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Structure and Cells in the Gills of The Three Air-Breathing Fishes *Clarias Batrachus, Heteropneustes Fossilis* and *Monopterus Cuchia*. A Light Microscopical Investigation

KEYWORDS: Gills, Histological, Histochemical

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ABSTRACT *Clarias batrachus and Heteropneustes fossilis have well developed gills with two sets of four holobranch, each set having a gill arch and two rows of primary filament or lamellae projecting caudolaterally. The gills of Monopterus cuchia is not much more prominent and are compact in nature. Histologically, the cells present in the gills of Clarias batrachus and Heteropneustes fossilis is almost similar. The suppression of gill lamellae in Monopterus cuchia are thought to be its adaptation to their environment which becomes muddy and semi terrestrial. The different cells present helps in number of gaseous exchange functions. Comparing the entire groups of air breathing fishes the shape, size and quantity is found to be reduced in Clarias batrachus, Heteropneusts fossilis and more reduced in Monopterus cuchia. Histochemically, the mucous goblet cells of gills showed strong reactions with AB pH 2.5 indicating the presence of glycoproteins with carboxyl groups and/ O sulphate esters.*

INTRODUCTION

Gills are the main respiratory organs in fishes and are the important site of gas exchange in almost all fishes. In addition to these, gills also play an important role in the maintenance of salt balance and excretion of certain waste products (Zayed and Mohammad, 2004). Besides gas exchange and respiratory homeostasis gills have other non respiratory functions such as, water and salt balance (Smith 1930, Key 1931,Hughes 1964, Munshi 1966); nitrogen metabolism (Goldstein 1982); acid-base balance (Heisler 1984); hormone metabolism (Nekvasil& Olson 1986); plasma detoxification (Munshi& Hughes 1991).

The gills of almost all the fishes have more or less similar structural organisation, even though some variations are being found. Gills undergo modifications to better adapt to the physic-chemical condition of its habits and habitats. Various structural changes have been taken place in general organisation of gills of air-breathing fishes in relation to their habitat environment (Hughes and Munshi, 1978; Dutta and Munshi, 1985)

Several works are being done upon gills,its structure and its function. In this context mention may be made of the work of Hughes and Munshi (1978), Dutta and Munshi (1985) upon the fine structure of some of the major Indian air-breathing fishes. Morgan and Towll (1973) reported the structure of salmon in detail. Fish gill morphology and its role in osmoregulation is being analysed by Laurent and Hebibi (1989). Gill dimensions of teleostian fishes are being studied by Hughes (1966) and Morgan (1973). Hughes (1966) did a comprehensive work on the dimension of fish gill in relation to their function. The morphology of gills and there role in acid base regulation is being studied by Goss *et al*. (1992). Changes in gill morphology and acclimation on rainbow trouts was studied by Balm(1996).

In the present study an attempt has been made to compare the gill structure of the three major air breathing fishes of India namely, *Clarias batrachus, Heteropneustes fossilis* and *Monopterus cuchia* histologically and histochemically.

METHODS AND MATERIALS

Live specimens of three species of air breathing fishes viz. *C. batrachus, H. fossilis and M. cuchia* were collected from fish landing centre, Dibrugarh, Assam. The fishes were acclimatized in the laboratory condition for one week. The fishes were cold anaesthetized following Mittal and Whitear (1978) and the gills of the fishes were dissected out accordingly and fixed immediately in alcoholic Bouins fluid for 24 hr.

The tissues are then transferred to 70% alcohol, the tissues are to be kept till the colour of the fixative gets disappeared. Next the tissues are kept in 90% alcohol for 24 hr ,then 100% alcohol. Then the tissues are kept in xylene for 2 hr with two changes of 1 hr duration. After this the tissues are kept in half xylene and half paraffin for 24 hr. Then tissues are transferred to paraffin with three changes every 1 hr and blocks were prepared. Serial sections were cut at a thickness of 5 µm using a Leica Rotary Microtome and were mounted on ethanol cleanded glass slides and were dried overnight in an oven at 37°C. Sections were deparaffinised in xylene, hydrated in a descending ethanol series and were stained with routine histological stain, Hhrlich's haematoxylin and eosin (H/E) (Bancroft and Gamble, 2008) to evaluate histological organization of the tissue. The stained sections were dehydrated in an ascending ethanol series, cleared in xylene and mounted in distrenedibutylphalate xylene (DPX). Examination of the stained sections of tissues were done using Leica ATC 2000 microscope and the results were recorded using a digital camera system Sony or Nikon Coolpix-5400. The histochemical techniques used are mentioned in the table 1.

