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# Operculum of peppered loach, *Lepidocephalichthys guntea* (Hamilton, 1822) (Cobitidae, Cypriniformes) : a scanning electron microscopic and histochemical investigation

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ABSTRACT. *Lepidocephalichthys guntea* frequently darts and burrows in mud or sand, and spends most of the time buried in the soft bottom of water bodies. The epidermis covering its outer surface (OE) and the epithelium lining the inner surface of the operculum (EISO) differ noticeably in their surface architecture seen under SEM, and in their glycoprotein secretions analysed histochemically in whole mounts. This indicates their important physiological activity as mediators for extrinsic factors to which these are exposed. Micro-ridges arranged compactly may impart firm consistency to the free surfaces of the epithelial cells in the OE as an adaptation to mechanical stress during burrowing. In the EISO, which is not prone to abrasion during burrowing, in contrast, widely spaced micro-ridges suggest less rigid surfaces. Mucous cells are of two types, A and B, in the OE, and only one, type C, in the EISO. Most mucous cells in the OE are type A, and elaborate mainly sulphated glycoprotein, which is associated with an increase in the viscosity of mucus providing protection against possible mechanical damage during burrowing and against pathogens. In contrast, glycoproteins with oxidisable vicinal diols and with carboxyl groups elaborated by type C mucous cells in the EISO are considered to lower viscosity of the mucus. Mucus with lower viscosity is washed away more easily by water currents, preventing accumulation on the surface that may obstruct or disturb the smooth flow of respiratory current across the branchial chamber. Presence of prominent taste buds in the OE is considered as an adaptation to locate food with increased efficiency. Presence of a large number of taste buds in the EISO, is regarded as an adaptation to detect other chemicals that could enter the buccal cavity during respiration. Possible functional significance of glycoprotein secretions in the taste buds are discussed.

KEY WORDS : *L. guntea,* opercular surfaces, SEM, whole mount, glycoprotein histochemistry.

## INTRODUCTION

The operculum in teleosts is an important structure covering the branchial chamber, primarily providing protection for the gills and preventing water entering the gill cavity during inspiration. The epithelium lining the inner surface of the operculum (EISO) in fishes is only in contact with the water entering the gill cavity, whereas the epidermis covering the outer surface of the operculum (OE) contacts all kinds of media, including the substrates into which fish burrow. This difference could impart modifications in the organisational patterns of the surfaces of the OE and the EISO.

A review of the literature reveals that comparative studies on aspects of the organisational patterns of the OE and the EISO have not drawn the attention of many researchers. GARG et al. (1995), using scanning electron microscopy, reported significant differences in the surface organisation of the OE and the EISO of an air breathing catfish *Clarias batrachus* Linnaeus, 1758.

The objective of this study was to compare the surface architecture of the OE and the EISO of *Lepidocephalichthys guntea* Hamilton, 1822 (Common names : Peppered loach, Guntea loach or Torential loach), using scanning electron microscopy, and to visualise glycoproteins, using histochemical methods, in whole mount preparations in light microscopy. Such a comparison may enable determination of whether or not the dichotomy in these tissues reflects differences in their functional organisation, and may promote better understanding of their roles in relation to the habit and habitat of the fish.

Loaches, in general, inhabit flowing or even stagnant waters, preferably parts that are not too deep and have a soft bottom. The fish has a habit of suddenly burrowing into the mud or sand on the bottom and in this way protects itself from predators. Food consists chiefly of worms and insect larvae, obtained by very sagacious hunting (GÜNTHER, 1989). *L. guntea* spends most of the time buried in the sandy bottom, but often comes to the surface with swift movements to gulp atmospheric air (MISHRA & AHMAD, 1986).

## MATERIAL AND METHODS

Live specimens of *L. guntea* (mean length 70 ± 5 mm, n = 10) were collected from the river Ganges at Varanasi, India and were kept in a laboratory aquarium, with a layer

of sand at the bottom, at 25 ± 2 ºC for up to 24 - 48h. The fish were cold anaesthetised, following MITTAL & WHITEAR (1978), to excise the opercular pieces for this study.

### Scanning electron microscopy

Opercular pieces were treated and prepared for scanning electron microscopy following PINKY et al. (2002). Critical point dried opercular pieces, attached to stubs with the OE or EISO facing upwards, were coated with gold and examined under a Scanning Electron Microscope (Leo, 435 VP, England).

