40000.

junctions described by us to be zonula occludens-like (Figs. 5, 7), are similar in appearance to the structures named 'tight junctions' by Brightman & Reese (1969). In order to investigate whether our observations of these junctions represent genuine zonulae occludentes, further studies using freeze-fracturing will have to be done. However, whatever the nature of these junctions, it is unlikely that the ependyma constitutes a significant barrier between the cerebrospinal fluid and the interstitial fluid of the nervous tissue.

A considerable number of gap junctions was seen at the lateral aspects of the ependymocytes. This indicates a pronounced ionic or metabolic coupling of the ependymal cells.

Luminal vesicles/apical cytoplasmic protrusions, interpreted as apocrine secretion, have been observed on the ventricular ependyma (Booz, 1975; Stumpf, Hellreich, Aumüller & Lamb, 1977; Hetzel, 1978; Gonzalez-Santander, 1979; Agnew, Alvarez, Yuen & Crews, 1980), the spinal cord ependyma (Agnew *et al.* 1980) and hormonally stimulated ventricular ependyma (Schechter & Weiner, 1972). However, other authors contend that such protrusions are mere fixation artefacts without any functional significance (Tennyson & Pappas, 1961; Davis, Milhorat & Lloyd, 1973; Shelton & Mowczko, 1978). In the present study, where luminal vesicles/apical protrusions were seen, the specimens were free of other signs acknowledged to be fixation artefacts (see Glauert, 1974; Hayat, 1981).

As far as secretion is concerned the cellular features reflecting this energy-consuming function are the presence of large amounts of rough endoplasmic reticulum and a prominent Golgi apparatus. Accordingly, when these organelles are found to be

inconspicious, secretion of any significance is hardly likely. In this study, insignificant amounts of rough endoplasmic reticulum and Golgi apparatus were observed in the ependyma so that we cannot support the assumption of apocrine secretion by the mouse spinal cord ependyma. What, then is, the significance of the luminal vesicles/apical protrusions that were observed? One may argue that this phenomenon is a subtle fixation artefact presenting itself before the occurrence of other acknowledged fixation artefacts. Another hypothesis is that the excretion is a 'passive' proteinaceous contribution to the cerebrospinal fluid.

The almost total lack of intracellular empty-looking vesicles and the sparse amount of intermediate filaments makes the hypothesis of ependymal transcellular transport unlikely.

The present study has demonstrated that the mouse spinal cord ependyma is essentially similar to the cuboidal, unspecialised, ventricular lining ependyma. No regional variations regarding ultrastructure were present along or around the central canal. Our observations do not support the hypotheses of secretion or transcellular transport by the ependyma, or that the ependyma represents a significant diffusion barrier.

Whether ciliary transport by the mouse spinal cord ependyma is its sole physiological function remains to be elucidated.

SUMMARY

This study was done in order to investigate the normal ultrastructure of well-preserved mouse spinal canal ependyma using light, scanning and transmission electron microscopy. The ependymal lining was found to consist of a simple, cuboidal epithelium essentially similar to the unspecialised cuboidal ependyma of the brain ventricles. Apart from great variation in kinociliary density, no intracellular difference was noted between the ependymal cells. In contrast to earlier findings, indications of the existence of zonulae occludentes between the apical part of the ependymal cells were observed. Our findings do not support the hypothesis of secretion or intracellular transport by the ependyma, or that the ependyma constitutes a significant diffusion barrier.

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