TABLE 1
Histochemical techniques employed to detect the various Glycoproteins (GP) in the gills of *C. batrachus , H. fossilis and M. cuchia*

Sl no	Histochemical techniques	References	Materials to be Demonstrated
1	Haemotoxylin/ eosin	Pearse (1968)	Cellular organisation

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Sl no	Histochemical techniques	References	Materials to be Demonstrated
2	Periodic acid/ Schiff (PAS)	Mc. Manus (1946)	Glycoproteins (GPs) with oxidizable vicinal diols&/ glycogen
3	Alcian Blue(AB) pH 1	Lev & Spicer (1964)	GPs with O sulphate esters
5	Alcian Blue(AB) pH 2.5	Steedman (1950)	GPs with carboxyl groups &/ O sulphate esters
6	AB/PAS	Mowey (1956)	GPs with oxidizable vicinal diols&/glycogen & O sulphate esters/carboxyl groups
7	Toluidine blue	Tock&Pearse (1965)	Metachromatic substances

RESULTS:

Clarias batrachus:

Clarias batrachus bears four pairs of well-developed gills, each having two rows of gill filaments or primary lamellae. On its two sides each primary lamella contains alternately arranged secondary lamellae. The vascular components of secondary lamellae are made up of alternately arranged pillar cells and blood channels (fig: a, b). The vascular element of secondary lamellae is lined by a thin layer of respiratory epithelium. The pillar cells system remains buried within epithelial layers of filament. Near the primary lamellae the epithelium is multilayered but becomes thinner towards the marginal channels. The epithelial cells of inner layers are cuboidal; externally they are covered by micro ridged outermost epithelium cell layer. The basement membrane is thinner compared to the epithelial layer.

C. batrachus is provided with specialised structures termed as the accessory respiratory organs to facilitate respiration. In this fish the accessory respiratory organs have a modified gill structure which develops as cauliflower like processes. The dendritic organs or respiratory tree or arborescent organs are projected into an extension of gill chamber

Histochemically, it is observed that the mucous cells in primary lamellae are weakly or moderately stained with PAS. The epithelial lining of secondary lamellae and primary lamellae stained moderately with PAS and strongly with AB pH2.5 indicating the presence of GPs with oxidizable vicinal diols and GPs with O sulphate &/ carboxyl groups. The thin layer of slime covering the gill surface is found to be moderately or strongly AB pH2.5 positive (fig: c).

Heteropneustes fossilis

The gills of *H. fossilis* are composed of two sets of four holobranch, each sets having a gill arch and two rows of primary filament or lamellae projecting caudolaterally. The inner most lining of the thin wall of the gill in a multilayered epithelium next to which are serially arranged basement membrane, connective tissue layer, a thin membrane and a thin mascular coat (fig: d, e). Vascular and non-vascular areas constitute the inner epithelial linings. The vascular areas are represented by secondary lamellae of various dimensions. Primary lamellae are formed by double row of modified secondary gill lamellae that hangs into the lumen of the gill. In transverse sections (T.S) this secondary lamellae appears as vertical strands penetrating perpendicularly into the multilayered epithelium. In the T.S this lamellae bulge out into lumen as finger like projections. Each secondary lamellae is made of stacks of serially arranged pillar cells. In between the pillar cells definite blood chanals are formed. Numerous blood capillaries were observed near the base of the primary lamellae and near the gill arch. The non-vascular area of epithelial lining is made up of several layers of epithelial cells and mucous cells. Both structures the gill lamellae and gill filament were found to be covered by specialised epithelial lamellae according to their locality; a stratified epithelium on the filament and a simple epithelium on gill lamellae. Pavement cells were distributed on the superficial layer of gill lamellae, the mucous cells were distributed along the filament and were rarely localised on the lamellae.

The histochemical studies revealed that the mucous cells were PAS negative and AB 2.5 positive, demonstrating the presence of acidic glycoconjugates (fig: f). The gill arch and the basement membrane were moderately PAS positive and toludine blue positive indicating the presence of neutral glycoconjugates and gamma meta chromatia.

