Scanning Electron Microscopy

Volume 1985
Number 1 *1985*

Article 24

10-5-1984

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SCANNING ELECTRON MICROSCOPY/1985/I (Pages 231-238)
SEM Inc., AMF O'Hare (Chicago), IL 60666 USA

0586-5581/85$1.00+.05

THE EPENDYMA OF THE CAT CENTRAL CANAL, WITH PARTICULAR REFERENCE TO ITS MITOCHONDRIA-CONTAINING BULBS

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(Paper received March 20 1984, Completed manuscript received October 5 1984)

Abstract

The ultrastructure of the ependyma in the central canal of adult cats was examined in both the scanning and the transmission electron microscopes (SEM and TEM).

The same morphological details were seen in the ependyma of the central canal as have so frequently been described in the ependyma of the brain ventricular system, for example bundles of cilia, single cilia, microvilli and occasional small cytoplasmic protrusions. The supraependymal cells and supraependymal nerve fibers found in the central canal also resembled those seen in the ventricular system.

The most striking feature of the canal ependyma were the large, spherical bodies containing numerous mitochondria. They are therefore called mitochondria-containing bulbs. In sections the bulbs were seen to be connected by long, slender stalks to neurons in subependymal position. In some respects the mitochondria-containing bulbs resemble the processes of cerebrospinal fluid-contacting neurons.

KEY WORDS: Ependyma, Spinal Cord Central Canal, Mitochondria-containing Processes, Cat, Scanning Electron Microscopy

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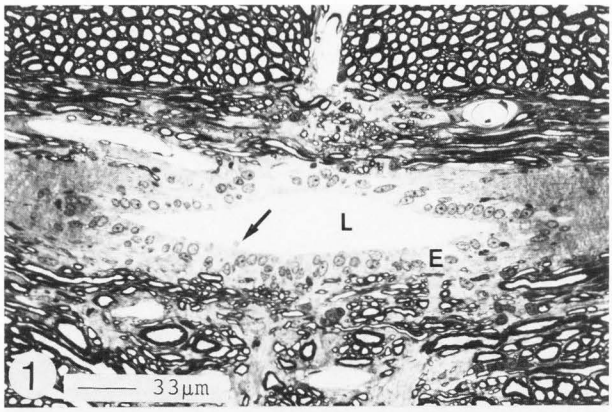
Introduction

Most scanning electron microscope studies of the ependyma have dealt with the lining of the brain ventricles (see extensive literature in Leonhardt 1980 and Low 1982). The ependyma of the central canal, however, has only rarely been subjected to intensive ultrastructural examination (Leonhardt 1976). One of the reasons for this relative lack of information on the central canal may be that its free surfaces are considerably more difficult to expose for observation in the SEM than are the surfaces of the ventricles. Another may be that the central canal has not very often been the subject of experimental and/or pathological research (Hall et al. 1975). The present study was undertaken in connection with an ongoing investigation of the alterations in the central canal caused by experimentally induced hydrocephalus in adult cats.

The ependyma of the cat central canal has many features in common with that of the ventricles of higher vertebrates and is quite similar to that of the rabbit central canal (Leonhardt 1976) but does differ in several respects from that of the rat canal (Nakayama and Kohno 1974) Both SEM and TEM revealed that the cat possesses spherical supraependymal structures containing numerous mitochondria. They could be identified as the processes of neurons lying in subependymal position. These neurons are therefore cerebrospinal fluid-contacting neurons. Their similarity to the well-known cerebrospinal fluid (CSF)-contacting neurons of lower vertebrates (Vigh and Vigh-Teichmann 1971) may give a clue to their possible function.

Materials and Methods

Adult cats were deeply anesthetized with nembutal (0.35g/kg) and artificially respirated before being transcardially perfused with a buffered mixture of 1% glutaraldehyde and 1% paraformaldehyde. The animals, which weighed between 2.3



