J. Anat. (1989), **165**, pp. 87–100
With 6 figures
Printed in Great Britain

Ultrastructural and cytochemical characterisation of the floor plate ependyma of the developing rat spinal cord

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(*Accepted* 18 *October* 1988)

INTRODUCTION

Ependyma lining the brain ventricles and the central canal of the spinal cord forms a wall separating the cerebrospinal fluid (CSF) from the nervous tissue (Rall, 1968). It is now considered that the ependymal epithelium contains a heterogenous population of cells which are actively involved in the absorption, transport and secretion of substances (Bruni, Del Bigio & Clattenburg, 1985; Fleischhauer, 1972). In addition, the ependymal cells of the central canal are responsible for the regenerative activity of the nervous tissue (Bernstein & Bernstein, 1967; Gilmore & Leiting, 1980; Gilmore, Sims & Leiting, 1984). From the standpoint of development, the ependyma is regarded as the final form of the neuro-epithelium after the genesis of the neuronal and glial cells; however, it is reasonable to assume that the ependymal cells must possess an unique developmental sequence throughout the development of the nervous system (Jordan, Reike & Thomas, 1987).

At an early stage of embryonic development, the spinal cord is subdivided into four different areas; the basal and alar plates of the lateral walls, and the floor and roof plates. Some investigators have reported that the floor plate ependymal cells display a specific type of metabolism of carbohydrates such as a form of glycogen different to that of the neuronal cell-producing cells of the lateral walls (McKay, Adams, Hertig & Danziger, 1955, 1956; Tanaka & Nishimura, 1974; Uehara & Ueshima, 1984). Recently, we observed that the floor and roof plates of the developing rat spinal cord had an increased activity of calcium-activated adenosine triphosphatase prior to neuronal differentiation (Yoshioka, Inomata & Tanaka, 1987). Although there is a suggestion that, as well as the roof plate, the floor plate is a source of non-neuronal cells such as radial glial cells during embryonic development (Altman & Bayer, 1984), the precise function of these regions during development still remains unclear.

Ultrastructurally, some observations are available for the embryonic and fetal development of the central canal ependyma of the spinal cord (Gamble, 1969; Malinsky & Brichova, 1967; Sturrock, 1981*a*). These observations have shown that the floor and roof plate ependymal cells differentiate during an earlier stage of embryonic development, and that, particularly, the floor plate ependymal cells are closely associated with the growth of decussating nerve fibres in the marginal layer, the 'tunnel epithelium' as it was named by Ramon y Cajal (1952). Sturrock (1981*a*) has also reported that the central canal ependyma cells of the developing mouse spinal cord exhibit cytological features characteristic of the choroid plexus, suggesting that these cells may relate not only to the internal medium of the central canal lumen but also to the external medium surrounding the developing spinal cord.

In our attempt to examine the correlation between the morphological and

histochemical characteristics of the developing spinal cord, we examined ultrastructurally the central canal ependyma cells, particularly the floor plate of the rat spinal cord during prenatal development. Many of our observations in rats were similar to those that have been made by Sturrock (1981*a*) in the developing mouse spinal cord. In the present report, particular mention is, however, made of a polarised development of the vesicular system, such as lysosomes, within the floor plate ependymal cells. A cytochemical demonstration of the activity of acid phosphatase (ACPase) was also applied to evaluate the distribution of lysosomes in the floor plate ependymal cells.

MATERIALS AND METHODS

Embryos of the Jcl:Wistar rat between embryonic day (E) 11 and 16 and of E18 were used in the present study. The day on which a positive vaginal plug was present was designated as E0. The pregnant animals were anaesthetised with sodium pentobarbitone, and the embryos were then obtained by Caesarian section. Earlier embryos from E11 to E13 were rapidly immersed in the appropriate fixative. Larger embryos and fetuses after E14 were briefly perfused by inserting a fine glass capillary into the cardiac ventricle and this was followed by immersing in the fixative.

Morphological examination

A mixture of 2·5% glutaraldehyde (GLA) and 2% paraformaldehyde (PFA) in 0·1 M phosphate buffer, pH 7·3, was used as a fixative. After fixation for one day or more, the brachial segments of the spinal cord were removed and sectioned transversely at 200–300 μm in thickness within a cold solution of 0·1 M phosphate buffer, pH 7·3, containing 4% sucrose, using a Microslicer (DK-1000, Dosaka Ltd, Kyoto). Tissue specimens were rinsed overnight and postfixed with 1% osmium tetroxide in the same buffer for 2 hours in ice. The osmicated specimens were then stained *en bloc* with an aqueous 2% uranyl acetate for 20 minutes, dehydrated in a series of graded ethanol solutions, treated with propylene oxide and embedded in Epon 812. Thin sections for electron microscopy were obtained by using a Reichert–Jung OmU_4 ultramicrotome, and stained with uranyl acetate and lead citrate (Reynolds, 1963). All specimens were examined and photographed with a JEM 1200EX (80 or 100 kV) or a Hitachi HS-9 (75 kV) electron microscope.

Cytochemical examination of ACPase activity

A mixture of 1% GLA and 2% PFA in 0·1 M sodium cacodylate buffer, pH 7·3, was selected for the demonstration of ACPase activity. Embryos and fetuses, perfused or not, were fixed for a total of one hour at 0–4 °C. After a rinse with 0·1 M cacodylate buffer, pH 7·3, containing 6% sucrose, 30–60 μm transverse sections of the brachial segment of the spinal cord were obtained with a Microslicer. Enzymatic reactions were carried out using two methods, that of Gomori (1952), using lead as a capture agent, and that of Robinson & Karnovsky (1983), using cerium as a capture agent. As control experiments, the substrate-free medium and the medium containing 10 mM sodium fluoride were used. After incubation for 30 minutes at 37 °C, specimens were postfixed with a 0·1 M cacodylate-buffered 2% osmium tetroxide for 40 minutes and processed for electron microscopy, as mentioned above. Thin sections, obtained for electron microscopy, were stained with uranyl acetate only and were examined.

