

THE DEVELOPMENT OF GUINEA PIG SPINAL CORD EPENDYMA: A LIGHT- AND ELECTRON-MICROSCOPIC STUDY

LO SVILUPPO DELL'EPENDIMA DEL MIDOLLO SPINALE DI CAVIA:
STUDIO AL MICROSCOPIO OTTICO ED ELETTRONICO

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SUMMARY

A morphological study on guinea pig spinal cord ependyma was carried out during growth because of a few observations were reported on the morphology of the spinal cord ependyma in guinea pig. From ten female Dunkin Hartley guinea pigs (five 1 day aged and ten 90 days aged) the Authors have utilized the vertebral column divided in cervical, thoracic, lumbar, sacro-coccygeal blocks. For light microscopy 10µm cross serially cut sections were stained with toluidine blue and a modification of Heidenhain method. For electron microscopy ultrathin cross sections stained with 2% uranyl acetate and lead citrate were utilized.

Cylindrical ependymal cells lined the central canal for most of its extent at P1 (new-born subjects). At P90 (adult subjects) these cells became cubical. At P1 the ependymal canal, which was oval at cervical level, showed a slit aspect from thoracic and lumbar levels to sacral one. At P90 the central canal maintained its oval shape. At the level of the terminal ventricle of the medullar cone the wall was reduced to thin layer formed by both ependymal cells and tanocytes (more evident in P1), whose basal processes divided bundles of axons and contributed to the formation of the stratum marginale gliae.

Along the guinea pig spinal cord length, both in new-born and in adult subjects, the ependymal cells showed similar ultrastructural features compared with those observed in rat and mouse.

Key words: guinea pig, spinal cord, ependyma, growth.

RIASSUNTO

È stato effettuato uno studio morfologico sull'ependima del midollo spinale di cavia durante l'accrescimento, poiché in letteratura sono riportati scarsi dati sull'argomento. Gli Autori hanno prelevato la colonna vertebrale da dieci cavie Dunkin Hartley di sesso femminile (cinque di 1 giorno di età e cinque di 90 giorni di età) da cui sono stati isolati i segmenti cervicale, toracico, lombare, sacro-coccigeo. Per la microscopia ottica,

sezioni di 10 μ sono state colorate con blu di toluidina e con una variante del metodo di Heidehain. Per la microscopia elettronica, sezioni ultrafini sono state colorate con uranil acetato e citrato di piombo.

Nei soggetti neonati (P1) il canale centrale del midollo spinale è circondato da cellule endimali cilindriche per la maggior parte della sua estensione.

Nei soggetti adulti (P90) queste cellule diventano cubiche. Allo stadio P1 il canale endimale, che risulta ovale a livello cervicale, mostra un aspetto a fessura a partire dai tratti toracico e lombare fino a livello sacrale. Allo stadio P90 il canale centrale mantiene la sua forma ovale. A livello del cono midollare, insieme alle cellule endimali che circondano il canale centrale, è stata osservata la presenza di taniciti (più evidente a P1).

Lungo tutto il midollo spinale di cavia, sia nei soggetti neonati che adulti, le cellule endimali hanno mostrato simili caratteristiche istologiche, anche in relazione a quelle riscontrate in letteratura nel ratto e topo.

Parole chiave: cavia, midollo spinale, endima, accrescimento.

INTRODUCTION

The morphology of the spinal cord ependyma was particularly examined in several studies in neonatal and adult rat (Rafols & Goshgarian, 1985; Bruni & Reddy, 1987) and mouse (Sturrock, 1981; Seitz et al., 1981; Bjugn et al., 1988; Bjugn et al., 1989) but a few observations were reported on the morphology of the guinea pig central canal ependyma.

To follow up our investigations on guinea pig spinal cord development (Marroni & Coli, 1999a, 1999b), the aim of this paper is to study the spinal cord ependyma in newborn (P1) and adult (90 days) subjects, focusing on the height of the ependymal cells during growth and along the rostrocaudal length of the spine.

Furthermore, the ultrastructural organisation of the spinal cord ependyma is reported to compare different morphological features during growth and to distinguish them from those typical of rat and mouse.

MATERIALS AND METHODS

Animals: ten female Dunkin Hartley guinea pigs (five 1 day Postnatal aged, named P1 and five 90 days Postnatal aged, named P90) were used. The Authors selected only female subjects because a

sexual dimorphism in relation to a different number of vertebrae between male and female guinea pigs occurred.

