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Histological study of pseudobranch in *Ctenopharyngodon idella*

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Abstract
In teleosts the pseudobranch is generally accepted as a modified posterior hemibranch of the mandibular gill arch that often fused to the anterior of the opercular cavity, but due to variation in size, form and location, presence or absence in different fish species, it has remained as an object of investigation for a long time.

This study was made on the 40 normal, mature and same size *Ctenopharyngodon idella*. After biometrical study, the samples were removed deeply from total epithelium of chambers. For microscopically study, the tissues were fixed in Bouin's solution and the sections were made on by routine paraffin embedding and stained by H&E, PAS methods.

Results showed that pseudobranches were existed under craniodorsal epithelium of chamber and were embedded deeply into the connective tissue of this area. The basic structure of the pseudobranch of *Ctenopharingodon idella* consists of several rows of parallel lamellae that are fused to each other throughout their length by a thin connective tissue. The lamellae comprise a central blood capillary that surrounded by large secretory cells. These cells have clear cytoplasm with abundant acidophilic granules and a large euchromatic nucleus. Pseudobranch was covered by a thick layer of connective tissue. The goblet cells, club cells and taste buds were seen in the stratified squamous epithelium that located on the connective tissue. The results suggest that location and structure of pseudobranch in *Ctenopharyngodon idella* have considerable species differences.

Keyword: histology, pseudobranch, *Ctenopharyngodon idella*

Introduction

More than 220 years ago, Broussonet (1785) has described that the pseudobranch is a small gill like structure and has a respiratory function, but later studies were discarded this function in adult fishes (Roy et al., 1997). In recent years, the literature have attributed endocrine (Bertin, 1958), neurosecretory in marine and estuarine teleosts (King, 1993 and Quinn, 2003), oxygen multiplier device (Munshi, et al., 1994), Maintenance of blood pressure of the eye as a vascular rete mirabile (Pelster & Randall, 1998 and Waser & Heisler, 2004), sensory (Bridges et al., 1998) and many other roles to the pseudobranch, but its overall role has still remained a mystery.

The pseudobranch in teleosts is generally accepted as a modified posterior hemibranch of the mandibular gill arch that often fused to the anterior of the opercular cavity (Quinn et al., 2003), but due to variation in size, form and location, presence or absence in different fish species, it has remained as an object of investigation for a long time (Roy et al., 1997). Although, it is now accepted that among the 238 teleostean families, the pseudobranch is found in almost all fishes barring few species of the order Anguilliformes, suborder Siluroidei and all the species of genus *Gymnarchus* and *Cobitis* (Roy et al., 1997).

However, in absence of sufficient work and experimental evidences, the structure of pseudobranch and particularly its microscopic structure in some species remain as yet ambiguous. The present histological study was taken to describe the structure of pseudobranch in grass carp.

Materials and methods

This study was made on the 20 normal, mature and same size grass carp, *Cetenopharingodon idella*, with 32.6 ± 0.52 cm in standard length. After biometrical study, the samples were removed deeply from epithelium of chambers. In order to light microscopic studies, tissue samples were fixed in Bouin's solution and then were processed by routine and standard

paraffin embedding and serially sectioned in 5-6 µ thickness. Tissue sections were deparaffinized and stained with hematoxylin–eosin and periodic acid schiff (Bancroft & Gamble, 2002). For micrometrical studies, thickness of connective tissue and outer epithelium that cover pseudobranch were measured and the number of taste bud, goblet cell and alarm cell has been counted along the 100µ length of its epithelium by calibrated lens.

