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STRUCTURAL MODIFICATIONS IN LIPS AND ASSOCIATED STRUCTURES OF INDIAN MAJOR CARP, *LABEO CALBASU*

Sateesh Chandra Dubey and J. P. N. Singh

Department of Zoology, R.H.S.P.G.College, Singramau, Jaunpur - 222 175, India

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ABSTRACT – In *Labeo calbasu* the mouth is subterminal and is bordered by thick and plicate lips thrown in to characteristic protuberances . The upper lip is associated on its ventral side with the horny upper jaw sheath and on its dorsal side with a conspicuous rostral cap having its apical end extending ventrally as a fold of flap partially shielding the upper lip and the fold of skin between the upper lip and rostral cap. The lower lip is associated on its dorsal side with horny lower jaw sheath on its ventral side with the fold of skin between the lower lip and skin on the ventral side of the head. In *Labeo calbasu* epithelia of fold of skin,rostrum and dorsal and ventral sides of upper lip and lower lip are mucogenic, while epithelia of horny upper and lower jaw sheath are keratinized. The unculi on horny jaw sheath associated with upper lip and lower lip, in this fish may be regarded as an adaptation to act as sharp cutting edges assisting the fish in feeding plants filaments and other plant materials on which it feeds. The mucous cells in the epithelium of fold of skin between the upper lip and the rostral cap and between the lower lip and the ventral head skin are more in number and secrete mucous at the surface. The mucous secretion in these regions may play a significant role in providing extra lubrication to the surface of the fold of skin. This reduces the resistance to surface drag during their stretching, enabling the jaw to protrude at the time of feeding with increasing efficiency and swiftness. The club cells are developed additionally to complement the mucous cells in the efficient functioning of the epithelium in protection against various hazards.

The presence of taste buds in upper and lower lip can be considered as an adaptation to assist the fish in selecting the food of its choice, available in surrounding medium through gustatory sense. It is interesting that the taste buds are, in general absent in rostrum, fold of skin and jaw sheath. It appears that the development of gustatory sense at these regions is not of desired significance.

Key words **:** Structural modification, lip, fish, *Labeo calbasu.*

INTRODUCTION

In fishes lips may be associated with the rostral cap (Rostral Kappe, Minzenmay, 1933), the horny jaw Sheath and the fold of skin between the upper lip and the rostoral cap, and between the lower lip and the skin on the ventral side of the head. The lips and associated structures show considerable adaptation in relation to the diversified nature of food, food procuring device and feeding habits of fish.

Studies on lips and structures associated with them in fishes are limited in main to their external morphology (c.f. Kapoor *et al,* 1975; Roberts, 1982). Studies on structural organization and histochemistry of epithelia of lips are limited (Agarwal and Mittal, 1991; Agarwal and Mittal 1992 a,b, Pinky *et al,* 2004; Singh and Gupta, 2006 a,b, Singh *et al*, 2009).

The aim of the present investigation has been to study the structural modification in the epithelia of lips and associated structures of a bottom feeder herbivorous fish *Labeo calbasu* belonging to the family cyprinidae and order cypriniformes.

MATERIALS AND METHODS

Live specimens of *Labeo calbasus* (approx. 21 cm in length) were collected from river Ganga at Mirzapur and were fed on rice bran powder. The upper and lower lips along with the structures associated with them were excised and were fixed in 10% neutral formalin, Helly's fluid and aqueous Bouins fluid. Paraffin sections were cut at 6µ and were stained with Ehrlichs haematoxylin eosin (HE), Verhoeffs haematoxicin eosin (VHE), Mallory's triple stain(MTS) and Papanicolaou's stain (PS) and with the methods to locate and differentiate protein and carbohydrate contents in cellular components following Lillie (1954), Gurr (1958), Bancroft and Stevens (1982) and Pearse (1985).