All animals have been maintained under an accredited animal care and use program. The animals were sacrificed with a lethal inhalation of ether.

Light microscopy: blocks of vertebral column (cervical, thoracic, lumbar and sacro-coccygeal segments) were fixed in Bouin solution for 3-5 days, after decalcification in a 5% HNO₃ solution for 1-4 days and rinsing under tap water for 24 hours. The segments were embedded in paraffin and 10µm cross serially cut sections were stained with toluidine blue and with a modification of Heidenhain method (Tolivia et al., 1988).

Electron microscopy: ultrathin sections stained with 2% uranyl acetate and lead citrate were examined with TEM.

Morphometrical data: the height of ependymal cells was obtained by processing 10 µm cross sections (1 over 100) with Leica Q Win Analysis software (Leica, Cambridge, United Kingdom). The counts were not corrected for shrinkage. The SD was calculated.

RESULTS

Light Microscopy

In the Table I the mean height of the ependymal cells in the segments of the vertebral column at P1 and P90 was reported.

At the level of *cervical spinal cord*, in P1 subjects the ependymal canal was oval in shape. The epithelium consisted in a single layer of cylindrical cells with nuclei displaced in a mediobasal position (Fig. 1). At P90 the cervical canal became elongated in shape and the height of the lining cells wasn't different from that of P1 subjects.

In the *thoracic spinal cord* a cylindrical epithelium surrounded an oval canal both at P1 and at P90, although at P90 the mean height was lower.

In *lumbar spinal cord* the ependymal canal was usually collapsed and eccentrically located to the floor plate at P1; at P90 the canal had an oval shape (Fig. 2). At P1 the mean height of the lumbar ependymal cells was the highest and it was always higher than that at P90.

Both at P1 and P90, the morphological features of the *sacro-coc-*

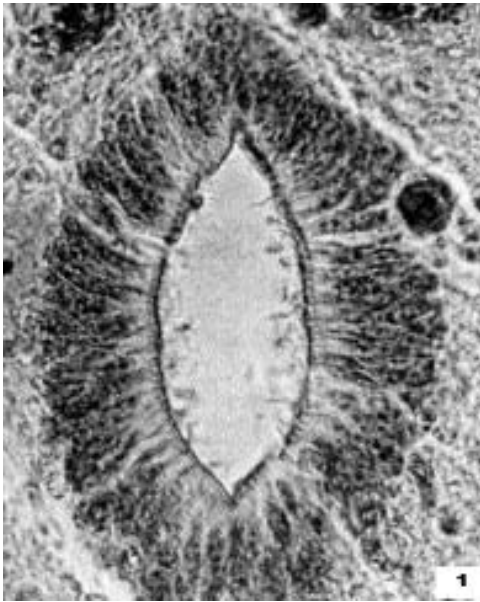


Fig. 1. Ependymal cells of cervical spinal cord (P1) (400X).

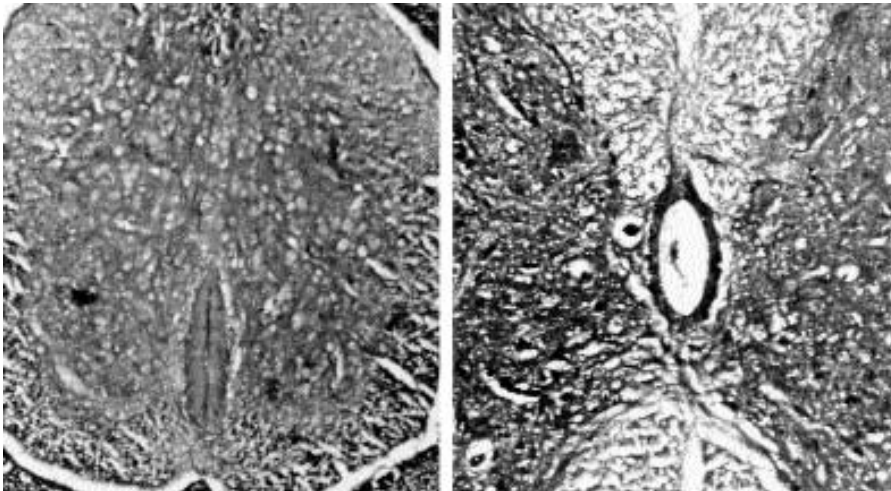


Fig. 2. Ependymal cells of lumbar spinal cord in P1 (sx) and in P90 (dx) (100X).

cygeal epithelium (Fig. 3) were in continuity with the lumbar one and the floor plate of the epithelium was near the pial covering. At P1 the ependymal canal was slit-like and lined by cylindrical cells while at P90 the oval ependymal canal was lined by cubic cells.