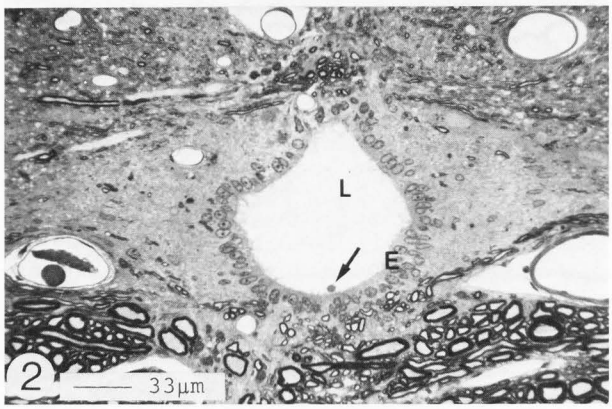
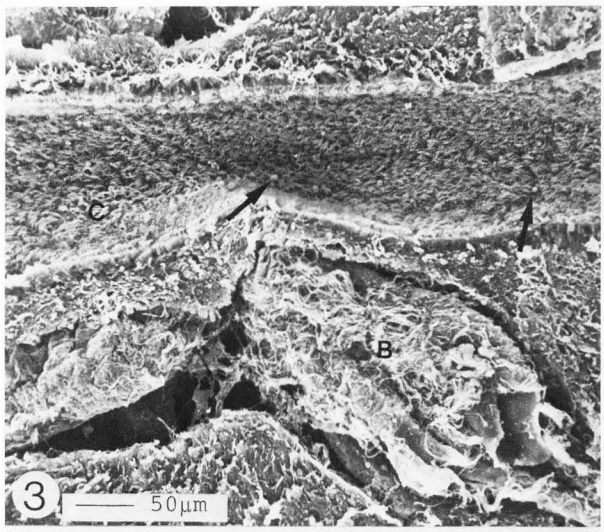


Figure 1. Semithin section of the central canal and its immediate surroundings in the cervical intumescence. L = lumen of the canal; E = ependyma; arrow = mitochondria-containing bulb.
Figure 2. Semithin section of the central canal in the upper lumbar region. L = lumen of the canal; E = ependyma; arrow = mitochondria-containing bulb.

and 3.0 kilograms, received 1 liter of warm perfusion fixative per kilogram body weight. The spinal cord was removed and postfixed in fresh cold fixative at least overnight before being dissected into tissue blocks for scanning and transmission electron microscopy. The samples for SEM were split to expose the ependyma and subsequently postfixed in a buffered solution of 1% osmium tetroxide. They were then rinsed in 2.4% NaCl and dehydrated in an ascending series of methanol. The tissue was transferred to Freon 11 and critical point dried in Freon 13 (Cohen et al. 1968) in a Polaron apparatus. The specimens were coated with gold in a Balzers sputtering chamber and examined in a Cambridge Stereoscan S4 at 20 kV.

The tissue samples for TEM were rinsed in phosphate buffer before being postfixed in 2% osmium tetroxide. They were then rinsed again in buffer. The samples were dehydrated in isopropanol, transferred to propylene oxide and embedded in an epoxy resin (ERL 4206, Spurr 1969).



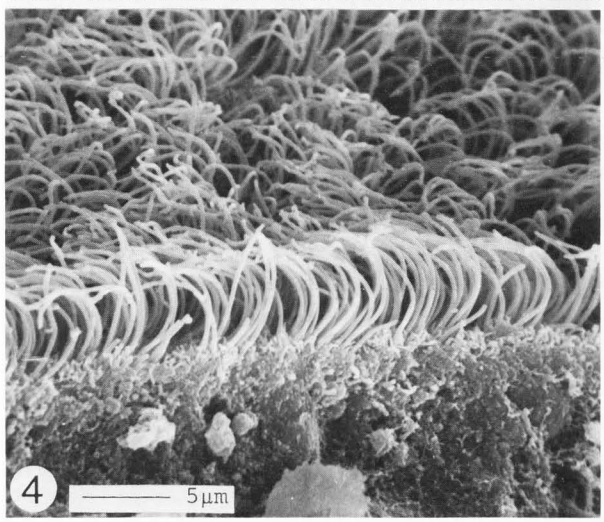


Figure 3. Overview of the ventral aspect of the central canal split longitudinally. Mid-thoracic region. C = heavily ciliated ependyma; arrow = mitochondria-containing bulbs; B = blood vessel with well-developed perivascular space.
Figure 4. Closeup of the cut edge of the ependyma illustrating the synchronous beat of the kinocilia. Caudal to the left.

Semithin and thin sections were cut on a Reichert microtome with glass knives; the thin sections were viewed in a Zeiss EM 9 at 60 kV.

