

The Epidermal and Inner Epithelial Lining of the Operculum in *Clarias batrachus* (Clariidae, Siluriformes)

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Abstract The operculum may be divided into the proximal, the distal and the intermediate regions. The epithelium lining the inner surface of the operculum (EISO) and the opercular epidermis (OE) of these regions in *Clarias batrachus* show significant differences in their thickness, in the density, in the dimensions of mucous cells and club cells, and in the distribution of lymphocytes, melanocytes, taste buds and ampullary organs. These differences in structural organization are associated with the different conditions prevailing at these locations. Rich vascularization observed in the tissues underlying the OE has been correlated with assisting the fish in accessory respiration. In contrast, the tissues underlying the EISO are poorly vascularized. Accessory respiration in this region may not be so advantageous.

Although there have been extensive studies on fish epidermis (Imaki and Chavin, 1984; Whitear, 1986; Whitear and Mittal, 1986; Singh and Mittal, 1990), studies on the epithelium lining the inner surface of the operculum (EISO) and its comparison with the opercular epidermis (OE) have not drawn the attention of many researchers. In one report, however, Karnaky and Kinter (1977) described the structural organization of EISO of *Fundulus heteroclitus* and *Hemibarbus americanus* with particular reference to ion transport across the epithelium.

The present investigation was undertaken to make a comparative study of the structural organization of the EISO and the OE of an air-breathing cat-fish *Clarias batrachus* inhabiting muddy and marshy waters (Günther, 1880) and belonging to the family Clariidae and order Siluriformes (Misra, 1962; Greenwood et al., 1966; Welcomme, 1988).

Materials and methods

Live specimens of *Clarias batrachus* (length 14.0 ± 1.0 cm, weight 32 ± 2 g) were collected from local ponds at Varanasi (Uttar Pradesh, India). The fish were maintained in the laboratory at a controlled room temperature ($25 \pm 1^\circ\text{C}$) and were fed with minced goat liver on alternate days.

After the fish were cold anaesthetized (Mittal and Whitear, 1978), the opercular pieces were excised, rinsed in physiological saline and then fixed in alcoholic Bouin's fluid, aqueous Bouin's fluid and 10% neutral formalin. Paraffin sections were cut at $5\ \mu\text{m}$

and were stained with Ehrlich's haematoxylin-eosin (HE), Verhoeff's haematoxylin-eosin (VHE) and Papanicolaou's stain (PS) to visualize histological organization, with periodic acid-Schiff (PAS) technique with and without prior diastase treatment to localize and differentiate mucopolysaccharides (= glycoproteins; Reid and Clamp, 1978) and glycogen contents, and with alcian blue (AB) at pH 1.0 and at pH 2.5 and AB and PAS to differentiate neutral, acidic sulphated and acidic non-sulphated mucopolysaccharides contents in different cellular components following Lillie (1954), Gurr (1962), Bancroft and Stevens (1982) and Pearse (1985).

Whole mount preparations of the EISO and the OE, separated from the underlying tissues following Mittal and Garg (1988) and stained with PAS, were also made for the statistical analysis of the density of the mucous cells.

Observations

The operculum may arbitrarily be divided into three regions—the proximal region (an area close to the cranium), the distal region (an area close to the opercular opening) and the intermediate region (an area between the proximal and distal regions) (Fig. 1). The thickness of the EISO and the OE in these three regions is different and is summarized in Table 1.

The EISO and the OE are stratified in nature and may be roughly divided into three principal layers—the superficial layer, the middle layer and the basal

layer.

The EISO and the OE are mainly composed of epithelial cells, interspersed with glandular—mucous and club cells, and with the intrusive cells—lymphocytes. The OE has additional pigment cells and sensory structures such as taste buds and ampullary organs.

Epithelial cells. In the EISO, the superficial layer epithelial cells, appear polygonal and are often vertically flattened in the distal region (Fig. 2), polygonal in the intermediate region (Fig. 3) and somewhat triangular in the proximal region with rounded central nuclei (Fig. 4). In the OE, these cells appear polygonal with rounded central nuclei in the distal region (Fig. 5), and columnar with rounded basal nuclei in the intermediate (Fig. 6) and proximal (Fig. 7) regions.

