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Research Article

LIGHT MICROSCOPICAL CHARACTERIZATION OF THE SACCUS VASCULOSUS OF FRESH WATER CATFISH, *EUTROPIICHTHYS VACHA* (HAMILTON, 1822)

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ABSTRACT
The histological features and histochemical characterization of saccus vasculosus in *Eutropiichthys vacha* (Hamilton, 1822) was investigated by optical microscopy. The saccus was pouch like protuberance, placed on the ventral side of the brain and quite separated from the hypophysis. Histologically, the saccus epithelium comprised of a large number of coronet cells and few supporting cells. The intense reaction of silver stain was discernible in the basal part of the coronet cells contained synaptic contact with blood vessels. The localization and detection of acid and neutral mucins in the different cells of the saccus vasculosus was studied by employing Periodic Acid Schiff's reaction in combination with Alcian Blue (PAS-AB) technique. Different shades of glycogen, basic protein and bound lipid were noted in the various cells of epithelial lining as well as in the blood vessels. The cytoarchitecture and different degrees of localization of neurons, mucopolysaccharides, glycogen, basic protein and bound lipid in the saccus vasculosus were correlated with the functional significance of the fish concerned.

Keywords: *Cellular Architecture, Histochemical Nature, Saccus vasculosus, Eutropiichthys vacha*

INTRODUCTION
The saccus vasculosus is highly vascular and situated mostly on the caudal infundibular wall of the diencephalon in bony fishes. The size of this circumventricular organ varies considerably as it may be well organized or poorly developed or even absent. It is a vascularized neuroepithelium, consists of characteristic coronet cells and supporting glial cells with interspersed liquor containing neurons (Joy and Sathyanesan, 1979; Sueiro *et al*., 2007). The saccus vasculosus serves as an umbilical link between cerebrospinal fluid and the blood vascular system. Its proximity to third ventricle, rich vascular epithelium and specialized microarchitecture, points to a very specialized role. The teleostean saccus vasculosus has been involved in sensory, secretory, ion transporting or osmoregulatory roles (Shimada, 1976). A number of researchers have studied the microarchitecture as well as functional significance of this ependymal organ through light and electron microscopes (Saksena, 1989; Corujo *et al*., 1990; Sanson and Kryvi, 1991; Bargmann, 2003; Gupta, 2007; Chakrabarti and Ghosh, 2009; Ghosh and Chakrabarti, 2010; 2013; 2014). However, the literature contains few investigations regarding the histochemical approaches of saccus vasculosus in Indian riverine teleosts.
Therefore, it would be naturally worthwhile to examine the detailed histoarchitecture and the chemical nature of mucins, localization and distribution of neuronal elements, glycogen, basic protein and bound lipid content in the saccus vasculosus of economically important food fish, *Eutropiichthys vacha* (Siluriformes, Schilbidae).

MATERIALS AND METHODS
Tissue Collection
Adult mature specimens of *E. vacha* (18 to 20 cm in total length) were collected from the river Ganga near Samudragar, Burdwan, West Bengal, India. The fishes were sacrificed by decapitation following the guidelines given by the Institutional Ethical Committee. The brain mass including the saccus vasculosus

was exposed from the ventral side of the brain and fixed *in situ* with 10% neutral formalin. After few minute the saccus vasculosus along with the rest of the brain was cautiously detached from the cranium and processed for histological and histochemical studies.

Histological Study

The saccus vasculosus was kept in aqueous Bouin's fluid for a period of 16-18 hour. After fixation the tissues were washed repeatedly in 70% ethanol, dehydrated with graded series of ethanol and cleared with xylene. Then the tissues were infiltrated in paraffin wax of 56-58˚C under a thermostat vacuum paraffin embedding bath for 1 hour and paraffin blocks were prepared. Thin serial sections were cut at 4 µm thick using a rotary microtome (Weswox). After routine histological procedure the deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain (Mallory, 1936).

Histochemical Study

The intact saccus vasculosus was fixed in 10% neutral formalin for 18 h. After dehydration in ascending 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 (Marsland *et al.*, 1954), Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for detection of neutral and acid mucins (Mowry, 1956), Best's Carmine (BC) method for detection of glycogen (Best, 1906), Mercuric Bromphenol Blue (MBB) method for detection of basic proteins (Mazia *et al.*, 1953) and Sudan Black B (SB) method for detection of bound lipid (Berenbaum, 1958).