Results

The central canal of the cat varies in shape depending upon the level of the spinal cord examined. In the upper cervical and in the sacral regions it is a narrow slit with the lateral walls almost touching; in the thoracic segments it is more or less round whereas in both the cervical and the lumbar intumescences it is again slit-like but with the dorsal

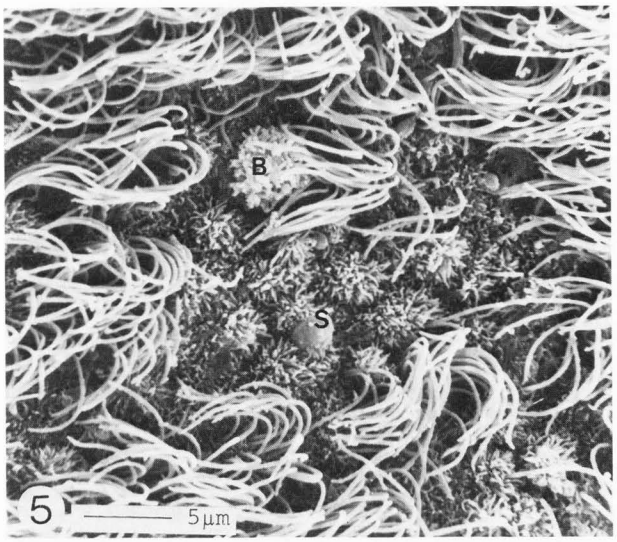
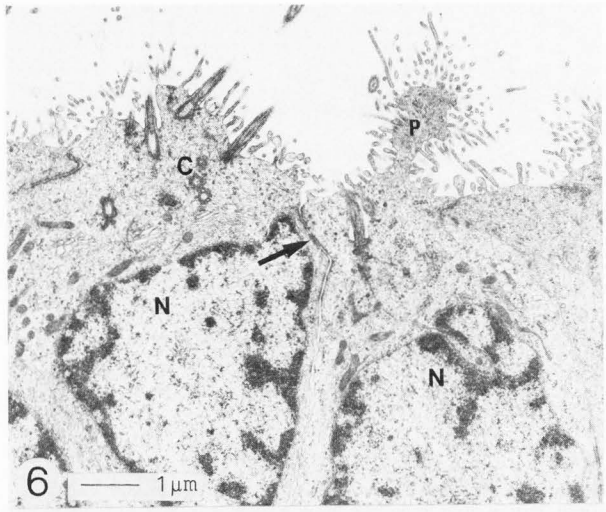


Figure 5. Detail of the surface of the less densely ciliated area along the dorsal cervical midline. Note the multitude of microvilli on the tall protrusions of the apical poles of the cells. B = mitochondria-containing bulb; S = smooth profile.

and ventral surfaces closely apposed (compare Figures 1 and 2). The shape of the canal may be slightly altered by large blood vessels with a well-developed perivascular space which penetrate into the subependymal tissue (Fig. 3). The SEM reveals that the ependyma of the canal has several ultrastructural features which are also characteristic of the ventricular ependyma. It is fairly densely ciliated throughout (Fig. 3). The cilia are grouped in bundles so close together that the cell surfaces of the ependymocytes can only rarely be seen. The cilia are bent in such a manner that their beat is apparently from rostral to caudal (Fig. 4). The carpet of cilia becomes sparser along the midline both dorsally and ventrally. These areas bearing fewer cilia are quite narrow in the upper cervical segments, broad in both intumescences and almost invisible in thoracic levels. The less densely ciliated areas show a multitude of microvilli on the cell apical poles. For the most part the latter protrude markedly into the lumen of the canal, extending their microvilli in all directions, thus giving the entire surface a mossy appearance (Fig. 5). In thin sections the protrusions of the apical poles were seen to contain a fine granular matrix and occasional microfilaments but no larger organelles (Fig. 6).

