ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(6): 112-117

www.fisheriesjournal.com
Received: 16-09-2016
Accepted: 17-10-2016

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Histological and surface ultrastructural observations on the saccus vasculosus of *Eutropiichthys vacha* (Hamilton)

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Abstract
The histological architecture and ultrastructural characteristics of saccus vasculosus in *Eutropiichthys vacha* (Hamilton) were investigated by employing light and scanning electron (SEM) microscope. The saccus vasculosus is small, oval shaped structure, situated on the ventral side of the diencephalon behind the pituitary. This circumventricular organ contained a typical large central lumen lined by characteristic coronet cells and supporting cells. The coronet cells possessed basally placed nuclei and intense apical secretion. The supporting cells were few in number and placed in between coronet cells. Under SEM observations the coronet cells were variable in shape and apical parts were provided with different shapes of globular protrusions. The supporting cells were fusiform in structure and arranged in between coronet cells. The coronet cells were attached with dense blood vessels. The intense reaction of silver stain was marked in the terminal end of coronet cells and nevers were attached with blood vessels. Under SEM observations the nerve fibres and blood vessels of different calibers were also found attached with the coronet cells. Various cells linining the epithelium of saccus vasculosus and their physiological activities in *E. vacha* have been discussed.

Keywords: Histology, ultrastructure, saccus vasculosus, *Eutropiichthys vacha*

1. Introduction
The saccus vasculosus is highly vascular ependymal sac like swelling, occupies a centrocaudal portion of the diencephalic infundibulum just behind the hypophyseal complex. The saccus vasculosus is mainly concerned with a secretory function [1] and works for an umbilical conjoin between cerebrospinal fluid and the blood vascular system [2]. Mecklenburg (1974) [3] has opined three possible groups for saccus vasculosus depending on the position of saccus in relation to pituitary – (1) Anteriorly, the saccus vasculosus joins the pituitary and posteriorly it ends at approximately the same level as the lobi inferiors. Most of the teleosts belong to this group. (2) Saccus vasculosus is separated by an interspace from the pituitary and ends usually ahead of the lobi inferiors. This is represented by members of Carangidae and Gobiidae. (3) Saccus vasculosus is hidden between the lobi inferiors. This is represented by the members of Cyprinidae. A number of authors have been published from time to time dealing with structure and function of saccus vasculosus in fishes [4-6]. The vascularized neuroepithelium of saccus vasculosus consists of characteristic coronet cells and supporting glial cells with interspersed liquor containing neurons [7, 8]. The highly specialized coronet cells lining the saccus epithelium are involved in homeostasis of cerebrospinal fluid by way of transporting low molecular weight substances into and from the fluid [9]. The cellular organization of the saccus vasculosus in teleosts have been investigated by many researchers using light and electron microscope [10, 11, 12, 13, 14]. Teleostean saccus vasculosus exhibits considerable morphological variability at both cellular and whole organ level of organization.
The aim of the present work is to perform histological and scanning electron microscopic studies of various cells lining the epithelium of saccus vasculosus of *Eutropiichthys vacha* to understand possible physiological role of the cells concerned.

2. Materials and Methods
Live mature fishes of *E. vacha* (16 to 18 cm in length) were procured from the river Damodar, Burdwan, West Bengal, India (23° 17' N, 87° 35' E). Fishes were anaesthetized with tricaine

methone-sulphonate (MS222) solution (4mg /L) and sacrificed following the guidelines given by the Institutional Ethical Committee. The brain together with the saccus vasculosus was disclosed from the ventral side of the head and was initially fixed in situ with 10% neutral formalin. After few minutes the saccus vasculosus together with the rest of the brain was carefully removed from the cranium and instantly processed for histological and scanning electron microscopic studies.

2.1 Histological study

The saccus vasculosus was fixed in aqueous Bouin's fluid for 16-18 hours. After fixation the tissues were washed repeatedly in 70% ethanol and dehydrated properly through ascending series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax of 56 °C-58 °C under a thermostat vacuum paraffin-embedding bath for a period of 1 hour and 30 minute. Serial sections were cut at 4 µm thick using a rotary microtom after routine histological procedure deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain, Chrome Alum Haematoxylin Phloxine (CAHP) after Gomori [15], Romies Azan (RA). Some tissues were immersed in 10% neutral formalin for 18 hours. Subsequent to dehydration the tissues were embedded in 52 °C-54 °C paraffin wax. Sections were cut at 8µm and stained in Silver impregnation Method (SI) [16].

