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EPENDYMAL LINING OF BRAIN VENTRICLES AND CENTRAL CANAL OF THE SPINAL CORD OF ADULT ALBINO RAT: A SCANNING ELECTRON MICROSCOPIC STUDY

By

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INTRODUCTION

Ependymal cells provide a unicellular epithelial lining for brain ventricles and central canal of the spinal cord **(Berry et al., 1995).** Embryologically, ependymal cells differentiate from the neuroepithelial cells, after the latter cease to produce neuroblasts and gliablasts **(Sadler, 1985).**

There is a marked regional variation in the ependymal lining of the brain ventricles in mammals. It was reported that the ependymal cells overlying areas of grey matter are cuboidal in shape and each cell presents numerous cilia in its apical centre, surrounded by short microvilli. However, where the ependyma lines white matter, the cells are markedly flattened up to the extent of being squamous and fewer of them are ciliated **(Scott et al., 1974; Page et al., 1979; Mestres et al., 1985; Mitro et al., 1989; Alvarez-Morujo et al., 1992).** Moreover, the ependyma of the ventricular choroid plexuses was noticed to be a low cuboidal epithelium with numerous microvilli but few cilia on the surface, and its underlying stroma was rich in fenestrated capillaries **(Clementi and Marini, 1972).**

In the adult human brain, it was reported that mature ependyma was not merely an inert lining but might regulate the transport of ions, small molecules, and water between the cerebrospinal fluid and nervous tissue, and it served an important barrier function that protected neural tissue from potentially harmful substances **(Bruni, 1998).**

Reviewing the literature, it was noticed that most of the studies of the fine surface structure of the ependyma in the rat brain dealt with that of the third ventricle **(Paull et al., 1977; Walsh et al., 1978).** However, information concerning the ependyma of other brain ventricles, as well as the central canal of the spinal cord, was relatively few.

Therefore, it became the aim of the present study to examine the ependyma of the adult rat brain ventricles with scanning electron microscopy in order to clarify the alteration in its surface fine structure throughout the whole ventricular system, and the central canal of the spinal cord.

MATERIALS AND METHODS

I. Obtaining the specimens of rat brain ventricles and spinal cord central canal:

Ten adult male Sprague-Dawley albino rats (150-200 gm) [obtained from the animal house, Faculty of Medicine, Ain Shams University] were used in the present study. All animals were anaesthetized by intraperitoneal injection of a single dose of phenobarbitone (6mg/100g body weight), and rapidly perfused transcardially in the left ventricle with heparinized phosphate-buffered saline (PBS; pH 7.4) followed by 1% glutaraldehyde (Fluka) - 2% paraformaldehyde (Sigma) in PBS (pH 7.4). In each animal, the cranium was cut from behind and opened to expose the brain. The whole brain was then carefully dissected out, and its different parts were visualized by the stereoscopic microscope. The spinal cord was immediately exposed in full length through dorsal laminectomy, and fixed in situ by 1% glutaraldehyde - 2% paraformaldehyde in PBS (pH 7.4). A series of horizontal, sagittal, and parasagittal sections were taken in each specimen to expose the lateral, third, and fourth ventricles as well as the cerebral aqueduct. Identification of the different segments of the spinal cord was achieved by counting the spinal nerves from the first cervical segment downwards. According to **Rowett (1968)**, the rat spinal cord presented thirty-four pairs of spinal nerves distributed as follows: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral, 3 caudal. In the present study, the cervical and lumbar segments of the cord were selected to be investigated.

II. Processing the rat brain ventricles and spinal cord central canal specimens for the scanning electorn microscopic study:

The obtained specimens of perfused brain ventricles and spinal cord central canal were washed twice in PBS (pH 7.4). The tissues were fixed for 60 min. in 1% glutaraldehyde - 2% paraformaldehyde in phosphate buffer at room temperature (pH 7.4). The tissues were then washed twice, 5 min. each, in buffered sucrose (0.1M phosphate buffer, 5% sucrose solution). Post fixation was performed at 4°C for 60 min. in 2% phosphate-buffered osmium. Following rinsing in several changes of cold distilled water, the tissues were dehydrated in a graded series of ethanols (40%, 50%, 70%, 80%, 90%, and twice in 100%). Further dehydration of specimens was carried out in absolute ethanol: acetone (1:1) solution for 30 min., followed by three changes of absolute acetone, 10 min. each. The specimens were then critically point dried in CO_2 drying apparatus CPD 030, mounted on stubs, and finally coated with gold in the sputter coater SCD005. Moreover, in order to expose subependymal tissues, some of the specimens were subjected to fracture freeze in liquid nitrogen. Fixed tissues were immersed in ethanol, quench-frozen in liquid nitrogen and cracked. The tissues were then processed to critical drying as usual. Examination and pho-

tography of specimens were carried out at the Scanning Electron Microscopy Center, Anatomy Department, Faculty of Medicine, Ain Shams University using Philips Scanning Electron Microscope XL 3 at 30 kv.

RESULTS

I. Scanning electron microscopic observations on the ependymal lining of the lateral ventricle:

In the present investigation, careful examination of specimens of the lateral ventricular wall showed areas of regular infoldings **(Figs. 1, 2)**. Two types of ependymal cells were seen; ciliated and non-ciliated. The ciliated cells expressed various patterns of ciliation, ranging from densely ciliated **(Figs. 2, 3)**, sparsely ciliated **(Fig. 4)**, and cells with single cilia **(Fig. 5)**. It was also noticed that the heavily ciliated cells constituted most of the ependymal lining of the lateral ventricle **(Figs. 2, 3)**, whereas the sparsely ciliated cells occurred in isolated groups **(Fig. 4)**. In several instances, the single cilia were relatively long and seemed to emerge from adjacent ependymal cells boundaries **(Fig. 5)**. The non-ciliated cells showed numerous surface microvilli **(Figs. 2, 3)**. Such microvilli were easily recognized in the sparsely ciliated and singly ciliated cells; however, they were obscured in the heavily ciliated ones.

In fracture freeze preparations, the ependymal cells appeared low columnar or cuboidal in shape **(Fig. 6)**. Terminal ciliary dilatation was observed. Moreover, a characteristic unidirection of cilia was identified **(Fig. 6)**. The average length of cilia was 6.6μm. In addition, few supraependymal (ranging from 0.9 to 1.3 μm in diameter) as well as numerous subependymal (ranging from 0.7 to 2.03 μm in diameter) vesicular structures were noticed **(Fig. 5)**. The subependymal vesicular structures were usually seen aggregated in clusters. Subependymal varicosed nerve fibers were also identified. Moreover, long cellular processes projecting from the base of ependymal cells were observed to extend deeply in the subependymal region **(Fig. 5)**.

II. Scanning electron microscopic observations on the ependymal lining of the third ventricle:

In the present study, examination of specimens of the third ventricular wall revealed variations in the ciliation density of the ependymal cell lining **(Fig. 7)**. Rows of densely ciliated ependymal cells were seen amidst singly ciliated cells **(Fig. 8)**. Similar to the findings in the examined specimens of the lateral ventricle, some of the single cilia appeared to project between adjacent ependymal cells. Furthermore, surface microvilli were observed in both non-ciliated and singly ciliated ependymal cells **(Fig. 8)**. Supraependymal fibers with characteristic varicosities were occasionally encountered **(Fig. 8)**. Areas of supraependymal globular vesicular structures of variable

sizes (ranging from 2.09 to 20 μm in diameter) were also observed **(Fig. 9)**. Many of these vesicles seemed to be arranged in a rather punch pattern. Moreover, thin fibers were seen in close association with such vesicles **(Fig. 9)**. According to their surface outline, three types of these vesicles could be identified; smooth, rough, or whorled **(Fig. 10)**.

Furthermore, supraependymal cells were seen overlying the ependymal lining of the third ventricle **(Fig. 11)**. Most of these cells were elongated, and their surface appeared irregular with few surface microvilli and blebs. Surface and side cellular processes were also noticed. Such cells were observed to span several ependymal cells. In addition, few smaller rounded cells with numerous thin processes were seen. Numerous rounded or irregular clumps of debris were observed in close association with the supraependymal cells **(Fig. 11)**.