The scanned specimens did not reveal any major ultrastructural differences between the dorsal and ventral surfaces of the canal, but thin sections consistently showed that the protrusions bearing



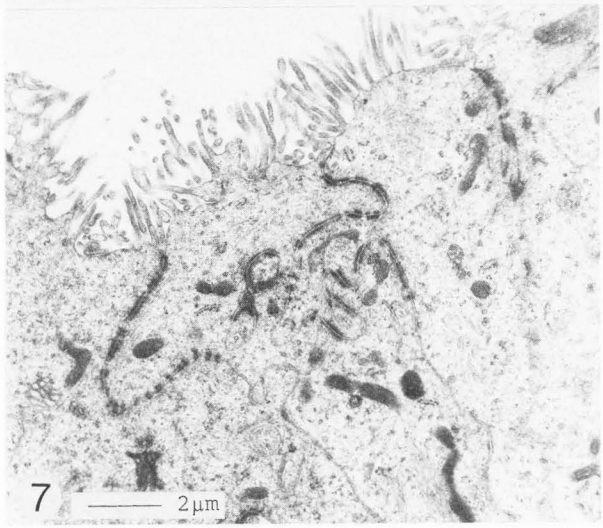
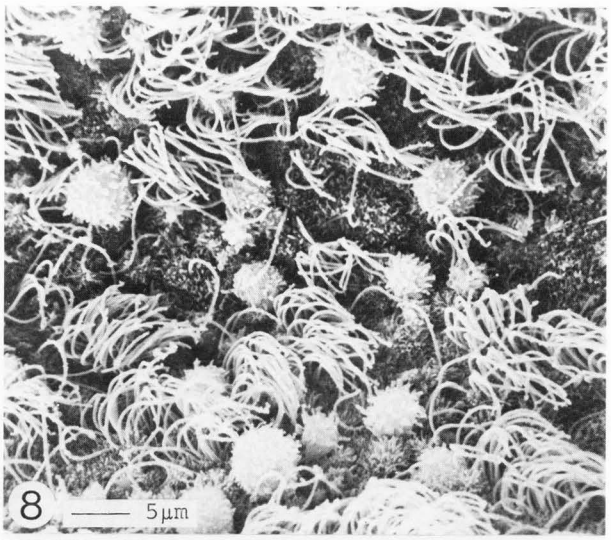


Figure 6. Thin section of area as in figure 5. P = apical protrusion with microvilli; C = basal bodies of a bundle of cilia; N = nuclei of ependymocytes; arrow= contacts between ependymocytes.
Figure 7. Apical parts of ependymocytes in ventro-median (cervical) position indicating the well-developed contacts and interdigitations between the cells.

microvilli were taller along the dorsal aspect than along the ventral. Another feature which distinguished the ventral from the dorsal ependyma of the midlines were the cell contacts. Those of the dorsal aspect were generally straight and consisted of only a few short desmosome-like contacts and an occasional apically located gap junction. The lateral cell borders of the ventrally positioned ependymocytes, however, had highly developed junctional complexes with long series of desmosome-like contacts. These cells interdigitated with one another giving the membranes a tortuous course (compare Figures 6 and 7).



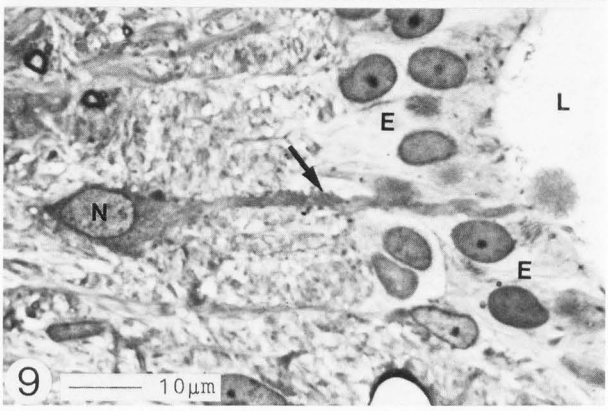
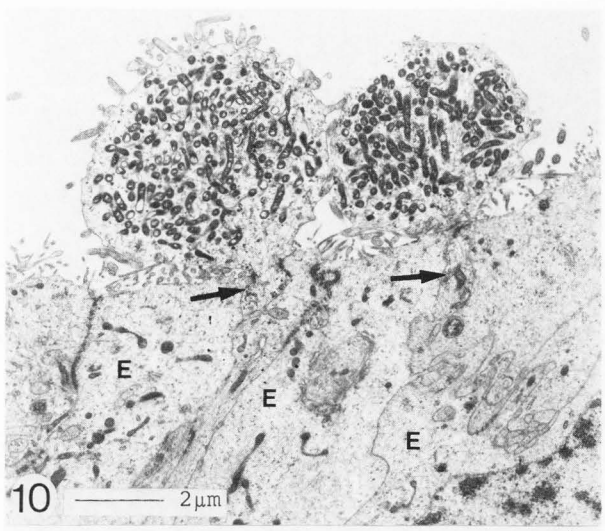


Figure 8. Several mitochondria-containing bulbs in ventral aspect of cervical canal. Note differences in size.
Figure 9. Semithin section of mitochondria-containing bulb with its neuron (N) and connecting stalk (arrow). E = ependyma; L = lumen of the canal.