Results

Microscopical results showed that pseudobranch was existed under craniodorsal epithelium of chamber and embedded deeply in to the connective tissue of this area. This structure was existed in 11.32 ± 4.66µ depth of epithelium surface. Generally, the basic structure of the pseudobranch of grass carp is similar to the gills and consists of several rows of parallel lamellae that are fused to each other throughout their length by a thin connective tissue (Fig. 1). The lamellae comprise a central blood capillary that forms a mesh work and surrounded by large secretory cells. These cells have clear cytoplasm with abundant acidophilic granules and a large euchromatic nucleus in center of cytoplasm and a prominent nucleolus. The cytoplasm of these cells has negative reaction to periodic acid schiff staining (Fig. 2 and 3). Pseudobranch was covered by a thick layer of connective tissue. The goblet cells, alarm cells and taste buds were seen in the non-keratinized stratified squamous epithelium that located on the connective tissue (Fig. 4). The numbers of these cells were 58.04 ± 2.18, 47.61 ± 4.18 and 1.00 ± 0.06 along the 100µ length of epithelium, respectively. The goblet cells were existed in superficial surface of epithelium while alarm cells were existed in middle to deeper layers of epithelium. Alarm cells contained one or two nucleus and have negative reaction to periodic acid Schiff staining, but goblet cells have positive reaction to this staining (Fig. 5).

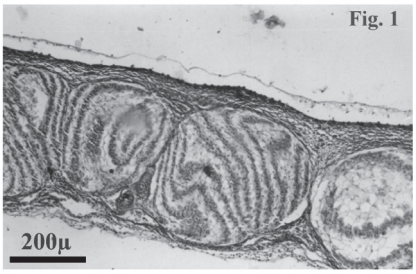

Fig. 1: the pseudobranch consists of several rows of parallel lamellae that are fused to each other throughout their length by a thin connective tissue, PAS staining.

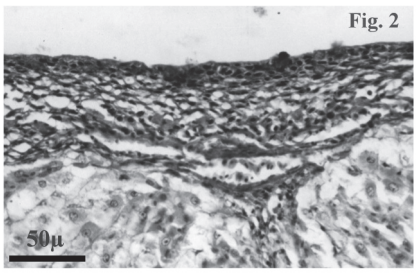

Fig. 2: Pseudobranch was covered by a thick layer of connective tissue, PAS staining.

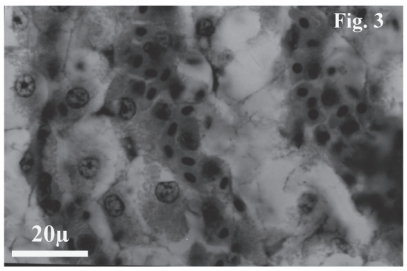

Fig. 3: The lamellae comprise a central blood capillary that forms a mesh work and surrounded by large secretory cells, H&E staining.

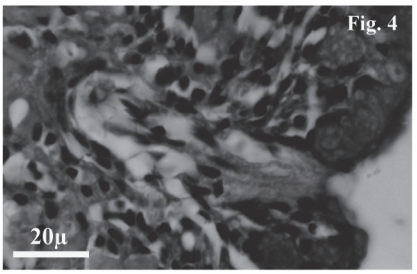

Fig. 4: The goblet cells and taste buds were seen in the non-keratinized stratified squamous epithelium that located on the connective tissue, PAS staining.

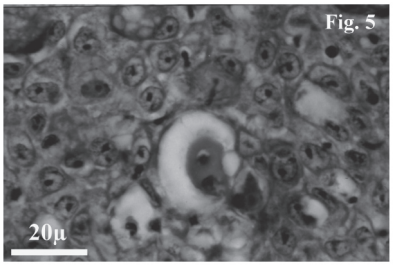

Fig. 5: The alarm cells were existed in middle to deeper layers of epithelium. These cells contained one or two nucleus and have negative reaction to periodic acid schiff staining, PAS staining.

Discussion

Although most teleosts have pseudobranch but its form and size varies in different species. The pseudobranch arises embryologically from the caudal hemibranch of the mandibular gill arch, is supplied with oxygenated blood and therefore has no respiratory function in adult fish; thus name pseudobranch (Stoskopf, 1993). The pseudobranch might be attached to the cranial side of the operculum in *Hilsa ilisha* or might be sunken deep into the connective tissue of the bucal roof in *Anabas testudineus* (Roy