OBSERVATIONS

In *Labeo calbasu* the mouth is sub-terminal (Plate-Ia) and is bordered by thick and plicate lips, thrown in to characteristics protuberances. The upper lip is associated on its ventral side with the horny upper jaw sheath and on its dorsal side with a conspicuous rostral cap having its apical end extending ventrally as a fold of flap partially

shielding the upper lip (Plate-Ia) and the fold of skin between the upper lip and rostral cap. The lower lip is associated on its dorsal side with horny lower jaw sheath and on its ventral side with the fold of skin between the lower lip and skin on the ventral side of the head (Plate-I.a).

The epithelia of the lips and the associated structures show great diversity in the distribution disposition and the histochemical nature of their cellular components. The epithelia are stratified. They may, in general, be divided in to three principal layers – the basal, the middle and the superficial layer and are mainly composed of the epithelial cells. In addition, the mucous cells, the club cells, the lymphocytes and the taste buds may also be discernible.

Upper Lip – The epithelium on dorsal side of upper lip differs from that of ventral side.

Epithelium on dorsal side of upper lip - The epithelium is mainly composed of epithelial cells with a few club cells distributed in the middle layer (Plate-Ib) and mucous cells distributed in the middle and surface layer. Lymphocytes are enclosed within, characteristics spaces in the basal and middle layer. These layers also include taste buds located in the deeper layer (Plate-I.c).

The epithelial cells in the surface layer appear to protrude some what at the surface giving it corrugated appearance.

The epithelial cells in the middle layer are arranged in several layers. The inter cellular spaces and cytoplasmic bridges are indistinct.

In general, these cells in the deeper layer appear vertically elongated and acquire a polygonal shape or some what flattened vertically as they are displaced towards the surface (Plate-Ic).

The epithelial cell in the basal layer are columnar and arranged in a row on a thin non cellular basement membrane. The nuclei in the epithelial cell in superficial layers and outer middle layer are centrally placed and appear rounded. In the basal layer and inner middle layer they appear elongated. In general they appear healthy with distinct chromatin material and nucleoli, and stain blue in HE and PS, black in VHE, reddish purple in MTS, deep blue in solochrome Cyanin R, and moderately purplish pink with the Feulgen reaction for DNA.

Epithelium on the ventral side of the upper lip :

In the epithelium on the ventral side of upper lip the taste buds are situated in the surface layers.The club cells with shrinked eosinophilic contents are located in the middle layer.The mucous cells occupy the middle layer of the epithelium(plate Id).

The superficial layer of epithelium is represented by very flattened epithelial cells arranged in the one or two layers. The cell boundaries are indistinct, the nuclei are either absent or very flattened, small and lightly stained. The free surface is thrown into characteristic papilla like unculi and appear corrugated.

The epithelial cells in the middle layer are arranged in three or four layers. They appear polygonal, with fine intercellular, cytoplasmic bridges and distinct inter cellular spaces and have centrally placed, rounded dilated nuclei, which appear healthy. These epithelial cells, which lie in the layer immediately below the superficial layer, are flattened with pyknotic nuclei (Plate-I.d).

The epithelial cells in the basal layer are short and columnar, each heaving centrally placed, healthy appearing nucleus.

Horny Upper Jaw Sheath :

The epithelial cells in the superficial layer of horny upper jaw sheath are modified in to strongly eosinophilic, thick, keratinized layer, which appear smooth. The nuclei are either absent or appear in different stage of degeneration(plate-I.e).

The epithelial cells in the middle layer are arranged in five to six layers. They appear polygonal with fine intercellular cytoplasmic bridges and distinct intercellular spaces (Plate-Ie) and have some what flattened centrally placed nuclei. The epithelial cells in the basal layer are low cuboidal each heaving centrally placed healthy nucleus.

Fold of skin between the Upper Lip and The rostral cap :

The epithelia of fold of skin, between the rostral cap and upper lip are composed of epithelial cells, mucous cells, and lymphocytes enclosed within characteristic lymphatic spaces. Club cells and taste buds are absent in the epithelium of skin fold (Plate-I-f.)

The epithelial cells appear flattened in superficial layer, polygonal and some what vertically compressed in the middle layer and cuboidal in the basal layer. The nuclei of epithelial cells appear healthy and are centrally placed and flattened the superficial and middle layer and rounded in the basal layer (Plate-I-g).