The stained slides were examined and photographed under Olympus-Tokyo PM-6 compound microscope.

RESULTS AND DISCUSSION

Histology

The saccus vasculosus of *E. vacha* is reddish sac like structure, located on the ventral profile of the diencephalon (Figure 1). It is encapsulated entity and is quite segregated from the pituitary.

Teleostean saccus vasculosus exhibits considerable morphological variability at both cellular and whole level of organization. In the present investigation, the saccus vasculosus of *E. vacha* is sac like protuberance on the ventral side of the brain and separated from the pituitary by an interspaced hence can be put into group II of Mecklenburg (1974).

The capsular wall in some regions penetrates the parenchyma of saccus to form villi like structure towards the lumen. These villi are lined by a large number of coronet cells as well as supporting cells, arranged in stratified manner (Figure 2). These cells are basally attached to either dividing septa or arranged along the vascular channel.

Prasada (1966) noticed the presence of haemopoietic tissue and high degree of accumulation of blood vessels in the posterior region of the infundibulum and secretory activities of the saccus vasculosus in some fishes. Van (1977) observed 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 saccus vasculosus often shows villi like projection towards the lumen. These infoldings increase the surface area of saccus epithelium to facilitate the absorption and/or secretion process. Similar infoldings of saccus epithelium have been observed by Jansen and Flight (1969) in rainbow trout. Dammerman (1910) opined that the capillaries are responsible to provide nutritive substances to the different cells ling the saccus epithelium. He suggested saccus vasculosus as benthic organ involved in the perception of depth of swimming.

The dominant coronet cells are pear shaped with basally located prominent nuclei (Figures 3, 4). These cells are provided with apical projections. The coronet cells show variation in size, shape as well as in the staining intensity indicating the differences in physiological state. Some of the coronet cells are contacted with nerve terminals (Figure 4). The supporting cells are less numerous and scattered among the coronet cells (Figures 3, 4). These cells are characterized by feeble cytoplasm and large nuclei in the middle zone

of the cells. The wide lumen is filled with basophilic secretory material (Figure 3). The deposition of the stainable material in some areas of the lumen is continuation with cytoplasmic outgrowths of coronet cells display identical tinctorial nature (Figure 4).

Kurotaki (1961) noted that the saccus epithelium of *Anguilla japonica* and *Cottus pullux* contained principal coronet cells and supporting cells. Furthermore, he 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 (1964) observed coronet and supporting cells alternating with each other in the saccus epithelium of *Mystus vittatus, Callichorus pabda* and *Bagarius bagarius*. They noticed different types of coronet cells which were presumed to be the stages of either formation or degeneration of coronet cells. In the present study, the saccus vasculosus of *E. vacha* represents the efficient type of coronet cells as well as elaborate system of blood vessels. The coronet cells in the experimental fish 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 (Ghosh and Chakrabarti, 2014). During the present investigation it is observed that characteristic coronet cells are beset with apical protrusions. Interestingly all the coronet cells are not provided with such extrusions. It can be suggested that cells with typical outgrowths indicate the active functional state while rest of the cells manifest the inactive state. Benjamin (1974) suggested that the coronet cells are secretory in nature, probably involved in osmoregulation. Not much attention has been paid to supporting cells although they are constant components of the saccus epithelium of the teleost studied so far. Supporting cells are closely associated with the coronet cells and hence it is likely that the essential role of these cells is to support (Shimada, 1976). Jansen *et al*., (1981) opined that not only the principal coronet cells but also the supporting cells of this circumventricular organ are involved in cerebrospinal fluid homeostasis.

Histochemistry

Detection of Neuron: In *E. vacha* intense reaction for silver stain is discernible in the basal part of the coronet cells which are connected with the blood vessels (Figures 5, 6). Some of the coronet cells connected with the network of nerve fibres exhibit maximum localization of silver stain. The luminal secretory part also shows intense silver stain (Figures 5, 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 in conformity with the findings of Chakrabarti and Ghosh (2009) in the saccus vasculosus of *Macrognathus aculeatum*.