2.2. Scanning electron microscopic study

The brain mass including the saccus vasculosus was immediately exposed and removed. The tissues were primarily fixed in 2.5% glutaraldehyde, buffered with cacodylate (pH 7.4) for 16 hours and post fixed in 1% buffered Osmium tetroxide (OsO4) for 2 hours. The tissues were dehydrated through ascending concentrations of acetone followed by amyl acetate and subjected to critical point drying method with liquid carbon dioxide. The dried tissues were mounted on metal stubs, coated with gold palladium with a thickness of approximately 20 nm and scanned in a Hitachi, S-530 SEM.

3. Results

In the histological sections the saccus vasculosus of *E. vacha* is well developed and oval sac like texture, located on the ventral side of the diencephalon near the third ventricle of the brain. The pituitary complex is separated from the saccus vasculosus by an interspace (Fig. 1). The saccus is encapsulated vascular organ and the blood vessels are existed interior to the capsule of saccus vasculosus in the form of vascular channels, exhibit intense red colour (Figs. 2, 3). In some places the capsular wall invades the parenchyma of saccus to form villi like outgrowths towards the lumen. These villi are lined by the single layer of stratified epithelium while the core is formed entirely by the blood vessels (Fig. 2, 3, 6). This arrangement appears to increase the inner surface area of saccus vasculosus. The inner epithelium of saccus of *E. vacha* is made up of mainly two types of cells: highly specialized coronet cells and the basal supporting cells (Figs. 2, 3, 6). The predominant coronet cells are tall columnar in shape and the basal portion is somewhat spindle shaped. The nuclei are large and conspicuous (Figs. 3, 6). The apical portion of the coronet cells are characterized by vigorous secretion which are projecting into the saccus lumen. The basal regions of the coronet cells are in close contact with blood vessels. The tinctorial properties of the cytoplasm of coronet cells are positive with the apical secretion (Figs. 3, 6, 7). The supporting cells are rather small and irregular in outline. They have deeply stained nuclei and scattered among the coronet cells (Figs. 3, 6, 7). The lumen of the saccus vasculosus contains appreciable amount of stainable materials which are continuous with the coronet cells (Figs. 3, 6, 7).

According to SEM studies, the saccus is composed of single layer of elevated and densely packed coronet cells interspersed with small sized supporting cells which are almost round in outline. These coronet cells are distributed along the basal membrane beneath which small depressions of blood vessels are present (Figs.4, 5). The SEM examination reveals that the apical part of the coronet cells are provided with different shapes of globular protrusions depending on different physiological activity of the cells (Figs. 4, 5, 8). Under SEM observation the saccus vasculosus of *E. vacha* represents the efficient elaborate system of blood vessels adjacent to the basal part of coronet cells. This system increase the surface area of saccus epithelium many folds (Figs. 9, 10). The saccus epithelium is contacted with nerve terminals as evidenced by the intense reaction of Marsland, Glees and Erikson technique. The nerve fibres of different calibers in the basal part of coronet cells are also connected with blood vessels (Figs.11). Under SEM observation the coronet cells along blood vessels are connected with the network of nerve fibres and some of the terminals show clear synaptic structure (Fig. 12).

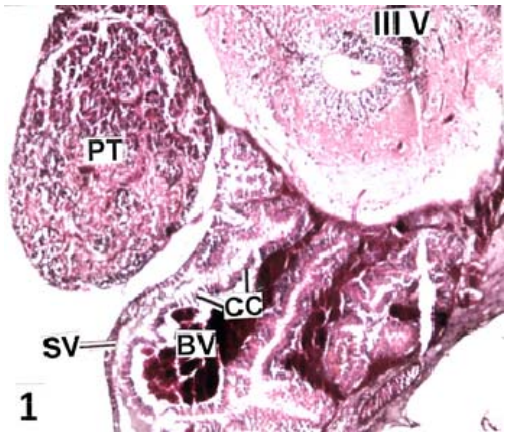

Fig 1: Oval shaped saccus vasculosus (SV) situated on the ventral side of the third ventricle (IIIV) of the brain beyond the pituitary (PT). Note blood vessels (BV) in the capsule of SV and series of coronet cells (CC). (HE) ×50 X.