Rostral Cap :

The epithelium of rostral cap is devoid of taste buds and is composed of club cells, more than that in the dorsal side of upper lip (Plate-II-a). Mucous cells are discernable mostly in the superficial layer. The basal layer epithelial cells are vertically elongated. The middle layer epithelial cells are polygonal with rounded nuclei.

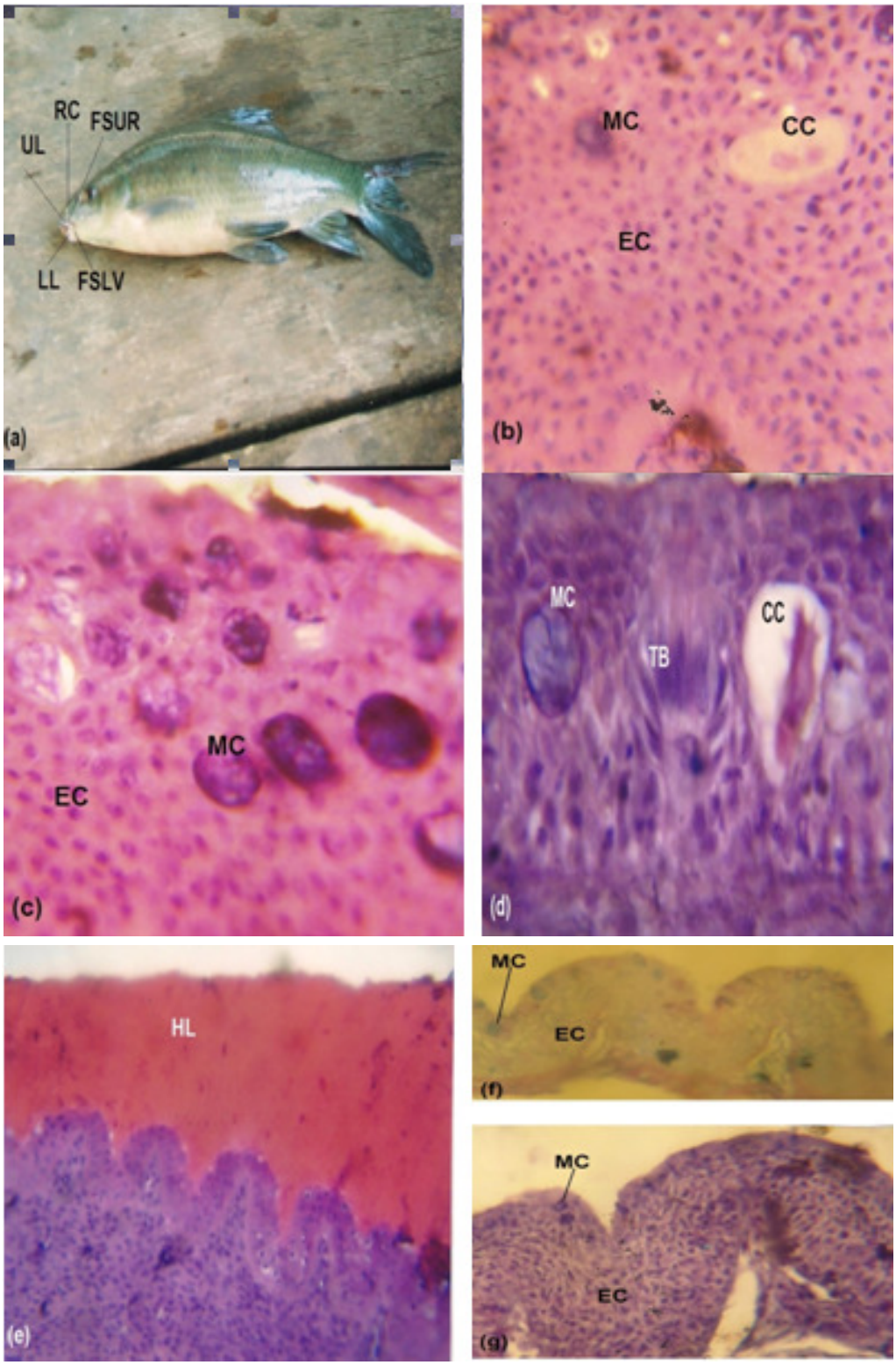

Plate I : (a) Photograph of head region of *Labeo calbasu*. The upper lip(UL) is associated with the rostral cap(RC) and the fold of skin between the UL and RC(FSUR). The lower lip(LL) is associated with the fold of skin between LL and ventral head skin(FSLV). **(b)** Showing structural organization of epithelium on the dorsal side of upper lip. Note columnar epithelial cells in basal layer and vertically elongated epithelial cells in the middle layer. Also note rounded basophilic mucous cells(MC) and binucleated club cells(CC)(HE)x600. **(c)** Showing distribution of mucous cells in the upper part of epithelium of dorsal side of upper lip(HE)x1000. **(d)** Showing distribution of mucous cells(MC),club cells(CC) and taste bud (TB) in the epithelium on the ventral side of upper lip(HE) x 1000. **(e)** Showing thick,eosinophilic,keratinized horny layer(HL) in upper horny jaw sheath (HE) x1000. **(f)** Showing distribution of mucous cells (MC) in the epithelium of fold of skin between UL and RC(AB/PAS)x600. **(g)** Showing structural organization of the epithelial cells(EC) and mucous cells(MC) in the epithelium of skin fold between UL and RC(HE)x1000.

The superficial layer epithelial cells having oval or flattened nuclei.

Lower Lip and associated Structures

Dorsal Sid of Lower Lip :

The basal layer epithelial cells are low columnar with rounded centrally placed nuclei. The middle layer, epithelial cells are arranged in 8-10 layers and are polygonal in shape with rounded centrally placed nuclei (Plate-II-b).

The superficial most and underlying two to three layers of surface layer epithelial cells are vertically compressed with flattened nuclei. Typical taste buds are observed which are found scattered (Plate-II-c).The mucous cells are mostly rounded occupying middle layer of epithelial cells.

Ventral side of Lower lip :