The middle layer epithelial cells, in the EISO at the distal and the intermediate regions and in the OE at the distal region, in general, appear compactly arranged and polygonal with rounded central nuclei (Figs. 2, 3, 5). In contrast, in the EISO of the proximal region and the OE of the intermediate and proximal regions these cells deeper in the middle layer appear less compactly arranged. They appear polygonal and sometimes vertically elongated in the EISO of the proximal region (Fig. 4), often vertically elongate in the OE of the intermediate region (Fig. 6) and very much elongate with elongate nuclei in the proximal region (Fig. 7). In these regions, in 3 to 4 rows immediately below the superficial layer, the epithelial cells acquire a polygonal shape with rounded central nuclei and appear compactly arranged (Figs. 4, 6, 7).

The basal layer epithelial cells, in all the three regions, appear cuboidal or like low columns with rounded central nuclei in the EISO (Figs. 2–4) and like tall columns with elongated basal nuclei in the OE (Figs. 6, 7). They rest on a thin non-cellular basement membrane which separates the EISO and

the OE from the underlying tissues.

The epithelial cells both in the EISO and in the OE, in general, have healthy appearing nuclei with distinct chromatin material and nucleoli. They stained dark blue in HE and PS, and blue black in VHE. The cytoplasm is homogeneous staining light pink in HE, PS and VHE. With the histochemical tests employed the epithelial cells in the basal layer and in the deeper middle layer remained unstained. Cells in the outer middle layer stained lightly: magenta with PAS, greenish blue with AB (pH 1.0 and 2.5) and purple with AB and PAS indicating low concentrations of a mixture of neutral and acid (both sulphated and non-sulphated) mucopolysaccharides (Figs. 8–13). A gradual increase in the intensity of the reactions appeared towards the surface and the superficial layer epithelial cells stained moderately.

Mucous cells. The mucous cells in both the EISO and the OE of the three regions of the operculum vary significantly in their density as well as in dimensions (Table 2). They open to the surface by narrow pores through which they void their secretions. These cells as seen in the whole mount preparations as well as in sections, appear few in number at the distal region (Figs. 8, 11). In contrast, their density is much higher in the intermediate region (Figs. 9, 12) and is still higher in the proximal region (Figs. 10, 13).

In the EISO, the mucous cells in the distal and intermediate regions, in general, appear like elongate sacs each having a rounded basal body and a relatively narrow neck often extending from the surface deep into the middle layer of the epithelium (Figs. 8, 9). In contrast, at the proximal region, the mucous cells, in general, appear relatively voluminous and are restricted mainly in the outer layers of the epithelium (Fig. 10; Table 2). The mucous cells in the EISO are filled with secretory contents which stain strongly, magenta in PAS, greenish blue in AB (pH 1.0 and 2.5) and purple in AB and PAS suggesting that they represent a mixture of neutral, acidic sulphated and acidic non-sulphated mucopolysaccharides. In addition at the proximal region, deeper in the middle layer and in between the basal layer epithelial cells small, rounded or irregular shaped cells of unknown identity may be observed only in histochemical preparations that stain light magenta in PAS and AB and PAS (Fig. 10) and remain unstained in AB both at pH 1.0 and at pH 2.5 indicating the presence of low concentrations of neutral mucopolysaccharides.

Table 1. Average thickness of the epithelium lining the inner surface of the operculum (EISO) and the opercular epidermis (OE) at the three regions of the operculum of *Clarias batrachus*.

