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Localization and Identification of Mucoprotein in the Skin and Gills Epidermal Mucous cells of Cynoglossus semifasciatus (Day, 1877)

Natarajan Elayaperumal

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502, India.

Abstract

Localization and characterization of skin and gill epidermal mucoproteins (SEM, GEM) in the mucous cells located in the gills of the fish Cyanoglossus arel were investigated. Using histological procedures pavement cells are the most abundant type of cell on both primary and secondary lamellae and there are a great number of mucous and chloride cells between them. Histochemical characteristics that included methods for localization and characterization of glycoproteins (GPs), showed no differences between the mucous cell contents of the primary and secondary lamellae. In the tongue sole skin, four morphologically distinct layers were identified: cuticle, epidermis, dermis and hypodermis. Neutral mucosubstances and/or glycoconjugates were observed in the epidermis, dermis and hypodermis of C.semifasciatus skin. Proteins rich in different amino acids, such as arginine and cysteine, reacted negatively or weakly positive in the epidermis, dermis and hypodermis. Blue at pH 2.5. When Alcian Blue pH 2.5–PAS reaction was performed, most mucous cells were stained blue (carboxylated mucins) and some mucocytes stained purple, indicating a combination of neutral and acid mucins. Proteins rich in cysteine-bound sulphydryl (-SH-) and cystine disulphide (-S-S-) groups were strongly detected in epidermal mucous cells, where as lysine, tyrosine and arginine containing proteins showed very weak staining in epidermal mucous cells. Protein reactions were strongly positive in the pillar cells, except for those rich in tryptophan. It is concluded from this study that cytohistochemical features of the tongue sole skin and Gill may serve as structures in natural systems to monitor or detect environmental stress responses at the histological level and histochemical level. The GPs were identified with (a) oxidizable vecinal diols; (b) sialic acids; (c) carboxyl groups and (d) sulphate groups. The distribution pattern of the mucous was identical in the primary and secondary lamellae. This work clearly demonstrates the heterogeneity of the mucous cell glycoconjugates, which could be involved in various functions, such as lubrication, protection, inhibition in microorganisms and a role in the regulation and diffusion.

Keywords: *Epidermal mucoprotein, Cyanoglossus semifasciatus, Glycoprotein, Mucocytes Glycoconjugates.*

1. Introduction

Cyanoglossus arel is a species of high commercial value, well adapted to warm climates and commonly exploited in east and west coat of India. Knowledge of normal features of tissular structures could indicate possible alterations in target organs, as a response to environmental change, which may be expressed as visible histopathological manifestations (e.g. necrosis, apoptosis, inflammation, hyperplasia, hypertrophy, pycnosis, oedema, cytohistochemical modifications, etc.). All of these abnormalities can be induced by changes in water quality (physicochemical stress, contaminants), infectious or parasitic diseases, nutritional deficiencies, pigmentation abnormalities, etc. As the external organs are in direct contact with the environment and cytohistochemical characteristics of skin of healthy

fish can be used as to study the stress responses in natural systems.

The structure and function of fish skin have been studied by different authors (**Hoar and Randall, 1984; Devi *et al*., 2006, Zoghby et al., 2016**). Skin secretes mucus, which serves as protection for the individual. Mucous secretion is also an important factor in disease resistance and in respiratory and osmoregulatory processes (**Shephard, 1994**), as well as in the natural defense against parasites and pathogens (**Fletcher, 1978**). Changes in the number and dimension of mucous cells may be indicators of pathological or inflammatory processes induced by adverse environmental conditions (**Ortiz *et al*., 1999, Zoghby et al., 2016**). Furthermore, sialitation and sulphation of glycoproteins, the main component of mucous secretions, may be important for increasing mucous resistance to bacterial degradation (**Rhodes *et al*., 1985**).