III. Scanning electron microscopic observations on the ependymal lining of the cerebral aqueduct:

In the current study, the examined specimens of the cerebral aqueduct revealed that it was lined by a thin layer of non-ciliated ependymal cells **(Fig. 12)**. The ependymal cells were polygonal in shape and had a convex dome-shaped surface studded with surface microvilli **(Fig. 13)**.

IV. Scanning electron microscopic observations on the ependymal lining of the fourth ventricle:

Careful examination of specimens obtained from the floor of the fourth ventricle showed that its ependymal lining was heavily ciliated near the median sulcus, while it was sparsely ciliated in its lateral part with a transition zone of moderately ciliated cells in between **(Figs. 14, 15)**. The heavily ciliated ependymal cells further exhibited two patterns of ciliary distribution; homogeneously densely ciliated ependymal cells **(Fig. 16)**, and rows of densely ciliated ependymal cells intermingled with non-ciliated ependymal cells with numerous surface micovilli **(Fig. 17)**. Infrequently, adherent tufts of cilia were seen among the heavily ciliated cells **(Fig. 18)**. Furthermore, in the laterally located sparsely ciliated zone, ependymal cells with few cilia, single cilia and even non-ciliated cells were identified **(Fig. 15)**. Numerous thin supraependymal fibers were commonly seen running between the cilia of the ependymal cells. Moreover, surface apical pits and numerous microvilli were observed on the surface of non-ciliated ependymal cells **(Fig. 19)**.

In fracture freeze preparations, the ciliated ependymal cells were noticed to extend long basal processes deep into the subependymal region **(Fig. 20)**. Moreover, varicosed nerve fibers together with thin ones were seen running beneath the ependymal cells **(Fig. 20)**. Occasionally, bullous protrusions were seen projecting between the ciliated ependymal cells **(Fig. 21)**.

V. Scanning electron microscopic observations on the ependymal lining of the central canal of the cervical part of the spinal cord:

In the present investigation, thorough examination of specimens of the central canal of the cervical part of the spinal cord revealed infolding of the ependymal lining. It was noticed that it was lined by a single layer of non-ciliated ependymal cells. The surface of the ependymal cells was studded with numerous microvilli and microblebs **(Fig. 22)**.

In fracture freeze preparations, the subependymal region presented several neural elements comprising cells with multiple processes, varicosed nerve fibers of variable thickness, and numerous vesicles variable in size and shape **(Fig. 23)**.

VI. Scanning electron microscopic observations on the ependymal lining of the central canal of the lumbar part of the spinal cord:

Examination of specimens of the central canal of the lumbar part of the spinal cord revealed regular ependymal infoldings. It was observed that the majority of the ependymal cells were non-ciliated with numerous apical surface microvilli. However, singly ciliated ependymal cells were constantly seen among the non-ciliated cells **(Fig. 24)**. The non-ciliated ependymal cells appeared dome-shaped **(Fig. 25)**. The cilia of the singly ciliated cells were of variable lengths (ranging from 1.92 to 7.44 μm). Commonly, the single cilia were seen emerging from adjacent ependymal cell boundaries **(Fig. 25)**.

Frequently, supraependymal club-shaped vesicular structures were also observed **(Figs. 25, 26)**. In addition, supraependymal fibers with characteristic varicosities were seen **(Fig. 26)**. Subependymal numerous vesicular structures of variable size (ranging from 0.9 to 3.06 μm) often with small numerous surface projections were also identified **(Fig. 27)**.

In fracture freeze preparations, some of the non-ciliated ependymal cells were seen to extend one or more long basal processes penetrating deeply in the subependymal region **(Fig. 28)**.

DISCUSSION

I. The ependymal lining of the lateral ventricle:

In the present study, it was shown that the ependymal lining of the lateral ventricle of the albino rat presented a folded pattern in the form of protrusions into the ventricular cavity separated by invaginations into the underlying brain tissue. Such ependymal folding was also recognized in the avian ventricular wall **(Korf and Fahrenkrug, 1984)**.

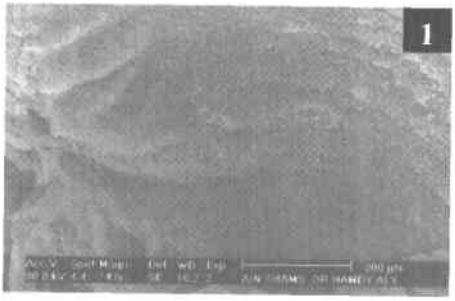

Fig. (1): Scanning electron micrograph of the lateral ventricle of albino rat showing regular infolding of its wall. **(x 147)**

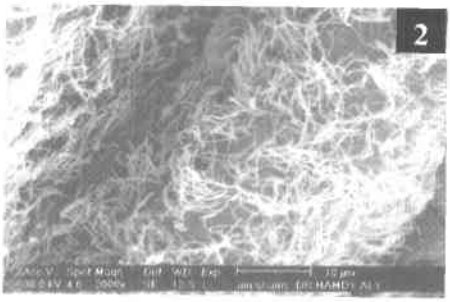

Fig. (2): Scanning electron micrograph of the lateral ventricle of albino rat showing the heavily ciliated ependymal folds. **(x 2000)**

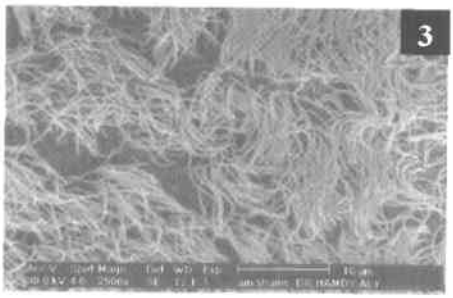

Fig. (3): Scanning electron micrograph of the lateral ventricle of albino rat showing that its heavily ciliated ependymal cells constitute most of the ependymal lining. Note that the non-ciliated cells present numerous surface microvilli. **(x 2500)**

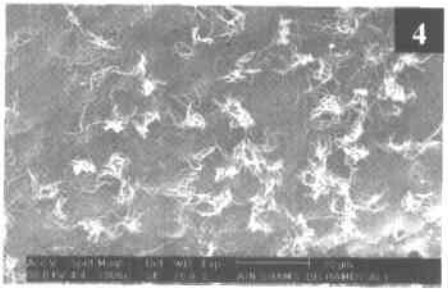

Fig. (4): Scanning electron micrograph of the lateral ventricle of albino rat showing isolated groups of sparsely ciliated ependymal cells with numerous surface microvilli. **(x 1000)**

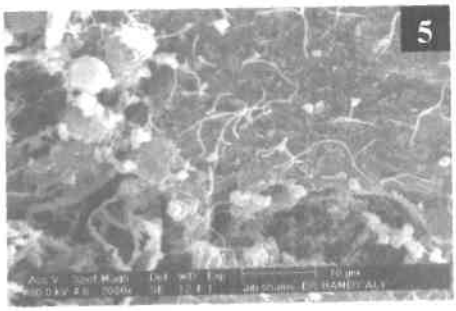

Fig. (5): Scanning electron micrograph of a fracture freeze preparation of the lateral ventricle of albino rat showing singly ciliated ependymal cells with numerous surface microvilli. Note the long single cilia emerging from adjacent ependymal cell boundaries. Few supraependymal and numerous clustered subependymal vesicular structures were noticed. Note also the subependymal varicosed nerve fiber (f) and the long cellular process extending from the base of ependymal cells (e) deeply in the subependymal region. **(x 2000)**

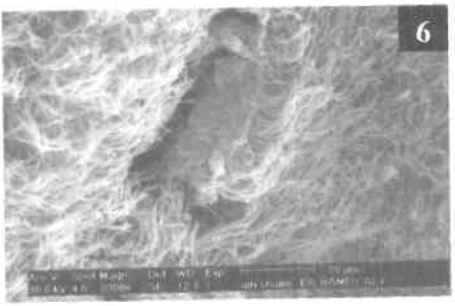

Fig. (6): Scanning electron micrograph of a fracture freeze preparation of the lateral ventricle of albino rat showing low columnar or cuboidal ependymal cells with a characteristic unidirection of cilia and a terminal ciliary dilatation. **(x 2000)**