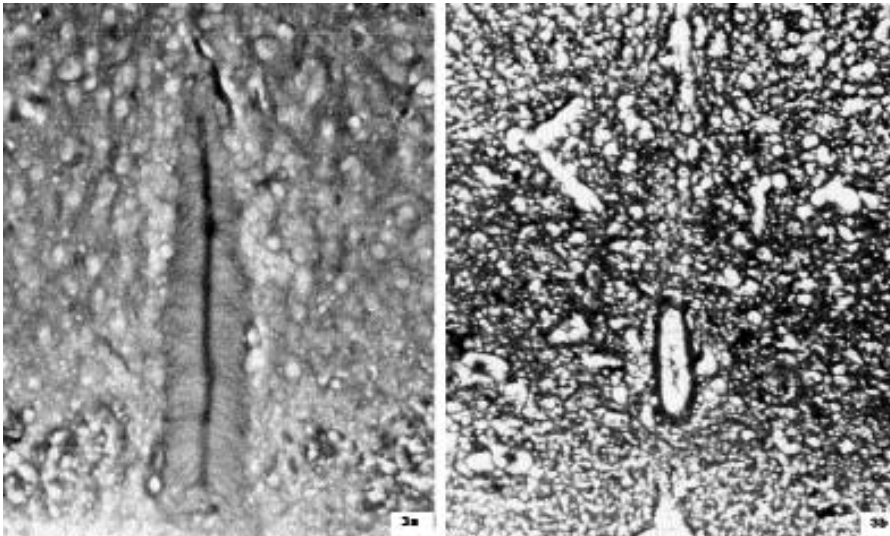


Fig. 3. Ependymal cells of sacral spinal cord in P1 (sx) and in P90 (dx) (200X).

At the medullar cone, the central canal occupied the largest area of the spinal cord cross sections and was irregular or triangular in shape (Fig. 4). While at P90 the lining cells were always cubical, at P1 the ependymal cells were more elongated and their height was double of that at P90.

Some cells with basal process were found. The basal processes of

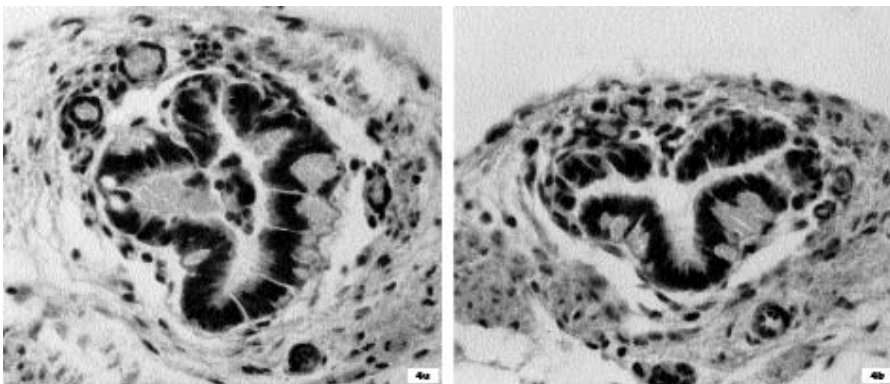


Fig. 4. Ependymal cells of medullar cone (P1): the central canal had irregular (sx) or triangular (dx) shape (400X).