The epidermis is a metabolically very active border tissue containing mucous, sensory, chloride (also called mitochondria rich cells or ionocytes) and club cells (**Ahmed et al., 2011**), and is covered by a mucous layer that forms an additional external barrier containing enzymes such as proteases, phosphatases and peroxidases (**Wendelaar Bonga, 1992**).

Many studies on the mucous cells of vertebrates and their secretion products, vis-a- vis histochemistry have been reported (**Suprasert et al., 1987, Salinas et al., 2011, Amira et al., 2017**). Among fish, those referred to mucous cells present in the epidermis reported that various types of mucous cells involved in glycoprotein secretion have been found in the epidermis of fish (**Fishelson, 1996**). Only a few studies have been carried out in the mucous cells of the gills of fish (Saboia-Moraes et al., 1996). Physiologically, mucous secretion has been mainly related to lubrication and protection against pathogenic micro-organisms (**Zaccone et al., 1989, Cinar et al., 2014, Bansari and Roy, 2015**). Among fish, this secretion also has an important role in the ion-regulation and diffusion of ions (**Handy et al., 1989, Esteban, 2012**).

The purpose of the present study was to establish cytohistochemical characteristics of the skin and gills of healthy adult tongue sole, *Cyanoglossus semifasciatus* specimens of an attempt to provide a baseline for comparison with pathological and stress conditions in natural or polluted environments.

2. Materials and Methods

Unless specified, chemicals were acquired from Sigma-Aldrich. All solutions were aqueous and prepared with deionized water (Elga), except when stated otherwise.

Live specimens of *C.semifasciatus* (mean ± SD standard length 60 ± 10 mm; n = 10) were collected from the aquarium, C.A.S in Marine Biology, TamilNadu, India and were reared in the laboratory at 25 ± 2^0 C. The fishes were cold-anaesthetized following the procedure described by Mittal and Whitear (1978). The skin and Gills structures were excised, rinsed in physiological saline and fixed in aqueous Bouin's fluid for histological studies and in Carnoy's fluid for glycoprotein histochemistry. The tissues were dehydrated through an ascending series of ethanol concentrations, cleared in cedar wood oil and embedded in paraffin wax. Sections were cut at a thickness of 6 µm using a Leica RM 2145 semi-motorized rotary microtome (Leica Microscopy and Scientific Instruments Group, Heerbrugg, Switzerland). The sections were mounted on ethanol-cleaned glass slides without any adhesive and were dried in an oven at 40ºC. Sections were deparaffinized in xylene and were hydrated through a descending ethanol series. Sections of Bouin's fluid-fixed tissues were stained with Ehrlich's haematoxylin and eosin (H/E) by routine protocol to study the general organization of the gills epithelium. Sections of Carnoy's fluid-fixed tissues were subjected to different histochemical methods (1–4), detailed in Tables 2.1, at room temperature unless specified otherwise, to identify different classes of GPs. These included GPs with oxidizable vicinal diols, GPs containing sialic acid residues with or without O-acyl substituents, GPs with O-sulphate esters and GPs with O-acyl sugars. The stained sections were dehydrated through ascending ethanol series, cleared in xylene, and mounted in Lendrum's distrene dibutylphthalate xylene (DPX).

Method 1 without oxidation (WO)/Schiff (S): The sections were, without prior oxidation with periodic acid, were rinsed in distilled water and treated with Schiff's reagent (prepared from basic fuchsin by the method of De Tomasi, 1936) for 10–15 min. The sections were then rinsed in three successive changes of the freshly prepared sulphite water (equal volumes of 1% potassium or sodium metabisulphite and 0.1 n HCl), washed in running tap water for 10 min and rinsed in distilled water.**Method 2 periodic acid Schiff (PAS):** The sections were oxidized in 0.5% aqueous periodic acid for 10 min, washed in running tap water for 10–15 min and then rinsed in distilled water. The sections were then treated, as per method 1, with Schiff's reagent. **Method 3 alcian blue at pH 2.5 (AB2.5):** The sections were stained in 1% Alcian blue 8GX in 3% acetic acid, pH 2.5, for 30

min, rinsed in 3% acetic acid and washed in running tap water. **Method 4 AB2.5/PAS:** The sections were stained with AB2.5 (method 3) followed by PAS (method 2).