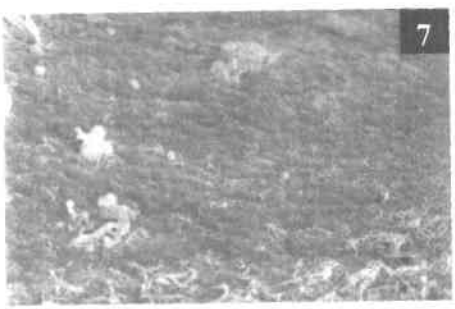

Fig. (7): Scanning electron micrograph of the third ventricle of albino rat showing variation in the ciliation density of the ependymal cell lining. **(x 650)**

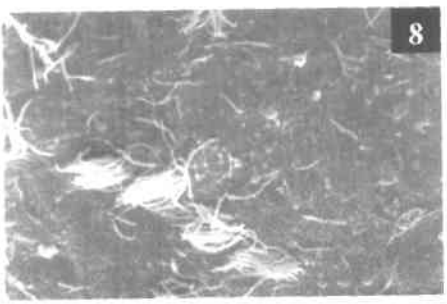

Fig. (8): Scanning electron micrograph of the third ventricle of albino rat showing rows of densely ciliated ependymal cells among singly ciliated cells. Some of the single cilia emerge between adjacent ependymal cells. Note the surface microvilli in both non-ciliated and singly ciliated ependymal cells, as well as the occasional presence of supraependymal nerve fibers (f) with characteristic varicosities. **(x 1452)**

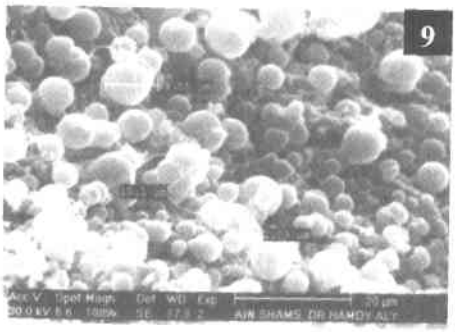

Fig. (9): Scanning electron micrograph of the third ventricle of albino rat showing supraependymal globular vesicular structures of variable sizes. Note their punch pattern arrangement and the thin fibers in close association with such vesicles.

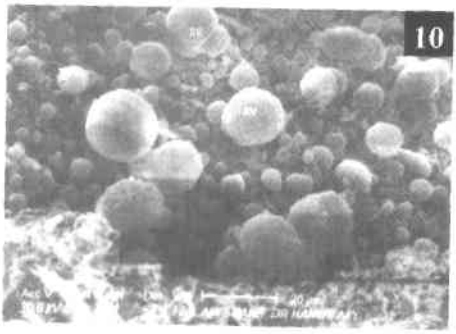

Fig. (10): Scanning electron micrograph of the third ventricle of albino rat showing the three types of the supraependymal globular vesicular structures; smooth (SV), rough (RV), or whorled (WV). **(x 1089)**

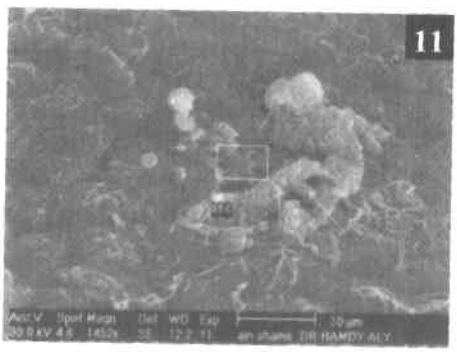

Fig. (11): Higher magnification of the field seen in Fig. (7) of the third ventricle of albino rat showing elongated supraependymal (SEC) non-ciliated cells with irregular surface. Note the surface microvilli and blebs, as well as the surface and side cellular processes (long arrows). Few smaller rounded cells (in the rectangular frame) with numerous thin processes were also seen. Note the clumps of debris (short arrows) in close association with the supraependymal cells. **(x 1452)**

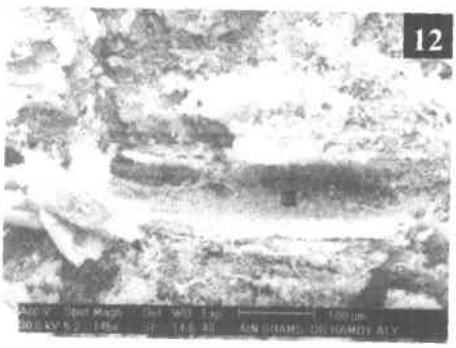

Fig. (12): Scanning electron micrograph of the cerebral aqueduct (ED) of albino rat showing a thin layer of non-ciliated ependymal cellular lining **(x 145)**

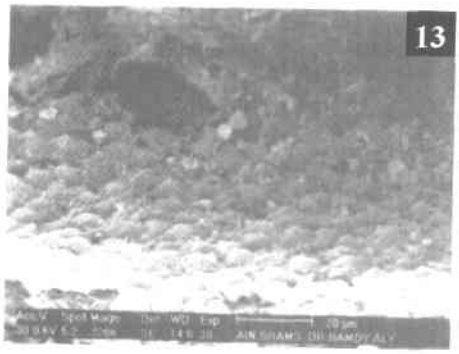

Fig. (13): Higher magnification of the previous field of the cerebral aqueduct of albino rat showing that its ependymal cells are polygonal in shape with convex dome-shaped surface studded with surface microvilli. **(x 726)**

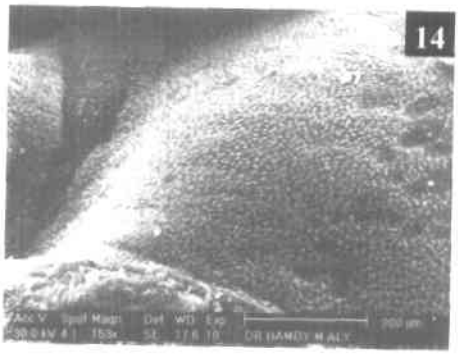

Fig. (14): Scanning electron micrograph of the fourth ventricular floor of albino rat showing heavily ciliated ependymal cells near the median sulcus. **(x 153)**

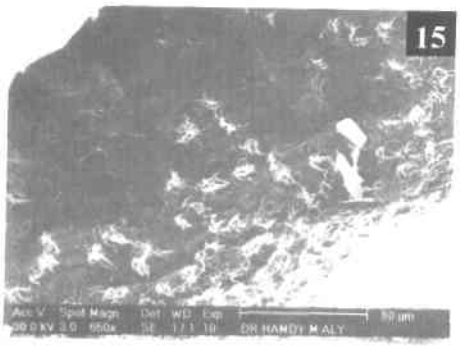

Fig. (15): Scanning electron micrograph of the ependymal lining of the floor of the fourth ventricle of albino rat showing a transition zone of moderately ciliated cells in between the medial zone of heavily ciliated cells and the lateral zone of sparsely ciliated cells. Note the presence of ependymal cells with few cilia, single cilia and even non-ciliated cells in the lateral sparsely ciliated zone. **(x 650)**

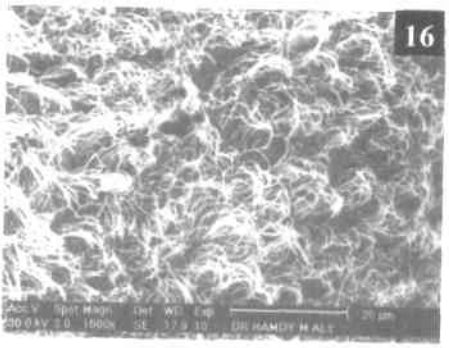

Fig. (16): Scanning electron micrograph of the heavily ciliated medial zone of the floor of the fourth ventricle of albino rat showing homogeneously densely ciliated ependymal cells. **(x 1500)**

