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FINE ANATOMICAL STRUCTURES OF THE INTESTINE IN RELATION TO RESPIRATORY FUNCTION OF AN AIR-BREATHING LOACH, *LEPIDOCEPHALICHTHYS GUNTEA* (ACTINOPTERYGII: CYPRINIFORMES: COBITIDAE)

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Background. The omnivorous freshwater loach, *Lepidocephalichthys guntea* (Hamilton, 1822) inhibiting unique ecological niche where oxygen content is low. Its alimentary canal is remarkably short, which is surprising considering the fact that this fish is an omnivore. This peculiarity can be correlated with the specific nature of food. Therefore, the aim of the present study was to investigate the mucosal adaptation of the intestine in adult *L. guntea* with respective digestive- as well as respiratory function.

Materials and methods. The surface design of the mucosa of different sections (anterior, mid, and posterior) of the intestine of *L. guntea* were studied using light and scanning electron microscope following commonly accepted procedures.

Results. The mucosa of the anterior intestine was provided with various types of mucosal folds and crowded with almost closely oval or rounded columnar epithelial cells with prominent microridges. The irregular mucosal surface of the mid intestine was exemplified by regularly spaced columnar epithelial cells with minute and prominent microridges/microvilli. On the contrary, irregular mucosal folds with stumpy villi were detected in the posterior intestine. The mucosal surface of that region was made up of columnar epithelial cells containing inconspicuous microridges. In that region the submucosa was provided with increasing number of blood vessels. Various cellular elements in the anterior and mid intestine were accordingly adapted to serve the important functions of absorption and gas exchange. However, the arrangement of low mucosal folds and dense blood capillaries in between the folds in the posterior intestine unequivocally suggested its respiratory role.

Conclusion. The micro-architectural pattern and cellular architecture of the anterior intestine and middle intestine of *L. guntea* unequivocally suggests that these two regions are adapted to serve major role of digestion and absorption process. However, major changes that appear in the posterior intestine are considerably reduction of the absorptive area and the penetration of blood capillaries in between the columnar epithelial cells. Therefore, the posterior intestine of *L. guntea* is probably adapted to suit its role for aerial respiration. However, further investigation is needed the detailed physiological processes involved for aerial respiration in the region concerned.

Keywords: intestine, ultrastructure, *Lepidocephalichthys guntea*, SEM

INTRODUCTION

In several members of Loricaridae, Cobitidae, and Callichthyidae air-breathing occurs by unidirectional ventilation of the alimentary canal, with air taken in at the mouth and simultaneous expulsion of air from the vent (Gee and Graham 1978, Graham and Baird 1982). There is extensive information on the topological characteristics of the gut epithelium of different teleosts (Clarke and Witcomb 1980, Sinha 1983, Chakrabarti and Sinha 1987, Chakrabarti and Ghosh 1990, Mandal and Chakrabarti 1996, de Oliveira Ribeiro and Fanta 2000, Mir and Channa 2010), but relatively little is known on the correlation of the surface ultrastructure and mucosal modification with the respiratory activities in intestinal air-breathing fish. Few authors have attempted histological analyses of the gastro-respiratory tract of air breathing teleosts (Huebner and Chee 1978, McMahon and Burggren 1987, Moitra et al. 1989) and correlated the regional cellular composition of the intestine with respective digestive and respiratory functions (Park and Kim 2001, Podkowa and Goniakowska-Witalińska 2002). There seems to be no record of topological architecture of the gut epithelium in the fish under study.

In the present study, therefore, the details of the mucosal modifications along with the morphology of the various cells lining the intestinal mucosa in the adult Guntea

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loach, *Lepidocephalichthys guntea* (Hamilton, 1822), have been investigated employing the resolving power of SEM.