Detection of Mucopolysaccharides: PAS-AB combined technique imparts bright purple colour for neutral mucopolysaccharides whereas AB produces a blue colour when it reacts with acid mucopolysaccharides. In *E. vacha* the apical zone of some of the coronet cells exhibits moderate to intense reaction (Figure 7). The cytoplasm of the coronet cells shows purple colour due to the presence of neutral mucopolysaccharides. The intensity of PAS-AB reaction in the cytoplasm of coronet cells also varies as some of the cells intensely react with PAS-AB while others are lightly stained. On the other hand, the blood vessels give very intense alcian blue reaction (Figure 7).

Chemically mucins are hexoseamine-containing polysaccharides which are bounded covalently with varying amounts of proteins. Jansen and Flight (1969) recorded PAS positive material in the saccus vasculosus in freshwater rainbow trout. Narshiman and Sundararaj (1971) also reported PAS positive materials in the saccus vasculosus of *Catla catla* and *Colisa fasciata*. Kulkarni and Sathyanesan (1982) 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 of the saccus vasculosus in *E. vacha*. This clearly indicates that perhaps the coronet cells are very actively engaged in the secretion and synthesis of neutral mucopolyaccharide. Singh and sathyanesan (1964) noted PAS-positive granules in the coronet cells of many fishes and suggested a secretory role.

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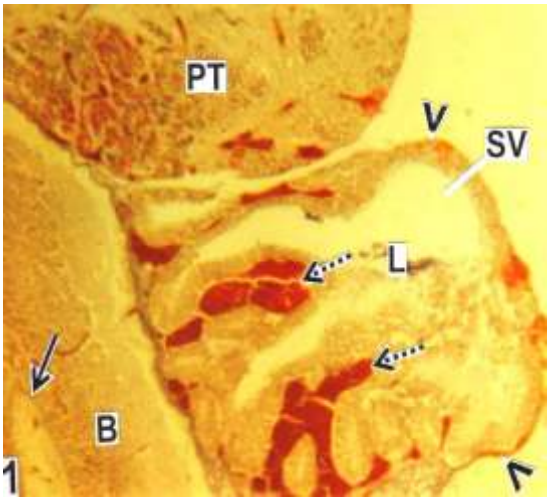

Figure 1: Showing the location of saccus vasculosus (SV) enclosed by connective tissue (arrow heads) on the ventral side of the brain (B) and is separated from the pituitary gland (PT). Note the presence of spacious lumen (L) and blood vessels (BV) (broken arrows). Solid arrow indicates third ventricle of B (MT) × 100 X.

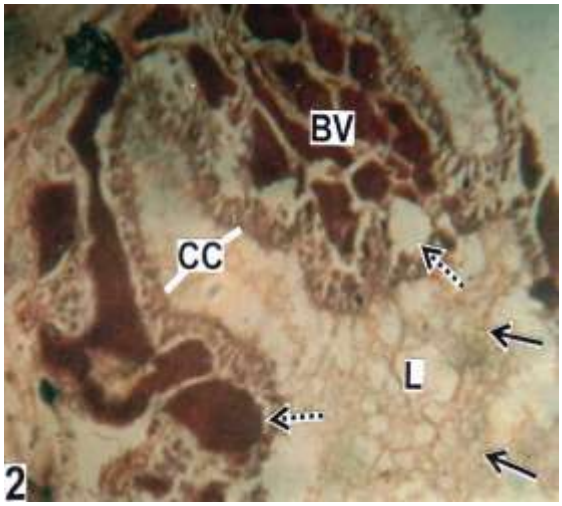

Figure 2: Villi like projections (broken arrows) of SV which are lined by coronet cells (CC). Note vascular packing (BV) under CC and stainable materials (solid arrows) in L (HE) × 200 X.