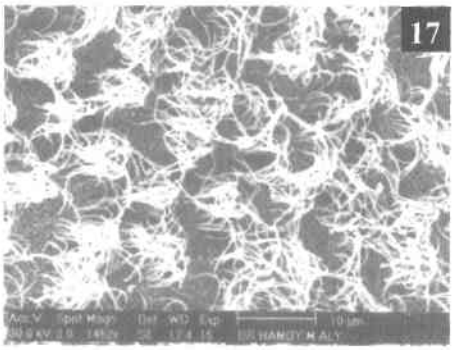

Fig. (17): Scanning electron micrograph of the heavily ciliated medial zone of the floor of the fourth ventricle of albino rat showing rows of densely ciliated ependymal cells intermingled with non-ciliated cells with numerous surface micovilli. **(x 1452)**

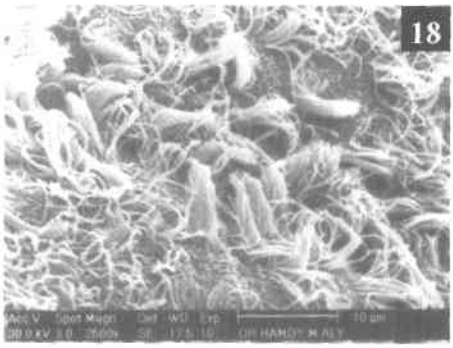

Fig. (18): Scanning electron micrograph of the heavily ciliated medial zone of the floor of the fourth ventricle of albino rat showing adherent tufts of cilia among the heavily ciliated cells. **(x 2500)**

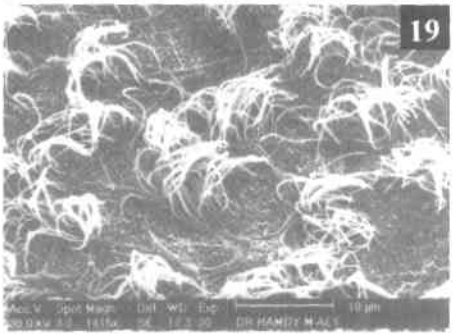

Fig. (19): Scanning electron micrograph of the floor of the fourth ventricle of albino rat showing numerous thin supraependymal fibers (arrows) between cilia of the ependymal cells, as well as the surface apical pits and the numerous microvilli of non-ciliated ependymal cells. **(x 1815)**

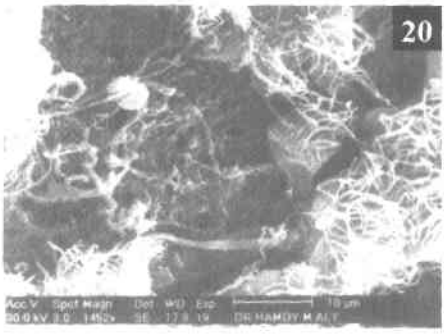

Fig. (20): Scanning electron micrograph of a fracture freeze preparation of the floor of the fourth ventricle of albino rat showing a ciliated ependymal cell (long arrow) extending long basal processes deep into the subependymal region. Note the varicosed (short arrows) and the thin (in the rectangular frame) nerve fibers running beneath the ependymal cells. **(x 1452)**

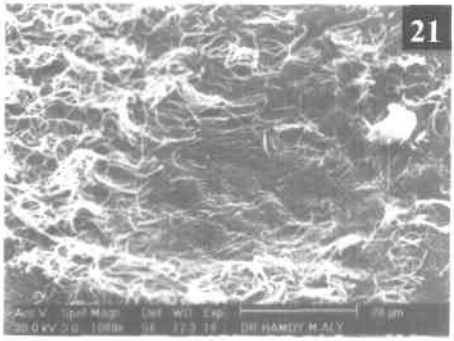

Fig. (21): Scanning electron micrograph of the floor of the fourth ventricle of albino rat showing a bullous protrusion (arrow) projecting between the ciliated ependymal cells. **(x 1089)**

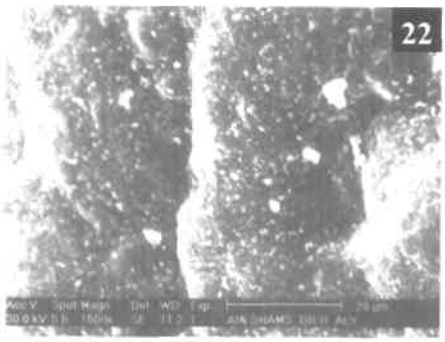

Fig. (22): Scanning electron micrograph of the central canal of the cervical part of the spinal cord of albino rat showing infolding of its ependymal lining that consists of non-ciliated ependymal cells studded with numerous microvilli and microblebs. **(x 1500)**

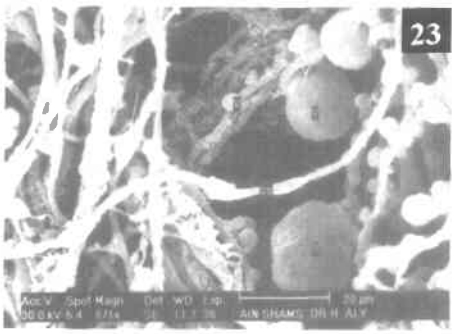

Fig. (23): Scanning electron micrograph of a fracture freeze preparation of the central canal of the cervical part of the spinal cord of albino rat showing the presence of cells with multiple processes (arrow), varicosed nerve fibers (VN), and numerous vesicles (V) in the subependymal region. **(x 871)**

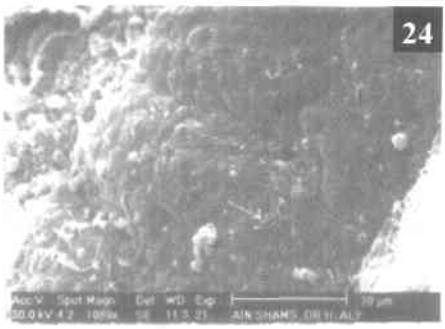

Fig. (24): Scanning electron micrograph of the central canal of the lumbar part of the spinal cord of albino rat showing regular infoldings of its ependyma. Note that the majority of the ependymal cells are non-ciliated with numerous apical surface microvilli but singly ciliated ependymal cells (arrows) are constantly seen among the non-ciliated cells. **(x 1089)**

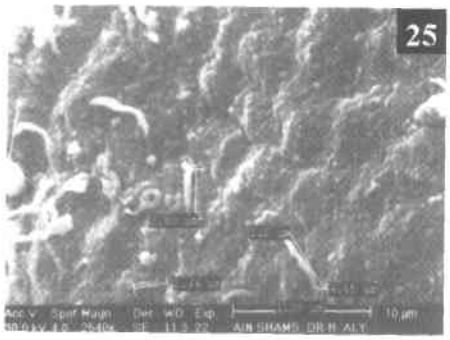

Fig. (25): Scanning electron micrograph of the central canal of the lumbar part of the spinal cord of albino rat showing the dome-shaped non-ciliated ependymal cells and singly ciliated cells with cilia of variable lengths that commonly emerge from adjacent ependymal cell boundaries. Note the supraependymal club-shaped vesicular structure (arrow). **(x 2540)**

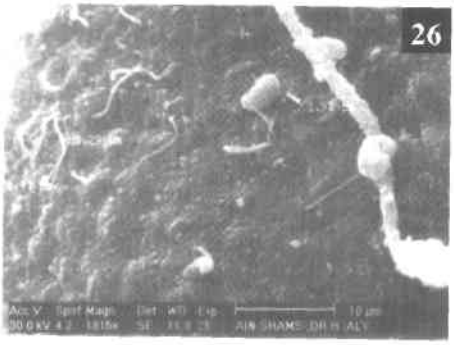

Fig. (26): Scanning electron micrograph of a nearby field of the specimen shown in the previous figure showing a supraependymal club-shaped vesicular structure (short arrow) and a supraependymal fiber with characteristic varicosities (long arrow). **(x 1815)**

