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Microanatomy and Cytochemistry of the Gastro-Respiratory Tract of an Air-Breathing Cobitidid Fish, *Lepidocephalichthys guntea*

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Abstract Gastro-respiratory tract of the loach, *Lepidocephalichthys guntea* has been studied with special reference to the nature of its mucus secreting epithelia. The mucous cells are strongly PAS-positive and their number per unit area (mm^2) in the mucosal layers of oesophagus, intestinal bulb, intestine and rectum are 733, 531, 223 and 540, respectively. The air-breathing segment of the gut is completely devoid of neutral mucosubstances, and there is a predominance of acidic mucosubstances over the neutral ones throughout the digestive tube. The air-blood pathway of the accessory respiratory organ is about 2.6 μm which is higher than the values of air-breathing organs of other fishes.

The loach, *Lepidocephalichthys guntea* (Hamilton-Buchanan) is an interesting intestinal air-breathing fish (Das, 1937) belonging to the family Cobitididae of the order Cypriniformes. The members of this species inhabit the stagnant backwater of hill streams. Such water bodies are prone to pollution and subjected to draught and luxuriant growth of macrophytes during summer. The water becomes hypoxic and hypercarbic. Under such adverse ecological habitat of Devonian period, the fish was forced to use intestine as air-breathing organ. The role of intestine in respiration seems to be a primitive air-breathing behaviour of the fish.

In addition to digestive function, the intestine of the loach affords to share respiratory responsibilities also. Dual function of intestine forms the basis of the present investigations on the cytochemical nature of the gastro-respiratory epithelium with special reference to its mucus secreting cells (glands).

Materials and methods

Live fishes were collected from Saharsha district of North Bihar, India, and maintained in the laboratory. The fishes were fed daily with plankton.

Various anatomically differentiated gastro-respiratory tube of the loach was fixed in 10% neutral formalin and aqueous Bouin's fluid. After proper processing, paraffin blocks were prepared, 5–6 μm sections were subjected to various histochemical tests to evaluate the chemical nature of mucin viz. PAS (McManus, 1946), Alcian blue (Steedman, 1950) dialysed iron method (Hale, 1946). In addition, PAS technique was employed in combination with AB and vice-versa for the detection of neutral and acidic mucins. Acetylation and deacetylation for the confirmation of hydroxyl group was performed following Lillie (1954). Methylation and demethylation (Spicer, 1960) were done to confirm the acidic nature of the mucins. Mucus secreting cells per unit area (mm^2) were also calculated from the PAS whole mount preparations.

Results

The gastro-respiratory tract of the loach is a straight tube. Unlike other fishes, the oesophagus is characterised by well developed mucosal folds (Fig. 1).

The true stomach is replaced by an intestinal bulb which is distinguished by comparatively short or low mucosal layer with gastric glands (Fig. 2). The short (low) mucosal layer of the intestine is provided with numerous capillaries (Fig. 3). The blood of the capillaries is separated from the air-contained in the intestinal lumen by a thin air-blood pathway (2.6 μm) separated by an outer epithelium, a thin basement membrane and an endothelium. The rectum bears typical piscian histological structures (Fig. 4).

The mucous cells per unit area (mm^2) in the mucosal layers of the oesophagus, intestinal bulb, intestine and rectum were 733, 531, 223 and 540,

respectively.

The mucous cells of the various units of the gastro-respiratory tract are strongly PAS-positive (Figs. 5, 6, 7). They give negative reaction with PAS after acetylation, deacetylation restores the colour. Negative response with Schiff's reagent was obtained without pretreatment to periodic acid. These cells are also negative towards Best's carmine stain for glycogen. The Alcian blue at pH 1.0 and 2.5 indicates the sulphated acidic nature of the mucous cells. With AB-PAS and PAS-AB staining, the mucous cells take up bluish purple to purple to purple-blue stain indicating the presence of neutral and acidic mucins. A few mucous cells stained red with AB-PAS technique (Table 1).

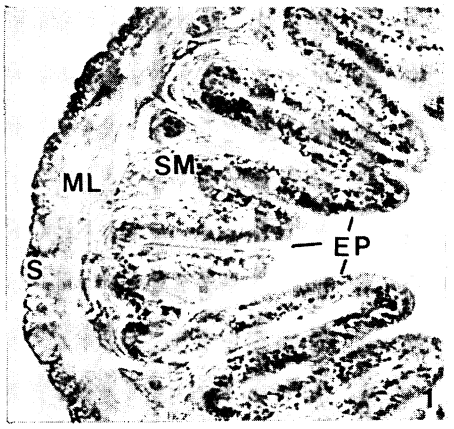
Table 1. A summary of the histochemical tests performed to show the nature of mucous cells of the alimentary canal of *Lepidocephalichthys guntea*. R, red; B, blue; RB, reddish blue; BR, bluish red; +, increasing intensity of reaction; ±, fairy present; −, absent.