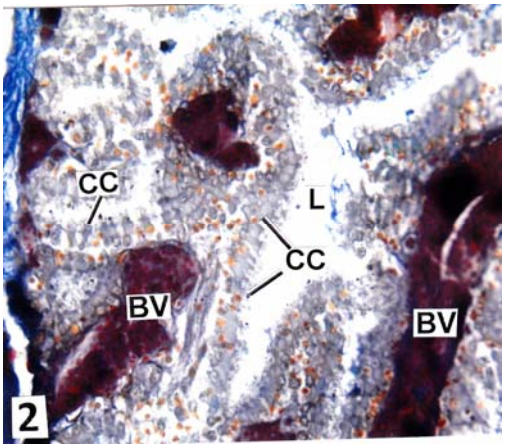

Fig 2: Villi like outgrowth of interior capsule of SV towards the Lumen (L). Note the presence of coronet cells (CC) attached with BV. (MT)× 100X.

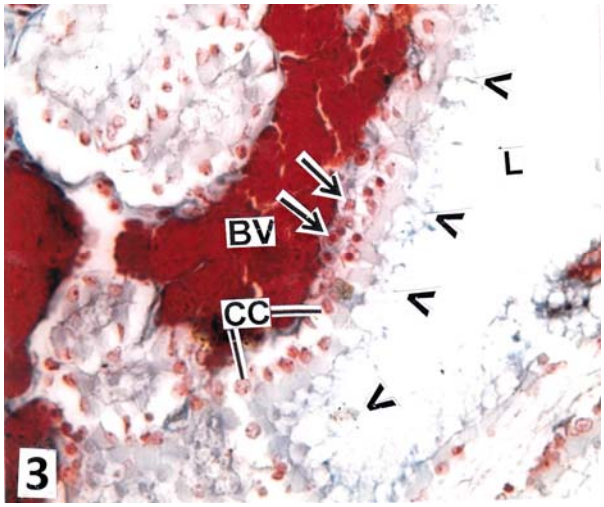

Fig 3: Inner epithelium of SV lined with CC attached with BV. CC possesses homogenous cytoplasm and basally placed nuclei. Note supporting cells (arrows) in between CC. Arrow heads indicate luminar protrusions from CC. (CAHP) × 600X.

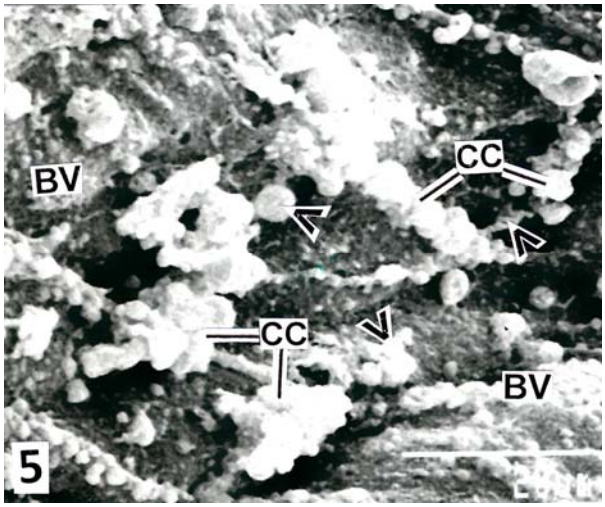

Fig 5: Linear fashion of CC adjacent to BV. Note apical secretion of CC (arrow heads). (SEM) × 2500X.