3. Results

Like other teleosts, the skin of the tongue sole, *C. semifasciatus*, is composed of three morphologically distinct layers: epidermis, dermis and hypodermis (Fig.2.1A). The epidermis and dermis are separated by a thin base membrane. The cuticle is a very thick cellular layer. The epidermis forms a stratified epithelium containing three cellular layers: the stratum germinativum or basal layer, the middle or fusiform layer and the outermost or mucosa layer (**Fig.3.1A**). The stratum germinativum is composed of a single layer of cuboidal or columnar basal cells on a thin base membrane. Small irregular lymphatic spaces are found together with basal cells that are interconnected with each other. The fusiform cells, arranged in several layers, are in general polygonal, whereas the outermost layer or mucosa is formed of cuboidal cells near the fusiform layer; the exterior region is formed by a double layer of columnar cells. The dermis is composed of collagen fibers and contains two layers: the upper stratum spongiosum, which is a network of collagen and reticulin fibres connected with the epidermal base membrane and contains pigment cells and scales (**Fig.3.1A),** and the stratum compactum, the dense collagenous matrix which provides the structural strength of the skin. Next to this layer is the hypodermis, which is mainly formed of fat and muscle tissue (**Fig.3.1A**).

The epithelium at the mucogenic regions of the skin is thick and consists of the epithelial cells, the mucous goblet cells and the club cells (Fig.2.1A) involved in the elaboration of the secretary contents. The epithelium of the skin at different locations in contrast, is relatively thin and is devoid of the club cells.

Analysis of results obtained by the combination of histochemical methods reveals that the epithelial cells in the superficial layers and underlying one to three layers in the mucogenic epithelium at different locations elaborate GPs with oxidizable vicinal diols in high concentrations, and traces of GPs with sialic acid residues. A strong positive reaction with PAS/ AB pH 2.5 (**Fig.3.1B**) indicates the presence of GPs with oxidizable vicinal diols in high concentrations and glycogen in trace amounts. Elaboration of GPs with oxidizable vicinal diols in high concentrations by these cells is further indicated by magenta reaction with methods AB 2.5/PAS (**Fig.3.1B**). A positive reaction with Alcian blue at pH 1.0 (AB1.0) however, the possibility of the presence of GPs with O-sulphate esters in these cells (Fig.2.1D). Presence of traces of GPs with sialic acid residues in these cells is confirmed by the reactions with a combination of the methods AB pH 2.5 (Fig. 2.1C). The epithelial cells in the middle and the basal layers of the epithelium, in contrast, elaborate only traces of GPs with oxidizable vicinal diols. Neutral mucosubstances and/or glycoconjugates are detected in epidermis, dermis and hypodermis. Periodic acid-Schiff (PAS) reactivity was moderately increased after the saponification process, suggesting presence of acetylated sialic acid in the goblet cells. Acid mucosubstances (sulphomucins and sialic acid) are also identified in the goblet cells (**Table 3.1**).

Table 3.1. Histochemical reactions for visualization and identification of Glycoconjugates (GC's) in mucous cells of *Cyanoglossus semifasciatus* skin.

Procedures	References	Interpretation of staining reactions	Mucous cells
PAS	McManus (1948)	Glycoproteins with oxidizable vicinal diols (or) glycogen.	+ +M
AB pH1.0	Lev and Spicer (1964)	Glycopreteins with O-sulphate esters.	+ + B
AB pH2.5	Lev and Spicer (1964)	Proteoglyans with carboxyl group and (or) with sulphate esters.	+ + B/P
ABpH2.5/PAS	Mowry (1956)	Proteoglyans with carboxyl group and glycoproteins	+ + P orB &M
PAS, periodic acid schiff; AB, Alcian Blue; GC's, Glycoconjugates; M, magenta; B, Blue; P, purple; (+ +) strong positive.			