### Whole mount study

Opercular pieces were rinsed in physiological saline, fixed in Carnoy's fluid or alcoholic Bouin's fluid, washed and stored in 70% ethyl alcohol. Intact sheets of OE and EISO were dissected from the opercular pieces, using fine forceps and needles, under a Nikon stereoscopic microscope (model SMZ - 1B) following MITTAL & GARG (1988). The sheets were hydrated in a descending ethanol series and stained with the histochemical methods along with their controls for glycoproteins summarised in Table 1. The stained sheets were dehydrated in graded ethanol in ascending concentrations, cleared in xylene, and mounted in Harleco synthetic resin (HSR) or in Kirkpatrick and Lendrum's distrene dibutylphthalate xylene (DPX). Observations were made on a Leitz microscope (model Laborlux S). Photomicrographs were taken with a Leitz camera system for automatic microphotography (model Vario Orthomat 2).

Densities of mucous cells and taste buds in the OE and the EISO were calculated using a stage micrometer (1 division = 0.01mm) and an eyepiece graticule with a square grid (5 x 5 = 25 squares) (Carl Zeiss, Jena). Ten randomly selected sites of each sample were analysed. Data thus obtained for each, the OE or the EISO, from five fishes were pooled separately and results were expressed as mean value ± SD throughout.

TABLE 1

Summary of the histochemical methods for visualisation of glycoproteins in different types of mucous cells and the taste buds in the OE and the EISO of *L. guntea* (Carnoy's fluid fixed tissues).

| Histochemical methods | Reactions | Interpretation of reactions | References | Mucous cells Types | | | Taste buds |
|---|---|---|---|---|---|---|---|
| | | | | A | B | C | |
| 1. WO/ S | M | Free aldehydes | | _ | _ | _ | _ |
| 2. PAS | M | GPs with oxidisable vicinal diols and/or glycogen | McMANUS, 1948 | 1-2M | 3-4M | 3-4M | 2M |
| 3. Acetylation/PAS | 0(M) | Same as '2' | LILLIE & FULLMER, 1976 | _ | _ | _ | _ |
| 4. α-amylase/PAS | M | GPs with oxidisable vicinal diols | SPICER et al., 1967 | 1-2M | 3-4M | 3-4M | 2M |
| | 0(M) | Glycogen | | | | | |
| 5. AB2.5 | T | GPs with carboxyl groups and/or with O- sulfate esters | MOWRY, 1956 | 3-4T | # | 3-4T | 2T |
| 6. Active methylation AB2.5 | 0 | GPs with carboxyl groups and/or with O- sulfate esters | SPICER et al., 1967 | - | - | - | - |
| 7. Active methylation/ saponification AB2.5 | T | GPs with carboxyl groups | SPICER et al., 1967 | 1-2T | # | 2-3T | 1T |
| | 0(T) | GPs with O-sulfate esters | | | | | |
| 8. AB1.0 | T | GPs with O-sulfate esters | LEV & SPICER, 1964 | 2-3T | # | 1-2T | 1T |
| 9. Active methylation AB1.0 | 0 | GPs with O-sulfate esters | SPICER et al., 1967 | - | - | - | - |
| 10. AB2.5/PAS | M | GPs with oxidisable vicinal diols and/or glycogen | MOWRY, 1963 | 3-4TP | 2-3M | 3-4P | 2P |
| | T | GPs with carboxyl groups and/or with O-sulfate esters | | | | | |
| | P | GPs with oxidisable vicinal diols and/or glycogen, GPs with carboxyl groups and/or with O-sulfate esters | | | | | |
| 11. AB1.0/PAS | M | GPs with oxidisable vicinal diols and/or glycogen | SPICER et al., 1967 | 2-3TP | 2-3M | 3-4MP | 2MP |
| | T | GPs with O-sulfate esters | | | | | |
| | P | GPs with oxidisable vicinal diols and/or glycogen, GPs with O-sulfate esters | | | | | |

Methods : WO/S, without oxidation Schiff's; PAS, periodic acid/Schiff; AB2.5, alcian blue at pH2.5; AB1.0, alcian blue at pH1.0.
Reactions : #, could not be distinguished; M, magenta; MP, magenta with purple tinge; P, purple; T, turquoise; TP, turquoise with purple tinge; -, no reaction; 1 to 4, feeble to very strong reactions.

