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Histological and histochemical observations on the saccus vasculosus of freshwater featherback, *Notopterus notopterus* (Pallas, 1769)

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ABSTRACT

The cytoarchitecture and histochemical characteristics of saccus vasculosus in Notopterus notopterus (Pallas, 1769) were investigated by employing histological and various histochemical techniques. Saccus vasculosus, the richly vascularized reddish, sac like organ bulging from the ventral wall of the diencephalon. Histologically, it contained several loculi lined with considerable number of specialized coronet cells and few supporting cells. The loculi were packed by blood sinusoids. The intense reaction of silver stain was marked in the free end of coronet cells and nerve terminals attached with blood vessels. The localization and chemical nature of acid and neutral mucins in the saccus epithelium was studied by employing PAS-AB technique. Different shades of glycogen were discernible in the apical globules of coronet cells and blood vessels. Intensity of protein and lipid reactions was found to be associated with the coronet cells and blood vessels also. The various intensities of the histochemical reaction in the coronet cells of saccus vasculosus in N. notopterus was correlated with their functional significance.

Keywords: saccus vasculosus, *Notopterus notopterus*, function, cellular architecture, histochemistry

INTRODUCTION

The saccus vasculosus is highly vascularized ependymal organ, found exclusively in certain fish species. This circumventricular organ appears to be a sac like protrusion of the caudal infundibular wall of the diencephalon, placed behind the hypophysis. The cavity of this organ is continuous to the third ventricle of brain [1]. From the earliest days of investigation on the saccus vasculosus two conflicting hypotheses, one involving a sensory and the other a secretory function were regarded in order to interpret the performance of this peculiar organ [2]. Saccus vasculosus has a dual role of transport and secretion [3]. The highly specialized coronet cells lining the saccus epithelium are involved in the homeostasis of cerebrospinal fluid by way of transporting low molecular weight substances into and from the fluid [4]. Comprehensive explorations and knowledge on the microstructure of the saccus vasculosus of different fishes using light and electron microscope are well documented [5-11] but studies relating to histochemical constitution and functional status of this enigmatic organ of teleosts are limited [12-16].
The objective of the present study was to determine more precisely the structural detailed, chemical nature and functional aspects of the saccus vasculosus of freshwater bottom dwelling teleost, *Notopterus notopterus* (Osteoglossiformes, Notopteridae) by histological as well as histochemical analysis.

MATERIALS AND METHODS

Living mature specimens of *N. notopterus* (20 to 22 cm in total length) were obtained from the local freshwater body of Burdwan, West Bengal, India. The fishes were anaesthetized with tricane methone-sulphonate (MS 222; Sigma Chemical Co.) solution (100 mg/L) and sacrificed following the guidelines given by the Institutional Ethical Committee. The brain including the saccus vasculosus was displayed from the ventral side of the head and primarily fixed *in situ* with 10% neutral formalin. After several minute the saccus vasculosus together with the rest of the

brain was attentively dissected out from the cranium and immediately processed for the histological and histochemical studies.

Histological study

The saccus vasculosus was fixed in aqueous Bouin's fluid for 16-18 hour. After fixation the tissues were washed repeatedly in 70% ethanol and dehydrated properly through ascending series of ethanol. Then the tissues were cleaned with xylene and embedded in paraffin wax of 56-58˚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 microtome (Weswox). After routine histological procedure deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain.

Histochemical study

The saccus vasculosus was fixed in 10% neutral formalin for 18 h. After dehydration in graded series of ethanol followed by clearing in xylene, the tissues were embedded in paraffin wax at 52-54˚C in vacuum embedding bath. Serial paraffin sections were cut at 8 µm thickness and then subjected to various histochemical techniques: Silver Impregnation Method (SIM) for detection of neurons [17], Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for detection of neutral and acid mucins [18], Best's Carmine (BC) method for detection of glycogen [19], Mercury-Bromphenol Blue (MBPB) method for detection of basic proteins [20] and Sudan Black B (SB) method for detection of bound lipid [21]. Staining slides were examined and photographed under Olympus-Tokyo PM-6 compound microscope.