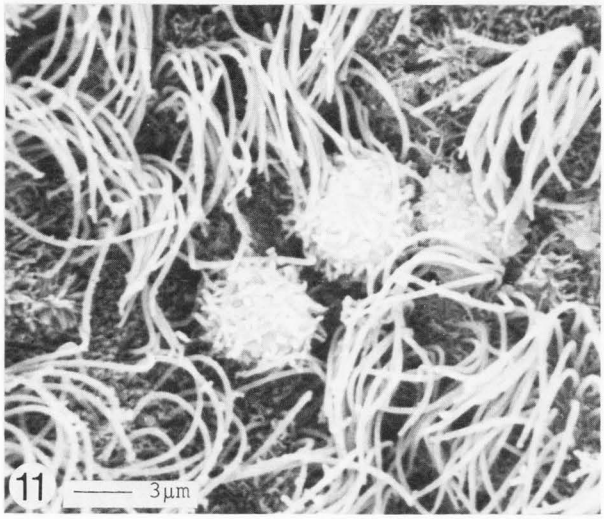


Figure 10. Two mitochondria-containing bulbs. Due to the thinness of the section their stalks (arrows) can be traced only for a short distance between the ependymocytes (E).
Figure 11. Closeup of three typical mitochondria-containing bulbs.

The most remarkable features of the cat central canal were the large, spherical structures which could be found throughout its entire length (Fig. 8). In light microscopic sections they could be easily recognized as round profiles with a granular cytoplasm (Figs. 1 and 2). Semithin sections sometimes showed the bulbs to be connected via long stalks to subependymally located cells. These cells had larger nuclei and nucleoli than the ependymal cells and fairly dark, homogeneously stained cytoplasm (Fig. 9). Thin sections revealed that these bodies are filled with mitochondria, some small vesicles and dense particles (Fig. 10). The abundance of mitochondria gives the structures their name: mitochondria-containing bulbs.

In serial thin sections the stalks of the bulbs can be traced for a short distance down between the ependymocytes. At the level of the apical cell contacts the stalks are constricted to a narrow neck which has desmosome-like contacts to the neighboring ependymal cells (Fig. 10). Neurotubules in more or less parallel array pass along the stalk, through the neck and fan out into the base of the bulb. The diameter of the bulbs varies between 2 and 10µm. Approximately 20% each belong to the size classes 2, 3, 4 and 5µm. The final 20% is distributed among the larger sizes. In the SEM the bulbs are covered with a multitude of microvilli; only rarely can a more smooth-surfaced one be seen (Figs. 5, 8, 11, 13 and 14). Their distribution pattern is

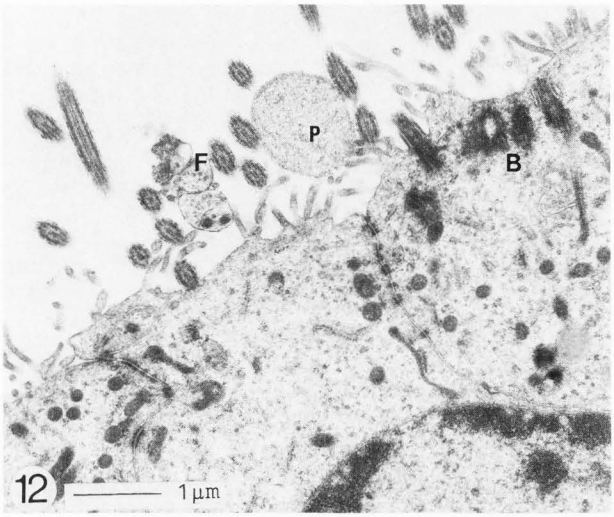
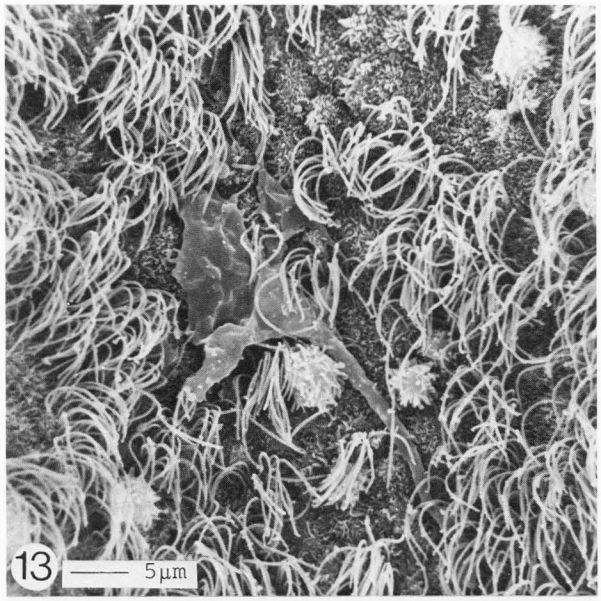