## RESULTS

### Scanning electron microscopy

The surfaces of the opercular epidermis (OE) and the epithelium lining the inner surface of the operculum (EISO) in *L. guntea* were a mosaic of irregularly polygonal epithelial cells of varied dimensions (Fig. 1 a, b). The free surfaces of the epithelial cells were differentiated into micro-ridges forming characteristic patterns. In the OE, the microridges were compactly arranged, sinuous with jagged surface, short and branched with abrupt ends

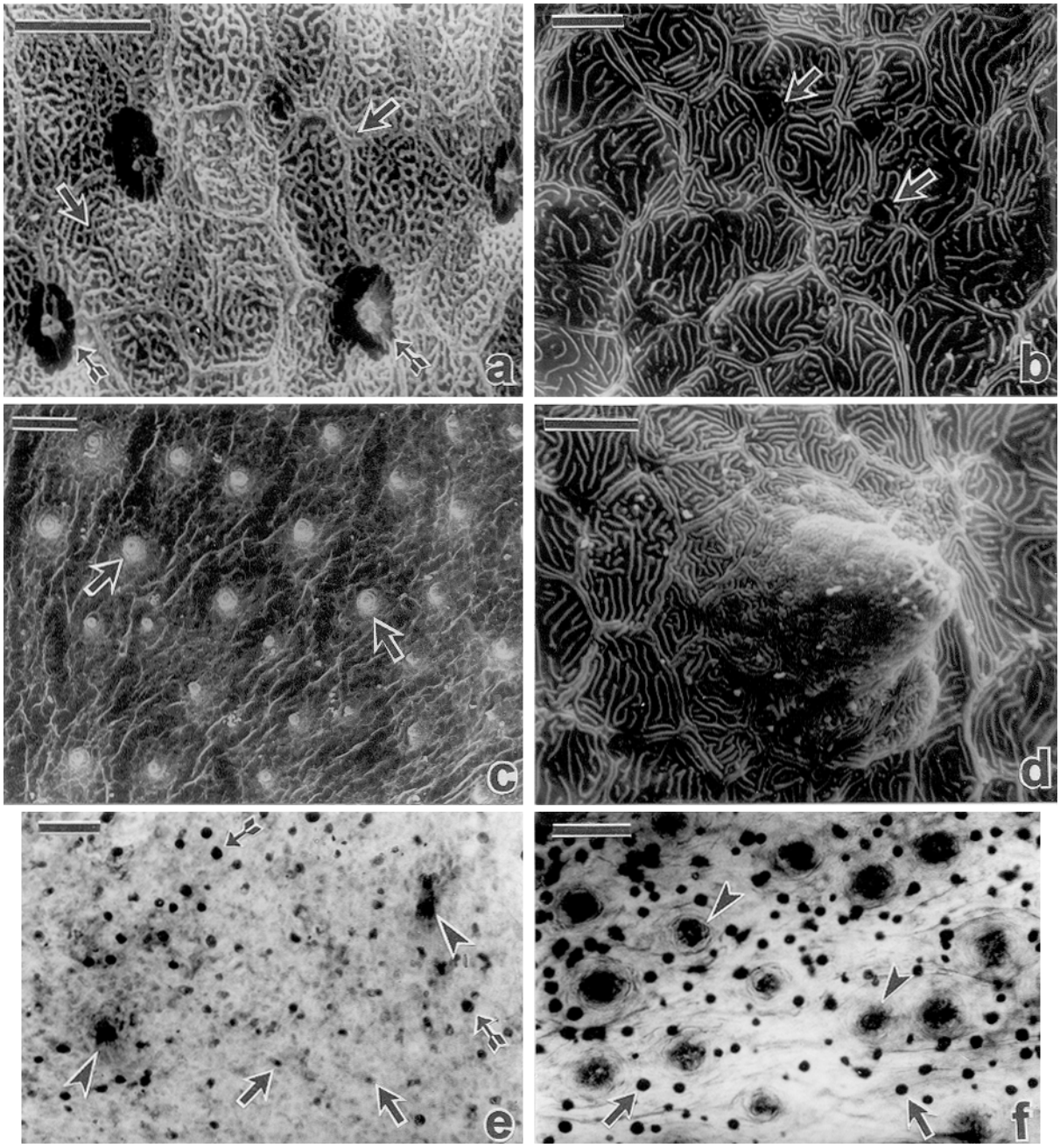


**Fig. 1 (a-d).** Scanning electron photomicrographs showing surface architecture of (a) OE and (b-d) EISO of *L. guntea*. **(a)** OE showing mosaic of epithelial cells with prominent boundaries. Microridges, often with fine microbridges (arrows) on the surface of the epithelial cells are compact and irregularly interwoven to form complex patterns. Note the presence of mucous cell apertures (winged arrows) laden with blobs of oozed mucus. (Scale bar = 5 μm). **(b)** EISO. Compare with OE (a). The microridges are without microbridges and are widely spaced to form simplified patterns on the surface of the epithelial cells. The mucous cell apertures are smaller (arrows). (Scale bar = 5μm). **(c)** The surface of the EISO is irregularly folded with high density of taste buds (arrows). (Scale bar = 40μm). **(d)** EISO showing conical epidermal elevation with a taste bud. Note microvilli, representing taste hairs, projected at the summit. (Scale bar = 5μm).

**Fig. 1 (e-f).** Photomicrographs of whole mount preparations of (e) OE and (f) EISO of *L. guntea*. **(e)** Showing a large number of type A mucous cells (arrows) (turquoise with purple tinge in original). Type B mucous cells are relatively few (winged arrows) (magenta in original). A few taste buds (arrowheads) (purple in original) are also discernible. (AB 2.5/PAS). (Scale bar=40μm). **(f)** Showing type C mucous cells (arrows) (deep purple in original) and a large number of taste buds (arrowheads) (purple in original). (AB2.5/PAS). (Scale bar = 40μm ).

or irregularly interwoven to form intricate mesh-like patterns (Fig. 1a). The microridges were often interconnected with fine transverse connections –microbridges (Fig. 1a). Boundaries between adjacent epithelial cells were demarcated by double rows of intimately associated microridges that often gave a braided appearance (Fig. 1a).