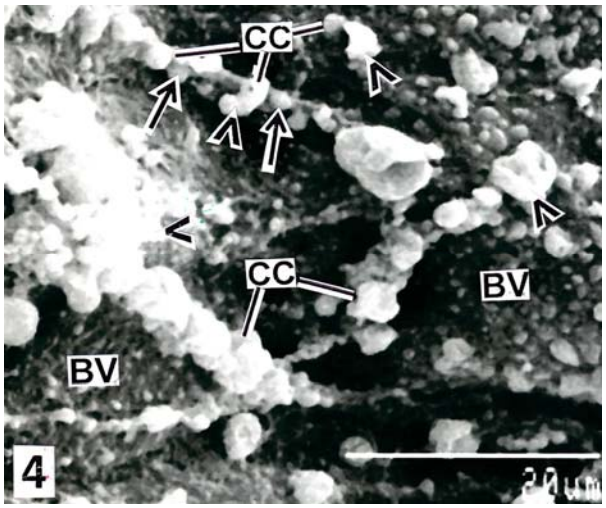

Fig 4: Luminal surface of SV showing linearly arranged closely packed CC leaving deep furrows of BV. Note the presence of supporting cells (SC) (arrows) in between CC. Arrow heads indicate apical secretion of CC. (SEM) × 2500X.

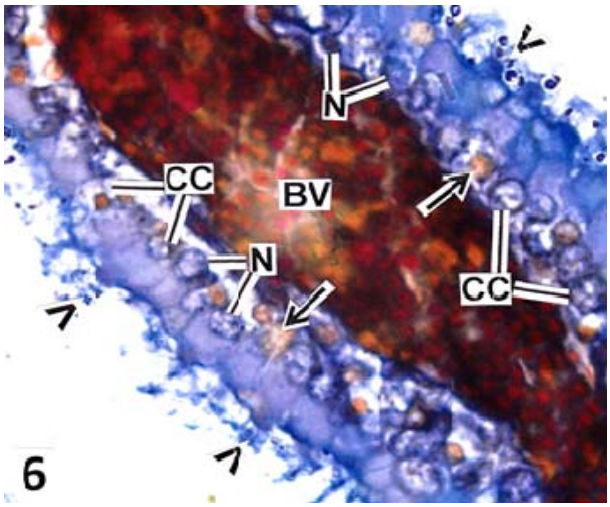

Fig 6: Higher magnification showing the alternate arrangement of CC and SC (arrows) firmly attached with BV. Note basally placed nuclei (N) and apical secretion of CC (arrow heads). (RA) × 1000X.

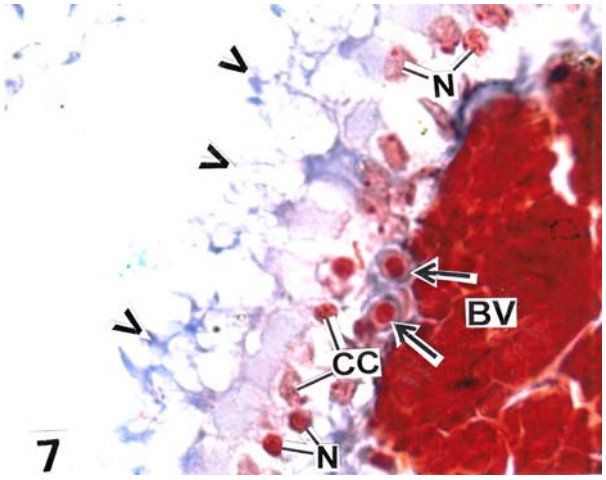

Fig 7: Higher magnification of saccus epithelium shows tall CC with prominent nuclei (N) and homogeneous cytoplasm. Note attachment of CC with BV and presence of SC (arrows) below the CC. Arrow heads indicate apical luminar secretion of CC. (CAHP) × 1000X.

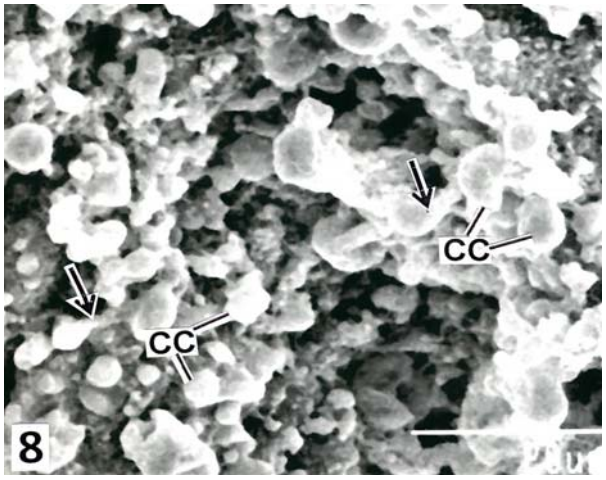

Fig 8: Surface view of saccus epithelium showing different shapes of CC. Note intense apical secretion from CC (arrows). (SEM) × 3000X.