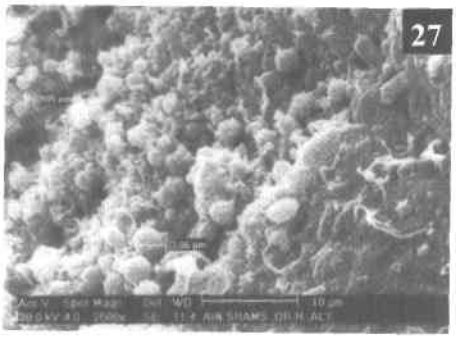

Fig. (27): Higher magnification of the field seen in Fig. (24) of the central canal of the lumbar part of the spinal cord of albino rat showing numerous subependymal vesicular structures of variable sizes often with small numerous surface projections. **(x 2500)**

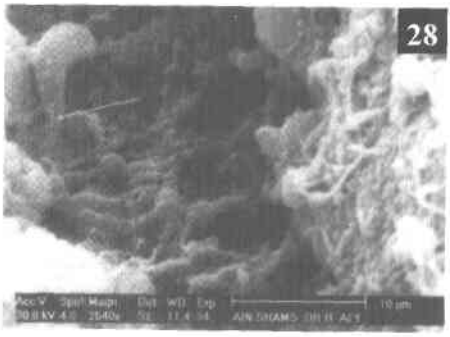

Fig. (28): Scanning electron micrograph of a fracture freeze preparation of the central canal of the lumbar part of the spinal cord of albino rat showing long basal processes (arrow) of a non-ciliated ependymal cell penetrating deeply in the subependymal region. **(x 2540)**

Characteristically, the present work revealed that most of the ependymal cells lining the lateral ventricle were heavily ciliated. However, areas of sparsely ciliated, singly ciliated or even non-ciliated ependymal cells were also seen. In man, **Mitro et al. (1989)** described similar variations in the distribution of ciliated and non-ciliated ependymal cells in the brain lateral ventricle. Moreover, different patterns of ciliation were identified in the pigeon lateral ventricle **(Mestres et al., 1985; Mestres and Rascher, 1994).** In that respect, **Scott et al. (1974)** mentioned that the ependymal cells were heavily ciliated over grey matter, while it was sparsely ciliated over white matter. **Berry et al. (1995)** mentioned that these differences might be related to a greater role in the exchange of metabolites between grey matter and CSF than the case for white matter. However, in pigeons, no correlation between a given surface pattern and a specific type of underlying nervous tissue could be identified **(Mestres et al., 1985).**

In the present investigation, it was noticed that the sparsely ciliated ependymal cells occurred in isolated groups. Similarly, ciliated ependymal cells were arranged in groups in the brain lateral ventricle of armadillo **(Jacobs and Monroe, 1977).**

In the current work, the non-ciliated cells showed numerous surface microvilli. Such microvilli were easily recognized in the sparse and singly ciliated cells. However, in case of the heavily ciliated cells, the microvilli were obscured by the cilia. Similar variations in microvilli distribution were noticed in ependymal cells of lateral ventricle in man **(Mitro et al., 1989)**, and in pigeons **(Mestres et al., 1985).**

On the other hand, the present work demonstrated that the cilia of the ependymal cells of the lateral ventricle had terminal dilatations. Such finding was recognized in sheep by **Rajtova (1988)** who noticed that the cilia of the ependymal cells of the lateral ventricle had club-like terminations.

II. The ependymal lining of the third ventricle:

The present investigation demonstrated variations in the density of ciliation of the ependymal cells that lined the wall of the third ventricle. In that respect, some areas showed rows of densely ciliated ependymal cells, while other areas were sparsely ciliated, singly ciliated or non-ciliated. Variation in the density of ciliation of ependymal cells was described in the rat **(Paull et al., 1977).** Moreover, in monkeys, **Coates (1977)** mentioned that ependymal cells lining the walls of third ventricle were heavily ciliated as compared to its floor. In addition, in female armadillo, the ependyma of the third ventricle was also densely ciliated except for the organum vasculosum and the infundibular recess **(Jacobs and Monroe, 1977).**

Furthermore, in the current work surface microvilli were observed in both non-ciliated and singly ciliated ependymal cells. Similar observations were reported in armadillo **(Jacobs and Monroe, 1977).**

In the present study, supraependymal fibers with characteristic varicosities were identified. By means of transmission and scanning electron microscopy, **Martinez and de Weerd (1977)** mentioned that such varicosities of supraependymal fibers represented synapses that might be either axoaxonic or axosomatic. These authors added that synapses between supraependymal fibers and ependymal cells were also identified.

Moreover, by means of enzyme immunohistochemistry and immunotransmission electron microscopy, **Hirunagi et al. (1995)** demonstrated that the majority of supraependymal fibers contained serotonin. **Cloft and Mitchel (1997)** identified a ganglion-like system of serotoergic supraependymal neuronal complex (SENC) in the floor of the third ventricle in the golden hamster. That SENC, formed of intraventricular neuronal perikarya and processes, was thought to be involved in neuro-endocrine regulation since it innervated the pituitary gland. In that respect, the presence of serotonin and its metabolites in the cerebrospinal fluid (CSF) was reported to be of clinical relevance in some psychiatric conditions such as depression **(Brusco et al., 1998).** A further possibility was that supraependymal neuronal processes were effectors regulating activity of ependymal cells **(Sancesario et al., 1996).**

In the present investigation, careful examination of the ependymal lining of the third ventricle revealed the presence of supraependymal globular vesicular structures of variable sizes (ranging from 2.09mm to 20 μm). **Ray and Choudhury (1985)** reported that such vesicular structures increased in size and number in response to experimental leakage of the cerebrospinal fluid in the rat, and they reflected an augmented activity of the ventricular ependyma to counteract the effects of such surgical trauma. In monkeys, ependymal cells displayed membrane specializations in the form of polymorphous membrane protrusions **(Coates, 1977).** Moreover, **Gonzalez-Santander (1979)** noticed cytoplasmic protrusions and isolated masses of cytoplasm in the third ventricle of the cat. In the present study, three types of supraependymal vesicles could be also identified: smooth, rough, or whorled. Similar whorled vesicles were described by **Schmidt (1978)** as concentric lamellated bodies in the third ventricle of the European mole rat.

Furthermore, the current investigation demonstrated two types of supraependymal cells (SEC) overlying the ependymal lining of the third ventricle. Most of these cells were elongated with irregular surface, and few surface microvilli and blebs. Surface and side cellular processes were also noticed. Such cells were observed to span several ependymal cells. Nu-

merous rounded or irregular clumps of debris were in close association with these SEC. Such characteristics might imply that these cells are macrophage-like cells lying in intimate association with some debris, which might be degenerated cells or secretory materials. The other type of the observed SEC was a smaller rounded cell with numerous thin processes that appeared as spider-like cells. **Paull et al. (1977)** reported similar findings in the rat third ventricle and identified two varieties of supraependymal cells; phagocyte-like and neuron-like. Based on their ultrastructural characteristics, supraependymal cells were reported to be classified into three categories; nerve cells, lymphocytes, and dense cells **(Martinez and de Weerd, 1977)**. In that respect, it was mentioned that the supraependymal cells were probably a mixed population of glia, neurons, and macrophages **(Berry et al., 1995)**. Furthermore, scattered supraependymal multipolar neurons containing nitric oxide synthetase activity were detected in the wall of the rat third ventricle using histochemistry for NADPH- diaphorase activity. These nitrergic neurons were further suggested to play an essential role in regulating the composition of CSF **(Sancesario et al., 1996).**

Again, supraependymal cells of chicken third ventricle were classified into two types: neuron-like cells, which might be unipolar or bipolar in appearance, and phagocyte-like cells **(Hirunagi et al., 1989).** Moreover, in the third ventricle of monkeys, supraependymal cells were more visible in non-ciliated regions **(Coates, 1977)**, whereas in Guinea pig, SEC were restricted to non-ciliated regions **(Mitchell, 1979).** In male goats, the supraependymal cells were always present with the exception of the "rest" period. However, in the female animals, the smallest number of the SEC was found during anestrus period **(Rajtova, 1990)**. Also, supraependymal cells were shown in great number in response to cerebrospinal fluid leakage **(Ray and Choudhury, 1985)**.