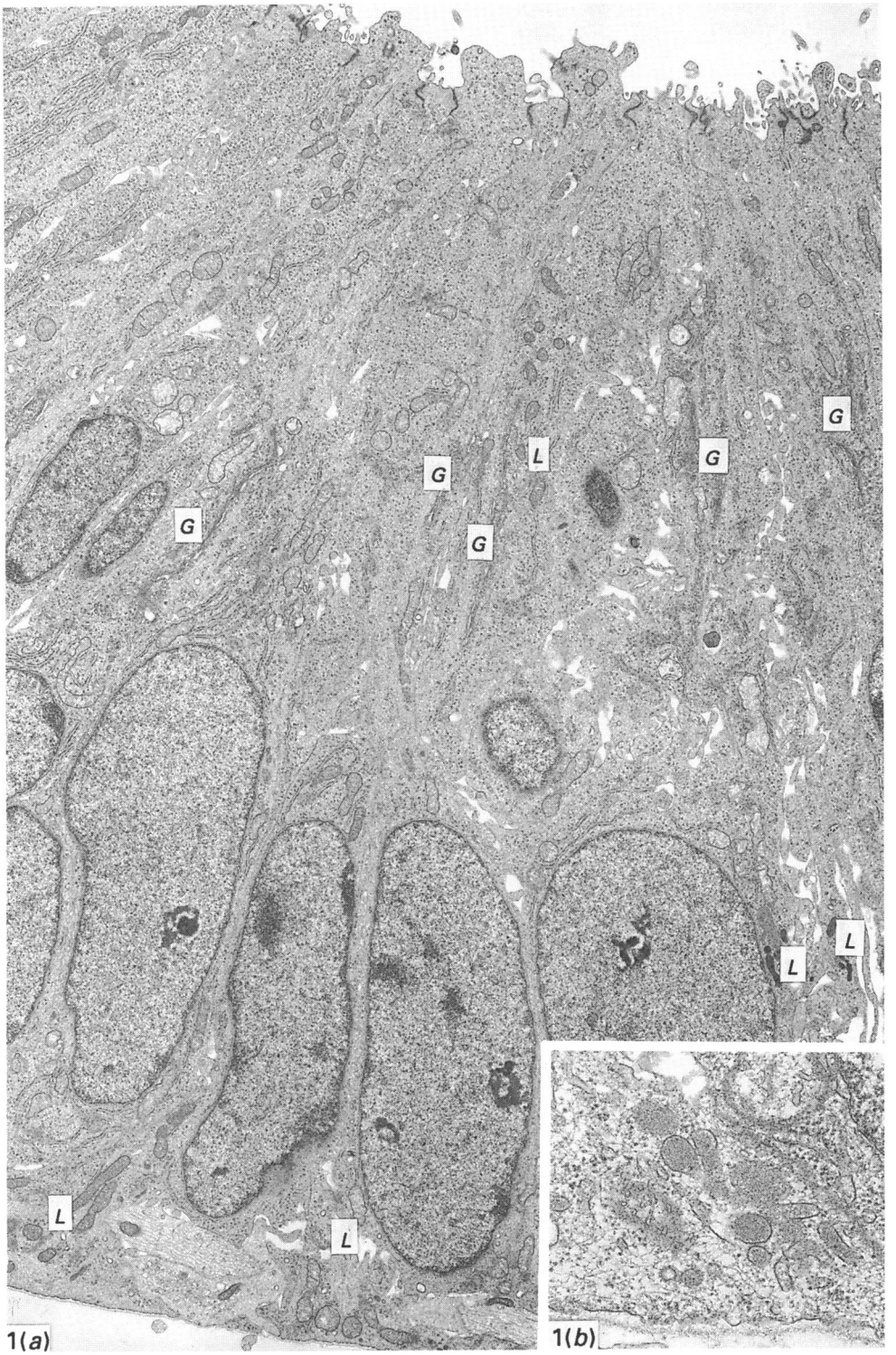


Fig. 1 (*a–b*). Floor plate ependyma at E12. (*a*) Well-developed Golgi apparatus (*G*) was found in the apical cytoplasm, wherease dense bodies (*L*) were rich in the basal cytoplasm. A small number of decussating nerve fibres were seen beneath the pial surface. × 5500. (*b*) High magnification of dense bodies in the basal cytoplasm near the ventral surface. × 25 500.