The ventral side of lower lip resembles to that of dorsal side of lower lip except distribution of mucous cells (Plate-II-d). The mucous cells are mainly distributed in surface layer (Player-II-e).

Fold of skin between lower lip and ventral head skin :

It resembles in structural organization to that of fold of skin between upper lip and rostral cap (Plate-If).

Horny Lower Jaw Sheath :

The epithelial cells in the superficial layer of horny lower jaw sheath are modified into strongly eosinophilic keratinized layer, which appears – corrugated due to presence of unculi. The nuclei are either absent or appear in different stages of degeneration (Plate-II-f).

The epithelial cells in the middle layer are arranged in three or four layers. They appear polygonal with fine intercellular cytoplasmic bridges and distinct intercellular spaces and have centrally placed, rounded, dilated nuclei which appear healthy (Plate-II-g).

The epithelial cells in basal layer are short and columnar, each heaving centrally placed healthy appearing nucleus.The staining properties of the superficial layer epithelial cells are similar to those of horny upper jaw sheath.

Histochemistry –

Epithelial Cell :

On the dorsal and ventral side of upper lip, rostral cap, the lower lip and the fold of skin between the upper lip and the rostral cap, and between the lower lip and the skin on the ventral side of the head, the superficial layer of epithelial cells, appear homogenous, are slightly eosinophilic in HE, PS and VHE stain purplish in MTS and show a moderate reaction to a mixture of neutral, acidic sulphated and acidic non sulphated mucopoly saccharide and general protein and a weak reaction to basic protein bound NH_2 groups. In the basal and middle layer epithelial cells, the reaction appears to be relatively weak.

On the horny lower jaw sheath and upper jaw sheath the superficial layer of epithelial cells and the specialized structures, the uncluli, appear strong eosinophilic in HE and PS(plate-II.f,g) dark grey in VHE and deep reaction with MTS show a strong reaction to general protein basic protein, protein, bound NH_2 group, tyrosine, tryptophan, cystine bound SS group and cystine bound SH groups. This indicates that the superficial layer of epithelial cells and the specialized structures in these regions are keratinized.