Region	Thickness (in $\mu\text{m} \pm \text{SD}$)	
	EISO	OE
Distal	31.65 \pm 3.28	76.65 \pm 8.16
Intermediate	41.06 \pm 7.76	77.54 \pm 6.82
Proximal	52.84 \pm 7.49	86.45 \pm 3.81

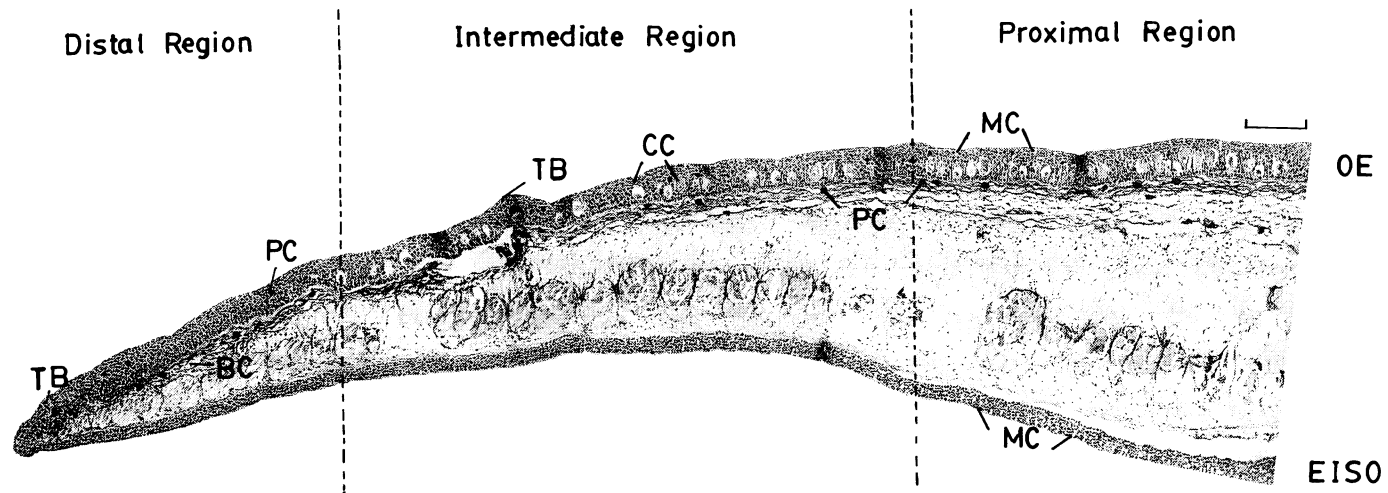


Fig. 1. Photomontage of the cross sections of the operculum of *Clarias batrachus* showing the general organization of the epithelium lining the inner surface of the operculum (EISO) and the opercular epidermis (OE) at the proximal, intermediate and distal regions. BC, blood capillary; CC, club cell; MC, mucous cell; PC, pigment cell; TB, taste bud. (HE stain) Scale bar = 50 μ m.

In the OE, the mucous cells from all the three regions, in general, appear elongated, each characterized by having a small elongate swollen apical part, a very much elongated slender middle part and a small rounded basal part (Figs. 11–13). They often extend up to the middle of the epidermis. In the proximal region the mucous cells, in general, appear relatively voluminous in dimensions (Fig. 13, Table 2).

The secretory contents of the mucous cells in the OE, as in the EISO, in general, show positive reactions for a mixture of neutral and acid (both sul-

phated and non-sulphated) mucopolysaccharides. At the distal and the intermediate regions, in general, the intensity of the reactions is very strong in the apical swollen part and weak in the narrow slender middle part and the rounded basal part of the mucous cells. At the proximal region, however, the mucous cells stain very strongly and do not show any significant differences in the intensity of the reactions in their different parts. The rounded basal parts of some mucous cells, as all the three regions, stain light magenta with PAS, and AB and PAS indicating the presence of neutral mucopolysaccharides.

Table 2. The density and the dimensions of the mucous cells, in the epithelium lining the inner surface of the operculum (EISO) and in the opercular epidermis (OE) at the three regions of the operculum of *Clarias batrachus*.