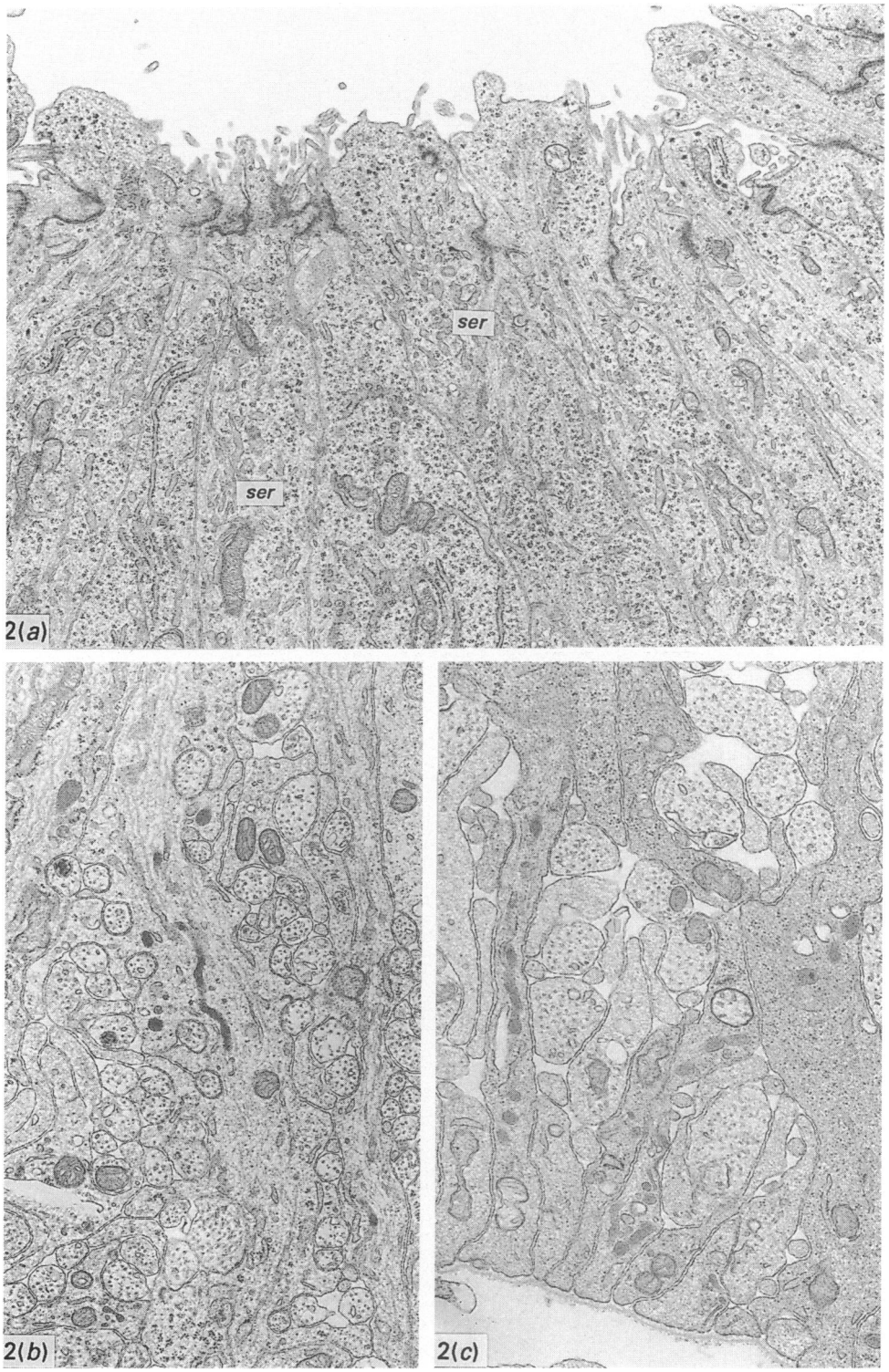


Fig. 2 (*a–c*). Apical and basal aspects of the floor plate ependymal cells at E13–14. (*a*) Abundant smooth ER (*ser*) in the apical cytoplasm at E14. The cytoplasmic protrusions at the apical surface bear a small number of cilia and microvillous projections. ×9500. (*b*) Basal processes of the ependymal cells passing through the marginal layer at E13. The processes contain abundant microtubules and typical dense bodies in tubular form. ×13000. (*c*) Basal terminals of the processes at the ventral pial surface. Terminals with a filamentous matrix contain many dense bodies. E13. ×18000.