The surface of the EISO, in contrast, was irregularly folded (Fig. 1c). The microridges on the surfaces of the epithelial cells appeared smooth, elongated, extensive or sometimes fragmented, and often oriented regularly almost parallel to each other with wide spaces between them to form simplified patterns (Fig. 1b). Microbridges were absent. Furthermore, the double row of microridges at boundaries between adjacent epithelial cells appeared smooth and extensive (Fig. 1b).

Interspersed between the epithelial cells, mucous cell apertures and prominent taste buds were distinguished on the surfaces of both the OE and the EISO. The mucous cell apertures, generally, were rounded and occurred at the border of three or four epithelial cells (Fig. 1a, b). These apertures in the OE, as compared to those in the EISO, appeared wide, often laden with blobs of oozed mucus (Fig. 1a). Each taste bud was situated on a characteristic epidermal elevation (Fig. 1c), which in general, appeared conical and jutted out at the surface (Fig. 1d). At the summit of each elevation, closely packed microvilli representing taste hairs projected through a rounded taste pore (Fig. 1d).

### Whole mount study

In the whole mount preparations of OE and EISO, the mucous cells, in general, appeared rounded (Fig. 1e, f), each opening to the surface through a narrow secretory pore. Histochemical methods employed to demonstrate glycoproteins differentiated three categories of mucous cells : for convenience designated as type A, B and C mucous cells (Table 1). In the OE, type A and type B mucous cells were distinguished (Fig. 1e), most belonging to type A, while type B mucous cells were sporadic. In the EISO, in contrast, only type C mucous cells were visualised (Fig. 1f). The density of mucous cells in the EISO was high, compared to that in the OE (Table 2).

TABLE 2

Density of mucous cells and the taste buds in the OE and the EISO of *L. guntea*

| Region | Density (number/mm$^2$) | | | |
|---|---|---|---|---|
| | Mucous cells | | | Taste buds |
| | Type A | Type B | Type C | |
| OE | 1392.00 ± 155.83 | 108.80 ± 95.64 | – | 35.20 ± 32.67 |
| EISO | – | – | 2443.64 ± 333.56 | 252.80 ± 73.34 |

Values = mean ± SD; N = 5 fish; –, absent

Analysis of reactions with the combination of histochemical methods and their controls (Table 1) indicate that type A mucous cells contain high amounts of glycoproteins with O-sulphate esters, together with relatively small amounts of glycoproteins with carboxyl groups and glycoproteins with oxidisable vicinal diols. Type B mucous cells contain high amounts of glycoproteins with oxidisable vicinal diols. Type C mucous cells contain high amounts of glycoproteins with oxidisable vicinal diols, and glycoproteins with carboxyl groups, together with low amounts of glycoproteins with O-sulphate esters.