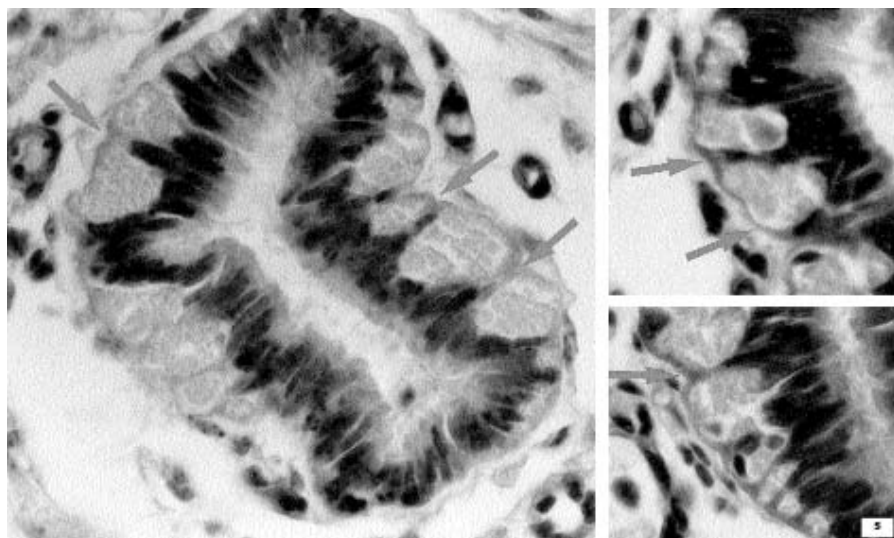


Fig. 5. Ependymal cells of medullar cone (P1): some tanycytes with basal processes divided bundles of axons (arrows) (800X).

these cells formed septa which divided bundles of myelinated axons (Fig. 5). These cells were considered as tanycytes.

Electron microscopy

The ultrastructural features of the ependymal cells of the guinea pig spinal cord resembled those described in rat (Bruni & Reddy, 1987) and mouse (Seitz et al., 1981).

Several lining ependymal cells with polymorphic nuclei displaced at various levels from the central canal were present (Fig. 6a). The nuclei contained heterochromatin near the nuclear envelope, but predominantly euchromatin, as pointed out in mouse spinal cord ependyma by Bjugn et al., (1988).

The cells showed well-developed Golgi apparatus, located in the apical pole of the cytoplasm. Small filamentous mitochondria were present particularly in a medio apical position; numerous clusters of free ribosomes were dispersed in the cytoplasm between scattered cisternae of rough endoplasmic reticulum; some glycogen granules were characteristic of the cells with a cilium (Fig. 6b).

At the pole of the cells the ciliary apparatus and numerous microvilli were visible (Fig. 6a).

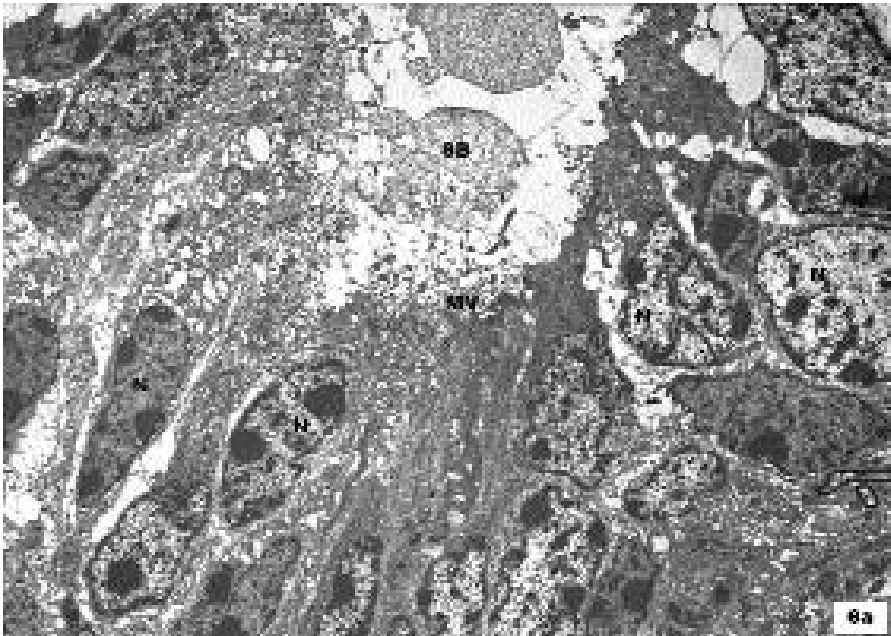


Fig. 6a. Ependymal cells of thoracic spinal cord (P1): several polymorphic nuclei (N) with prevalent euchromatin, microvilli (MV) and some supraependymal bulbs (SB) were visible (2500X).

Adjoining cells were connected by zonulae adherentes (Fig. 6b).