Techniques employed	Regions of the gut				Remarks
	Oes	Int. bulb	Int.	Rec.	
PAS	⧺	++	+	++	R
PAS after digestion in malt diastase	++	±	+	+	R
Alcian blue (1.0)	⧺	+	±	+	B
Alcian blue (2.5)	⧺	++	+	±	B
Alcian blue PAS	++	⧺	±	++	B, BR
PAS-Alcian blue	⧺	⧺	±	±	B, BR, R
Dialysed iron	+	±	−−	±	B
Dialysed iron-PAS	±	±	−−	±	BR
Acetylation followed by PAS	−	−	−	−	confirmation of 1, 2 glycol group
Acetylation followed by Saponification-PAS	++	±	−−	±	R
Methylation followed by AB	−	−	−	−	confirmation of sulphate & carboxyl group of mucosubstance
Methylation followed by Saponification-AB	++	++	+	+	B

Table 2. Diffusion distance in the air-breathing organs of dual breathing fishes. [1] Hughes et al., 1973; [2] Hughes et al., 1974; [3] Hakim et al., 1978; [4] Biswas et al., 1981; [5] present authors.

Species	Air-breathing organ	Diffusion distance (μm)
Anabas testudineus[1]	Suprabranchial chamber and labyrinthine organ	0.21
Heteropneustes fossilis[2]	Air-sac	1.6
Channa punctata[3]	Suprabranchial chamber	0.78
Boleophthalmus boddaerti[4]	Opercular chamber	1.22
Lepidocephalichthys guntea[5]	Intestine	2.6

Fig. 1. Cross section of oesophagus showing its histological details (hematoxylin and eosin.) ×160.
Fig. 2. Cross section of intestinal bulb showing its various histological units (H/E). ×400.
Fig. 3. Cross section of intestine showing fine blood capillaries at the epithelial layer, large blood vessel in the lamina propria and reduced muscularis layer (H/E). ×400.
Fig. 4. Cross section of rectum showing its histological details (H/E). ×160.
Fig. 5. Cross section of oesophagus showing mucous cells (PAS technique). ×160.
Fig. 6. Cross section of intestinal bulb showing mucous cells (PAS technique). ×400.
Fig. 7. Mucous secreting cells in the intestine (PAS technique). ×400. Abbreviations used: BM, basement membrane; BV, blood vessel; EP, epithelial layer; LP, lamina propria; ML, muscularis layer; NMC, non-secretory mucous cells; RBC, red blood corpuscles; S, serosa; SMC, secretory mucous cells.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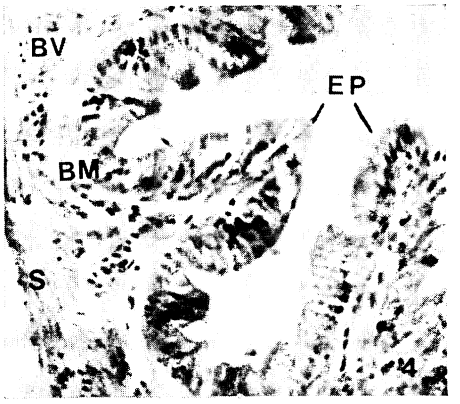
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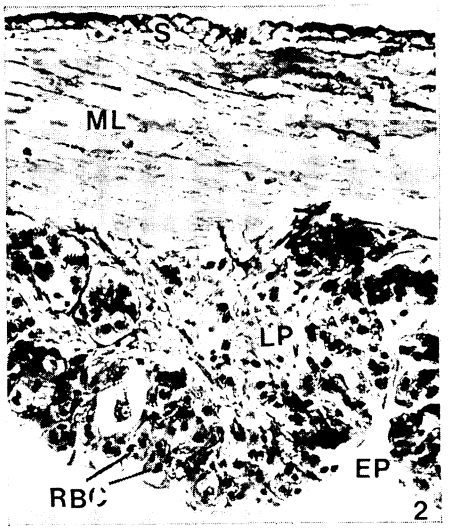
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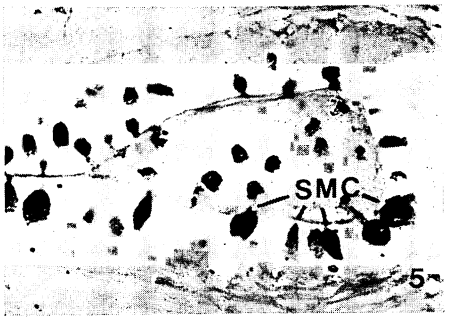
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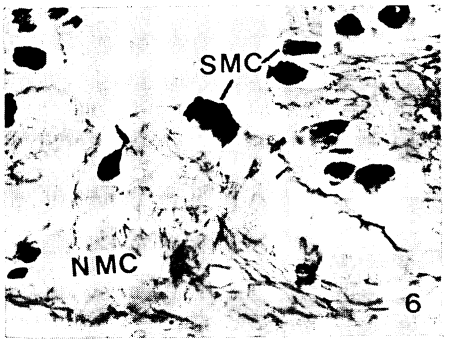
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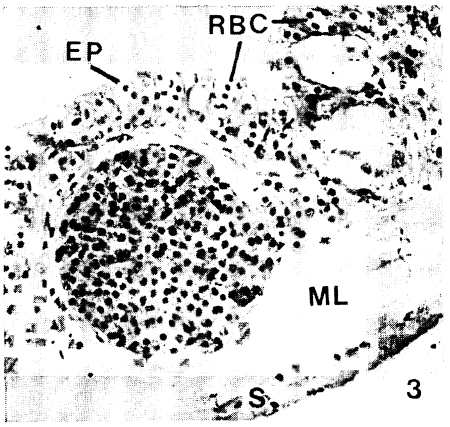
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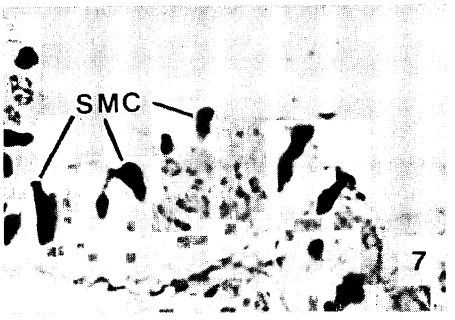
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Discussion