The epithelial cells in the middle layer appear slightly eosinophilic in HE, PS and VHE; stain purplish in MTS and show a positive reaction to the protein end groups(plate-II.f,g), which however is intense than of the superficial layer of epithelial cell. In addition cells they show a weak reaction to mixture of neutral acidic sulphated and acidic non-sulphated mucopolysaccharide. The flattened epithelial cells in the layer immediately bellow the superficial layer show similar staining properties to earpohydrate and proteins and appear basophilic. The epithelial cells of the basal layer resemble in their staining properties, those of the epithelium on the dorsal side of upperlip.

Mucous cells :

The mucous cells from the different locations appear light blue in HE, PS and MTS and unstained in VHE, and do show a positive reaction to protein end groups. The peripheral cytoplasmic region of these cells. However show a moderate reaction to general proteins. The secretary content of these cells in the epithelium of the upper lip show a strong reaction to acidic mucopolysaccharides (both sulphated and non sulphated). The mucous cells in the epithelia of rostral cap, lower lip and the fold of skin between the upper lip and the rostral cap and between the lower lip and the skin on the ventral side of the head show a positive reaction to mixed neutral and acidic (sulphated and non-sulphated) mucopolysaccharide.

In the deeper epithelial layer of the fold of skin between the upper lip and the rostral cap a few irregularly shaped cells, probably differentiating mucous cells are discernible, staining magenta with Periodic acid- Schiff (PAS) and alcian blue (AB)- PAS, indicating the presence