Taste buds were conspicuous in the whole-mounts of OE and EISO. The epithelial cells around each taste bud were characteristically arranged in concentric whorls (Fig. 1f). The cluster of sensory cells and the tuft of taste hairs of each taste bud were stained positively for glycoproteins (Fig. 1e, f). Analysis of these reactions (Table 1) shows that the taste buds contain moderate amounts of glycoproteins with oxidisable vicinal diols together with glycoproteins with carboxyl groups, and low moieties of glycoproteins with O-sulphate esters. Density of taste buds was much higher in the EISO than in the OE (Fig. 1 e, f) (Table 2).

## DISCUSSION

Noticeable differences exhibited in the patterns of microridges on the epithelial cells, composition of glycoproteins elaborated by mucous cells and distribution of taste buds on the surfaces of the OE and the EISO of *L. guntea* may be considered as modifications relating to possible difference in the functional requirements at the two locations.

Microridges have been reported to vary considerably in configuration and disposition, constituting varied patterns at different locations in different fish species, and have been implicated to play variable roles. These include to retain mucous secretions to the cell surface, to increase the surface area for excretion and absorption through the skin, to facilitate the spread of mucus away from goblet cells, to aid in producing laminar flow, to provide reserve surface area for stretching, and so on (see review of Whitear, 1990). In the OE of *L. guntea,* compactly arranged microridges forming intricate mesh-like patterns, may in addition impart firm consistency or rigidity to the free surfaces of the epithelial cells. This could be considered as an adaptation to withstand mechanical stress and protect the surface of the fish, which has the characteristic habit of frequently darting and burrowing in sand or mud. Furthermore, these microridges may gain a firm base and support from a dense network of fine filaments - the terminal web, which, as shown by Schliwa (1975), Mittal et al. (1980) and Whitear (1986), is differentiated characteristically at the apical regions of the surface epithelial cells. The microbridges interconnecting the microridges may further reinforce renitency of the surfaces of epithelial cells in the OE of *L. guntea*. Microbridges, variously named as cross connections (Whitear, 1990) or interconnections (Reutter et al., 1974) in fish epidermis, and as microbridges (Karlsson, 1983) or crossbridges (Avella & Ehrenfeld, 1997) in gill epithelia, have also been reported previously. In the EISO, in contrast, widely spaced microridges forming simplified patterns and the absence of microbridges between them

suggest less rigid surfaces of the epithelial cells. This is interesting since the EISO, unlike the OE is not prone to abrasion during burrowing.

The characteristically folded surface of the EISO in *L. guntea* may further be considered to impart stretchability to the epithelium, required for expansion of the branchial chamber during respiratory movements of the operculum. Furthermore, it may account for increased surface area as well for respiration in the fish.

Acid glycoproteins have been shown to coincide with increased viscosity of mucus in the alimentary tract of fish *Arrhamphus sclerolepis krefftii* Steindachner, 1866 (Tibbetts, 1997), in airway epithelia of mammals (Jones et al., 1973; Iravani & Melville, 1974) and in corals (Miekle et al., 1988). The elaboration of mainly sulphated glycoproteins by type A mucous cells, the most common type in the OE in *L. guntea*, could thus be related to increased viscosity of the mucus and lubrication of the surface of the fish. This could play a vital role in providing protection to the body against mechanical damage to which these fishes are highly vulnerable during burrowing in mud or sand. Mittal et al. (1994 a, b) also correlated the release of large amounts of epidermal glycoproteins with O-sulphate esters to provide heavy lubrication to protect the body against mechanical damage in *Monopterus cuchia* Hamilton, 1822 and *Mastacembelus pancalus* Hamilton, 1822 during their peculiar wriggling movements on moist grass and during burrowing in mud. Elaboration of sulphated glycoproteins by superficial layer epithelial cells and most mucous cells in the epidermis of semi-amphibious *Blenny sanguinolentus* Pallas, 1811 (Zaccone, 1983) and *Blennius pholis* Linnaeus, 1758 (Whitear & Mittal, 1984) both with the habit of creeping about with the aid of paired fins, further supports this view.