RESULTS AND DISCUSSION

Histology

The size of the saccus vasculosus in *N. notopterus* is notable capacious and situated on the ventral aspect of the diencephalon just behind the pituitary complex (Fig. 1). The saccus is separated from the pituitary gland by a distinct interspaced.

Teleostean saccus vasculosus shows considerable morphological variability at both cellular and whole level of organization. In the present study, the saccus vasculosus in *N. notopterus* is sac like protuberance on the ventral side of the brain and detached from the pituitary by an interspaced hence can be classified under Mecklenburg [22] group II.

In histological section the saccus vasculosus is made up of a massive number of loculi having different fashion and magnitude (Figs. 1 and 2). These loculi are surrounded by blood sinusoids/vessels. The lumen of the loculi invents inter communicating system of tubes or channels which divergence to a considerable degree as evident by presence of simple or branched loculi. The saccus of *N. notopterus* lack a common central cavity, rather the cavity of the saccus consist of a system of branch tubes which does not communicate directly with the cavity of the brain. It appears that all the terminal or distal branches unite to form a few large collecting channels which ultimately fused and end into larger duct which open communication with the diocoel (Fig. 1).

Rao [23] observed the degree of vascularisation especially at the area around the infundibulum and secretory activities of the saccus vasculosus of some teleosts. Van de Kamer [24] noticed rich vascular supply and interpreted saccus vasculosus as a gland of brain and assigned a secretory role of unknown function. In *N. notopterus* the saccus vasculosus consists of several loculi having coronet cells and supporting cells, bathed with blood from encircling sinusoids, provide nutritive substances to the various cells lining the saccus epithelium. This corresponds to the findings of Dammerman [25] on the saccus vasculosus of fish.

The loculi of saccus are composed of a heterogenous population of characteristic coronet cells as well as supporting cells. These cells are distributed along the basal membrane beneath which lies the vascular elements (Figs. 2 and 3). The predominant coronet cells are tall columnar or pear shaped having centrally or basally placed conspicuous nuclei (Figs. 3 and 4). The coronet cells show variation in size, shape and cytoplasmic density indicating the differences in physiological state. Some of the coronet cells are provided with apical globular protrusions (Fig. 4). The size of these protrusions also varies from cell to cell. Some of the coronet cells are contacted with nerve terminals show cleared synaptic contact (Fig. 3). The supporting cells (tanycytes) are almost triangular in outline, less numerous and scattered among the coronet cells (Figs. 2, 3 and 4). These cells are closely associated with the basal zone of the coronet cells. Both types of coronet and supporting cells are characterized by scanty cytoplasm. The lumen of the loculi in some areas contains stainable materials (Figs. 2 and 3).

The coronet cells are specialized ependymal cells with a characteristic apical cytoplasmic outgrowth, so called head bearing radially arranged flask or fibre like protrusions, so called globules. The supporting cells are also called as tanycytes [26] are small and present between the coronet cells. Kurotaki [27] during his histological studies on the saccus vasculosus of *Anguilla japonica* and *Cottus pullux* noted that the saccus epithelium is made up of principal coronet cells and supporting cells. He further reported that elongated coronet cells with cytoplasmic projections at the apex provided with large ovoid nuclei in the basal region. The supporting cells occupy the spaces among the coronet cells and have irregular outline. Singh and Sathyanesan [28] noticed coronet and supporting cells alternating with each other in the saccus epithelium of *Mystus vittatus, Callichorus pabda* and *Bagarius bagarius*. They have encountered different types of coronet cells which are presumed to be the stages of either formation or degeneration of coronet cells. In the present observation, the saccus vasculosus of *N notopterus* represents the most efficient of structural pattern and orientation of coronet cells as well as elaborate system of blood vessels. This system increased the surface area of saccus epithelium many folds to facilitate the absorption and/or secretion process. The coronet cells in the experimental teleost are contacted with nerve terminals. Some of the terminals show clear synaptic structure, suggesting the cholinergic nerve. Further, the coronet cells probably function as a chemoreceptor monitoring the composition of the cerebrospinal fluid. During the present investigation it is noted that typical coronet cells in *N. notopterus* are located in the loculi and are provided with characteristic apical globules. Interestingly all the cells of the loculi are not provided with apical globular head as some of the cells are devoid of such globules. It can be suggested that cells with typical protrusion represent the active functional state while rest of the cells, the inactive state. Most of the investigators have also paid attention to the supporting cells which are present among the coronet cells either at neck level or near the basal end of the coronet cells. Although their structural organization, do not indicate the morphological signs of intense activity which may be correlated to secretory or resorptive functions.