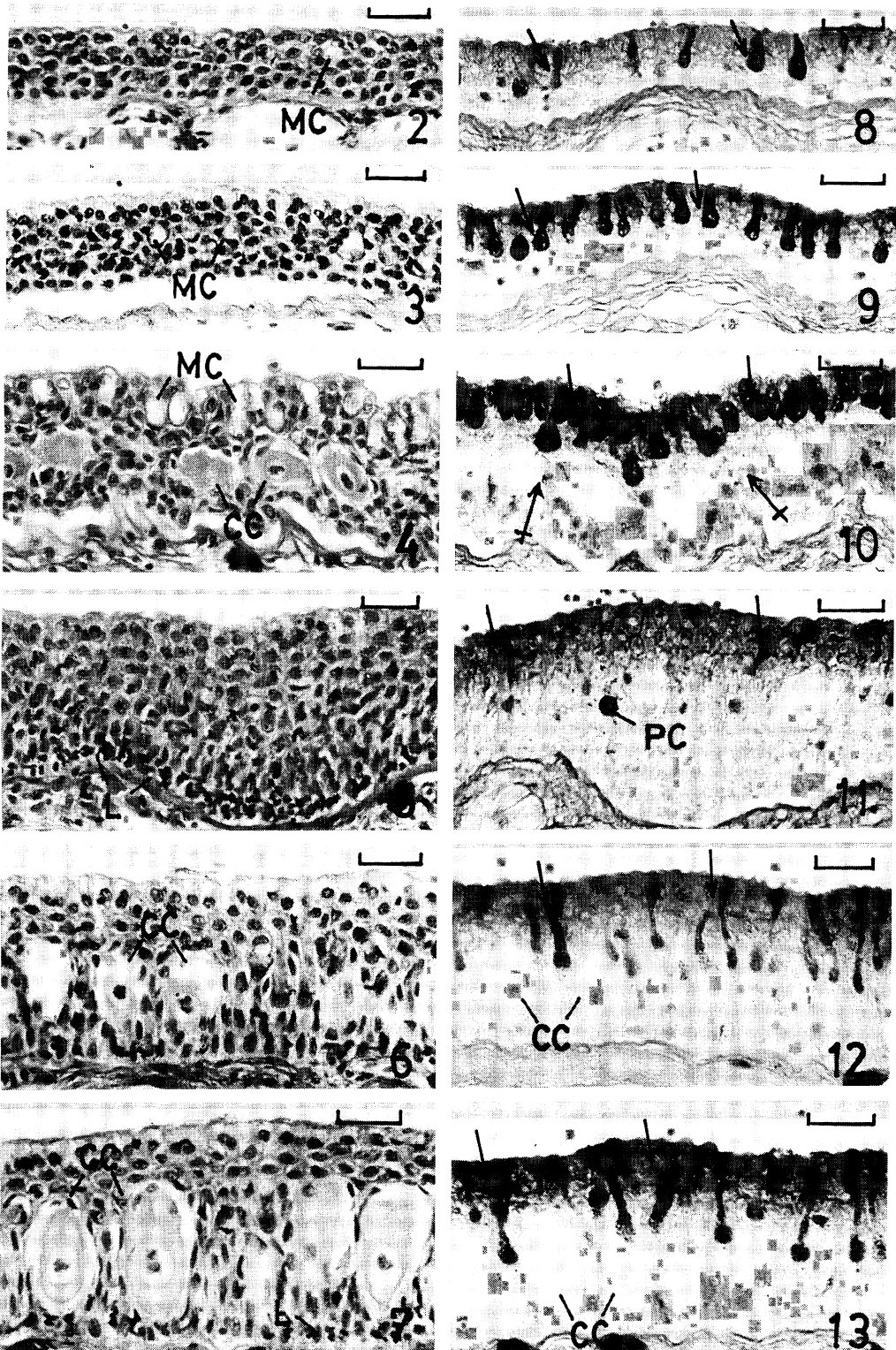
Tissue	Region	Density of mucous cells (no./mm ² ±SD)	Dimensions of mucous cells (in µm±SD)	
			Height	Width
EISO	Distal	2728.30±743.41	20.10±1.27	3.28±0.65 (apical part) 6.42±0.95 (basal part)
	Intermediate	5120.89±417.98	22.44±2.10	3.77±0.78 (apical part) 6.84±1.27 (basal part)
	Proximal	7849.66±242.42	20.61±2.85	5.13±0.71 (apical part) 7.88±1.01 (basal part)
OE	Distal	3238.36±383.82	35.36±4.37	4.78±0.88 (apical part) 3.11±0.83 (middle part) 5.61±0.55 (basal part)
	Intermediate	5252.39±333.14	43.66±4.72	6.20±0.62 (apical part) 3.44±0.65 (middle part) 6.44±0.73 (basal part)
	Proximal	7209.36±322.48	47.75±3.81	6.65±0.94 (apical part) 4.44±0.62 (middle part) 6.44±0.51 (basal part)