Furthermore, sulphation of complex carbohydrates has also been shown to result in increased resistance to their enzymatic breakdown by bacterial glycosidases (Mian et al., 1979; Tsai et al., 1992), to play a role in defence against pathogens (Solanki & Benjamin, 1982) and to prevent the proliferation of pathogenic microorganisms (Tsukise & Yamada, 1981; Suprasert et al., 1986, 1987). Thus high proportions of sulphated glycoproteins in the mucous cell secretions on the surface of the OE in *L. guntea* may also confer high resistance against pathogens and protect the fish.

Sibbing & Uribe (1985) reported that sialomucins are less viscous than sulphomucins produced by mucous cells in the pharynx of *Cyprinus carpio* Linnaeus, 1758. Further, Tibbetts (1997) has shown that neutral glycoproteins are less viscous than the acid glycoproteins produced by mucous cells in the alimentary tract of *A. sclerolepis krefftii.* Elaboration of glycoproteins with oxidisable vicinal diols (neutral glycoproteins) and glycoproteins with carboxyl groups (sialomucins) in high concentrations by the type C mucous cells in the EISO of *L. guntea,* would thus lower the viscosity of the mucus in this region. Mucus with lower viscosity is considered to be fairly easily washed away with the respiratory water current. This prevents the accumulation of mucus on the surface of the EISO that could otherwise obstruct or disturb the smooth flow of respiratory current across the branchial chamber. Generally, visibility is poor at the muddy bottoms inhabited by *L. guntea*, owing to depth and increased turbidity, caused by the disturbance of the bottom mud due to the characteristic habit of the fish. The presence of prominent taste buds in the OE might be considered to increase the probability of accurately detecting and locating prey concealed by darkness or turbidity, and may also permit the accurate location of small food particles, which would be missed otherwise. Presence of a large number of conspicuous taste buds on the interior face of the operculum i.e. the EISO as well, could play a role in detecting other chemicals that could enter the buccal and gill cavity during respiration and that could potentially be noxious to gills. Garg & Mittal (1990), however, reported the absence of taste buds in the EISO of C. batrachus. Barrington (1957) reported that taste buds are often present even in fish oesophagus and postulated that it is an indication of the probable importance of this region in the selection and rejection of food.

Characteristic concentric whorls of epidermal cells encircling the taste buds in *L.* guntea are interesting. Such arrangements of epithelial cells have also been reported in *C. batrachus* (Garg et al., 1995) and in cod (Harvey & Batty, 1998). Harvey & Batty (1998) stated that the characteristic ring of epithelial cells may make it possible to locate and count taste buds even when their apex was damaged or missing.

Presence of glycoproteins indicates a secretory function of the taste buds of *L. guntea*. Grover-Johnson & Farbman (1976) and Reutter (1971), using transmission electron microscopy, reported two types of gustatory cells - light and dark - in the taste buds of *Ictalurus punctatus* Rafinesque, 1818 and *Ameiurus nebulosus* LeSuer, 1819. Grover-Johnson & Farbman, (1976) postulated that the dark cells were secretory in function. Witt & Reutter (1990) have shown, using lectin histochemistry for the detection of carbohydrates, that the dark sensory cells in the taste buds in European catfish *Silurus glanis* Linnaeus, 1758 are secretory. Glycoproteins with sialic acids have also been demonstrated in the taste buds in rabbit tongue (Witt & Miller, 1992). The secretions in the taste buds have been postulated to play different roles e.g. to maintain and regulate the chemical microenvironment (Grover-Johnson & Farbman, 1976), to protect mucus or other glycoproteins in the taste bud cells and inside the taste pore from premature enzymatic degradation and to have a hormone-like paraneuronal function (Witt & Miller, 1992), and to play a role in recognition phenomena on the plasmalemmata of the taste bud sensory cells and recognition processes directed to bacteria or viruses (Witt & Reutter 1990). Fujita (1994) and Zaccone et al. (1999) also suggested a paraneuronal role of the gustatory cells in taste buds.

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## REFERENCES


Avella, M. & J. Ehrenfeld (1997). Fish gill respiratory cells in culture : A new model for $Cl^-$- secreting epithelia *J. Membrane Biol.,* 156 : 8-97.

Barrington, E.J.W. (1957). The alimentary canal and digestion. In : Brown M.E. (ed), *The physiology of fishes,* Academic Press, New York : 109-161.