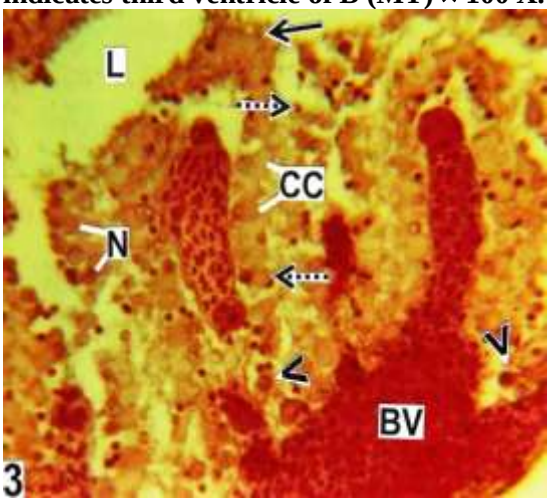

Figure 3: Showing tinctorial properties of the cytoplasm of CC with prominent nuclei (N) and apical protrusions (broken arrows) of CC. Note few supporting cells (SC) scattered among CC and secretory deposition (solid arrow) in L (HE) × 400 X.

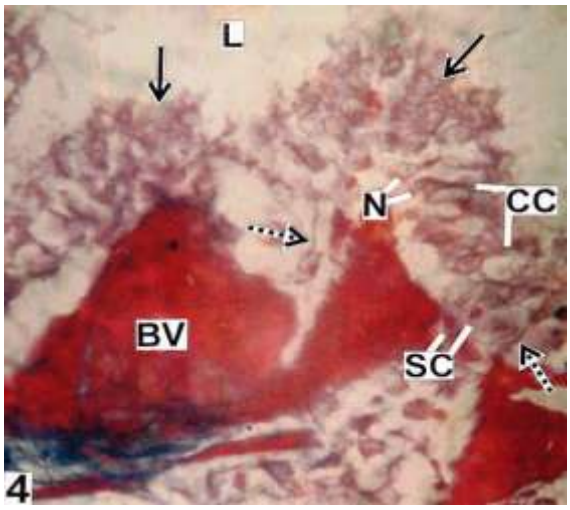

Figure 4: CC and SC in higher magnification showing underlying BV. Note the conspicuous N of CC and stainable secretory materials (solid arrows) in the L. Broken arrows mark nerve from CC (MT) × 600 X.

Figures 1-4: Photomicrographs of the sagittal section of saccus vasculosus of *E. vacha* showing histological architecture stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain.

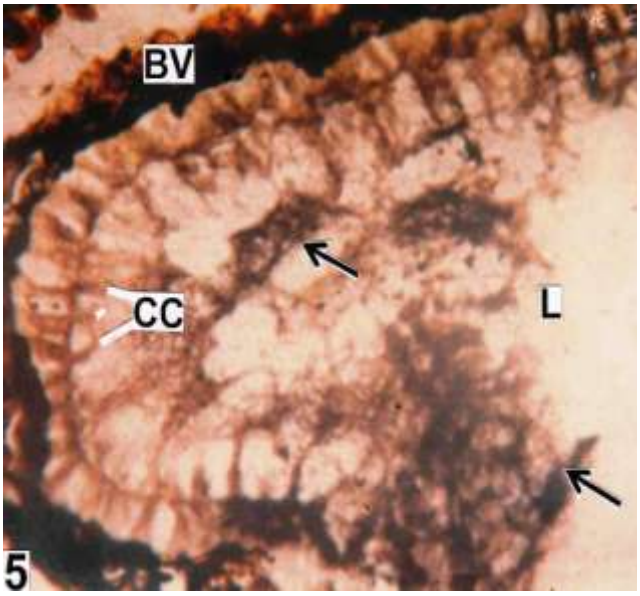

Figure 5: Showing silver reaction in coronet cells (CC), blood vessels (BV) and luminar (L) secretion (solid arrows) (SIM) × 440 X.