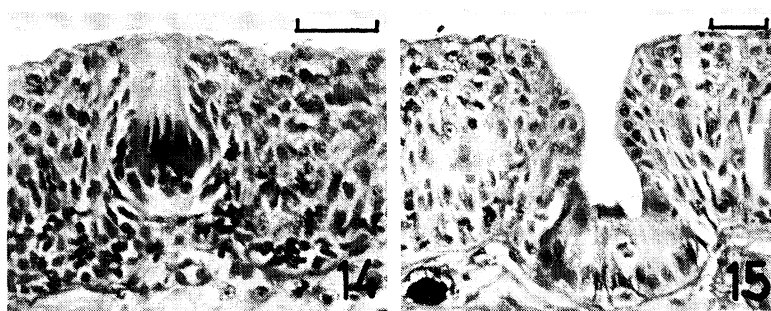
Figs. 2–4. Cross sections of the EISO showing its structural organization at different regions. 2: The distal region. 3: The intermediate region. 4: The proximal region. Note the difference. CC, club cell; MC, mucous cell. (HE.) Scale bar=10 µm.

Figs. 5–7. Cross sections of the OE showing its structural organization. 5: The distal region. 6: The intermediate region. 7: The proximal region. Note the difference. CC, club cell; L, lymphocyte. (HE.) Scale bar=10 µm.

Figs. 8–10. Cross sections of the EISO showing the distribution of the mucous cells (arrows) (deep purple in original). 8: The distal region. 9: The intermediate region. 10: The proximal region. Note the difference in their density and dimensions. Positive reactions are seen in the superficial layer epithelial cells (purple in original) and in the outer middle layer epithelial cells (light purple in original). The underlying epithelial cells and the club cells remain almost unstained. Small rounded or irregular shaped cells of unknown identity (barred arrows) (light magenta in original) are discernible in the proximal region. (AB and PAS.) Scale bar=10 µm.

Figs. 11–13. Cross section of the OE showing the distribution of the mucous cells (arrows; deep purple in original). 11: The distal region. 12: The intermediate region. 13: The proximal region. Note the difference in their density and dimensions. Positive reactions are seen in the superficial layer epithelial cells (purple in original) and in the outer middle layer cells (light purple in original). The underlying epithelial cells and the club cells (CC) remain almost unstained. PC, pigment cell. (AB and PAS.) Scale bar=10 µm.





Figs. 14-15. Cross sections of the OE. 14: A pear-shaped taste bud. 15: An ampullary organ. (HE.) Scale bar = 10 μ m.

Club cells. In the EISO, at the proximal region, typical binucleated club cells, as in the epidermis of other bony fishes are located. They, in general, are few (20.66 ± 1.10 per mm length of the epithelium), appear rounded or vertically compressed and are arranged in a row, mainly in the lower middle layer of the epithelium (Fig. 4). Their average height is $18.32 \pm 1.67 \mu$ m, and width is $17.76 \pm 5.32 \mu$ m. In the distal and intermediate regions, no club cells could be located.

In contrast, in the OE, at the proximal region, the club cells, in general, appear more in density (28.66 ± 2.80 per mm length of the epidermis) and relatively closely approximated. They appear rounded, oval or vertically elongated and voluminous (average height $50.21 \pm 2.75 \mu$ m and width $28.18 \pm 3.10 \mu$ m), often occupying the major portion of the epidermal thickness and are arranged in a row immediately above the basal layer epithelial cells (Fig. 7). In the intermediate region, these cells are relatively small in dimensions ($36.18 \pm 6.04 \mu$ m in height, $22.45 \pm 4.74 \mu$ m in width) and are located at longer intervals (17.00 ± 7.00 per mm length of the epidermis) (Fig. 6). At the distal region the club cells are, in general, absent. However, in some sections few (one or two) such cells could be located in this region.