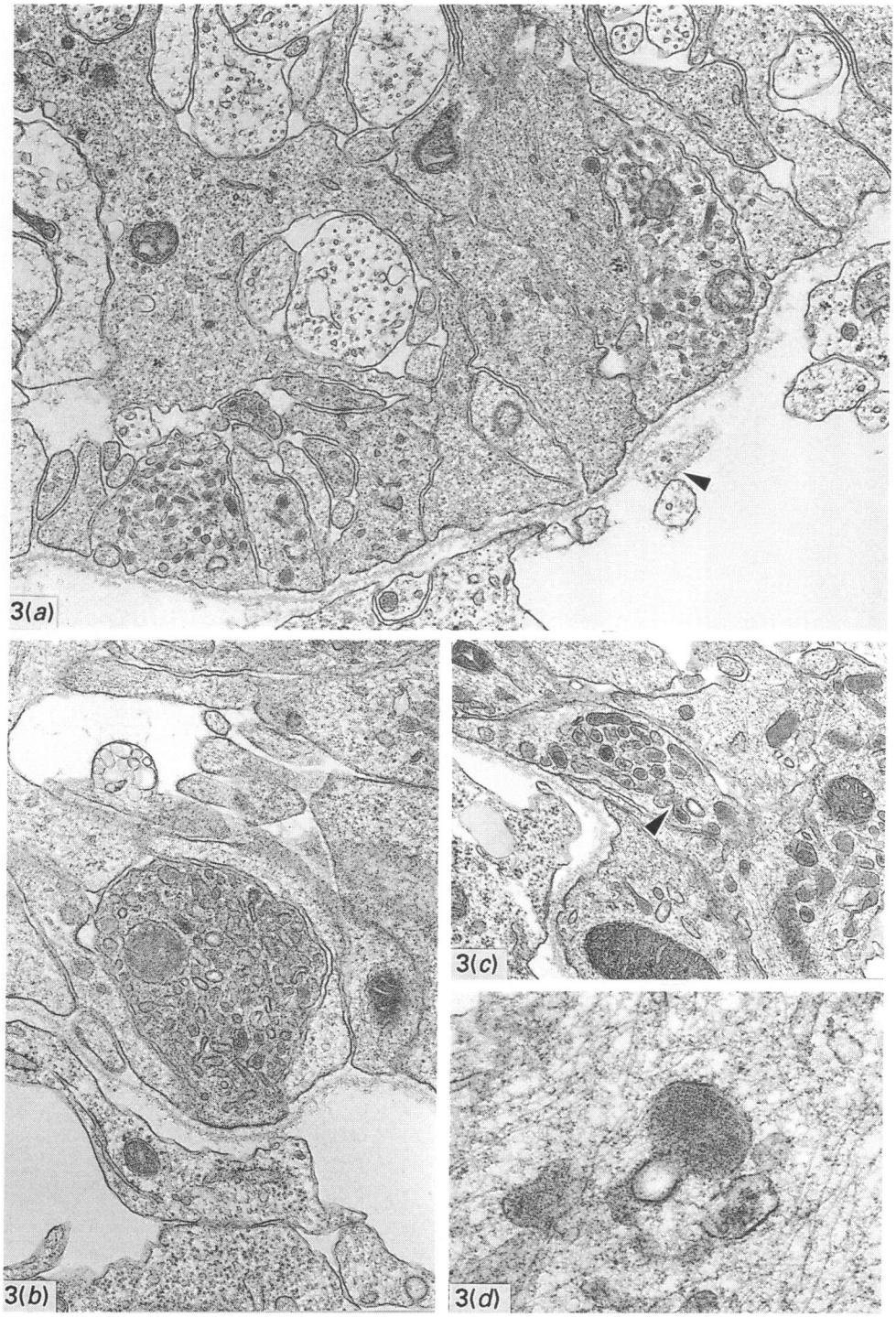
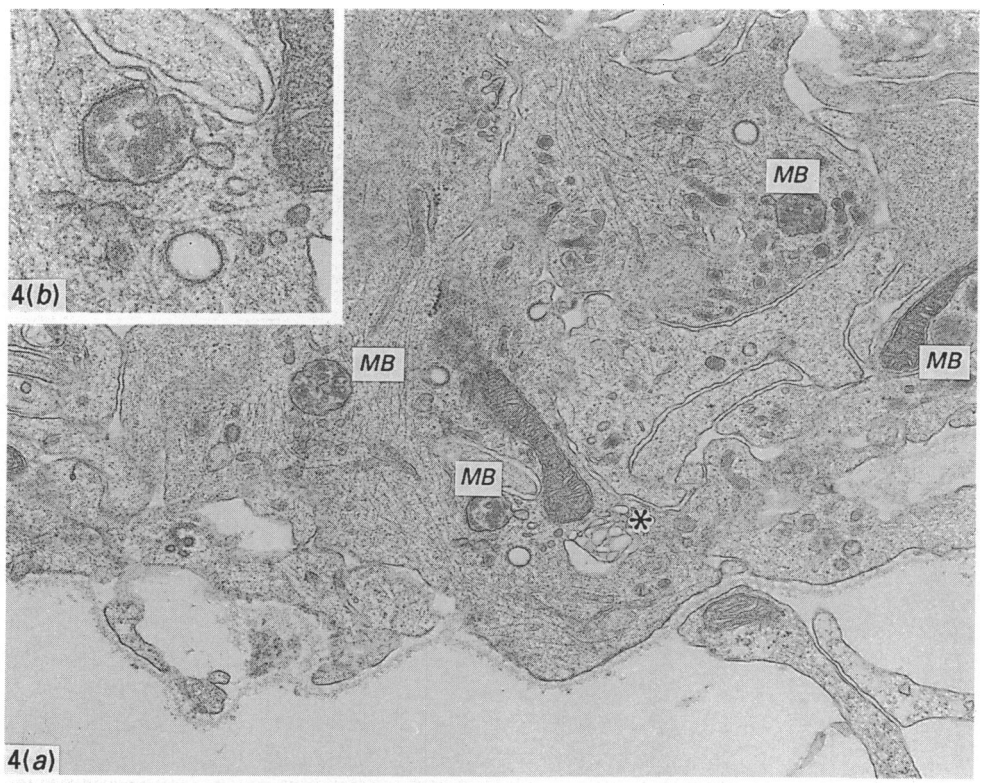


Fig. 3 (*a–d*). Basal aspects of the floor plate ependymal cells after E15. (*a*) Many, but not all, basal terminals contain numerous dense bodies and other vesicles. Note clustering of small vesicles in the extraparenchymal space (arrowhead). E15. ×20500. (*b*) A basal terminal with numerous and packed vesicles seen at E18. ×26000. (*c*) Within the terminals, a dense body appears to be in close association with other vesicles (arrowhead). E16. ×22000. (*d*) High magnification of fusion of the dense body to the pinocytotic vesicle in the terminal cytoplasm. E16. ×33000.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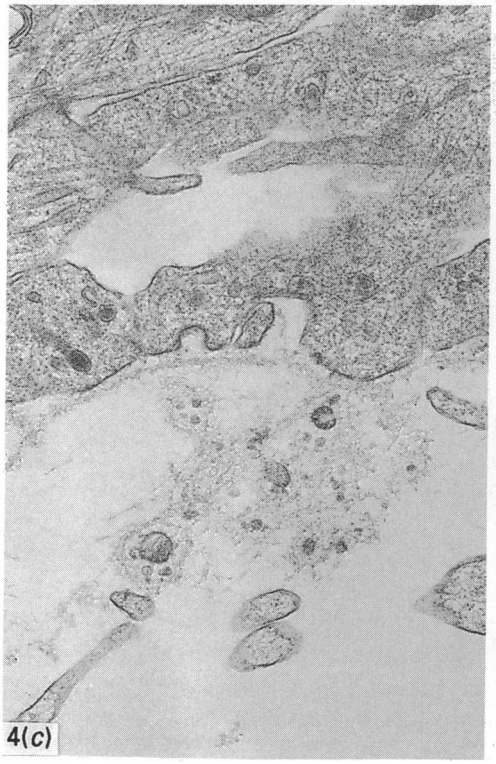
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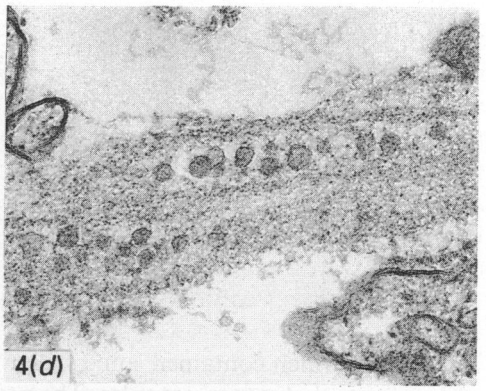
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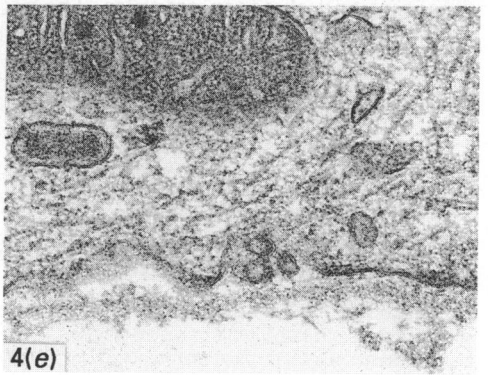