On the surface of the ependymal cells some cytoplasmic extensions were observed. These supraependymal bulbs can be in contact with ependymal epithelium or free in the central canal (Fig. 6a).

DISCUSSION

In the present study the Authors point out that guinea pig spinal cord ependyma shows lining cells of different height surrounding a central canal variously expanded.

During growth, the height of the ependymal cells progressively decreased: at P1 cylindrical lining cells around central canal were present from cervical to sacro-coccygeal segments; on the contrary, at P90 a columnar epithelium progressively decreased in height caudally until it showed as cubical at coccygeal level.

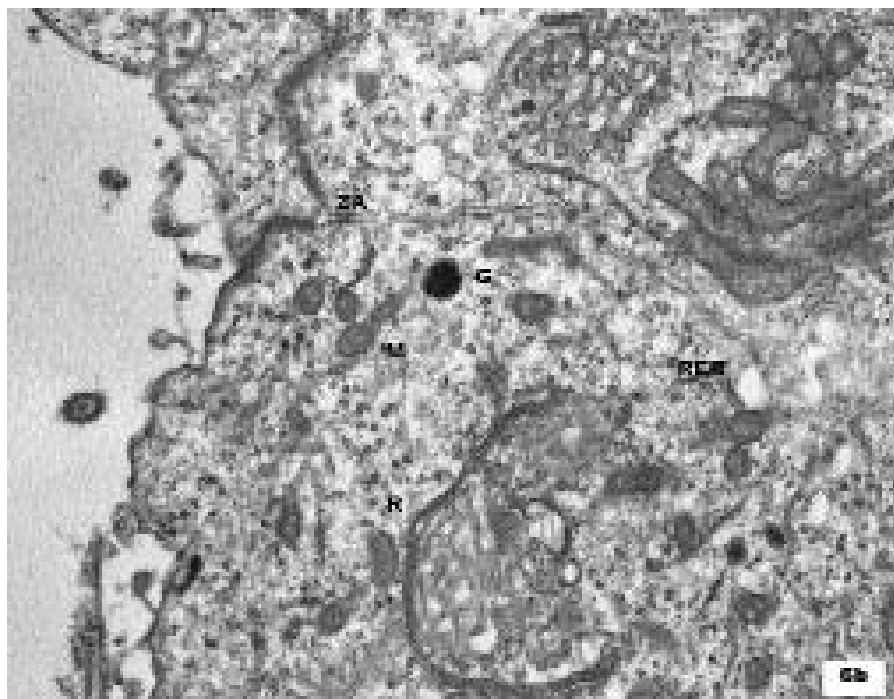


Fig. 6b. Ependymal cells of thoracic spinal cord (P90): small filamentous mitochondria (M), clusters of free ribosomes (R), scattered cisternae of rough endoplasmic reticulum (RER), glycogen granule (G), zonulae adherents (ZA), cilium (C) (15000X).

The shape of the central canal was oval in the cervical and thoracic ependyma both in P1 and P90 subjects; only in P1, the lumbar and upper sacral central canal was collapsed, as described in the mouse (Sturrock, 1981) and rat (Bruni & Reddy, 1987).

At the level of the medullar cone the central canal was irregular or triangular in shape, as pointed out by Seitz et al. (1981) in mouse spinal cord. This terminal part of the medullar cone, called as “terminal ventricle” (Warwick et al., 1985) was triangular in shape also in man. Around the central canal of the medullar cone the wall was reduced to a thin layer formed by both ependymal cells and tanocytes (more evident in P1 subjects) whose basal processes divided bundles of axons. Peripherically these processes or tails, bound each other, lied near the pial surface and blood vessels.

The Authors verify that these processes contribute to the formation

of the stratum marginale gliae (Seitz et al., 1981) and the ependymal coat at the level of the medullar cone.

Our observations on the presence of tanycytes confirmed those of Seitz in mouse and of Rafols and Goshgarian in rat, that reported those cells to be a transport system between the central canal and the subarachnoid space.

The ultra structural features of guinea pig spinal cord ependyma resembled those reported in rat spinal cord (Bruni & Reddy, 1987)

The supraependymal bulbs observed are interpreted as neurosecretory terminals and are also observed in mouse and rat spinal cord (Sturrock, 1981; Bjugn et al., 1988; Cards & Rafols, 1978).

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