MATERIALS AND METHODS

Adults of *Lepidocephalichthys guntea* weighing 25 to 30 g (6 to 7 cm in length) were captured from local bodies of freshwater, killed by an overdose of tricaine methone-sulphonate (MS 222) following the guidelines given by the Institutional Ethical Committee and representative portions of the intestine were removed. To expose the luminal surface, intestines were cut longitudinally, spread open and pinned onto cork sheets with the luminal surface facing up. The adhering mucus of the surface was removed by heparinized saline (heparin sodium salt 10 000 IU mixed in 0.67% NaCl solution). After rinsing the representative portions of gut in 0.1 M cacodylate buffer, tissue was infiltrated with 2.5% glutaraldehyde for 24 h at 4°C, post-fixed in 1% O_SO_4 in 0.1 M cacodylate buffer for 2 h, dehydrated through ascending series of acetone and subsequently acetone followed by amyl acetate and subjected to critical-point drying. After drying the serosal surface of each tissue was mounted on metal stubs, coated with gold and viewed under Hitachi, S-530 SEM.

For histological purpose tissue was fixed in Bouin's fluid, dehydrated in an ethanol series and embedded in paraffin. Tissue was sectioned at 4 to 5 mm thickness and stained with Mallory's triple stain.

RESULTS

The intestine of *Lepidocephalichthys guntea* was a short and straight tube having RLG 2 : 1. The mucosal folds showed clear differences in the anterior, mid and posterior intestine. Histologically, the anterior intestine consisted of mucosa, submucosa, muscularis, and serosa. The mucosal layer of the anterior intestine was thrown into finger-like villi covered by an apical top plate. The mucosa was made up of simple columnar epithelial cells with basally placed nuclei. The mucous cells were scattered between the columnar epithelial cells. The submucosa layer was formed of a network of connective tissue, collagen fibres, and blood capillaries which projected into the mucosal folds forming the lamina propria (Figs. 1, 2). The muscularis was composed of an inner thick circular muscle layer and an outer thin longitudinal muscle layer. The serosa was made up of peritoneal cells traversed by blood vessels.

Under the scanning electron microscope (SEM) the mucosal surface of the anterior intestine exhibited various folds forming many discrete but irregular concavities. The inner surface of the intestine was typified by the presence of oval or rounded elevations measuring about 4–5 mm and conforming to the apical surface of the columnar epithelial cells (Fig. 3). The apical plasma membrane of these columnar epithelial cells exhibited prominent microridges (Fig. 3). The mucosa of anterior intestine was made up of compactly arranged columnar epithelial cells with basally placed nuclei (Fig. 2). When viewed under SEM, no space between the columnar cells was observed on the luminal surface (Fig. 3). Mucous cells were few in number and located between columnar epithelial cells. Adhering mass of mucin were also found to be adhering to the epithelial surface (Fig. 3).

The basic histological feature of mid intestine was similar to that of anterior intestine, though the number of mucous cells was greater. The submucosa was thick and consisted of tightly arranged collagen fibres traversed by blood vessels (Fig. 4). An important feature of the mucosa of mid intestine was the presence of irregular wavy folds that did not anastomose with each other. The internal mucosa of the mid intestine was supported by regularly packed rounded or oval columnar epithelial cells measuring 5–6 mm in diameter (Fig. 5). SEM revealed the apices of said cells were exhibited densely packed fine microvilli (Fig. 5). The packing of columnar cells was interrupted in some areas by opening of the mucous cells (Fig. 5). Mucin droplets of varying shapes and sizes were found on the epithelial cells (Fig. 5).