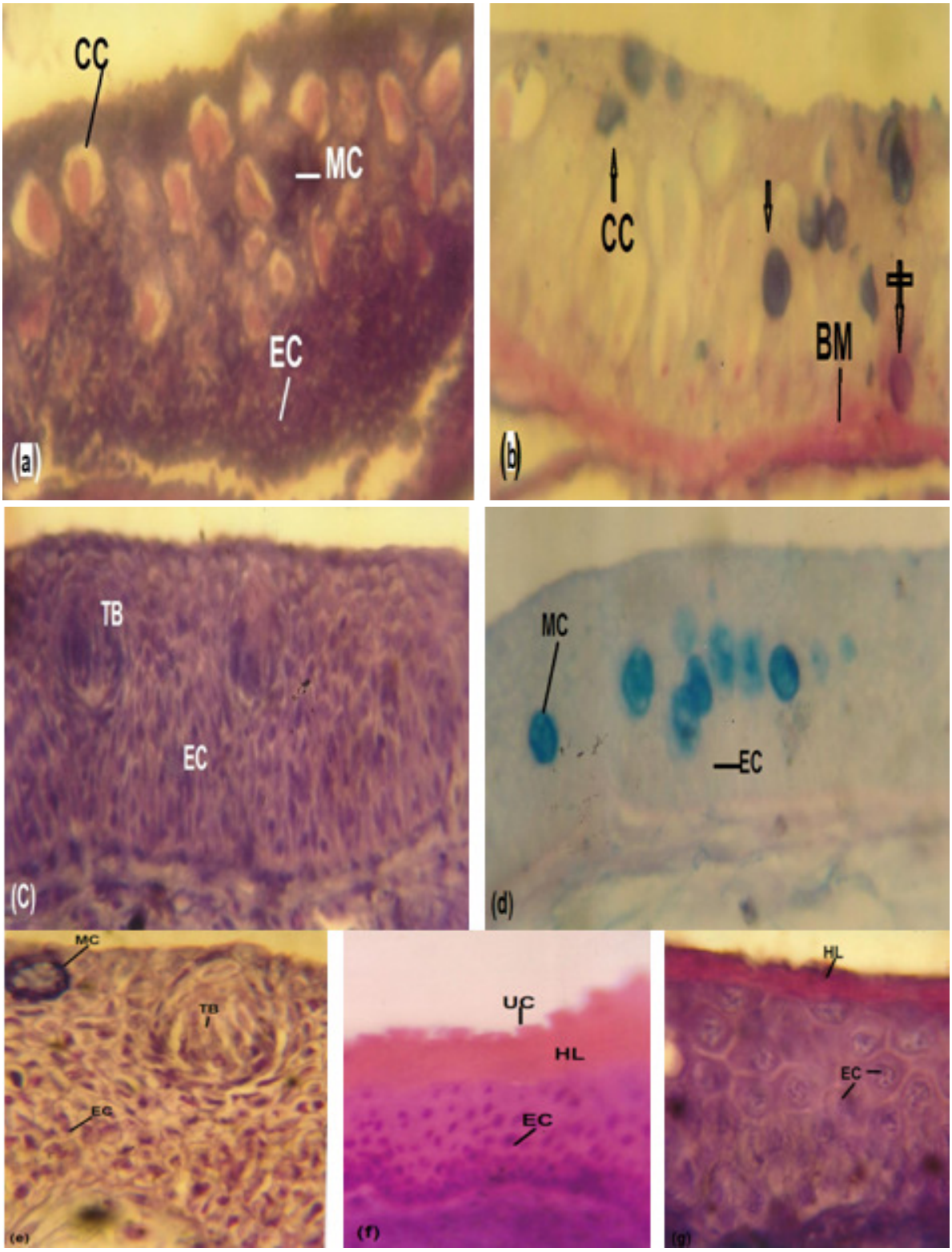

Plate II : (a) Showing distribution of basophilic mucous cells(MC),eosinophlic club cells(CC) and epithelial cells(EC) in epithelium of rostrum(HE)x600. **(b)** Showing club cells(CC)weakly stained for mixed glycoprotein.The mucous cells in middle and superficial layers(arrow)stained turquoise blue for acidic glycoprotein and mucous cells arising from basal layer (barred arrow)stain for neutral glycoprotein.Basement membrane(BM) stain strong magenta for neutral glycoproteins in epithelium of rostrum(AB/PAS)x600. **(c)** Showing taste buds and epithelial cells in epithelium of dorsal side of lower lip(HE)x600. **(d)** Showing mucous cells staining blue for acidic glycoproteins in epithelium of dorsal side of lower lip(AB/PAS)x600. **(e)** Showing mucous cells(MC),taste buds(TB) and epithelial cells(EC)in epithelium of ventral side of lower lip(HE)x1000. **(f)** Showing eosinophilic keratinized horny layer(HL) with unculi(UC) in superficial layer. The middle layer epithelial cells(EC) with distinct intercellular spaces(HE)x600. **(g)** Showing superficial layer cells modified into eosinophilic keratinized horny layer(HL) .The middle layer epithelial cells (EC)with distinct inter cellular spaces (HE)x1000.

of neutral mucopolysaccharides. These cells could not be located with the use of HE, PS, VHE, MTS or the other histochemical technique employed.

Club cells :

The club cells appear slightly eosinophilic and show a strong reaction to general protein and a moderate reaction to basic protein, protein bound NH_2 groups, and tyrosine. The periphery of the cells show a weak reaction to tryptophan. These cells do not show a positive reaction to carbohydrates.

DISCUSSION

Cytology of the epithelial cells supplemented by cytochemistry reveals that the epithelial of the lips and associated structures of *Labeo calbasu* show considerable modifications and represent two diverse developmental potentials one related to mucogenesis and other to keratinization. The epithelia of fold of skin, rostral cap the dorsal and ventral sides of the upper and lower lips are mucogenic and the epithelial of the horny upper and lower jaw sheath are keratinized. These basic differences in structural organization may be considered as an adaptation in relation to food and feeding habits of these fish species.

The unculi on horny jaw sheaths associated with lower lip in this fish may be regarded as an adaptation to act as sharp cutting edges assisting the fish in feeding plant filaments and other plant materials on which it feeds. Ceirgis (1952) observed horny jaw sheath associated with both upper and lower lip in *Labeo horie* and co-related with securing good groups of plant material on which it feeds.

Roberts (1982) reported that in the cyprinoides, the jaw quite commonly have well developed horny sheath which lie immediately to the lips and separate them from bony support of Jaws. Agrawal and Mittal (1992a) reported that in *Cirrhina mrigala* on omnivorous bottom feeder fish, only the lower lip has horny jaw seath.