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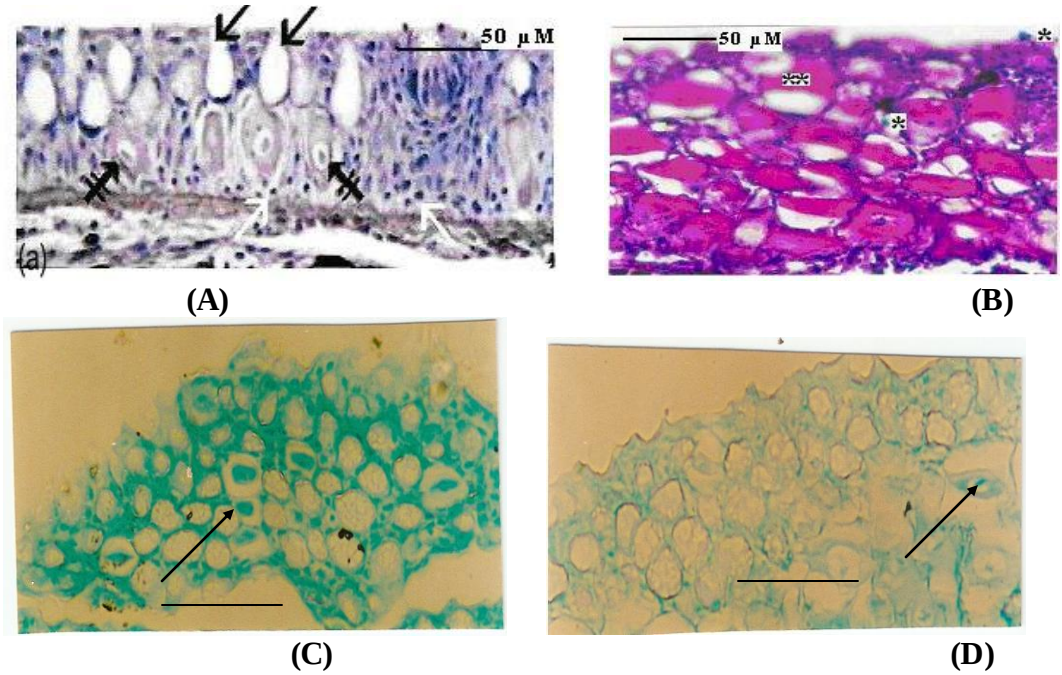


Fig.3. 1. Photomicrographs of the cross section showing the general organization of the epithelia of the mucogenic region of the dorsal skin of tongue sole, *Cyanoglossus semifasciatus* (**H/E**); showing reactions for GPs in the cellular components. **A)**. Secretary Cells, the epithelial cells in the superficial layer, voluminous sac like mucous cells (arrows) and club cells (barred arrows). The epithelial cells arranged in the middle and basal layer, the lymphocytes (white arrows) in between the epithelial cells are also discernible **X 100**. **B)**. Type A mucous cells are stained blue (single *) and the epithelia cells in the superficial layer and the underlying two to three layers were stained magenta (double **) AB pH 2.5 /PAS. **X 100. C).** Alcian blue p H 2.5 **X 100**. D. Alcian blue pH 1.0 **X 100. Scale Bar** = 50 µm.

Each gill consisted of two rows of gill filaments (primary lamellae), which on both sides containing a series of alternately arranged respiratory (gill) lamellae (secondary lamellae) (**Fig. 3a-b).** Alternately arranged Pavement cells (PVCs) and Sinus venus cells (CVCs), through which usually one or two cells can pass, constitute the vascular component of the gills (Fig.la). A very thin barrier layer of respiratory epithelium covers the vascular component of the gill lamellae. Goblet mucus cells were always present in the interlamellar epithelial lining as well as on the distal tip of the gill filament. The gill lamellae have very few (**Fig.3.1b**) or no goblet cells in their epithelial coverings. Chloride cells are regularly present at different locations on the gills. Mucous cells were detected mostly among other epithelial cells of the primary and secondary gill lamellae. The contents of mucous cells of H & E-stained preparations remained unstained.