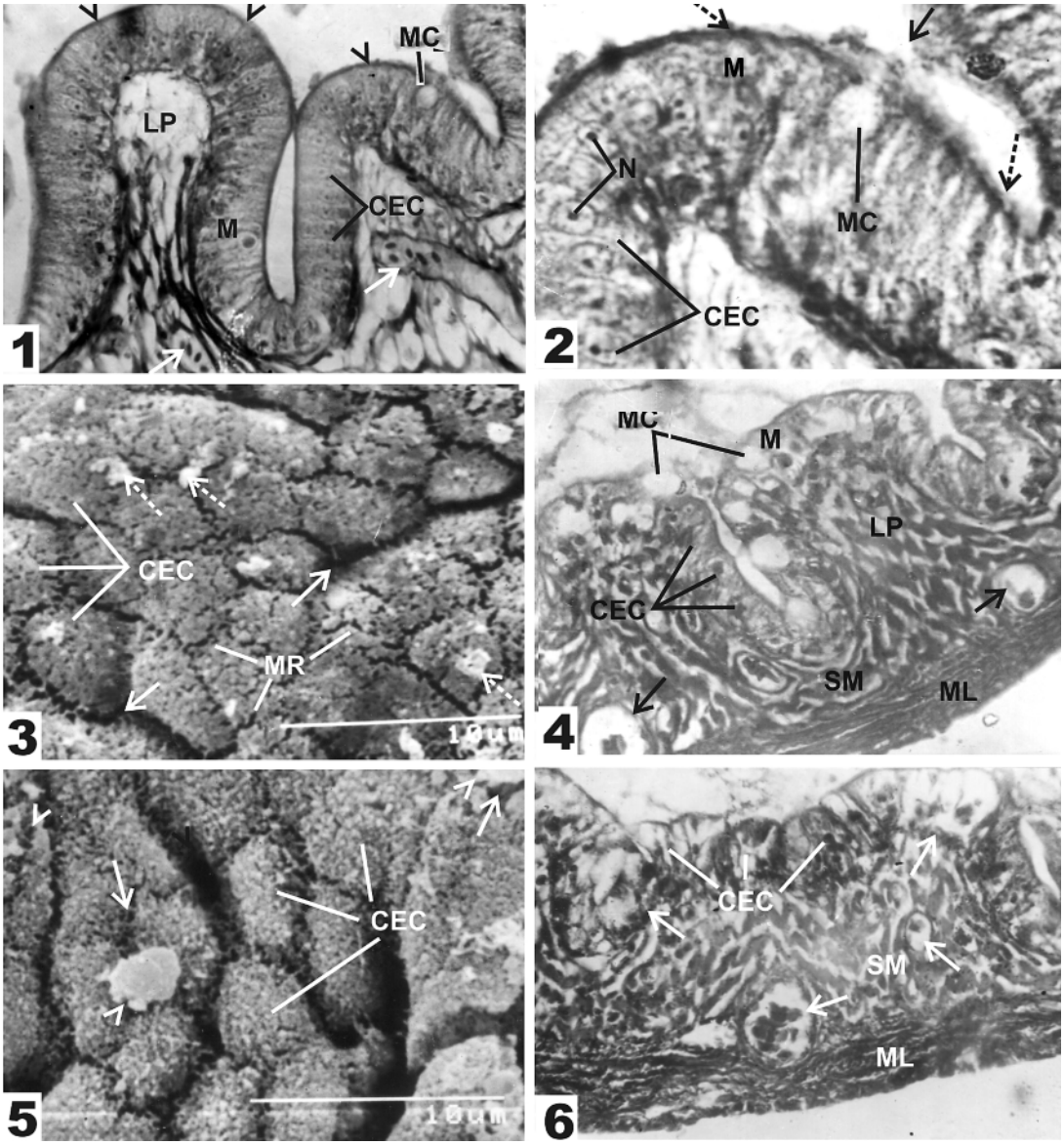
In the posterior intestine the mucosal villi were short and thick than those present in the anterior and mid intestine. The mucosa consisted of undifferentiated short columnar epithelial cells. Few mucous cells were dispersed between columnar cells. The submucosa was comprised of a network of connective tissue and was highly vascularized. The muscularis was thin in comparison to anterior and middle intestine and consisted of inner circular and outer longitudinal muscle layers. The serosa was well defined (Fig. 6). SEM study revealed that the mucosa of the posterior intestine to exhibit various types of mucosal folds. These folds were comparatively low than those of other two regions previously stated. However, prominent blood capillaries were associated between the mucosal folds (Figs. 7, 8), which were not prominently discernible in anterior and mid intestine. At higher magnification, the mucosal surface of the posterior intestine was demarcated into oval or round structures representing the luminal surface of columnar epithelial (Figs. 9, 10). The luminal plasma membrane of said cells presented inconspicuous microridges. Minute blood capillaries were also evident between the epithelial cells (Figs. 9, 10).