Fujita, T. (1994). Gustatory cells as paraneurons. In : Kurihara, K., N. Suzuki & H. Ogawa (eds.), *Olfaction and Taste*, XI, Springer-Verlag, Tokyo : 2-4.

Garg, T.K. & A.K. Mittal (1990). The epidermal and inner epithelial lining of the operculum in *Clarias batrachus* (Clariidae, Siluriformes). *Japan. J. Ichthyol.,* 37 : 149-157.

Garg, T.K., V. Deepa & A.K. Mittal (1995). Surface architecture of the opercular epidermis and epithelium lining the inner surface of the operculum of a walking catfish, *Clarias batrachus. Japan. J. Ichthyol.,* 42 : 181-185.

Grover- Johnson, N. & A. I. Farbman (1976). Fine structure of the taste buds in the barbel of the catfish, Ictalurus punctatus. Cell Tiss. Res. ,169 : 395-404.

Günther, S. (1989*). Fresh water fishes of the world Vol. I & II* (Translated and revised by Tucker, D. W.), Falcon books, Cosmo Publications, New Delhi.

Harvey, R. & R.S. Batty (1998). Cutaneous taste buds in cod. *J. Fish Biol.*, 53 : 138-149.

Irawani, J. & G.N. Melville (1974). Long term effects of cigarette smoke on the mucocilliary function in animals. *Respiration*, 31 : 358-366.

Jones, R., P. Bolduc & L. Reid (1973). Goblet cell glycoprotein and tracheal gland hypertrophy in rat airways : the effects of tobacco smoke with or without the anti-inflammatory agent phenylmethylaxadiozole. *Br. J. exp. Path.,* 54 : 229-239.

Karlsson, L. (1983). Gill morphology in the zebra fish, *Brachydanio rerio* (Hamilton- Buchanan). *J. Fish Biol.,* 23 : 511-524.

Lev, R. & S.S. Spicer (1964). Specific staining of sulphate groups with alcian blue at low pH. *J. Histochem. Cytochem*., 12 : 309.

Lillie, R.D. & H.M. Fullmer (1976). *Histopathologic Technic and Practical Histochemistry, 4th* ed., McGraw-Hill, New York.

McManus, J.F.A. (1948). Histological and histochemical uses of periodic acid. *Stain Technol*., 23 : 99-108.

Mian, N., C.E. Anderson & P.W. Kent (1979). Effect of O- sulphated groups in lactose and N-actyl- neuraminyl lactose on their enzymatic hydrolysis. *Biochem. J.*, 181 : 387-399.

Miekle, P., G.N. Richards & D.Yellowlees (1988). Structural investigations on the mucus from six species of coral. *Marine Biology*, 99 : 187-193.

Mishra, K.P. & M.F. Ahmad (1986). On the structural adaptation of an intestinal breather *Lepidocephalichthys guntea* (Ham.), a torrential loach. *Curr. Sci.*, 55 : 210-211.

Mittal, A.K. & T.K. Garg (1988). An improved method of separation of intact epidermal sheets in fish. *J. Fish Biol*., 32 : 803-804.

Mittal, A.K. & M. Whitear (1978). A note on cold anaesthesia of poikilotherms. *J. Fish. Biol.*, 13 : 519-520.

Mittal, A.K., M. Whitear & S.K. Agarwal (1980). Fine structure and histochemistry of the epidermis of the fish *Monopterus cuchia. J. Zool., Lond*., 191 : 107-125.

Mittal, A.K., T. Ueda, O. Fujimori & K. Yamada (1994a). Histochemical analysis of glycoproteins in the epidermal mucous cells and sacciform cells of an Indian swamp eel *Monopterus cuchia* (Hamilton) (Synbranchiformes, Pisces). *Acta Histochem. Cytochem.*, 27 : 193-204.

Mittal, A.K., T. Ueda, O. Fujimori & K. Yamada (1994b). Histochemical analysis of glycoproteins in the unicellular glands in the epidermis of an Indian freshwater fish *Mastacembelus pancalus* (Hamilton). *Histochem. J.*, 26 : 666-677.

Mowry, R.W. (1956). Alcian blue techniques for the histochemical study of acidic carbohydrates. *J. Histochem. Cytochem*., 4 : 407-408.

Mowry, R.W. (1963). The special value of methods that colour both acidic and vicinal hydroxyl groups in the histochemical study of mucins with revised directions for the colloidal iron stain, the use of alcian blue 8GX, and their combination with the periodic acid-Schiff reaction. *Ann. NY. Acad. Sci*., 106 : 402-423.