4(e)

RESULTS

Fine structures of the floor plate ependyma

In E11 rat embryos, the floor plate consisted of a single layer of bottle-shaped neuro-epithelial cells whose nuclei were located basally. The cells, with a dense cytoplasm, had cytoplasmic protrusions and some microvillous projections at the apical surface and the cells were lying on a thin basal lamina ventrally. The intracytoplasmic organelles were not well-developed except for the Golgi apparatus. At E12, the cells were elongated ventrally and were strikingly constricted at their apices. The Golgi apparatus, which was arranged ventrodorsally, was better developed and the rough endoplasmic reticulum (rER) was increased in the perinuclear cytoplasm (Fig. 1*a*). Dense bodies, seen in small vesicular and/or tubular forms, appeared in the cytoplasm; some of them were located in the basal cytoplasm near the ventral pial surface (Fig. 1*a*, *b*). A small number of decussating nerve fibres which, in contrast, exhibited a pale cytoplasm, appeared beneath the pial surface; however, the marginal layer became clearly identified after E13. Mitotic cells were often encountered in the lateral regions of this plate, but not in the medial region.

At E13–14, the floor plate ependymal cells were more elongated and their nuclei were arrayed in two or three layers. In the cytoplasm of these cells, the Golgi apparatus and the rER were remarkably developed. Occasionally, rER was seen to encircle the mitochondria. The smooth endoplasmic reticulum (sER) was also increased and was concentrated in the apical and perinuclear cytoplasm (Fig. 2*a*). In addition, dense bodies were increased in the apical and perinuclear cytoplasm and a population of dense bodies appeared in the basal processes (Fig. 2 *b*, *c*). The basal processes of the ependymal cells extended into the marginal layer to divide the newly formed decussating nerve fibres into funiculi and they terminated on the ventral pial surface. The basal processes through the marginal layer were rich in microtubules, whereas the terminal cytoplasm contained abundant microfilaments. Thin lamellar processes, which branched from the main stem process, were seen to ensheath the nerve fibres. Dense bodies, found basally, were encountered in the large processes, but less frequently in the thin lamellar processes (Fig. 2*b*). At the ventral pial surface, the basal terminals, though not all of them, contained a number of vesicular and tubular dense bodies and sER-like cisterns (Fig. 2*c*). Pinocytotic vesicles, associated with the terminal plasma membranes, were often observed. On the other hand, the apical surface of the cells showed abundant microvillous projections and cytoplasmic protrusions which contained some sER-like cisterns in addition to mitochondria, free ribosomes and glycogen granules. The apical protrusions bore a small number of cilia and microvillous projections (Figs. 1*a*, 2*b*).

As the floor plate became narrowed along the midline with the rapid development of the ventral column after E15, the elongated ependymal cells contained a condensed cytoplasm. Well-developed Golgi apparatus and rER were prominent in the cytoplasm

Fig. 4 (*a–e*). Multivesicular bodies and extraparenchymal vesicles seen at the ventral pial surface. (*a*) Increased number of multivesicular bodies (*MB*) at E18. The ependymal cells also contain a cluster of clear vesicles (*), resembling those in the growth cones of dendrite and axon of growing neuronal cells. × 16000. (*b*) High magnification of a multivesicular body seen in (*a*). The multivesicular body contains a small number of vesicles and was surrounded by 'bristles'. Note close association of this body with a smooth ER-like vesicle. × 49000. (*c*) Small vesicular structures seen extraparenchymally at E18. These vesicles were always found in a dense matrix similar in appearance to basal lamina. × 27000. (*d*) High magnification of extraparenchymal vesicles seen at E15. These were 40–60 nm in diameter. × 67000. (*e*) Vesicles seen under the lining basal lamina at E18. This figure suggests exocytosis of the multivesicular bodies at the ventral pial surface. × 65000.