DISCUSSION

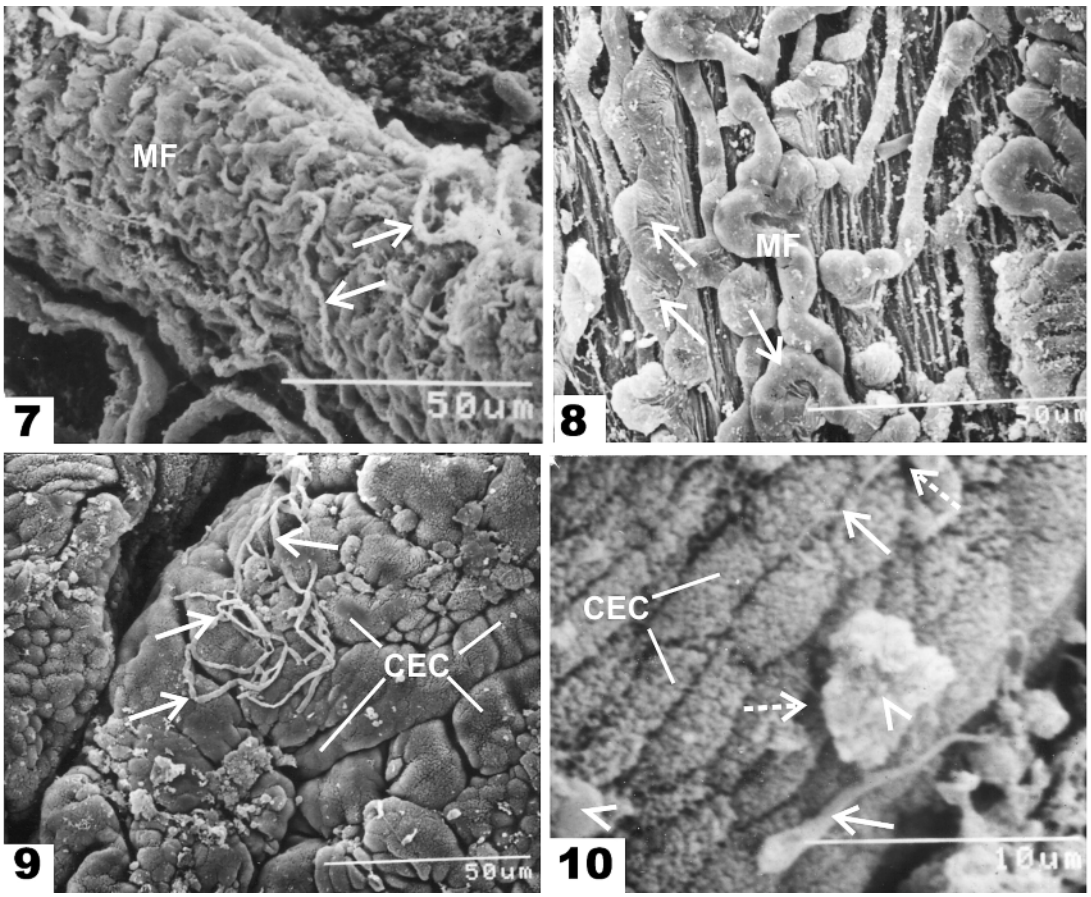
The adult *Lepidocephalichthys guntea* is an omnivorous Indian freshwater fish with a remarkably short alimentary canal (Mishra et al. 1991). In the present study, the main anatomical and morphological adaptations of the alimentary canal of the said fish are the short intestine, modifications of the mucosal surfaces, and complexity of the intestinal villi. It has been observed that the mucosal folds along with villi in the various regions of the intestine get modified in different ways. As for example, the villi of the anterior intestine are comparatively long and provided with columnar epithelial cells which are covered by top plate. However, major feature of the anterior intestine is the casting of the luminal plasma membrane of the columnar epithelial cells into stubby microridges. In the anterior intestine the microridges by virtue of their nature would serve to hold and spread a film of mucus which is secreted by the adjacent mucous cells and probably performs a number of functions such as easy transport of

ingested material and reduction of surface friction. However, similar function of microridges on the intestinal epithelium also reported on a variety of fish (Yamamoto 1966, Krementz and Chapman 1975, Sinha 1983, Mandal and Chakrabarti 1996).