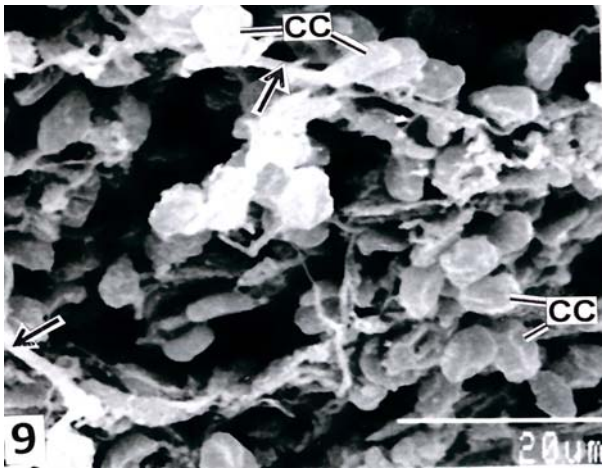

Fig 9: Showing most elaborate system of blood vessels (arrows) attached to the CC in the surface epithelium of saccus. (SEM) × 3000X.

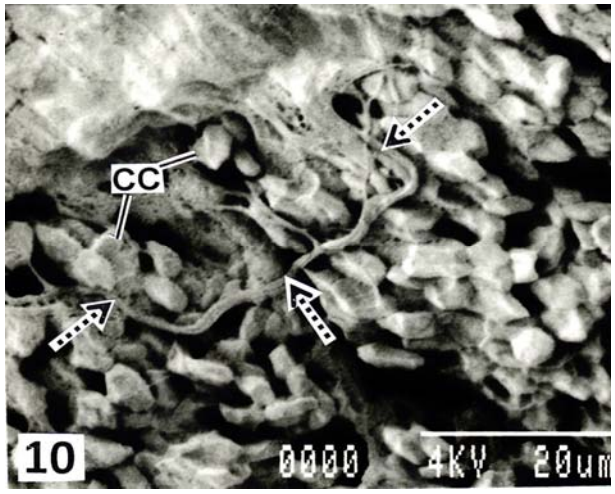

Fig 10: Showing disposition of blood vessels (broken arrows) supplying blood to the CC. (SEM) × 3000X.

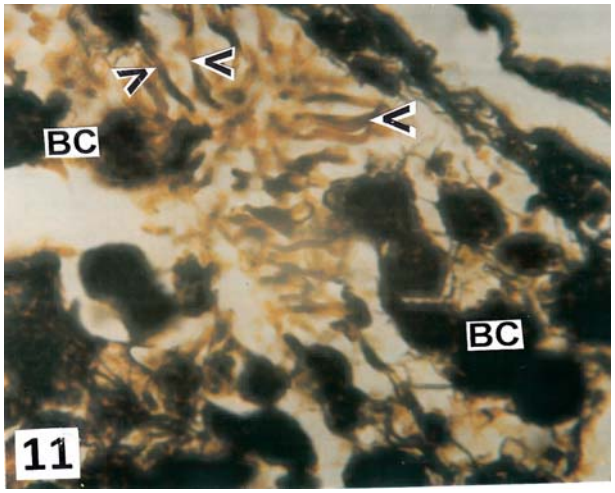

Fig 11: Showing intense silver reaction of nerve terminals (arrow heads) adjacent to intense coloured blood capillaries. (MGE) ×1000 X.

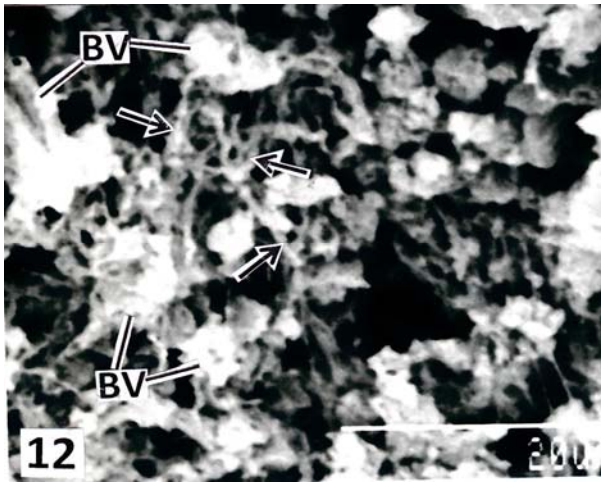

Fig 12: Showing different calibers of nerve terminals (arrows) connected with blood vessels (BV) in saccus vasculosus. (SEM) × 3000X.