Agrawal and Mittal (1992b) noticed horny jaw sheath associated with both upper and lower lips in *Labeo rohita*, a herbivorous bottom feeder fish. Pinky *et al* (2004) observed horny jaw sheath associated with upper and lower lips of *Garra lamta,* a hill stream fish. Spine like curved unculi have been also observed on the rostral cap and adhesive pad of *Garra lamta* by these workers. Singh *et al* (2009) have also observed the unculi in horny jaw sheath associated with the lips of *Aspidoporia morar.*

In *Cirrhina mrigala* (Agrawal and Mittal, 1992a) unculi on horny lower jaw sheath have been associated to help the fish to scrape the substrate, serving as a cutting edge and assisting in grasping food. In *Labeo rohita* (Agrawal and Mittal, 1992b), unculi on both horny upper jaw sheath and horny lower jaw sheath have been associated as an adaptation to act as sharp cutting edges assisting the fish to browse upon and to aid in scraping the surface of substrates during feeding. In *Garra lamta* (Ojha and Singh, 1992; Pinky *et al*, 2004) the spine like curved unculi at the rostral cap and adhesive pad may be considered and adaptation to assist firm anchorage on the substratum and prevent the fish from being swept away in fast flowing turbulent stream. The unculi in addition could also function as food scuppers. The unculi on horny jaw sheath may serve as sharp, cutting tooth like structures.

The club cells are observed in the lips and associated structures of *Labeo calbasu*. They appear binucleted with eosinophilic secretory contents which stain strongly for protein. The clubs cells are more in numbers as compared to dorsal and ventral side of upper lip. The uninucleated club cells have been observed in the epithelia of lips and associated structures of *Catla catla* (Agrawal and Mittal, 1991) and *Cirrhina mrigala* (Agrawal and Mittal, 1992a) while binucleated club cells have been noticed in the epithelia of lips and associated structures of *Rita rita* (Agrawal and Mittal, 1992b).

Information regarding functional singificance of club cells is available mainly from the studies on the epidermis over general body surface of the fish. It appear that club cells are developed additionally to complement the mucous cells in the efficient functioning of the epithelium in protection against various hazards.

It is significant to note that in unkeratinized area of the epithelium on the upper lip, lower lip, rosttrum and fold of skin in *Labeo calbasu* the lymphocytes are in general, observed in appreciable number and epithelial cells in the middle layer are arranged with less distinct intercellular spaces and indistinct intercellular cytoplasmic bridges.

In contrast, in keratinized epithelia at horny upper and lower jaw sheath, the lympthocytes are in general either very few or absent and epithelial cells is the middle layer are arranged with distinct intercellular spaces and prominent inter-cellualr cytoplasmic bridges. Electron microscopic studies undoubtedly are needed to reach any firm conclusion regarding their co-relation.

The mucous cells in the epithelium of the fold of skin between the upper lip and the rostral cap and between the lower lip and the ventral head skin are more in number and secrete mucous at the surface. In addition to their primary role to protect and to keep the surface of the

epithelium clean by preventing the deposition of foreign matters in the groove. The mucous secretion in these region may play a significant role in providing extra lubrication to the surface of the fold of skin. This reduces the resistance to surface drag during their stretching, enabling the jaw to protrude at the time of feeding with increasing efficiency and swiftness.

Rosen and Conford (1971) suggested the lubricating effect of the mucous secreted on the body surface, in reducing water friction around the body of the fish and enabling movement at a greater speed with less expenditure of energy.

In *Labeo calbasu* the taste buds are located mainly in the dorsal and ventral side of upper and lower lip. The ventral side of upper lip and the dorsal side of lower lip both come in direct contact of food material. The presence of taste buds in these regions can be considered as an adaptation to assist the fish in selecting the food of its choice, available in surrounding medium through gustatory sense. It is interesting that the taste buds are, in general absent in rostrum, fold of skin and jaw sheath. It appears that the development of gustatory sense at these regions is not of desired significance.

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