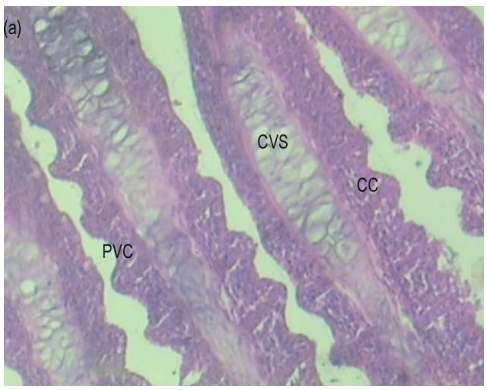
The mucous cells appeared characteristically depressed in the epithelium surface of the epithelial cells which covered them almost completely (**Fig3.1a and b**). They were chiefly occupied by tightly packed membrane-bound mucous globules, which were large, polygonal- or round-shaped.

The histochemical staining properties of glycoproteins (GPs) present in mucous cells are summarized in Table 1. No histochemical differences were detected between the mucous cells of the primary and secondary lamellae. The secretory contents of those cells showed a positive reaction to mixed neutral and acidic mucopolysaccharides. Mucous cells with periodic acid Schiff (PAS) reaction showed a strong positive response, in which the coloration disappeared after acetylation and were recovered after saponification. Further, the purple coloration of the mucous cells in an Alcian Blue-PAS sequence also indicated the presence of neutral and acid mucopolysaccharide groups. A sequence of procedures utilizing Alcian Blue at different pH values and molarities displayed the presence of

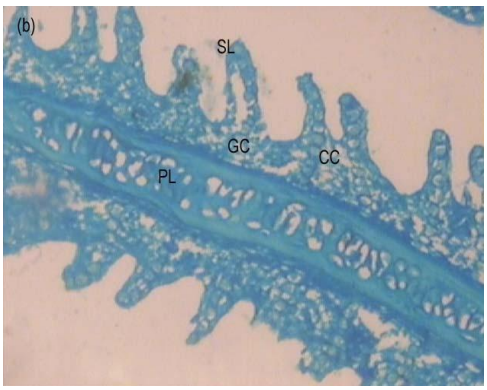
strong and weakly sulphating GPs and some sialic acids.

Table3. 2. Histochemical reactions for visualization and identification of Glycoconjugates GC's in mucous cells of *Cynoglossus semifasciatus* gills.

Procedures	References	Interpretation of staining reactions	Mucous cells
PAS	Mc Manus (1948)	Glycoproteins with oxidizable vicinal diols (or) glycogen.	+ +M
AB pH1..0	Lev and Spicer (1964)	Glycopreteins with O-sulphate esters.	+ + T
AB pH2.5	Lev and Spicer (1964)	Proteoglyans with carboxyl group and (or) with sulphate esters.	+ + T
ABpH2.5/PAS	Mowry (1956)	Proteoglyans with carboxyl group and glycoproteins	+ + P
PAS, periodic acid schiff; AB, Alcian Blue; GC's, Glycoconjugates; M, magenta; T, turquoise; p, purble. (+ +) strong positive.			



(a). Histological section of gills stained with Hematoxylin /Eosin. CC chloride cells; PVC- Pavement cells; CVS- Central venous sinus.



(b) Histological section of gills stained with Alcian blue pH2.5. Primary lamellar (PL) and secondary lamelia (SL) are observed; unstained chloride cells and alcinopohilic goblet cells (gc) are observed.