Histochemistry

Detection of neuron

Silver reaction furnishes deep brown to black colour, localized in the various regions of the saccus epithelium. In *N. notopterus*, intense reaction of silver stain is discernible in the free anterior part of the coronet cells and the basal part which are connected with the blood vessels. In the basal part, some of coronet cells are contacted with network of nerve fibres show clear synaptic contact (Figs. 5 and 6).

The presence of neuronal elements indicates its credible sensory role. Therefore, our findings suggest that the function of the coronet cells for the metabolism of the cerebrospinal liquor is controlled by the cholinergic nerve. This is conformity with the findings of Chakrabarti and Ghosh [10] in the saccus vasculosus of *Macrognathus aculeatum*.

Detection of mucopolysaccharides

The combined PAS-AB reaction produces purple-bluish colour of varying intensities in accordance with the neutral and acid mucin content of the various cells in the saccus vasculosus. This combined test imparts a bright purple colour due to PAS for neutral mucin and bright blue colour for AB reaction due to the presence of acid mucin exclusively. The cytoplasm of the coronet cells exhibits intense purple colour confirming the presence of exclusively neutral mucopolysaccharides. The coronet cells show the clear gradient of staining intensity increasing from the base of the cells towards the apical protrusion (Figs. 7 and 8). The apical protrusion and globules also contain intense purple-bluish colour. However, the blood vessels exhibit bluish colour confirming the presence of acid mucins (Figs. 7 and 8).

Chemically mucins are hexoseamine-containing polysaccharides which are bounded covalently with varying amounts of proteins. The presence of acid mucopolysaccharides was first established by Kamer et al. [29]. They detected the presence of glycogen in the apical protrusion of coronet cells and propagated the hypothesis that this glycogen is used in the formation of an acid mucopolysaccharide. Jansen and Flight [30] noted the presence of PAS positive material in the saccus vasculosus in freshwater rainbow trout. Narshiman and Sundararaj [13] also recorded PAS positive materials in the saccus vasculosus of *Catla catla* and *Colisa fasciata*. Kulkarni and Sathyanesan [15] noted both PAS positive and alcianophilic materials in the coronet cells of *Mystus vittatus*. The present study reveals the predominance of neutral mucopolysaccharides in the cytoplasm of coronet cells including the apical protrusions of the saccus vasculosus in *N. notopterus*. This clearly indicates that perhaps the coronet cells are very actively engaged in the secretion and synthesis of neutral mucopolyaccharide. Singh and sathyanesan [28] noted PAS-positive granules in the coronet cells of many fishes and suggested a secretory role.

Detection of glycogen

Results of Best's carmine test indicate an intense to moderate content of glycogen in the coronet cells lining the saccus epithelium of *N. notopterus*. The apical protrusions and globules contain different shades of staining

deposition of glycogen (Fig. 9). This indicates that there are individual differences in the physiological activity of the cells. The basal membrane is also positive to glycogen reaction. However, the blood vessels exhibit moderate reaction for glycogen (Fig. 9).

In the present histochemical investigation mainly the coronet cells lining the saccus epithelium exhibit intense to moderate reaction for glycogen probably for metabolic as well as physiological activities. Sundararaj and Prasad [12] noticed that coronet cells of the saccus vasculosus of *N. chitala* contain glycogen in the apical protrusion. They have suggested that glycogen is converted to glucose which thereafter is delivered to cerebrospinal fluid. Khanna and Singh [31] suggested that glycogen in coronet cells is converted to acid mucopolysaccharide before extrusion. In the present observation glycogen reaction in the coronet cells of saccus vasculosus advocates that the experimental fish may accumulate least amount of glycogen in the coronet cells as energy source but directly utilize glucose for the purpose from the blood which is richly supplied in saccus vasculosus.