Pinky, S. Mittal, J. Ojha & A.K. Mittal (2002). Scanning electron microscopic study of the structures associated with lips of an Indian hill stream fish *Garra lamta* (Hamilton) (Cyprinidae, Cypriniformes). *Europ. J. Morph.*, 40 : 161-169.

Reutter, K. (1971). Die Geschmacksknospen des Zwergwelses *Ameiurus nebulosus* (Lesueur). *Morphologische und Histochemische Untersuchungen. Z. Zellforsch.*, 120 :280-308.

Reutter, K., W. Breipohl & G.J. Bijvank (1974). Taste bud types in fishes. II Scanning electron microscopical investigations on *Xiphophorus helleri* Heckel (Poeciliidae, Cyprinodontiformes, Teleostei). *Cell Tissue Res*., 153 : 151-165.

Schliwa, M. (1975). Cytoarchitecture of surface layer cells of the teleost epidermis. *J. Ultrastruct. Res*., 52 : 377-386.

Sibbing, F.A. & R. Uribe (1985). Regional specialisations in the oropharyngeal wall and food processing in the carp (*Cyprinus carpio* L.). *Neth. J. Zool.*, 35 : 377-422.

Solanki, T.G. & M. Benjamin (1982). Changes in the mucous cells of the gills, buccal cavity and epidermis of the nine-spined stickleback, *Pungitus pungitus* L., induced by transferring the fish to sea water. *J. Fish Biol.*, 21 : 563-575.

Spicer, S. S., R. G. Horn & T. J. Leppi (1967). Histochemistry of connective tissue mucopolysaccharides. In : Wangner, B. M. & D. E. Smith (eds.), *The Connective Tissue,* Williams & Wilkins, Baltimore : 251-303.

Suprasert, A., T. Fujioka & K. Yamada (1986). Glycoconjugates in the secretory epithelium of the chicken mandibular gland. *Histochem. J.*, 18 : 115-121.

Suprasert, A., T. Fujioka & K. Yamada (1987). The histochemistry of glycoconjugates in the colonic epithelium of the chicken. *Histochemistry*, 86 : 491-497.

Tibbetts, I.R. (1997). The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *J. Fish Biol.*, 50 : 809-820.

Tsai, H.H., D. Sunderland, H. Gibson, C.A. Hart & J.M. Rhodes (1992). A novel mucin sulphatase from human faeces : its identification, purification and characterization. *Clin. Sci.*, 82 : 447-454.

Tsukise, A. & K. Yamada (1981). The histochemistry of complex carbohydrates in the scrotum of the boar. *Histochemistry*, 72 : 511-521.

Whitear, M. (1986). Epidermis. In : Bereiter-Hahn, J., A. G. Matoltsy, & K. S. Richards (eds.), *Biology of the Integument*. Vol. 2, *Vertebrates*, Springer-Verlag, Berlin : 8-38.

Whitear, M. (1990). Causative aspects of microridges on the surface of fish epithelia. *J. Submicrosc. Cytol. Pathol.*, 22 : 211-220.

Whitear, M. & A.K. Mittal (1984). Surface secretions of the skin of *Blennius (Lipophrys) pholis* L. *J. Fish Biol.*, 25 : 317-313.

Witt, M. & Jr. I.J. MILLER (1992). Comparative lectin histochemistry on taste buds in foliate, circumvallate and fungiform papillae of the rabbit tongue. *Histochem*, 98 : 173-182.

Witt, M. & K. Reutter (1990). Electron microscopic demonstration of lectin binding sites in the taste buds of the European catfish *Silurus glanis* (Teleostei). *Histochem*,. 94 : 617-628.

Zaccone, G. (1983). Histochemical studies of acid proteoglycans and glycoproteins and activities of hydrolytic and oxidoreductive enzymes in the skin epidermis of the fish *Blennius sanguinolentus* Pallas (Teleostei : Blenniidae). *Histochemistry*, 78 : 163-175.

Zaccone, G., A. Mauceri, L. Ainis, S. Fasulo & A. Licata (1999). Paraneurons in the skin and gills of fishes. In : Saxena, D.N., (ed.), *Ichthyology. Recent research advances*, Oxford and IBH, New Delhi, Calcutta : 417-447.

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