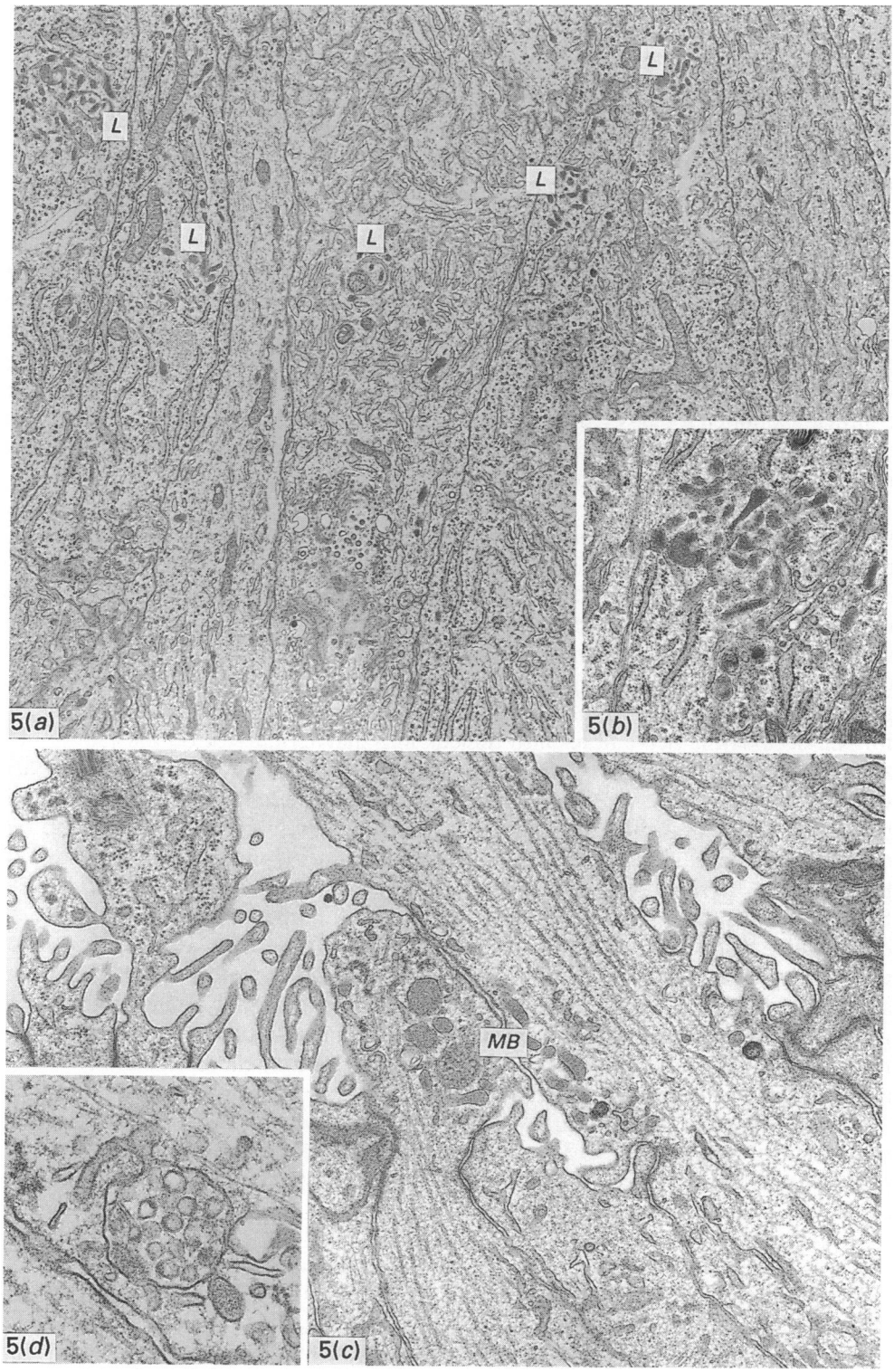


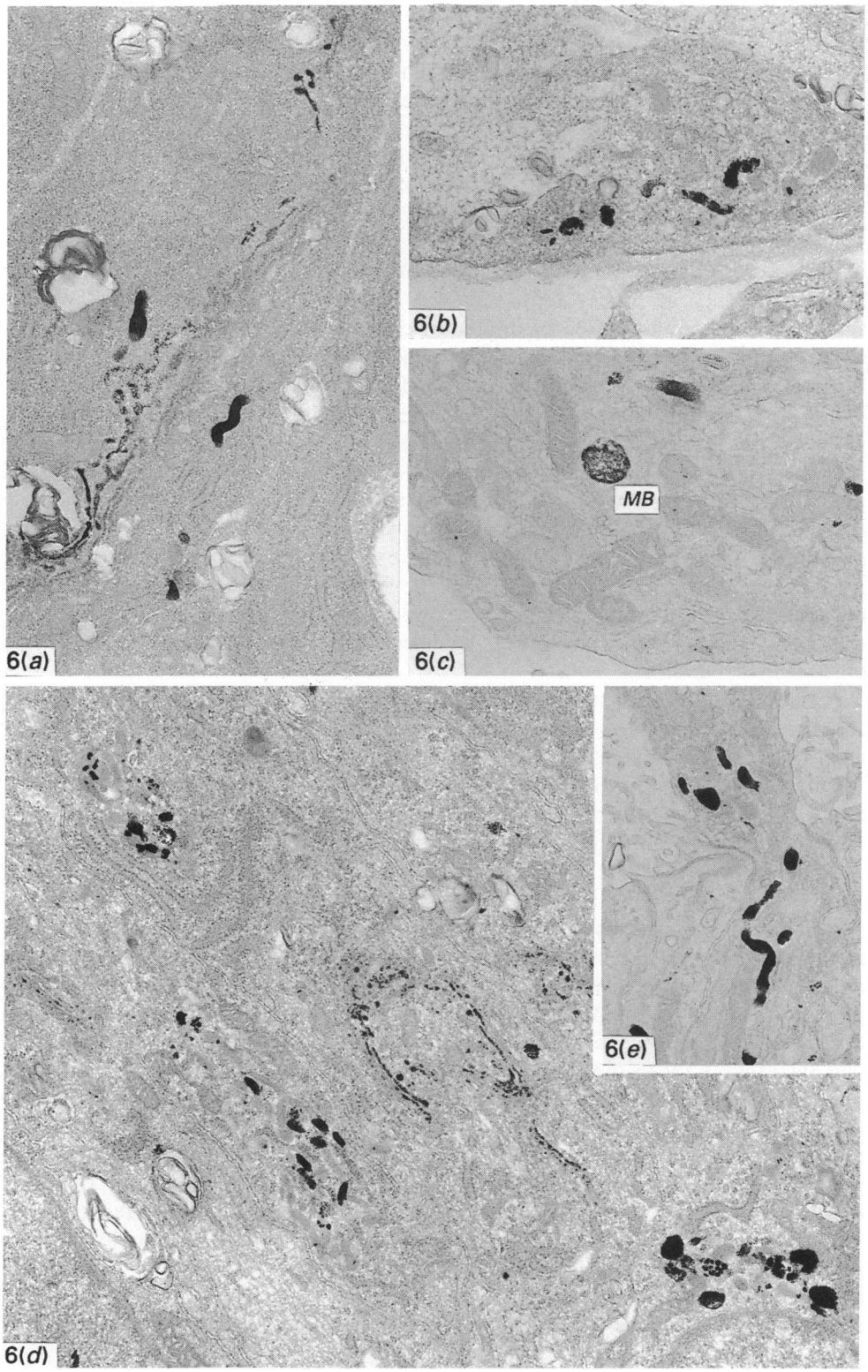
Fig. 5 (*a–d*). Apical aspects of the floor plate ependymal cells at E18. (*a*) Numerous dense bodies (*L*) in clusters in the apical cytoplasm. ×7000. (*b*) High magnification of a cluster of dense bodies. ×16500. (*c*) Dense bodies and multivesicular body (*MB*) within the cytoplasmic protrusions at the apical surface. ×21500. (*d*) High magnification of a multivesicular body within the apical protrusion. ×40000.