Detection of basic protein

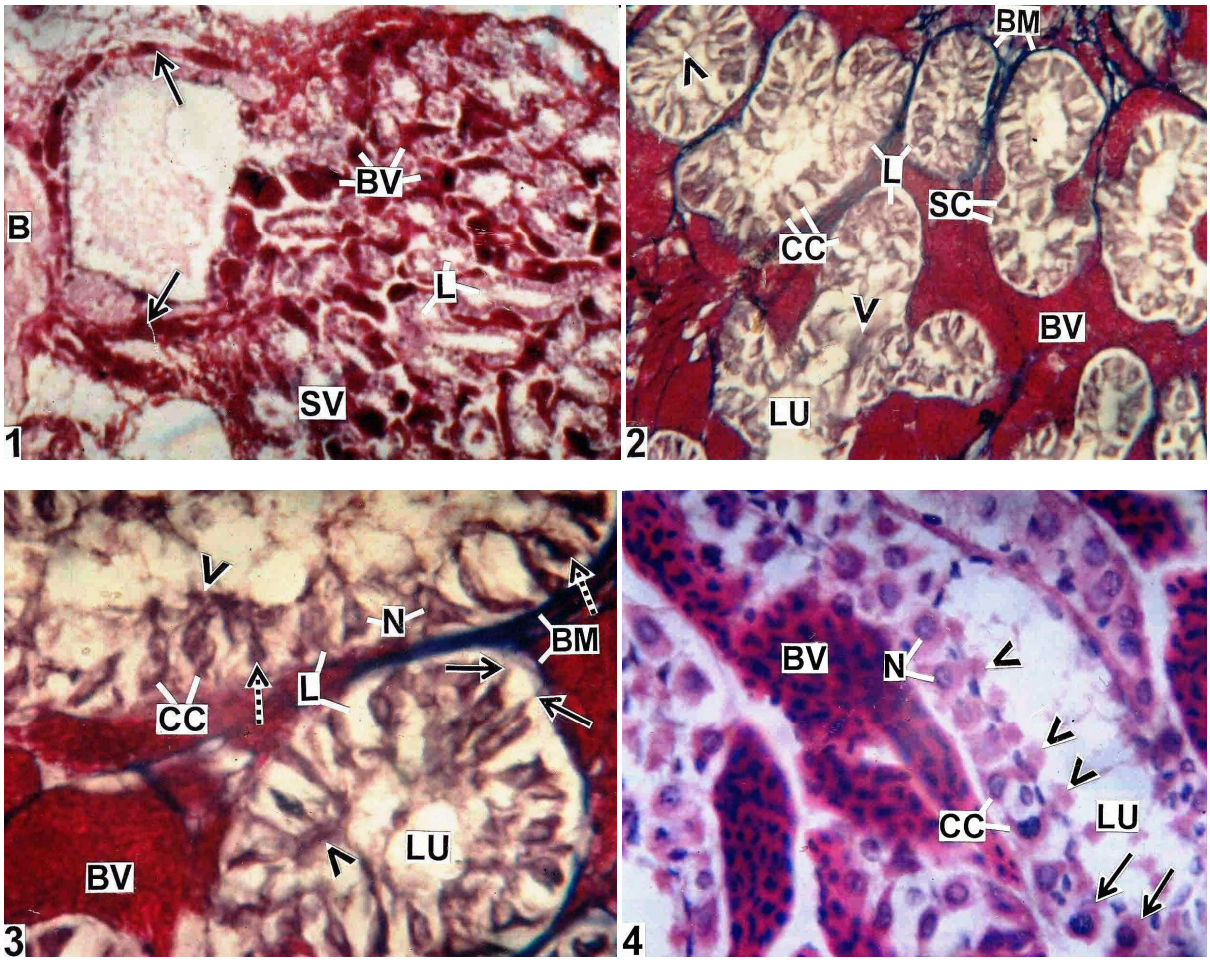
An intense reaction for protein is discernible in the coronet cells of the saccus vasculosus. The apical protrusion and globules of coronet cells exhibit strong reaction for protein. However, maximum reaction for protein is recorded in the blood cells (Fig. 10).