III. The ependymal lining of the cerebral aqueduct:

In the present study, it was shown that the cerebral aqueduct was lined with a thin layer of polygonal non-ciliated ependymal cells. The ependymal cells had a convex surface studded with surface microvilli. **Meller and Dennis (1993)** reported that, in the rabbit, the ependyma of the cerebral aqueduct was so heavily ciliated that most of the ependymal surface was obscured. The authors added that certain specialized supraependymal structures could be discerned lying on (or embedded within) this matt of cilia. Such features were not met with in the present study, and such difference might be attributed to species differences. Moreover, **Rajtova, (1988)** stated that, in ewes and rams, small foci without microvilli and cilia ("bare") could be observed in the ependymal lining of the cerebral aqueduct. Again, in armadillo, the cilia of aqueduct ependyma are evenly placed over the cellular surfaces **(Jacobs and Monroe, 1977)**.

IV. The ependymal lining of the fourth ventricle:

The present study demonstrated that the floor of the fourth ventricle was lined with a single layer of ependymal cells that were heavily ciliated near the median sulcus. More laterally, the ependymal lining was sparsely ciliated with a transition zone of moderately ciliated cells in between. Similar results were described in the mouse **(Yamadori and Yagihashi, 1975)**. On the contrary, each half of the floor of the fourth ventricle of the adult rhesus monkey **(Singh et al., 1982)** and the pigeon **(Mestres and Rascher, 1994)** exhibited dense ciliation that decreased towards the median sulcus. **Hirunagi and Yasuda (1979)** reported that the ependymal cells lining the floor of the median sulcus of the fourth ventricle in the domestic fowl had a solitary cilium, whereas on both sides of the sulcus, the cilia were densely distributed.

In the present work, the heavily ciliated ependymal cells showed two patterns of ciliary distribution: homogeneously densely ciliated ependymal cells medially and rows of densely ciliated ependymal cells intermingled with non-ciliated ependymal cells with numerous surface micovilli laterally. Infrequently, adherent tufts of cilia were seen among the heavily ciliated cells. Furthermore, in the sparsely ciliated zone, ependymal cells with few cilia, single cilia and even non-ciliated cells were identified. In that respect, **Singh et al. (1980)** recognized regional variation in the surface fine structure of the ependymal cells lining the floor of the rat brain fourth ventricle in the form of dense ciliation, sparse ciliation, central tufts of cilia, and solitary cilia.

In addition, the present investigation revealed the presence of numerous thin supraependymal fibers that were commonly seen running between the cilia of the ependymal cells forming a network among the ependymal cilia. **Mathew (1998, 1999)** found networks of nerve fibers of similar distribution and suggested that they might have a role in the coordination of ciliary movement. Embryologically, the supraependymal fibers of the rat fourth ventricle was found to originate mainly from neurons in the dorsocaudal region of the raphe dorsalis nucleus **(Didier-Bazes et al., 1997).**

In the present work, occasional bullous protrusions were seen projecting in between the ciliated ependymal cells. By means of scanning and transmission electron microscopy, such bullous protrusions were also identified by **Yamadori and Yagihashi (1975)** who further reported that they were projections from subependymal nerve cells that contained numerous mitochondria. **Kiss and Mitro (1978)** suggested that such protrusions played a certain role in the exchange of various materials between the CSF, ependyma, and neuropile.

V. The ependymal lining of central canal of the spinal cord:

In the present study, detailed examination of the central canal of the cervical and lumbar regions of the rat spinal cord showed regular infoldings of the ependymal lining that comprised a single layer of uniformly organized ependymal cells. In both regions of the cord, the ependymal cells were non-ciliated with numerous surface microvilli and microblebs. However, in the lumbar region, cells with single cilia were identified lying among the non-ciliated cells. These findings were different from those reported in the mouse, and in the cat, where bundles of cilia as well as single cilia, were noted **(Rascher et al., 1985; Bjugn et al., 1988)**. Moreover, the luminal surface of the ependymal cells of the central canal of monkey spinal cord beared many microvilli and cilia that were regularly arranged **(Erhardt and Meinel, 1986)**. The absence of dense ciliation in the ependymal lining of the central canal demonstrated in the present work might be attributed to the lack of demand to the propelling action of cilia needed for the circulation of CSF. Such suggestion was based on the work of **Cifuentes et al. (1992)** who reported that, by means of applying horse radish peroxidase (HRP) in the central canal, the CSF was traced to penetrate through the luminal surface of the ependymal cells as well as the intercellular spaces reaching the subependymal neuropile, and then to the basement membrane of local capillaries.

In the present investigation, supraependymal varicosed nerve fibres were seen running on the ependymal surface of the lumbar region of the cord. A similar finding was noticed in the central canal of the rabbit spinal cord **(Leonhardt, 1976)**, and in the central canal of spinal cord of adult cat **(Rascher et al., 1985)**. Moreover, in the present study, supraependymal club-shaped vesicular structures were observed in the lumbar region. **Rascher et al. (1985)** said that the most striking feature of the central canal ependyma of cat was the large, spherical bodies that were connected by long, slender stalks to neurons in subependymal position.

In the present study, it was noticed that the non-ciliated ependymal cells of the central canal of the lumbar region of the spinal cord appeared dome-shaped with indistinct cell boundaries. **Bruni and Reddy (1987)** mentioned that ependymal cells lining the rat central canal were columnar. **Bruni and Anderson (1987)** further added that these ependymal cells were often radially elongated. Furthermore, ependymal cells lining the human central canal were reported to be simple columnar or cuboidal in the second decade of life **(Kasantikul et al., 1979)**. In addition, ependymal cells of the mouse spinal canal were observed to be simple cuboidal similar to those of its brain ventricle **(Bjugn et al., 1988)**.

In fracture freeze preparations of the lumbar region, the current work showed that one or more long basal cytoplasmic processes originated from

the base of some of the non-ciliated ependymal cells and penetrated deep into the subependymal region. Such long basal processes were also identified in the rat by **Bruni and Anderson (1987),** and by **Bruni and Reddy (1987)**.

On the other hand, in fracture freeze preparations of the cervical region, the present investigation recognized several neural elements subependymally comprising multipolar neurons, varicosed nerve fibers of variable thickness, and numerous vesicles variable in size and shape. In the rabbit spinal cord, similar bipolar or multipolar nerve cells were seen below the ependymal cells of the central canal **(Leonhardt, 1976)**.

VI. Surface Specializations:

In the present study, certain surface specializations were identified emerging between adjacent ependymal cells of brain ventricles and central canal of spinal cord. Such specializations were in the form of projecting single cilia and bullous protrusions. In that respect, a system of cerebrospinal fluid-contacting neurons was described in various periventricular brain regions that seemed to have a special role in taking up, transforming, and emitting non-synaptic signals mediated by the CSF and intercellular fluid of the brain **(Vigh et al., 2004)**. Most of the CSF-contacting neurons were reported to send dendritic processes into brain ventricles and the spinal cord central canal where they formed terminals bearing a solitary cilium **(LaMotte, 1987; Vigh et al., 2004)**. Cytologically, such neurons resembled the known sensory cells of the chemoreceptor type, while others appeared to be sensitive to the pressure or flow of CSF **(Vigh et al., 2004)**.

SUMMARY

The present study aimed at clarifying the alteration in the surface fine structure of the ependyma throughout the whole brain ventricular system and the central canal of the spinal cord. Ten adult male albino rats of the Sprague-Dawley strain, weighing 150-200 gm, were used in the present study. The animals were anaesthetized by intraperitoneal injection of a single dose of phenobarbitone, and fixed with 1% glutaraldehyde - 2% paraformaldehyde in phosphate-buffered saline at room temperature (pH 7.4). In each animal, the brain and spinal cord were carefully dissected out and the obtained specimens of brain ventricles and central canal of cervical and lumbar regions of the spinal cord were processed for scanning electron microscopy.