of such cells. The increased sER was seen not only in the apical cytoplasm but also in the basal processes. The basal terminals, packed with numerous dense bodies and other organelles such as sER-like vesicles and cisterns, were frequently seen at the ventral pial surface (Fig. 3*a–c*). Some of the vesicles had a dense matrix centrally, thus resembling a dense-cored vesicle. In the terminal cytoplasm, dense bodies were seen to fuse to other vesicles and cisterns and occasionally to pinocytotic vesicles (Fig. 3*c*, *d*). Dense bodies also appeared to be associated closely with the terminal plasma membrane but no distinct fusion of the two was identified in the present study. Multivesicular bodies were also increased in the basal terminals after E15 (Fig. 4*a*). This increase did not appear to be accompanied by an increase of multivesicular bodies in the basal processes that passed through the marginal layer. When a multivesicular body appeared in the terminals its membrane was often observed to connect with the membrane of sER-like vesicles and cisterns containing a moderately dense material (Fig. 4*b*). Multivesicular bodies were, in part, coated with bristles (Fig. 4*b*). In line with the increase of multivesicular bodies in the basal terminals, small vesicles similar to those in a multivesicular body were found in the extraparenchymal space (Figs. 3*a*, 4 *c*). These extraparenchymal vesicles were 40–60 nm in diameter and were always embedded in a basal lamina-like material (Fig. 4*c*, *d*). Figure 4*e* suggests that the contents of the multivesicular body may be released into the extraparenchymal space by means of exocytosis. In the apical cytoplasm at E18, dense bodies were seen in large clusters which first appeared in the cytoplasmic protrusions at the apical surface (Fig. 5*a–c*). Multivesicular bodies were also found in the apical protrusions and were closely related to other vesicular and cisternal structures within the protrusions (Fig. 5*c*, *d*).

Besides the vesicular system mentioned above, the ependymal cells of the floor plate contained clear clustered vesicles which were similar to those of the axonal and dendritic growth cones (Fig. 4*a*). Occasionally, the slender processes from the ependymal cells passed through the lining basal lamina of the ventral surface and came into close contact with the extraparenchymal cells, whereas no penetration of the extraparenchymal cell element into the spinal cord was observed.

ACPase activity in the floor plate ependymal cells

In the early stages of development, the activity of ACPase was found prominently in the Golgi apparatus, in which reaction products were localised in the trans-face cisterns and in the vesicles associated with them (Fig. 6*a*). A small number of lysosomes also gave a positive reaction and this lysosomal activity was seen in a tubular form (Fig. 6*a*). With an increase in embryonic age, the activity was widely demonstrated in the growing Golgi apparatus and in the lysosomes in the apical and perinuclear cytoplasm. At E18, almost all the lysosomes clustered in the apical cytoplasm contained intense ACPase activity (Fig. 6*d*). The activity was also found in lysosomes within the apical protrusions (Fig. 6*e*). On the other hand, the activity in the basal processes and terminals was positive in a limited number of lysosomes (Fig. 6*b*, *c*). In many cases, lysosomes containing enzyme activity appeared in tubular form within the terminals. Multivesicular bodies also showed enzyme activity, the reaction products of which filled up their matrices but enzyme activity was not observed in the vesicular contents (Fig. 6*c*). In a control experiment, the activity of ACPase in the floor plate ependymal cells was inhibited by the addition of sodium fluoride to the incubation medium.


6(a)
6(b)
MB
6(c)
6(d)
6(e)

DISCUSSION

The early differentiation of the ependymal cells of the floor plate, as well as of the roof plate, during embryonic development is a common feature of the mammalian spinal cord (Altman & Bayer, 1984; Gamble, 1969; Malinsky & Brichova, 1967; Sturrock, 1981*a*). Present observations in rats revealed that the differentiating ependymal cells of the floor plate contained two populations of dense bodies, which were found related to the apical and basal surfaces of cells, and also that the increased number of multivesicular bodies, following the increase of dense bodies, might be involved in the secretory activity of these cells on both surfaces. In human embryos, such secretory activity into the central canal lumen has been shown in the floor plate ependymal cells (Malinsky & Brichova, 1967; Tanaka, Yoshioka & Shinohara, 1988). In rats examined in the present study, the apical protrusions and the basal processes of the floor plate ependymal cells contained numerous dense bodies and other vesicles which appeared to originate from the Golgi apparatus or sER. The cytochemical activity of ACPase was positive in almost all dense bodies, that is, in the lysosomes, in the apical cytoplasm, whereas it was positive in only a limited number of lysosomes and in the multivesicular bodies within the basal terminals.