Intrusive cells. In the EISO, in general, at all the three regions, lymphocytes could hardly be seen. In contrast, in the OE, at the proximal and at the intermediate regions they are easily located. They are rounded or irregular, stained deep blue in HE, PS and black in VHE and enclosed within characteristic lymphatic spaces in between the basal layer epithelial cells. In the OE, at the distal region, the lymphocytes are located in a large number often overcrowding the basal layer (Fig. 5) and even pene-

trating the lower middle layers.

The pigment cells, i.e., the melanocytes, appear rounded and are distributed at random in different layers of the OE at all the three regions. Such cells, however, could not be located in the EISO (Fig. 1).

Sensory structures. In the OE, of all the three regions, typical sensory structures, such as the pear-shaped taste buds (Fig. 14) and the ampullary organs (Fig. 15) are distributed. In general, the ampullary organs are encountered at long intervals and are not easily located. In contrast, in the EISO of all three regions neither of these sensory structures could be located.

Interspersed among the tissues immediately underlying the OE, quite a good number of melanocytes and blood capillaries are located. In the tissue underlying the EISO, however, the pigment cells are, in general, absent and the blood capillaries appear very few and small in dimensions (Fig. 1).

Discussion

The primary function of the epithelium together with its cellular components is protection against various hazards in the surrounding medium. It is interesting to note that at all the three regions of the operculum of the present cat fish, *Clarias batrachus*, the EISO in contrast to the OE is very thin. Further, in the EISO, the mucous cells, the secretions of which provide protection in various ways (Mittal and Banerjee, 1980; Imaki and Chavin, 1984), are significantly less in their density as well as small in dimensions; the club cells, the secretory contents of which also contribute to the surface slime and appear to play an additional role in defence against various hazards (Whitaker and Mittal, 1983), are, in general,

either absent or very few and small in dimensions; and the lymphocytes which may be involved in immunological defence (Mittal et al., 1980) are very few and may be located only with difficulty. It appears that these dissimilarities are mainly due to different conditions to which they are exposed. An increased thickness, the presence of the mucous cells and the club cells in larger numbers and dimensions and the lymphocytes in high density are not necessitated in the EISO, since, being on the inner surface of the operculum, as compared to the OE, it is not exposed directly to various hazards in the environment surrounding the fish and thus appears well protected.

The mucous cells are variable in their density as well as in their dimensions at the three regions of both the EISO and the OE. In the proximal region, they appear voluminous and high in density suggesting profuse mucus secretions. In the intermediate region, they are relatively few and less voluminous indicating a moderate degree of secretion and in the distal region they are very few and small in dimensions or at intervals even absent showing much less mucus secretions. Thus, there is a gradient in the mucous secretions that suggests that the necessity of secreting mucus appears gradually reduced from the proximal region to the distal regions of the operculum. A high degree of mucus secreted at the proximal region tends to migrate through the intermediate region towards the distal region. Thus, the intermediate and the distal regions will receive mucus from the proximal region in addition to the mucous cells in these regions; resulting in the formation of a surface mucous layer of desired thickness at all the three regions of the operculum for the efficient performance of their functions. This corroborates with the view of Pickering (1974) who observed a high concentration of the mucous cells in the anterior regions, the density of which gradually declined towards the posterior region where they were in low concentrations in *Salmo trutta* and *Salvelinus alpinus* and suggested that this distribution of mucous cells might ensure an even layer of slime on the body as the mucus would tend to migrate backwards with the forward movement of the fish through the water.