Examination of the obtained specimens with scanning electron microscopy revealed various patterns of ciliary arrangement and distribution as well as microvilli, blebs, protrusions, supraependymal and subependymal structures along the ependymal lining of brain ventricles and central canal of the spinal cord.

In that respect, the lateral, third, and fourth ventricles showed areas of densely ciliated, sparsely ciliated, singly ciliated and even non-ciliated ependymal cells. On the other hand, the ependymal lining of the aqueduct and the cervical part of the central canal was non-ciliated, whereas that of the lumbar region exhibited single cilia among non-ciliated cells. Commonly, single cilia of the lateral ventricle, third ventricle, and of the central canal of the lumbar cord seemed to emerge between two adjacent cells. Occasionally, bullous protrusions were seen projecting between the ciliated ependymal cells of the fourth ventricle and the lumbar region of the spinal cord.

Two types of supraependymal cells (phagocyte-like and cells with multiple processes) were seen overlying the ependymal lining of the third ventricle. Supraependymal fibers with characteristic varicosities were seen running over the ependyma of third ventricle, fourth ventricle, and lumbar region of the central canal. Furthermore, subependymal varicosed nerve fibers were noticed in the lateral ventricle, fourth ventricle, and the cervical and lumbar regions of the spinal cord. Supraependymal vesicular structures variable in size and shape were noticed in the lateral ventricle, in the third ventricle, and in the lumbar region of central canal. Moreover, subependymal vesicular structures variable in size and shape were seen in the lateral ventricle as well as the cervical and lumbar regions of the spinal cord.

The above mentioned findings were discussed in relation to the probable role of the ependyma in the secretion (vesicular structures), movement (cilia), absorption (microvilli), and homeostasis (surface specializations) of the cerebrospinal fluid.

REFERENCES

1. Alvarez-Morujo, A. J.; Toranzo, D., Blazquez, J. L.; Pelaez, B., Sanchez, A., Pastor, F .E.; Amat, G. and Amat, P. (1992): The ependymal surface of the fourth ventricle of the rat: A combined scanning and transmission electron microscopic study. Histol. Histopathol., **7 (2)**: 259-266.

2. Berry, M.M.; Standring, S.M. and Bannister, L.H. (1995): Nervous system. In: Gray's Anatomy. 38th ed., Williams, P.L., Berry, M.M., Collins, P., Dyson, M., Dussek, J.E. and Ferguson, M.W.J. (eds.), Churchill Livingstone, New York, Edinburgh, London, Tokyo, Madrid and Melbourne, 939.

3. Bjugn, R.; Haugland, H. K. and Flood, P. R. (1988): Ultrastructure of the mouse spinal cord ependyma. J. Anat., **160**: 117-125.

4. Bruni, J. E. (1998): Ependymal development, proliferation, and functions: A review. Microsc. Res. Tech., **41(1)**: 2-13.

5. Bruni, J. E. and Anderson, W. A. (1987): Ependyma of the rat fourth ventricle and central canal: Response to injury. Acta Anat. (Basel), **128(4)**: 265-273.

6. Bruni, J. E. and Reddy, K. (1987): Ependyma of the central canal of the rat spinal cord: A light and transmission electron microscopic study. J. Anat., **152**: 55-70.

7. Brusco, A.; Lopez-Costa, J. J.; Tagliaferro, P. and Pecci Saavedra, J. (1998): Serotonergic ependymal fibres in rat and monkey: light and electron microscopic immunocytochemical study. Biocell, **22**(2): 115-122.

8. Cifuentes, M.; Fernandez-LLebrez, P.; Perez, J.; Perez-Figares, J. M. and Rodriguez, E. M. (1992): Distribution of intraventricularly injected horse radish peroxidase in cerebrospinal fluid compartments of the rat spinal cord. Cell Tissue Res., **270(3)**: 485-494.

9. Clementi, F. and Marini, D. (1972): The surface fine structure of the walls of cerebral ventricles and of choroid plexus in the cat. Z. Zellforsch Mikrosk. Anat., **123**: 82–95.

10. Cloft, H.J. and Mitchell, J.A. (1997): Serotonergic innervation of the supraependymal neuronal complex of the golden hamster. Brain Res., **761**: 210- 216.

11. Coates, P. W. (1977): The third ventricle of monkeys: Scanning electron microscopy of surface features in mature males and females. Cell Tissue Res., **177(3)**: 307-316.

12. Didier-Bazes, M., Voutsinos, B., Aguera, M., Peyron, C., Akaoka, H. and Belin, M.F. (1997): Specific potentialities of the embryonic rat serotonergic neurons to innervate different periventricular targets in the adult brain. J. Comp. Neurol., **382**: 29- 45.

13. Erhardt, H. and Meinel, W. (1986): Comparative scanning and transmission electron microscopic studies of the ependyma of the central canal in the spinal cord of primates. I. Electron optical image of the ependyma in the central canal of the spinal cord of the callithrix monkey (*Callithrix jacchus*, Linne 1758). Gegenbaurs Morphol. Jahrb., **32(4)**: 535-554.

14. Gonzalez-Santander, R. (1979): Electron microscopic study of the secretion of the ependymal cells in the domestic cat (ependymin-beta cells). Acta Anat. (Basel), **103(3)**: 266-277.

15. Hirunagi, K.; Rommel, E. and Korf, H. W. (1995): Ultrastructure of cerebrospinal fluid-contacting neurons immunoreactive to vasoactive intestinal peptide and properties of the blood-brain barrier in the lateral septal organ of the duck. Cell Tissue Res., **279(1)**: 123-133.

16. Hirunagi, K.; Uryu, K. and Fujioka, T. (1989): Supraependymal cells and fibers in the third ventricle of the domestic chicken. A scanning electron microscopic study. Z. Mikrosk. Anat. Forsch., **103(4)**: 529-539.

17. Hirunagi, K. and Yasuda, M. (1979): Scanning electron microscopic analysis of the linings of the fourth ventricle in the domestic fowl. Cell Tissue Res., **197(1)**: 169-173.

18. Jacobs, J. J. and Monroe, K. D. (1977): A scanning electron microscopic survey of the brain ventricular system of the female armadillo. Cell Tissue Res., **183(4)**: 531-539.

19. Kasantikul, V.; Netsky, M. G. and James, A. E. Jr (1979): Relation of age and cerebral ventricle size to central canal in man. Morphological analysis. Neurosurg., **51(1)**: 85-93.

20. Kiss, A. and Mitro, A. (1978): Ependyma and supraependymal structures in some areas of the fourth ventricle in the rat. Acta Anat (Basel), **100:** 521- 531.

21. Korf, H. W. and Fahrenkrug, J. (1984): Ependymal and neuronal specializations in the lateral ventricle of the Pekin duck. Anas. platyrhynchos. Cell Tissue Res., **236**(1): 217-227.

22. LaMotte, C. C. (1987): Vasoactive intestinal polypeptide cerebrospinal fluid-contacting neurons of the monkey and cat spinal central canal. J. Comp. Neurol., **258**(4): 527-541.

23. Leonhardt, H. (1976): The cerebrospinal fluid contact processes in the central canal of the spinal cord. A scanning and transmission electron microscopic study of the rabbit. Z. Mikrosk. Anat. Forsch., **90(1)**: 1-15.

24. Martinez, P. M. and de Weerd, H. (1977): The fine structure of the ependymal surface of the recessus infundibularis in the rat. Anat. Embryol. (Berl.), **151(3)**: 241-265.

25. Mathew, T. C. (1998): Supraependymal neuronal elements of the floor of the fourth ventricle in adult rat: A scanning electron microscopic study. J. Submicrosc. Cytol. Pathol ., **30(1)**: 175-181 .

26. Mathew, T. C. (1999): Association between supraependymal nerve fibers and the ependymal cilia of the mammalian brain. Anat. Histol. Embryol., **28(3)**: 193-197.