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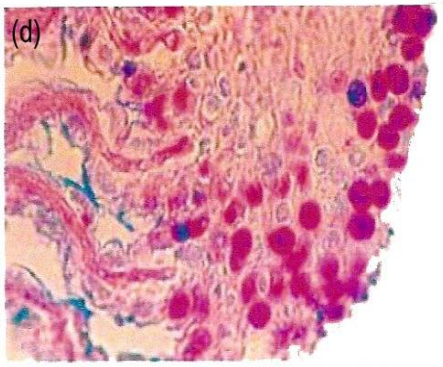
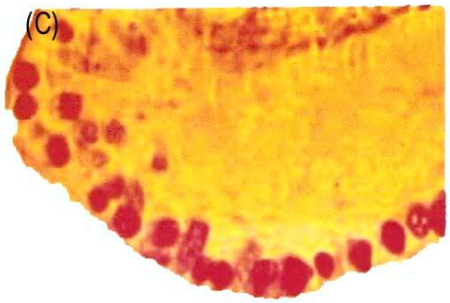
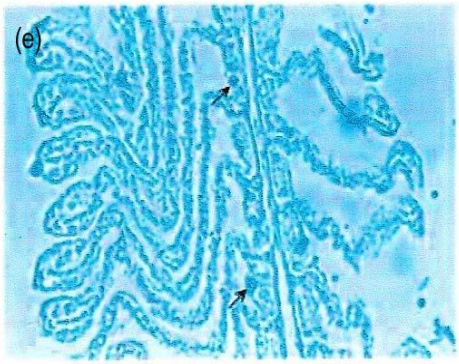


(d) AB 2.5 / PAS- Stained mucous cells in the primary filament of the branchial epithelia



(c) PAS- Positive mucous cells in primary filament.



(e) AB. 10 mucous cells are stained turquoise (arrows)

Fig. 3.2 a-e. Histological section of gills, *C. semifasciatus*

4. Discussion

The skin of many air breathing fishes also acts as an efficient water breathing organ. The skin is made up of three main layers: epidermis, dermis, and sub cutis (fig.2.1A).The epidermis is a stratified epithelium, which very often remains covered with a protective a protective coating of slime containing different types of glycoproteins. The structure of the epidermis of the nonscaly skin of *C. batrachus* and *H.fossilis* are more less identical and consists of polygonal epithelial cells, club cells, goblet cells, and wandering blood cells (mostly leucocytes (fig.2.1A).The epidermis of *C.striatus* consists of epithelial cells, sacciforms granular cells, chloride cells (**Mittal and Banerjee, 1975, Esteban, 2012, Amira et al., 2017**) and migrating leucocytes. The mucous cells and sacciform granulated cells are mostly present in the outermost layer, extending deep into the middle layer. The goblet cells in different species stain differently for different types of glycoproteins. The contents of the sacciform granulated cells are mainly proteinaceous in nature. The dermis bears scales, if present. The highly vascularzed outer loose layer of the dermis of many fishes contains a large quantity of sulfated glycoproteins which are known to prevent desiccation during drought (**Mittal *et al.*, 1976**)

The structure of the skin of *C.arel* is similar to that of other teleost species (**Devi *et al.*, 2006, Cinar et al., 2014, bansari and Roy, 2015)** and consists of epidermis, dermis and hypodermis.The epidermis of fish contains epithelial, club, chloride cells, lymphocytes, etc. Mucus-secreting cells are found in the epidermis of all fish species; these cells usually originate in the middle layer of the epidermis, increasing in size and producing secretions (mainly glycoproteins) as they approach the surface. Two morphological goblet cell types were distinguishable