Early evidence that the floor plate ependymal cells of the developing spinal cord show intense ACPase activity was mentioned in a histochemical study by Schoenen (1978); however, Schoenen's descriptions were mainly focused on the neuronal differentiation of the spinal cord. Recently, Wilson (1986) has reported that some cytoplasmic processes in the midventral marginal layer of the mouse hindbrain during embryonic development contain ACPase-positive reaction products. Notwithstanding Wilson's doubt that the activity may be artefactual, it is possible to say that the ACPase activity may belong to small lysosomes in the basal processes of the floor plate ependymal cells. It is now believed that, as in other tissues, the lysosomal ACPase is related to a variety of cellular functions such as degeneration and cellular interaction in the nervous tissue (Inomata & Yoshioka, 1979; Pannese, Luciano, Iurato & Reale, 1976; Wender, Kozik & Sniatala-Kamasa, 1976). As suggested in the myelination process (Inomata & Yoshioka, 1979; Wender *et al.* 1976), lysosomes may play a role in the close association of the basal processes with the nerve fibres of the marginal layer. However, this does not seem to explain the presence and accumulation of lysosomes at the apical and basal surfaces of the ependymal cells. In addition to their role in intracellular digestion, lysosomes are also involved in extracellular digestion and/or modification of materials, by their release of enzymes into the surrounding medium (De Robertis & De Robertis, 1987; Poste, 1971). Within the basal terminals and the apical protrusions of the ependymal cells, dense bodies appear to fuse with the pinocytotic vesicles and also to be integrated with the multivesicular bodies. Lysosomes, characterised by the positive reaction of ACPase, may function in both internal and external media surrounding the developing spinal cord by being released through the multivesicular bodies.

Enrichment of glycogen granules in the cytoplasm is a feature characteristic of

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Fig. 6 (*a–e*). ACPase activity in the floor plate ependymal cells. (*a*) Activity in lysosomes and Golgi apparatus in the apical cytoplasm at E13. Cerium method. ×18500. (*b*) Activity in the basal terminals at E14. Reaction products were seen in a limited number of tubular lysosomes. ×25500. (*c*) Activity in the basal terminals at E18. Activity was positive in the multivesicular body (*MB*) and lysosomes. ×25500. (*d*) Activity in the apical cytoplasm at E18. Intense activity was confined to lysosomes in clusters and the Golgi apparatus. ×16000. (*e*) Activity in lysosomes within an apical protrusion at E18. ×16000. Figure 6(*b–e*) – lead method.

radial glial cells in the developing central nervous system (Henrikson & Vaughn, 1974; Sturrock & Smart, 1980; Uehara & Ueshima, 1984), suggesting that glycogen may be important as an energy source in these cells in supporting neuronal growth (Brückner & Biesold, 1981). It is likely that the floor plate may be a source of such radial glial cells during embryonic development of the spinal cord (Altman & Bayer, 1984). Tanycytes, a specialised population of ependymal cells with a transporting capacity (Bruni *et al.* 1985; Fleischhauer, 1972), may be the closest derivative of radial glial cells in the mature brain (Hajos & Basco, 1984). These ependymal cells are present along the length of the central canal in the mouse and rat spinal cord and have also been observed in clusters at the poles of the central canal in mice (Bruni & Reddy, 1987; Seitz, Lohler & Schendemann, 1981). In addition to involvement in neurohormonal transport between the ventricular and neurovascular spaces, tanycytes may have the ability to transport material from the brain surface (Gaze & Watson, 1968; Hinds & Hinds, 1972).

From another point of view the floor plate ependymal cells presented here exhibit some cytological features characteristic of the so-called CSF-contacting neuron, a specialised neuronal cell that faces the ventricular space with its axonal or dendritic processes (Bruni & Reddy, 1987; Fleischhauer, 1972; Vigh & Vigh-Teichmann, 1973; Vigh-Teichmann & Vigh, 1983). The CSF-contacting neurons contain well-developed rough and smooth ER in addition to the dense-cored vesicles, and a certain type of neuron found in the mature spinal cord also has abundant granular vesicles, resembling secretory granules, within an axonal terminal on the basal lamina (Vigh & Vigh-Teichmann, 1973; Vigh-Teichmann & Vigh, 1983). As compared with those of the CSF-contacting neurons, the floor plate ependymal cells appear to have a synthesising ability since they have a well-developed Golgi apparatus and ER in the apical cytoplasm. In addition, abundant vesicles, perhaps originating from such organelles, are concentrated within the basal terminals. Unfortunately, however, except for a possible pathway of lysosomes and multivesicular bodies which are characterised by ACPase activity, we have no understanding of the nature of many of the other vesicles nor of their exact fate. Apart from a possibility that the floor plate ependymal cells may be a type of CSF-contacting neurons during the development of the rat, the physiological significance of these ependymal cells in the developing rat spinal cord remains to be resolved.

In conclusion, the present study has revealed that the ependymal cells of the early differentiated floor plate may possess secretory functions by means of the polarised development of the vesicular system such as lysosomes and other vesicles. If it is true, the primitive CSF and extraparenchymal fluid can be the most effective media mediating the transport of released substances and such a function of the ependymal cells may consequently relate to the close network of the extraparenchymal vascular plexus surrounding the developing brain and spinal cord during early embryonic development (Evans, 1909; Simón-Marín, Vilarova, Aguinagalde & Barberá-Guillem, 1983; Sturrock, 1981*b*).

SUMMARY

The ependymal cells, particularly of the floor plate of the developing spinal cord of prenatal rats, ranging from embryonic Day 11 to Day 18, were examined at the ultrastructural level. A cytochemical evaluation of ACPase activity was applied to characterise the lysosomes in the developing ependymal cells.

During embryonic and fetal development of the rat spinal cord, the floor plate

ependymal cells contain abundant dense bodies and other vesicles, which perhaps originate from the well-developed Golgi apparatus and the rough and smooth endoplasmic reticulum in the apical cytoplasm. Lysosomes, characterised by acid phosphatase activity, appear to be transported through the basal processes of the ependymal cells and accumulate in the terminals on the ventral pial surface; lysosomes are also numerous in apical protrusions at later stages. Within the basal terminals, dense bodies can be found in close association with pinocytotic and other vesicles. Multivesicular bodies, containing acid phosphatase activity, develop in the terminal cytoplasm. Their contents may be released into the extraparenchymal space surrounding the developing spinal cord by means of an exocytosis – small vesicles with dense materials are found to increase extraparenchymally. The present findings suggest that the floor plate ependymal cells, which were seen to be synthetic during prenatal development, possess possible functions of secretion into not only the cerebrospinal fluid but also the extraparenchymal fluid surrounding the spinal cord.

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