Figure 12. Supraependymal nerve fibers (F) containing microtubules and small mitochondria. Next to them a cytoplasmic protrusion (P) of an ependymal cell, in this section not connected to it. B = basal bodies of cilia.

much more easily recognized in the SEM than in serial sections. They are somewhat more frequent in the above-mentioned less densely ciliated midlines, especially in the ventral, than in lateral areas. There is an average of 45-80 bulbs per millimeter canal; only in the lumbar intumescence does the number rise to 90-160 per millimeter. The mitochondria-containing bulbs differ in size, contents and surface ultrastructure from the small, smooth profiles which could occasionally be detected between the cilia and microvilli of the apical surfaces (Fig. 5). These profiles contained no mitochondria, but were filled with a homogeneous, lightly staining fine granular matrix. They had no stalks but were rather protrusions of the ependymocytes themselves (Fig. 12).

Supraependymal elements were also found in the central canal. Supraependymal cells could be found at all levels of the canal, but were most frequent in the cervical region, particularly in the intumescence. They occurred on both the dorsal and the ventral surfaces. These cells were extremely polymorphic, but all had broad lamellar processes which were at times closely associated with the cilia and mitochondria-containing bulbs (Figs. 13 and 14).

In TEM sections occasional very thin unmyelinated nerve fibers were observed close to the apical surfaces of the ependymocytes, between the cilia and microvilli (Fig. 12). They contained synaptic vesicles and a few small dense-cored vesicles, but no contacts between the fibers and ependymocytes or other supraependymal structures were detected in our material.



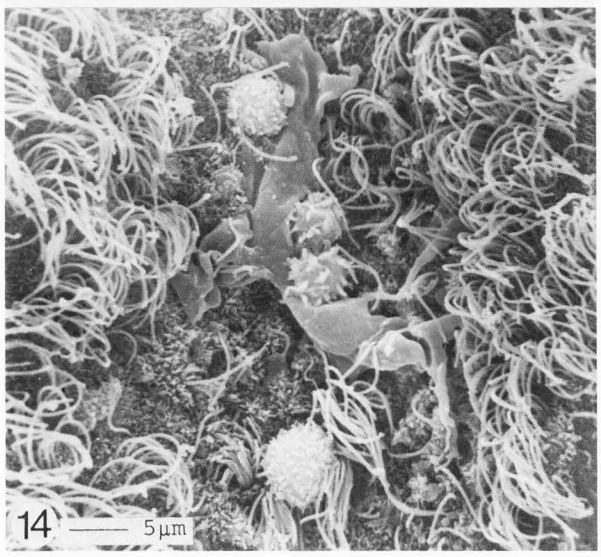


Figure 13. Supraependymal cell in cervical region.
Figure 14. Supraependymal cell in thoracic region.

This may be due to the limitations of TEM sampling.

Discussion

The central canal of the spinal cord is the caudal continuation of the ventricular system of the brain; its ependymal cells develop in basically the same manner as those of the ventricles and it is therefore not surprising that the ultrastructure of the two are very similar. The linings of the ventricles of numerous mammalian species have been extensively described (see Leonhardt 1980 and Low 1982 for literature). One of the very first SEM studies to be done on the ependyma

dealt with that of the cat ventricles (Clementi and Marini 1972). Quite in contrast to the vast body of research on ventricular ependyma, that of the central canal has received much less attention.