The cytoplasm content of coronet cells of saccus vasculosus of *N. notopterus* shows positive reaction for protein, therefore, it is concluded that the content of coronet cells is at least in part proteinaceous. Therefore, the acute reaction for protein confirming the elaborate secretion of glycoprotein from the coronet cells of saccus vasculosus [16].

Detection of bound lipid

In *N. notopterus* the coronet cells afford moderate to weak lipid content. The intensity of bound lipid reaction in the apical protrusions of the coronet cells varies greatly due to the varying amounts of secretory products. However, the blood vessels have been found to be more reactive to this bound-lipid reaction (Figs. 11 and 12).

Sundararaj and Prasad [12] observed the localization of traces of phospholipid in the apical protrusion and globules of coronet cells in the saccus vasculosus of *N. chitala*. In the present study the variation of sudanophilic materials in the coronet cells of *N. notopterus* have been observed. It is quite likely that necessary energy is needed for the physiological activities and secretion of the secretory product of the coronet cells which is mainly derived from the accumulated lipid material of the cell concerned. This is conformity with the findings of Kulkarni and Sathyanesan [15] in the saccus vasculosus of *Mystus vittatus*.



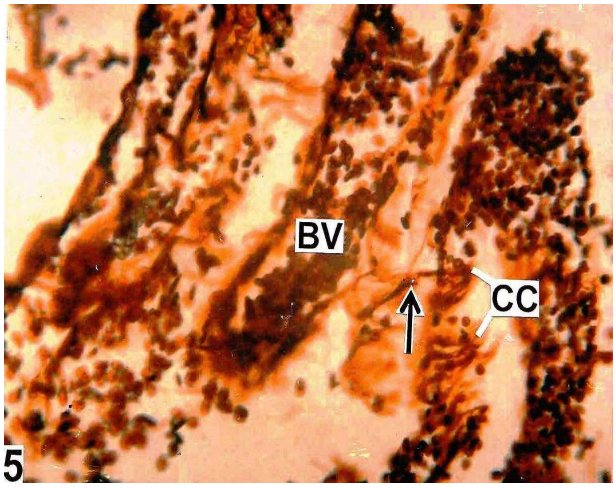
Figs. 1-4. Photomicrographs of the histological sections of saccus vasculosus of *N. notopterus* stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain.

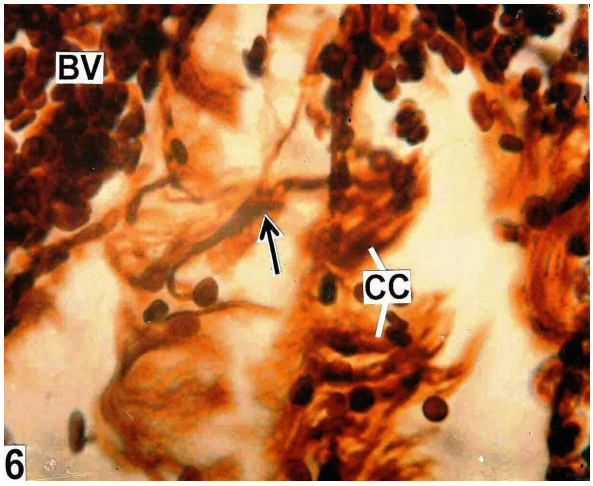
Fig. 1. Showing saccus vasculosus (SV) having numerous loculi (L) surrounded by blood vessels (BV) attached with the brain (B). Arrows indicate connection of SV with the diocoel of brain (HE) × 50 X.

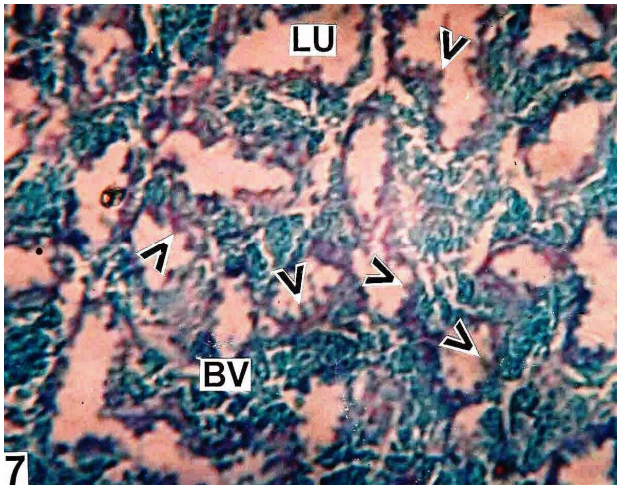
Fig. 2. Showing various shapes of L packed with coronet cells (CC) and supporting cells (SC) along with basal membrane (BM), surrounded by BV. Arrow heads indicate luminal (LU) extrusion from CC (MT) × 100 X.