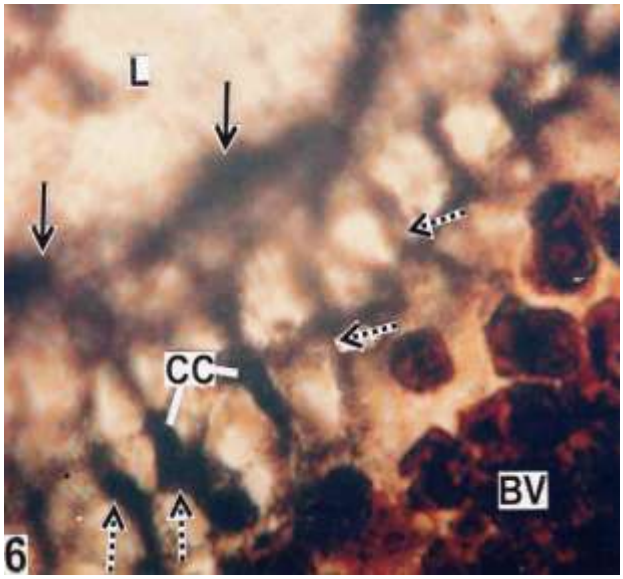

Figure 6: Higher magnification exhibits intense silver reaction in CC and synaptic contact (broken arrows) of nerves with BV. Note also positive reaction in luminar extrusion (solid arrows) of CC. L marks lumen (SIM) × 1000 X.

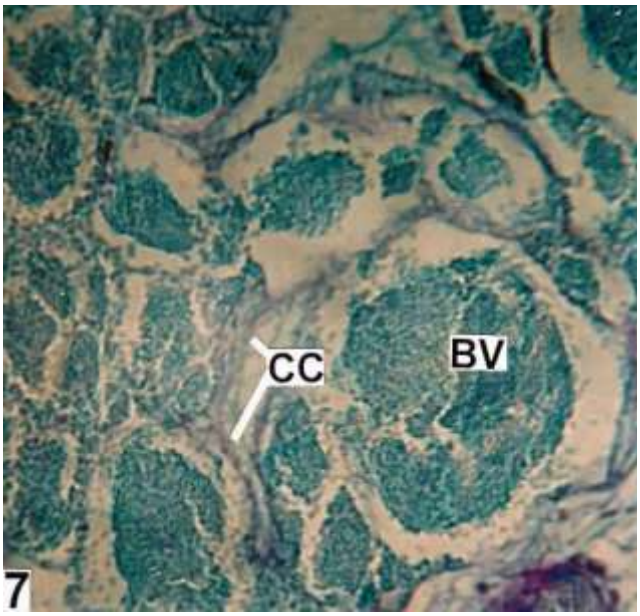

Figure 7: Showing intense purple colour in CC and blue colour in BV (PAS-AB) × 100 X.

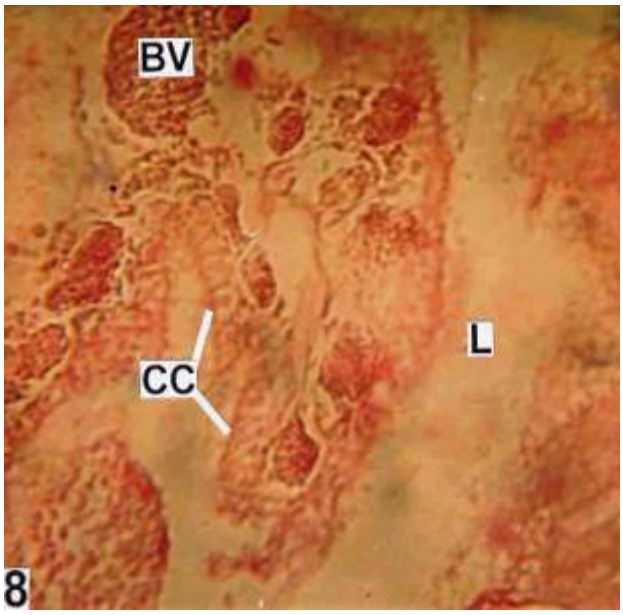

Figure 8: Moderate reaction of glycogen in CC. Note also positive reaction in BV. L denotes lumen (BC) × 100 X.

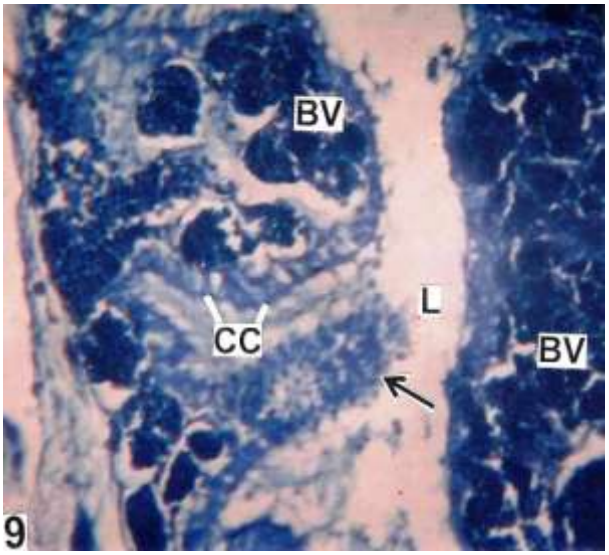

Figure 9: Showing strong protein reaction in CC, BV and secretory material (solid arrows) of L (MBB) × 100 X.