Various units of the gastro-respiratory tube of the present loach are modified to suit its dual role of digestion and respiration.

Higher density of mucous glands in the oesophagus is obviously related to greater mucus secretion which in turn acts as lubricant for smooth passage of food to the intestinal bulb and its mastication. Absence of capillaries from the luminal surface of the mucosa of the oesophagus rules out its role in gaseous exchange.

The gastric glands in the mucosal layer of the bulbous intestine supports its role in digestion but the absence of capillary bed in the mucosal surface does not support its role in gaseous exchange.

Numerous capillaries were seen near the surface of the mucosa of intestine. The distance between the blood of capillaries and the intestinal lumen is about 2.6 μm. This vlaue is lower than the value reported for the diffusion distance in the secondary lamellae of this fish (Singh et al., 1981) and higher than the values reported for air-breathing organs of other fishes (Table 2). 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. Sparsely distribution of mucous glands in the respiratory epithelium of the intestinal mucosa is an adaptation to expose greater surface area for effective gaseous exchange, because the vascular regions of the accessory respiratory organs of air-breathing fishes also lack mucous glands (Hughes and Munshi, 1978, 1986; Munshi, 1985; Munshi and Hughes, 1986). The mucus produced by some of the glands of the intestine perhaps help to clean the respiratory surface and facilitates the gaseous exchange. The acidic mucosubstances have the water binding properties (Singh et al., 1974) which is necessary to keep the surface of intestine moist, for gas exchange. Oxygenation of moist surface is renewed during ventilation. Such mucus lining perhaps also protects delicate respiratory epithelium from the passage of digested food meterial. The role of mucosal capillaries in absorption of digested materials by the intestine is not ruled out.

Bullock (1967) reported the presence of a mixture of mucopolysaccharides and mucoprotein in the free border of anterior intestine of *Gambusia affinis*. Occurrence of neutral mucopolysaccharides from the different epithelial layers of the digestive tract of the rainbow trout has been reported by Weinreb and Bilstad (1955). Mucous cells in the luminal surfaces of oesophagus, intestinal bulb, intestine and rectum of the loach contain acidic or a mixture of neutral and acidic mucins. Furher, the acidic mucosubstance predominates throughout the gut. Similar observations have also been made by Sinha and Chakravarty (1982) in the alimentary canal of the catla *Catla catla*.

In the siluroid fish, *Saccobranchus fossilis*, the respiratory membrane of the air-breathing organ (airsac) bears a large number of mucous cells. These cells are equipped with both sulphated and non-sulphated acid mucopolysaccharides (Guha et al., 1967). Singh et al. (1974) have also observed acidic mucosubstances in the airsac of this fish. The acidic mucoproteins predominantly with carboxyl functional group seems to be characteristic of the gastro-respiratory tube of the loach.

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Literature cited

Biswas, N., J. Ojha and J. S. D. Munshi. 1981. Morphometric of the respiratory organs of an estuarine goby, *Boleophthalmus boddaerti*. Japan. J. Ichthyol., 27(4): 316–326.