Most work on canal ependyma was done in connection with investigations on experimental hydromyelia and syringomyelia (Becker et al. 1972). Our findings agree with the light microscope observations of these authors as well as with the earlier TEM results of Takeichi (1966). Takeichi's paper, however, does not mention the protrusions of the cell apical poles bearing microvilli. Similar specializations have apparently been seen only in the choroid plexus (el Gammal 1983). The supraependymal structures, free cells and unmyelinated nerve fibers, are a frequent finding in the ventricular system (Leonhardt and Lindemann 1973; Coates 1973; Scott et al. 1974). The supraependymal cells seen in the present material are most likely macrophages; their appearance in the SEM does not differ from what has already been described of the epiplexus cells of the choroid plexus and of other free cells seen on the ventricular walls, particularly in the infundibular recess (Mestres 1976; Mestres and Breipohl 1976; Bleier 1977).

The supraependymal nerve fibers also resemble others described on the ventricular lining (Lorez and Richards 1982; Ribas 1977). Judged by their content of microtubules and small mitochondria the fibers are axons. They seem to be much thinner and less common in the cat central canal than in that of the rabbit (Leonhardt 1976). In our material we have up to now not found any myelinated fibers nor contacts between the fibers and other structures. Nor have we been able to determine the location of the neurons from which they originate.

The ependymal protrusions seen in the SEM as well as in thin section can readily be classified into two markedly different groups: 1) the usually small, smooth protrusions which can only rarely be seen between the microvilli and cilia and are a part of the ependymal cells themselves. Neither the contents nor the surface membrane of this type of protrusion is specialized in any way. Similar protrusions have been observed in the ventricular wall of fetal animals (Booz 1975; Hannah and Geber 1977). What role they may play on the ependyma of the central canal in adult animals could not be determined with the present methods. 2) the considerably larger mitochondria-containing bulbs. They have been described in the central canal of the rabbit (Schwanitz 1969; Leonhardt 1976), but were apparently seen for the first time in the kitten (Takeichi 1966). Takeichi (1966) does not provide any information about the number or location of the bulbs, but our findings as to their ultrastructure agree entirely with his, indicating that the bulbs appear long before maturity in the cat. Their frequency and distribution in the adult cat has been quantified in serial semithin sections (Boger 1980). Our assessment of their frequency and distribution in SEM micrographs also agrees with these earlier findings.

The mitochondria-containing bulbs (Mitochondrienkolben) seen in the central canal and in the fourth ventricle of the rabbit (Leonhardt 1967; Leonhardt and Prien 1968; Lindemann and Leonhardt 1973) are similar to, but do differ in some respects from those seen in the cat. The stalks of the bulbs contain cytoplasmic elements which indicate that they are the processes of neurons; the contacts between the stalks and the ependymal cells are identical in the two species. The position of the neurons around the central canal of the rabbit seems to be close to the ependyma or even intraependymal. In the cat, however, neurons in the immediate vicinity of the ependyma are the exception and a direct connection between bulbs and perikaryon could not be unequivocally established in TEM sections. In the cat the neurons are so few and far between that they may be part of a hypendymal plexus, a given neuron contributing several bulbs, each on a long stalk. Another distinct difference between the bulbs of the two species lies in their content of mitochondria. Leonhardt (1976) divided the bulbs he observed in the rabbit into two main groups, the first with many mitochondria but few microvilli, the second with fewer mitochondria but countless microvilli. This distinction is not possible among the bulbs of the cat canal. Here they all appear to belong to a different group altogether: They have many mitochondria *and* microvilli. Bulbs with fewer microvilli were the exception.

The possible function of the bulbs has been of particular interest because they are the CSF-contacting terminals of neuronal processes. CSF-contacting neurons have been described in the central canal in all vertebrate classes (Vigh and Vigh-Teichmann 1971). These authors found that the number of such neurons decreases with ascent on the phylogenetic ladder. On the basis of morphological features they proposed that the CSF-contacting neuronal processes might be dendrites belonging to receptor-like neurons. This seems to hold true for the lower vertebrates. On the other hand, the corresponding cells in the rabbit have been interpreted to be secretory neurons (Leonhardt 1967) or have been seen to possess characteristics which belong either to receptor cells or to neurons capable of electrolyte exchange with the CSF (Leonhardt 1976). In the

cat the bulbs pose an even more intriguing problem than in the rabbit because the neurons to which they belong are so difficult to locate. The bulbs do not contain any cytoplasmic elements which would clearly indicate that they are either secretory or receptor-like. Nor does the surface ultrastructure provide conclusive evidence for one or the other function. For the present the question as to their function must remain unanswered.