Monopterus cuchia

The gills of *Monopterus cuchia* is not much prominent and are much compact in nature. The second branchial arch has a few small blades like filaments with no supporting gill rays. There is no differentiation of secondary lamellae which are represented by short elevated protruberances on the margin of filaments (fig: g). Near the base of the gill filaments where they remain fused with branchial arch, holes or slits like apparatus are found which represents inter lamellae spaces. The lamellae or filament are represented by small bud like structures having loops that are lined by endothelial cells and sometimes penetrate the outer epithelium to come fairly close to the outer surface and so reduces water blood distance. Pilar cells are absent. The surface of filament or lamellae are covered by micro ridged multilayered epithelial cells. In between micro ridged cells are found triangular shaped micro villi bearing areas which represent the apical parts of chloride cells. Histochemically it gave moderately positive reaction for PAS and AB 2.5 pH revealing the presence of both GPs with oxidizable vicinal diols and GPs with carboxyl group & O sulphate esters.

DISCUSSION

In teleosts, only four pairs of holobranchs are present, and the interbranchial septum is much reduced compared with elasmobranchs. The septum usually extents to the base of the filaments and thus the filaments of teleosts are much more freely moving. The water enters the pharynx from the mouth, then passes over the filaments and follows the inner wall of the operculum until it exits via a caudal opening of the operculum. The gills are bilaterally situated on either side of the pharynx and gill arches are composed in a series that provide support to the delicate gill filaments or primary lamellae. The surface of the primary lamellae is increased by folding into secondary lamellae where gas exchange takes place between the water and blood (Schottle, 1931).

The fishes under this study belong to the air breathing fishes. These fishes are found to come near the water surface and ventilate their gills with the waters of relatively high oxygen tension. Fish that habitually live in deeper waters must swim to the surface to gulp air, with a consequent increase in oxygen consumption. The fishes uses some forms of accessory organs for respiration in extreme conditions. In air breathing fishes the accessory respiratory structures can easily cope up with the serving of oxygen demand, but are unable to function efficiently in the elimination of carbon dioxide (Hughes & Singh 1970, Singh &

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Hughes 1971, 1973). In general the gills or skin are used for CO_2 elimination and ion water and pH regulation (Johansen 1970).

As compared to the entire group of air breathing fishes the size of gill filament and its quantity is found to be reduced in *C. batrachus, H. fossilis* and even more reduced in *M. cuchia.* The secondary lamellae have become stumpy and their number considerably reduced, the inter lamellar space has increased leading to reduction in gill resistance to water flow (Munshi and Hughes 1973). In *C. batrachus* and *H. fossilis* pillar cell system forming blood channels of lamellae remains buried within epithelial layer of gill filaments this helps in reducing the blood water diffusion exchanges as in Arapaima gills (Hulbert, Moon & Hochachka, 1978). In *C. batrachus* and *Anabus t*he epithelium at the base of secondary lamellae becomes several layers thick, which increases the thickness of water blood barrier it is assumed that it may be related with the oxygen deficient environment in which they live and thus helps in reduction of loss of oxygen from blood to water. In *M. cuchia*, the pillar cells are not present in adult gill lamellae and all the blood capillaries are lined with endothelium. The suppression of gill lamellae in *M. cuchia* is thought to be an adaptation to their environment which becomes muddy and semi terrestrial Its rigid shortened gill filaments and lamellae do not collapse readily when gills are not supported by aquatic medium i.e in the muddy condition. The thickened epithelium helps to cut down diffusion of gas from gills