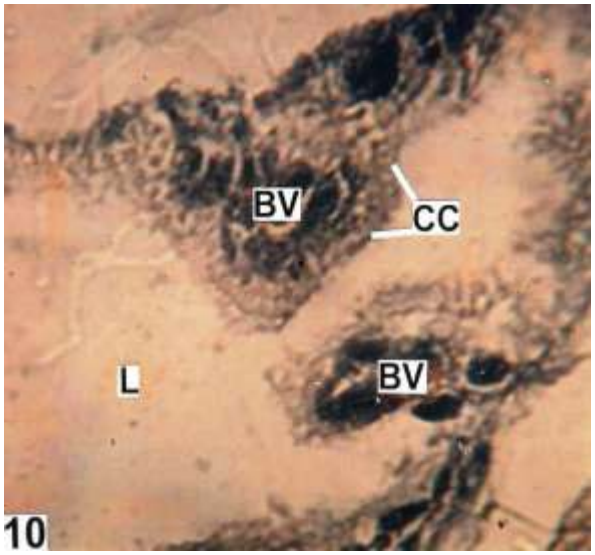

Figure 10: Showing moderate lipid reaction in CC and intense reaction in BV. L marks lumen (SB) × 100 X.

Figures 5-10: Photomicrographs of the section of saccus vasculosus of *E. vacha* 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 mucopolysaccharides, Best's Carmine (BC) reaction for glycogen, Mercuric Bromphenol Blue (MBB) reaction for basic protein and Sudan Black B (SB) reaction for bound lipid.

Detection of Glycogen: Best's carmine assay denotes an acute to moderate quantity of glycogen in the cytoplasm of coronet cells and apical border of the coronet cells (Figure 8). However, utmost glycogen reaction is observed in the blood cells of blood vessels of *E. vacha*.

The coronet cells show intense to moderate reaction for glycogen probably for metabolic as well as physiological activities. Sundararaj and Prasad (1964) noted that coronet cells of the saccus vasculosus of *N. chitala* contain glycogen in the apical protrusion. They opined that glycogen is converted to glucose which thereafter is delivered to cerebrospinal fluid. Khanna and Singh (1967) 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 teleost 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: The cytoplasm of the coronet cells in the saccus of *E. vacha* stain more intensely due to proteinaceous nature of these cells (Figure 9). The apical secretion of the coronet cells along with luminal secretory products also shows intense reaction for protein. On contrary, maximum protein reaction has been noticed in the blood vessels (Figure 9).

The cytoplasm content of coronet cells of saccus vasculosus of *E. vacha* shows positive reaction for protein, therefore, it is concluded that the content of coronet cells is at least proteinaceous in nature. Therefore, the acute reaction for protein confirming the elaborate secretion of glycoprotein from the coronet cells of saccus vasculosus (Ghosh and Chakrabarti, 2013).

Detection of Bound Lipid: The coronet cells which are oriented in the basal region facing toward the lumen respond to sudan black B reaction. The rim of cytoplasm of the coronet cells shows moderate to weak reaction (Figure 10). The apical extrusions of coronet cells contain primarily bound lipid material.

However, the blood vessels have been found to be more reactive to this reaction than that of the coronet cells (Figure 10).

Sundararaj and Prasad (1964) observed the localization of traces of phospholipid in the apical protrusions of coronet cells in the saccus vasculosus of *N. chitala*. In the present investigation the deposition of sudanophilic materials is observed in the coronet cells of *E. vacha*. 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 in compliance with the findings of Kulkarni and Sathyanesan (1982) in the saccus vasculosus of *Mystus vittatus.*

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