Acknowledgements

The authors gratefully acknowledge the skillful technical assistance of Mr. N. Pütz, Ms. S. Brundaler and Ms. G. Kühnreich. We thank Ms. G. Haag for her help with the typing. This project was financed in part by the Sonderforschungsbereich 38, Homburg/Saar.

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Discussion with Reviewers

F. N. Low: Since numerous authors have related ciliation and/or the lack of it in ependyma to underlying structures (nuclei, etc.), could you comment on this for the central canal in the spinal cord?
Authors: The distribution pattern of cilia in the cat central canal might be seen in connection with the nature of the underlying tissue. The less densely ciliated midlines are closer to white matter and the heavily ciliated lateral areas cover gray matter. This is comparable to what Page observed in the lateral ventricles of the rabbit (Page RB, 1975, Scanning electron microscopy of the ventricular system in normal and hydrocephalic rabbits. J Neurosur 42:646-664) In the lateral ventricles of other species, however, Scott et al. (1974) observed the reverse situation. The latter authors point out that "The degree of cilial sparsity is not consistent from animal to animal, or species to species but appears random." Indeed, we have compared the surface of the canal ependyma of the cat, the rabbit and the rat (Rascher K, Booz KH, Nacimiento AC, 1984, Comparative ultrastructure of the ependyma in the cervical central canal of cat, rabbit and rat. An SEM study. Beitr Elektronenmikroskop Direktabb Oberfl 17:239-246) and seen that there are major differences between the species, for example, the rat canal ependyma does not bear any bundles of cilia at all below the level of cervical I. In this species there are only one or two cilia per ependymal cell, which confirms the findings of Nakayama and Kohno (1974).

G. P. Kozlowski: Why is the neuron in figure 9 so dark? Is it consistent with other examples of similar neurons with mitochondria-containing bulbs?
Authors: Work on the CSF-contacting neurons is still in progress, but they do seem to have in common a darker-staining cytoplasm. This may be due to their comparatively numerous mitochondria and/or other osmophilic components of the cytoplasm. Similarly darker-staining cells with mitochondria-containing bulbs have also been seen in the rabbit (Leonhardt 1976).

G.P. Kozlowski: Could the perivascular space in figure 3 be an artifact?
Authors: At first we also considered this possibility, but in SEM and light micrographs the perivascular space was a consistent finding surrounding the larger vessels in the gray matter. TEM analysis showed that there were pericytes and collagen fibers within the perivascular space which is isolated from the tissue around it as well as from the vessel wall by a basement membrane. Some of these elements may be seen in figure 3; they are even more easily recognized in figure 15. These perivascular spaces do not appear to be unique to the cat spinal cord. We have seen identical ones in both the rat and the rabbit cord.

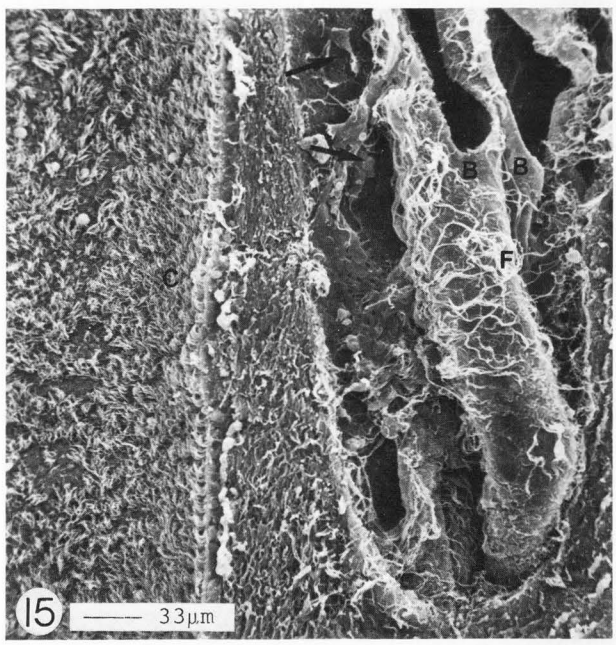

Figure 15. Blood vessels (B) with well-developed perivascular space next to the central canal (C). Note abundance of collagen fibers (F) and several pericytes (arrows).