Figs. 1 to 12. Photomicrograph of the histological sections and scanning electron micrographs (SEM) of saccus vasculosus of *Eutropiichthys vacha*.
(Delafield's haematoxylin-eosin: HE), (Mallory's Triple: MT), (Chrome alum haematoxylin phloxin: CAHP), (Romie's Azan: RA), (Marsland, Glees and Erikson: MGE).

4. Discussion

The saccus vasculosus forms part of the floor of the diencephalon in most of the fishes and is considered as saccular protrusion of the caudal infundibular wall of the diencephalon; hence it always lies in the vicinity to the hypophyseal complex. Teleostean saccus vasculosus exhibits considerable morphological variability at both cellular and whole level of organization (Tsuneki) [17]. In the present investigation, the saccus vasculosus in *E. vacha* is oval sac like structure, situated ventral side of the brain and is separated from the pituitary gland by an interspace and it seems to have no functional connection with the gland hence, can be classified under Mecklenburg [3] group II. Kamer [18] has noticed rich vascular supply and interpreted saccus vasculosus as a gland of brain and assigned a secretory role of unknown function. In *E. vacha* the vascular channels consisting of capillaries and the wall of the saccus vasculosus often shows infoldings into the lumen. This system increased the surface area of saccus epithelium to facilitate the absorption and/or secretion process and may also be responsible to provide nutritive substances to the different cells lining the saccus epithelium. Similar infoldings of saccus epithelium have been observed by Jansen and Flight [19] in rainbow trout. In the present study, the single stratified epithelium of saccus of *E. vacha* is composed of heterogeneous population of coronet cells and supporting cells. The coronet cells are tall, columnar having basally placed conspicuous nuclei. These cells are highly specialized ependymal cells with a characteristic apical cytoplasmic outgrowth or secretion projected into the saccus lumen. The apical secretion from the coronet cells in the lumen of saccus may have some role to supply cerebrospinal fluid through the third ventricle. Rossi and Palmbi [20] have investigated the structure of the coronet cells of the saccus vasculosus at different stages of the life cycle of *Anguilla anguilla*. They opined that during marine larval stage, the narrow lumen of the saccus and vesicle within the apical globules are filled with electron dense material while in freshwater forms the saccus lumen appears expanded, the apical globules are very much developed and electron dense material has disappeared. According to Shimada [10] in the cytoplasm of coronet cells smooth endoplasmic reticulum composed of reticularly connecting tubules in the zone near the basal places. These tubules in the coronet cells must open into the saccus lumen to discharge their contents. Lanzing and Lennep [21] have demonstrated acid mucopolysaccharide in the apical protrusions of the coronet cells in teleosts which was first stored in the globules and ultimately secreted into the ventricle of the brain.

In present study the supporting cells are present among the coronet cells near the basal end of coronet cells. Although their structural feature, do not indicate the morphological signs of intense activity which may be correlated to secretory or resorptive function. In the present observation, the saccus vasculosus of *E. vacha* represent the degree of vascularization in the infoldings of villi like projections. This system probably increased the surface area of saccus to facilitate the absorption and/or secretion process.

The coronet cells in the experimental teleost are contacted with nerve terminals as evidenced by light and scanning electron microscopy. Some of the terminals show clear synaptic structure. It can be suggested that the coronet cells probably function as a chemoreceptor and maintaining the composition of the cerebrospinal fluid. Ryochi and Keiji [22] during their electron microscopic study in *Cyprinus carpio* noted that the terminals on the coronet cells contained only cleared synaptic vesicles, suggesting the cholinergic nerve and emphasized that the function of the coronet cells for metabolism of the cerebrospinal liquour is controlled by the cholinergic nerve.

5. Acknowledgements

One of the authors Rubina Khatun is grateful to the authorities of UGC-Moulana Azad National Fellowship sponsored by University Grants Commission, New Delhi.

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