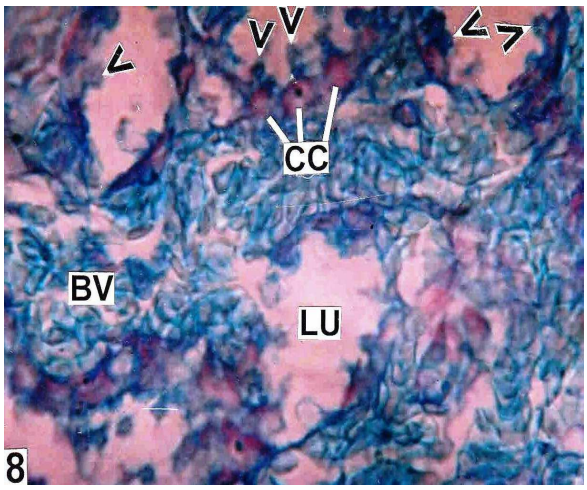
Fig. 3. Showing L lined with single layer of columnar CC having conspicuous nuclei (N) and few SC (broken arrows), encircled by BV. Solid arrows indicate the attachment of CC with BM. Note the presence of secretory materials (arrow heads) in the lumen (LU) (MT) × 400 X.

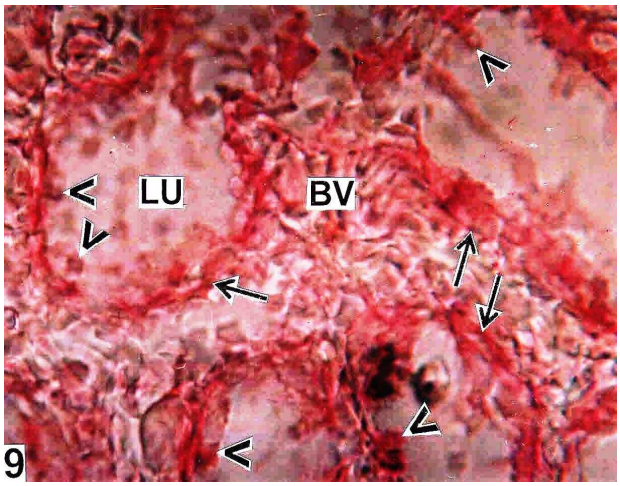
Fig. 4.Higher magnification of CC with basal N and SC (solid arrows). Note the presence of BV and globular protrusion (arrow heads) of CC towards the LU (HE) × 600 X.