Under SEM the luminal surface of the mid intestine was lined with columnar epithelial cells provided with well developed microvilli. These microvilli probably perform various functions including absorption. The anchorage of mucus on the epithelial cells by the adjacent mucous cells probably performs the reduction of surface friction during partial retention of semi digested food, reaching from anterior intestine. Few authors reported that the intestinal microvilli of teleosts to be of a greater



Figs. 1–6. Scanning electron micrographs (SEM) and histological photomicrographs of different regions of the intestine of *Lepidocephalichthys guntea*; **Fig. 1.** Villi of anterior intestine showing compactly arranged columnar epithelial cells (CEC) in the mucosa (M) layer. Note the presence of top plate over mucosal border (arrow heads); Solid arrows indicate blood vessels (BV) in lamina propria (LP) and MC indicates mucous cell in between CEC; (Mallory's Triple Stain) × 400; **Fig. 2.** Mucosa of anterior intestine showing CEC with prominent nuclei (N) and top plate (broken arrows) over CEC; Note the presence of MC and secreted mucus (solid arrow) from MC; (Mallory's Triple Strain) × 1000; **Fig. 3**. Inner surface of anterior intestine showing oval or rounded CEC with prominent microridges (MR); Note MC (solid arrows) in between CEC; Broken arrows indicate mucin mass over CEC; (SEM) × 3200; **Fig. 4.** Blunt and wide villi of middle intestine showing undifferentiated CEC and the presence of MC in between CEC. Note compactly packed collagen fibres in LP and submucosa (SM); Note also BV (solid arrows) in SM and prominent muscularis layer (ML); (Mallory's Triple Stain) × 400; **Fig. 5.** CEC of middle intestine showing minute and well defined microridges (MR). Note the presence of MC (solid arrows) in between CEC and overlying mucin mass (arrow heads); (SEM) × 3200; **Fig. 6.** Low mucosal villi of the posterior intestine with compactly arranged CEC and presence of blood vessels (solid arrows) in between CEC; Note packed collagen fibres and prominent BV (solid arrows) in SM. ML indicates muscularis layer (Mallory's Triple Stain) × 400



Figs. 7–10. Scanning electron micrographs (SEM) and histological photomicrographs of different regions of the intestine of *Lepidocephalicthys guntea*; **Fig. 7.** Mucosal folds (MF) of posterior intestine showing prominent blood capillaries (arrows) in between MF; (SEM) × 50; **Fig. 8.** Irregular MF of posterior intestine showing distribution of minute blood capillaries (arrows) in the MF; (SEM) × 200; **Fig. 9.** Luminal surface of posterior intestine showing distribution of blood vessels (arrows) on the apical surface of the columnar epithelial cells; (SEM) × 1000; **Fig. 10.** Oval or elongated elevations provided with microridges represent the apical surface of CEC in the posterior intestine; Note the presence of MC (broken arrows) in between CEC and blood vessels (solid arrows) on the surface of the CEC; Arrow heads indicate overlying mucin mass. (SEM) × 3200

length than those of mammals (Yamamoto 1966, Krementz and Chapman 1975). In the present observation the basal part of the submucosa of the mid intestine in *L. guntea* was provided with numerous patches of blood vessels indicating its possible respiratory function.

Drastic reduction in the thickness of mucosal epithelium and the penetration of an elaborate capillary bed into the mucosal epithelium and submucosa are the peculiarities of the posteriors intestine in *L. guntea*. The posterior intestine plays a negative and/or minor role regarding the food storage, digestion and absorption. Therefore, the presence of low mucosal folds bear a close relationship with its functional aspects in the present study. In the present SEM study the mucosal epithelium of the posterior intestine was made up of columnar epithelial cells having inconspicuous microridges. The unique arrangement of mucosal folds and presence of blood capillaries in the apical surface of the columnar epithelial cells in the posterior intestine of *L. guntea* advocated the reduction in absorptive process but increases diffusion path for respiratory gases in the intestine of *L. guntea*. The gaseous exchange probably takes place between the air contained in the intestine and the blood circulating in the respiratory epithelium of the intestinal mucosa. Park et al. (2003) also reported short mucosal folds, thinner wall and extensive vascular capillary network in the mucosa of the intestine and the rectum of pond loach, *Misgurnus anguillicaudatus*. The mucin secreted by mucous glands perhaps facilitates gaseous exchange and also protects delicate respiratory epithelium from mechanical abrasion. Huebner and Chee (1978) have also observed that regional cellular composition and ultrastructural features are correlated with respective digestive and respiratory functions of the digestive tract of the intestinal air breather "*Hoplosternum thoracatum*" (=*Megalechis thoracata*).

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