The present histochemical reactions show that in both the EISO and the OE, the epithelial cells in the superficial layer and the outer middle layers and the mucous cells are involved in the synthesis of the neutral and acid (both sulphated and non-sulphated)

mucopolysaccharides contributing to surface epithelial secretions. The mucus synthesized in the epithelial cells may form an extracellular cuticular coat as in several fish species investigated ultra-structurally (Whitear, 1986). The mucous cells and surface layer epithelial cells in the epidermis of different fish species investigated by previous workers show variable histochemical properties which may be neutral and acidic (both sulphated and non-sulphated) mucopolysaccharides or a mixture of both (Whitear, 1986). The functional significance of these differences in histochemical reactions of mucus is not understood and needs further investigation.

The club cells under normal conditions, neither extend apically to the free surface nor open on the surface to secrete their contents. In some circumstances, however, the club cells may discharge part of their outer cytoplasm and could even become confluent to form a protective layer (Mittal and Munshi, 1970; Whitear and Mittal, 1983). Zaccone (1980) reported that in eels, the club cells shed at the surface of the epidermis in stressed fish contributing to the formed elements of the slime. Ingram (1980) observed that mechanical protection and lubrication were alternative functions of the exposure of club cell cytoplasm. Whitear and Mittal (1983) suggested that the primary function of the club cells was protection in some way which was not yet experimentally established and that the recognition of specific pheromones (Pfeiffer et al., 1971) was a secondary phenomenon. The exact chemical nature of the club cell secretions is still unknown. However, it has been reported histochemically that they are mainly proteinaceous in nature (Mittal and Banerjee, 1980; Whitear, 1986).

The presence of the taste buds, which are associated with the gustatory system in fishes and the ampullary organs to which the function of electroreception has been attributed (Srivastava and Seal, 1981; Bullock, 1982; Andres et al., 1988), in the OE of *Clarias batrachus* is significant. These sensory structures may be associated with the normal perception of the surroundings in this fish, in which the eyes are poorly developed and in which the visibility is poor owing to its habit to live in the muddy bottom of water bodies and increased turbidity. In the EISO, in contrast, these structures could not be located where their functional significance may be ambiguous.

The presence of melanocytes in the OE as well as

in the tissue immediately underlying it in *Clarias batrachus* may be associated with the function of camouflaging the bottom dwelling fish with the dark surroundings. In contrast, the melanocytes are, in general, entirely absent in the EISO and in the tissues immediately underlying it where they may not be of much significance.

The rich vascularization of the tissues underlying the OE in *Clarias batrachus* is significant. It may be correlated with the function of assisting the fish in accessory cutaneous respiration to cope up with the oxygen-depleted conditions of weed-infested swamps and derelict ponds which it inhabits. Mittal and Munshi (1971) and Mittal and Banerjee (1975) also suggested that the skin in *Amphipnous cuchia* and *Channa striata* was an important organ of respiration in which the epidermis was thin and the tissues immediately underlying it were richly vascularized. Krogh (1904) and Jeuken (1957) reported that *Anguilla anguilla* and *Misgurnus fossilis*, in which the epidermis was very thick, were able to meet nearly all their oxygen demand through their skin. The blood capillaries are relatively few and much smaller in dimensions in the tissue immediately underlying the EISO. It may be due to the reason that the accessory respiration in this region may not be of much use as most of the oxygen is extracted from the water during its passage through the gills before it reaches the opercular cavity.

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ナマズの一種 *Clarias batrachus* の鰓蓋内表上皮と鰓蓋上皮との比較研究

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Clarias batrachus の鰓蓋は、近位、遠位および中間の3部位に分けられる。これら3部位における鰓蓋内表面を裏打ちする上皮 (EISO) と鰓蓋上皮 (OE) とは、厚さ、粘液細胞および棍棒細胞の密度と容積、リンパ球・メラニン細胞・味蕾並びに瓶器の分布において顕著な相違がみられる。これら構成上の相違は、EISO と OE の部分を支配している諸条件と関連している。OE 下に存在する組織内にみられた豊富な血管分布は、本種の副呼吸に役立っている。これとは対照的に、副呼吸がそれほど有効ではない EISO 下の組織には、血管分布が乏しい。