et al., 1997 and Singh et al., 1986). The microscopic structure of pseudobranch varies among the species of fishes. In *Glossogobius giuris*, it is similar to the gills, while it has a glandular structure in *Notopterus chitala* (Roy et al., 1997). Bertin (1958) described three morphological types of pseudobranch in fishes; I) these with distinguishable lamellae in contact with the water that the secondary lamellae are completely free. II) Those that were covered with the opercular membrane and the connective tissue. This kind can be subcategorized into three types based on its location and its degree of isolation from water: A- A thin layer of connective tissue due to which lamellae can not be separated but the filaments are free. B- Connective tissue layer thicker than type A due to which both the filaments and the lamellae can not be separated. The lamellae, rays and the vessels are well organized. C- A thick layer of connective tissue in which the structure is so covered that all the elements lose their identity. III) Those that were completely reduced and embedded in the tissue. In this type pseudobranch is separated from the opercular chamber by the folds of connective tissue. This causes sinking of the pseudobranch in the roof of the buccopharynx or opercular cavity.

On the basis of classification of Bertin, it can be said that the pseudobranch of grass carp comes under the category 'C' of covered type. Mattey et al. (1980) described the covered type of pseudobranch in trout, embedded in carp, free in bass, and semifree in mullet. Harder (1975) discovered that in the larvae of *Cyprinus carpio* measuring 11mm, the pseudobranch situated at the base of the operculum is of the free type. At a 12 mm stage, it changes to the covered type and is transformed into the embedded type. On the basis of evidence available on the structure of the pseudobranch it can be concluded that its presence, absence or nature can not be related in any way to the systematic position of the fish concerned.

Two different types of pseudobranchs, free and embedded have been recognized on the basis of their gross microscopical structure. The epithelium of the pseudobranch is heterogeneous in composition and consists of pavement cells, acidophilic mitochondria rich cells, mucous cells and specialized pseudobranchial cells (Roy et al., 1997).

The pavement, mucous and chloride cells are generally present in the free type of pseudobranch as in *Hilsa ilisha* (Singh et al., 1986). In semifree and covered types of pseudobranchs, chloride cells are replaced by pseudobranchial cells. Earlier both the cell types were considered to have a similar structure and function, but later on it was realized that these are two different cell types with a common characteristics (Roy et al., 1997).

Singh et al. (1986) described that in *Hilsa ilisha*, the pseudobranch consists of only one row of parallel filaments attached to the opercular epithelium at the based of the gill arch and the filaments are free. Leaf-like secondary lamellae are arranged on both sides of the filaments throughout their length. These investigators also reported that in *Gadusia chapra,* the general structure and location of the pseudobranch is similar to that of *Hilsa ilisha* but the hemibranch bears a row of filaments with secondary lamellae on both sides. The lamellae are fused throughout the filament length except the tip of region, where only a few lamellae remain free.

In *Glossogobius giuris*, the pseudobranch is represented by few bud-like structures present at the base of the operculum. Each bud-like structure represents a filament in which the lamellae of both sides are folded, arranged like petals and covered externally with an epithelial layer. In *Notopterus chitala*, a pair cylindrical glandular body is fond embedded in the connective tissue and muscular far anterior in the head region just behind the eyes (Roy et al., 1997).

Munshi and colleagues (1994) have indicated that the pseudobranchs are paired structures which have migrated from the opercular cavity to the roof of buccopharynx and are embedded deeply into the connective tissue. Each pseudobranch is a three lobed structure surrounded by a thick membrane in *Channa punctata* and *C. gachua*, but multilobed in *C. marulius*. It is encircled by connective and fatty tissues.

In salmonid species, the pseudobranch is covered by an epithelium in the anterior opercular cavity. It is structurally similar to the gill, with primary and secondary filaments. The pseudobranch cell type bears some resemblance to the gill chloride cell, being packed with mitochondria (Quinn et al., 2003). The histological picture of the pseudobranch of *Anabas sp.* and *Channa sp.* is different from other fish species. Each lobe of the pseudobranch is clearly divisible in two parts: inner medulla and outer cortex. In *Anabas* the two parts are distinct, but not so much as in *Channa sps*. (Roy et al., 1997). The basic structure of the pseudobranch of *H. ilisha*, *G. chapra* and *G. giuris* is similar to the gills. The lamellae comprise a central vascular layer surrounded by thin basement membrane and an outer epithelial coverage on both sides. In *Hilsa*, the epithelium is double layered (Singh et al., 1986). It can be concluded that there is a considerable species variation in structure and location of pseudobranch in teleosts.

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