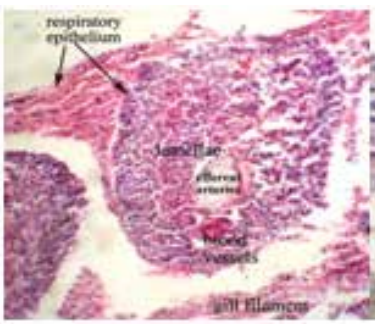

Fig. a

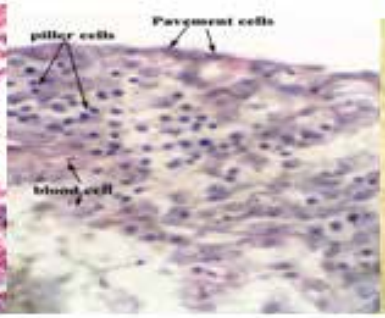

Fig. b

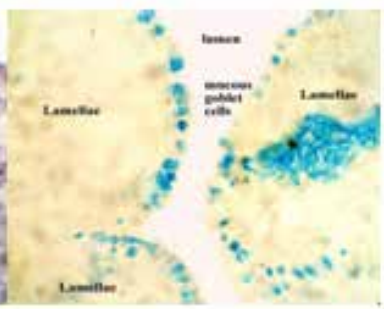

Fig. c

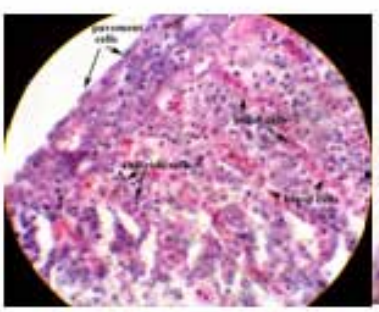

Fig. d

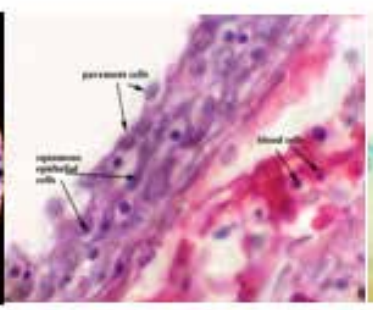

Fig. e

Fig. f

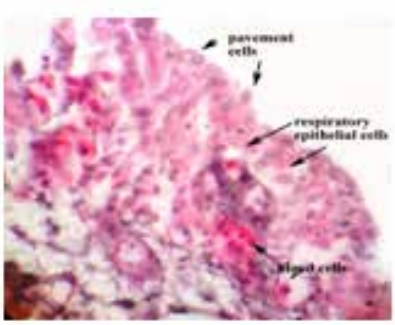

Fig. g

Figs.Photomicrograph (a-b) cross section of the gill of the fish *C. batrachus*, with H/E stain . Scale bar : 30µm (c) mucous

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

goblet cells stain strongly AB pH 2.5. Scale bar : 30µm (d-e) H/E stain of the cross section of the gill of the fish, *H. fossilis*. Scale bar: 30 µm (f) mucous goblet cells stain strongly with AB pH 2.5.Scale bar: 30µm (g) cross section of the gill of the fish *M. cuchia* with H/E stain. Scale bar: 30µm. into water and if water is severely deoxygenated, this may serve as an oxygen conserving device preventing diffusion of oxygen from blood to water (Hughes 1970)

The cells that composes the gill epithelium are very much similar among the different groups of fishes (Wilson & Laurent ,2002) .The gills comprises of cells such as , mucous cells, pavement cells, chloride cells and blood vessels. Typical goblet type mucous cells are present in large number in most fresh water fishes (fig) The acid mucopolysaccharide possess the capacity of water binding which helps the mucous cells of gills to meet dehydration between blood and water where the fishes live (Singh ,Guha, Munshi 1974). The pavement cells are the cells covering the epithelium. Its apical surface are usually large and polygonal and may have micro ridges or micro villi (Laurent,1984; Crespo,1982; Olson, 1996; Wilson *et al*. ,2002) (fig). These cells represents the ultrastructural characteristics indicative of high metabolic activity (Olson & Fromm, 1973; Laurent &Dunnel 1980; Laurent 1984) and also in acid base regulation (Perry, 1997; Goss *et al.,* 1998; Wilson *et al*. 2000). Chloride cells are present in the epithelium of gill filament, in the interlamellar region and on the trailing edges of the filaments (fig). Chloride cells are found in gills of many air breathing fishes (Munshi 1964, Hughes &Munshi 1973, Hughes &Munshi 1979). They may be in active or inactive forms. The active Chloride cells found in *M. cucchia* are rich in mitochondria and enveloped in a tubular system (Mushi& Hughes,1973). Other than these some squamous cells forms external boundary of the gill. These cells cover the gill arch and gill racker surface and their surface ridge often forms whorls. The gills are having blood vascular system to enhance the respiratory process.

CONCLUSION

From the study it can stated that in these air breathing fishes under consideration, there exist a clear relationship between the gill structures and its habitat. *C. batrachus* are found in all types of water more abundant in derelict and swampy shallow waters. *H. fossilis* also have the same kind of habitat as the C. *batrachus*, they are found to survive in muddy condition when the water level and oxygen content of the water is sufficiently low. *M. cuchia* is an obligatory air breather , which inhabits holes and crevices in the muddy banks of swamps, lakes, ponds and slowly running rivers. Due to these extreme kind of environment in which they live they are provided with special modifications for breathing in atmospheric air also in combination with the air dissolved in the water according to the necessity. Therefore there gill structures are found to be different from the other water breathing fishes. Along with respiration the gills also helps in immunological activity of the fishes, they are provided with mucous cells that acts as a first line of defense to protect themselves from pathogens and other toxic chemicals present in the water.

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