Bullock, W. L. 1967. The intestinal histology of the mosquito fish, *Gambusia affinis* (Baird & Girard). Acta Zool., 48: 1–17.

Das, B. K. 1937. Some further observations on the structure and physiology of an air-breathing loach, *Lepidocephalus* (syn. *Lepidocephalichthys*) *guntea* (Ham.-Buch.) found in Hyderabad. Proc. 24th Indian Sci. Cong., Hyderabad, p. 305.

Guha, G., B. R. Singh and J. S. D. Munshi. 1967. Histochemical observations on the respiratory mucosa of air-chamber of *Saccobranchus fossilis* (Bloch). Mikroskopie, 22(78): 194–198.

Hakim, A., J. S. D. Munshi and G. M. Hughes. 1978. Morphometrics of the respiratory organs of the Indian green snake-headed fish, *Channa punctata*. J. Zool. Lond., 184: 519–543.

Hale, C. W. 1946. Histochemical demonstration of acid polysaccharides in animal tissue. Nature, 157: 802.

Hughes, G. M. and J. S. D. Munshi. 1978. Scanning electron microscopy of the respiratory surfaces of *Saccobranchus* (=*Heteropneustes*) *fossilis* (Bloch.) Cell Tissue Res., 195: 99-109.

Hughes, G. M. and J. S. D. Munshi. 1986. Scanning electron microscopy of the accessory respiratory organs of the snake-headed fish, *Channa striata* (Bloch) (Channidae, Channiformes). J. Zool., Lond., 209: 305-317.

Hughes, G. M., S. C. Dube and J. S. D. Munshi. 1973. Surface area of the respiratory organs of the climbing perch, *Anabas testudineus*. J. Zool., Lond., 170: 227-243.

Hughes, G. M., B. R. Singh, G. Guha and J. S. D. Munshi. 1974. Respiratory surface areas of an air-breathing siluroid fish, *Saccobranchus* (*Heteropneustes*) *fossilis* in relation to body size. J. Zool., Lond., 172: 215-232.

Lillie, R. D. 1954. Pages 190-191 *in* Histopathologic technique and practical histochemistry. 3rd ed. Blackiston.

McManus, J. F. A. 1946. Histochemical demonstration of mucin after periodic acid. Nature, 158: 202.

Munshi, J. S. D. 1985. The structure, function and evolution of accessory respiratory organs of air-breathing fishes of India. Pages 353-366 *in* Duncker/Fleischer, eds. Vertebrate morphology. Gustav Fisher-Verlag, Stuttgart.

Munshi, J. S. D. and G. M. Hughes. 1986. Scanning electron microscopy of the respiratory organs of juveniles and adult climbing perch, *Anabas testudineus*. Japan. J. Ichthyol., 33(1): 39-45.

Singh, B. R., G. Guha and J. S. D. Munshi. 1974. Mucous cells of the respiratory sac in an air-breathing fish, *Saccobranchus fossilis* (Bloch). La Cellulae, 70(1): 1-15.

Singh, B. R., A. N. Yadav, J. Ojha and J S. D. Munshi. 1981. Gross structure and dimensions of the gills of an intestinal air-breathing fish (*Lepidocephalichthys guntea*). Copeia, 1981 (1): 224-229.

Sinha, G. M. and P. Chakraverty. 1982. Characterisation and distribution of neutral and acidic mucins in the alimentary canal of an Indian freshwater major carp, *Catla catla* (Ham.) by histochemical methods. Gegen. Morph. Jb. Leipzig, 128(2): 188-200.

Spicer, S. S. 1960. Histochemical differentiation of sulphated rodent mucin. Histochem. Ann., 7: 23.

Steedman, H. F. 1950. Alcian blue 8GS: a new stain for mucin. Quart. J. Micr. Sci., 91: 477-479.

Weinreb, E. L. and N. M. Bilstad. 1955. Histology of the digestive tract and adjacent structures of the rainbow trout. Copeia, 1955(3): 194-204.

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空気呼吸ドジョウの一種 *Lepidocephalichthys guntea* における腸呼吸管の微細解剖と細胞化学

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ドジョウの一種の腸呼吸管を，粘液分泌上皮の性質を主眼にして研究した．粘液細胞は強PAS陽性で，1 mm² 当りの数は食道粘液層で 733 個，腸管球では 531 個，腸および直腸の粘液層で 223 と 540 個であった．消化管の呼吸節は，完全に中性粘液物質を欠き，消化管全体にわたって酸性粘液物質の方が卓越している．副呼吸器官の空気一血液径路は，2.6 μm ほどで，他の魚類における空気呼吸器官よりも大きな値であった．