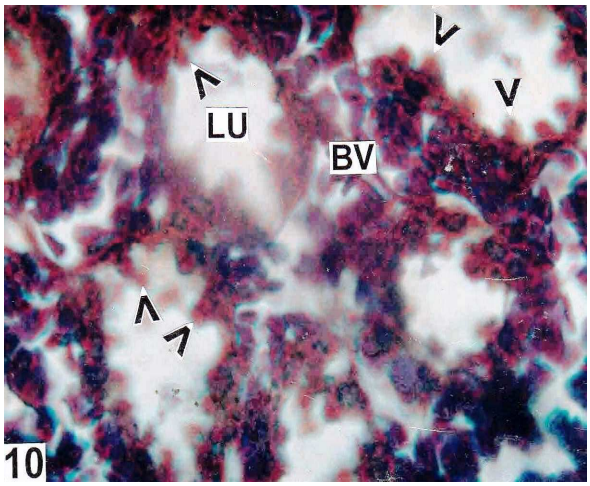

BV
CC
5

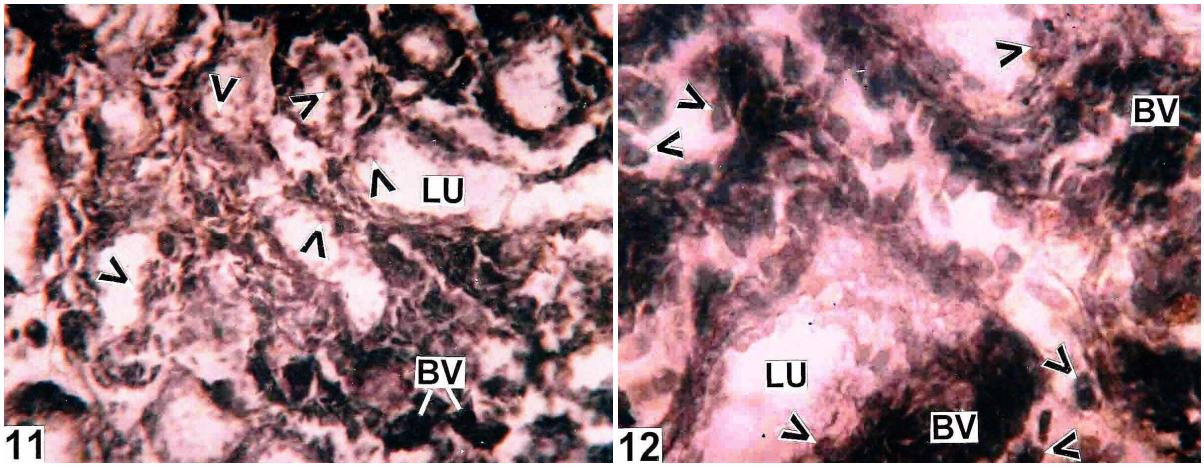

BV
CC
6


LU
BV
7


CC
BV
LU
8


LU
BV
9


LU
BV
10



Figs. 5-12. Photomicrographs of the section of different regions of saccus vasculosus of *N. notopterus* showing silver deposition in the neurons by Silver Impregnation Method (SIM), Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for detection of neutral and acid mucins, Best's Carmine (BC) reaction for glycogen, Mercury-Bromphenol Blue (MBPB) reaction for basic protein and Sudan Black B (SB) reaction for bound lipid.

Fig. 5. Showing silver reaction in free end of coronet cells (CC) and nerve terminals (arrow) attached with blood vessels (BV) (SIM) × 100 X.

Fig. 6. Higher magnification showing intense silver reaction in the anterior part of CC. Note positive reaction in the terminal nerves (arrow) which are in contact with BV (SIM) × 400 X.

Fig. 7. Showing purple colour in the CC (arrow heads) advocates presence of neutral mucopolysaccharides. BV exhibit blue colour due to acidic mucopolysaccharides; LU marks lumen (PAS-AB) × 100 X.

Fig. 8. Higher magnification exhibiting intense purple colour in the globular apical protrusion (arrow heads) of CC towards the LU while BV show blue colour (PAS-AB) × 400 X.

Fig. 9. Showing different shades of staining deposition of glycogen in the apical protrusions and globules of CC (arrow heads) towards the LU. Note the positive reaction in basal membrane (solid arrows) and moderate reaction in BV (BC) × 400 X.

Fig. 10. Showing intense protein reaction in the globular extrusion of CC (arrow heads) towards the LU. Note maximum reaction in BV (MBPB) × 400 X.

Fig. 11. Showing moderate lipid reaction in CC (arrow heads) and maximum reaction in blood vessels (BV). LU indicates lumen (SB) × 100 X.

Fig. 12. Showing moderate lipid reaction in the apical protrusion of CC (arrow heads) towards the LU. Note intense reaction in BV (SB) × 400 X.

CONCLUSION

The present observations reveal that the structural pattern, cellular orientation and chemical content of the cells lining the saccus epithelium of *N. notopterus* may be involved in both secretory and sensory functions. The present structural data points a high degree of vascularity of saccus vasculosus and eminent metabolic function of the coronet cells due to bottom dwelling habit of fish concerned. However, further studies of electron microscopy will be useful in corroborating the present findings.

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