27. Meller, S. T. and Dennis, B. J. (1993): A scanning and transmission electron microscopic analysis of the cerebral aqueduct in the rabbit. Anat. Rec., **237(1)**: 124-140.

28. Mestres, P. and Rascher, K. (1994): The ventricular system of the pigeon brain: A scanning electron microscope study. J. Anat., **184 (1)**: 35-58.

29. Mestres, P.; Rascher, K. and Delius, J. D. (1985): Scanning electron microscopy of the lateral ventricle of the pigeon brain. Scan. Electron Microsc., **(Part 1)**: 239-246.

30. Mitchell, J. A. (1979): Morphology and distribution of Type II supraependymal cells in the third ventricle of the Guinea pig. J. Morphol., **159**(1): 67-80.

31. Mitro, A.; Polak, S. and Pogady, J. (1989): A scanning electron microscopic study of the ependyma in the lateral cerebral ventricle in humans. Bratisl Lek. Listy., **90(11)**: 814-819.

32. Page, R. B.; Rosenstein, J. M.; Dovey, B. J. and Leure-du Pree, A. E. (1979): Ependymal changes in experimental hydrocephalus. Anat. Rec., **194**: 83-103.

33. Paull, W. K.; Martin, H. and Scott, D. E. (1977): Scanning electron microscopy of the third ventricular floor of the rat. J. Comp. Neurol., **175(3)**: 301-310.

34. Rajtova, V. (1988): Ependyma in sheep. 5. Ependymal lining of the cerebral ventricles using scanning electron microscopy. J. Hirnforsch., **29 (2)**: 221-235.

35. Rajtova, V. (1990): Ependyma of the goat. I. The third cerebral ventricle: Scanning electron microscopy study. J. Hirnforsch., **31(4)**: 415-421.

36. Rascher, K.; Booz, K. H.; Nacimiento; A. C. and Donauer, E. (1985): The ependyma of the cat central canal, with particular reference to its mitochondria-containing bulbs. Scan. Electron Microsc., **(Part 1)**: 231-238 .

37. Ray, P. K. and Choudhury, S. R. (1985): Changes in the surface fine structure of ependyma of the rat third ventricle following operative leakage of cerebrospinal fluid. J. Anat., **140 (1)**: 1-11.

38. Rowett, H. G. Q. (1968): The spinal cord. In: The rat as a small mammal, revised 2nd ed., John Murry, London, Great Britain, 66.

39. Sadler, T. W. (1985): Langman's Medical Embryology. 5th ed., Williams & Wilkins, Baltimore, London, Los Anglos, Sydney, 342.

40. Sancesario, G.; Morello, M.; Massa, R.; Fusco, F.R.; D'Angelo, V. and Bernardi, G. (1996): NADPH-diaphorase neurons contacting the cerebrospinal fluid in the ventricles of the rat brain. Cereb. Blood Flow Metab., **16**: 517- 522.

41. Schmidt, M. L. (1978): Concentric lamellated psammoma-like bodies in the third ventricle of the European mole rat (*Spalax leucodon*, Nordmann 1840). Acta Neuropathol. (Berl.), **43(3):** 255-258.

42. Scott, D. E.; Dudley, G. K. and Knigge, K. M. (1974): The ventricular system in neuroendocrine mechanisms. 2. In vivo monoamine transport by ependyma of the median eminence. Cell Tissue Res., **154**: 1-16.

43. Singh, D. R.; Bajpai, V. K.; Maitra, S. C.; Shipstone, A. C. and Hasan, M. (1982): Scanning and transmission electron microscopy of the ependyma of the fourth ventricle in the monkey brain. Acta Anat. (Basel), **112(4)**: 365-375.

44. Singh, D. R.; Hasan, M.; Bajpai, V. K. and Maitra, S. C. (1980): Surface fine structure of the ependymal lining of the rat fourth ventricle. Acta Anat. (Basel), **107(2)**: 198-204.

45. Vigh, B.; Manzano, E.; Silva, M. J.; Frank, C. L.; Vincze, C.; Czirok, S. J.; Szabo, A.; Lukats, A. and Szel, A. (2004): The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. Histol.Histopathol., **19(2)**: 607-628.

46. Walsh, R. J.; Brawer, J. R. and Lin, P. L. (1978): Early postnatal development of ependyma in the third ventricle of male and female rats. Am. J. Anat., **151(3)**: 377-407.

47. Yamadori, T. and Yagihashi, S. (1975): A scanning and transmission electron microscopic observation of the fourth ventricular floor in the mouse. Arch. Histol. Jpn., **37 (5)**: 415-432.

الملخص العربى

البطانة العصبية المبطنة لبطينات الدماغ والقناة المركزية للحبل الشوكي للجرذ الأبيض البالغ : دراسة بواسطة الميكروسكوب الإلكتروني الماسح

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تهدف هذه الدراسة إلى توضيح التباين في التركيب السطحي الدقيق للبطانة العصبية لبطينات الدماغ والقناة المركزية للحبل الشوكي. استخدم في هذه الدراسة عشرة من ذكور الجرذان البيضاء البالغة، تم تخديرها بواسطة الفينوباربيتون، وتم استئصال الدماغ والحبل الشوكي بعناية. ثم تم التعرف على بطينات الدماغ والقناة المركزية للحبل الشوكي حيث أخذت منها عينات تم تمريرها وإعدادها للفحص بالمجهر الإلكتروني الماسح.

أظهر الفحص المجهري الإلكتروني الماسح وجود أنماط متعددة لترتيب و توزيع الأهداب و كذلك الزغيبات، والنفطات، والتركيبات الموجودة فوق وتحت البطانة العصبية لبطينات الدماغ والقناة المركزية للحبل الشوكي. وقد تم التعرف علي مناطق من البطانة العصبية تكون فيها الخلايا ذات أهداب كثيفة، ومناطق أخرى تكون فيها الأهداب متناثرة، ومناطق ثالثة تحمل فيها الخلية هدب مفرد، هذا وقد تخلو الخلايا من الاهداب تماما. ومن ناحية أخرى أظهر الفحص المجهري الإلكتروني الماسح أن البطانة العصبية للقناة الدماغية والجزء العنقي من القناة المركزية للحبل الشوكي كانت تخلو من الأهداب بينما تلك التي تبطن الجزء القطني للحبل الشوكي كانت تحوى خلايا تحمل أهدابا مفردة. هذا وقد لوحظ أن الأهداب المفردة الموجودة في الجزء القطني للحبل الشوكي أو تلك التي توجد في بطينات الدماغ كانت تبرز بين خليتين متجاورتين. كما تم

التعرف علي بروزات فقاعية الشكل تظهر بين خلايا البطانة العصبية للبطين الرابع والجزء القطني من الحبل الشوكي.

كما أظهرت هذه الدراسة وجود نوعين من الخلايا فوق البطانة العصبية للبطين الثالث. النوع الأول شبيه بالخلايا البلعمية، والنوع الثاني شبيه بالخلايا العصبية. وتم التعرف كذلك على ألياف عصبية دالية فوق البطانة العصبية للبطينين الثالث والرابع، والجزء القطني من الحبل الشوكي. وقد لوحظت مثل هذه الألياف العصبية الدالية تحت البطانة العصبية للبطينين الوحشي والرابع، وأيضا في الجزئين العنقي والقطني للحبل الشوكي. وقد تم التعرف على تركيبات حويصلية مختلفة الشكل والحجم فوق البطانة العصبية للبطينين الوحشي والثالث، وكذلك الجزء القطني من الحبل الشوكي، كما وجدت تركيبات حويصلية مختلفة الشكل والحجم تحت البطانة العصبية للبطين الوحشي، والجزئين العنقي والقطني للحبل الشوكي.

هذا وقد نوقشت النتائج سالفة الذكر من حيث علاقتها بدور البطانة العصبية في إفراز ودوران، وامتصاص، والاستقرار المتجانس للسائل المخي الشوكي.