in the skin of *C.arel*: type A cells, and type B cells, but larger than those found in type A cells. In the *Oreochromis niloticus* oesophagus, **Morrison and Wright** (1999) observed two types of goblet cells which differed in size and in tinctorial affinity when PAS/Alcian Blue reactions were performed. Two types of mucous cells were also described by **Mittal *et al.* (1980**) in *Monopterus cuchias* epidermis; one of these cells was ultrastructurally similar to mackerel mucous cells. As pointed out by previous authors, we also can conclude that there are differences in the morphology of the mucus-secreting cells, depending on their localization in the skin. A mucous coat, which is often present on the epithelial surface (**Hughes and Munshi 1979, Salinas et al., 2011, Amira et al., 2017**) is sloughed off continuously into the surrounding medium perhaps facilitates the removal of trapped or bound pathogens, toxicants and foreign matter. According to **Rajan and Banerjee (1991**), copious secretion of mucus is perhaps a common response of the fish epidermis to a large number of irritants present in the environment. **Zaccone *et al*. (1989**) also reported that the slime of stressed fish contains a mixture of neutral and acidic complex carbohydrates, the latter including 0-acetylated sialic acids.

Glycoproteins with sialic acid residues elaborated by both the type A and the type B mucous cells as well as by the epithelial cells in the superficial layer and in the underlying one to three layers in the epithelium of lips and associated structures in G. lamta may be associated to function as protective shield for cells and act as receptor sites for the binding of pathogenic agents such as toxins, bacteria, viruses, protozoa and other exogenous macromolecules following. In infectious processes colonizing of bacteria can be limited to sialic acids coat covering the host cell surface. This could prevent the invasion of pathogenic agents in the underlying epithelial cells and thus play an important role in the organism's host defense mechanisms. The role of GPs with sialic acid residues has also been implicated with the protection of the tissues against bacterial degradation and with the inhibition of the invasion of viruses.

In fish species, acidic and/or neutral glycoconjugates are the main component of mucous cells. When Alcian Blue pH 2.5 PAS reactions were performed in *C.arel*, most mucous cells of skin stained blue (acid mucins) and some mucocytes stained purple, indicating a combination of neutral and acid mucins. Similar results were observed in skin from *Sparus aurata*, *S. senegalensis* and *Acipenser baeri* larvae and juvenile specimens (**Sarasquete *et al*., 2001, Zoghby et al., 2016**). According to the scheme proposed by **Harrison *et al*. (1987**), variability in staining within a given cell could be attributed to a temporal sequence granule. Biosynthesis of mucin glycoconjugate includes at least two post-transcriptional modifications to the secretory protein: glycosylation of the protein followed by modifications to the sugar moiety (**Phelps, 1978; Laboisse, 1986, Zoghby et al., 2016**). Accordingly, those granules that did not stain with PAS contained only protein; PAS-positive secretory granules could be related to the stage when the cells were mainly producing glycoproteins. Secretory granules would stain with Alcian Blue pH 2.5 when the glycoproteins had been carboxylated and the presence of sulphated glycoproteins (Alcian Blue pH 2.5) would coincide with the stage when sulphated groups had been conjugated to the glycoproteins.

Glycoproteins with O-sulphate esters in high concentrations may as well be associated to prevent the proliferation of pathogenic microorganisms on the epithelial surfaces.. An increase in proportions of sulphated GP secreting mucous cells in the epidermis of Colisa lalia and Trichogaster species and suggested that such an increase could be an adaptation for protection against microorganisms. Nevertheless, **Whitear and Mittal (1984)** conceived that such a shift could be related to a greater likelihood of infection in the medium inhabited by the fish. Without taking into the account of the nature of the GPs in mucous secretions postulated that mucus secreted on the surface inhibits the invasion and proliferation of pathogenic micro-organisms and prevent their colonization in fish epidermis. However, correlated increased production of sulphomucins in fish skin with the maintenance of ionic equilibrium and osmoregulation.

In fish gills, the mucous cells are generally located on the gill filaments (**Kendall and Date 1979, Ahmed et al., 2011**), or rarely on the gill lamellae, but also appear metaplastically on the gill lamellae during disease and ambient stresses. A mucous coat, which is often present on the epithelial surface (**Hughes and Munshi 1979**) is sloughed off continuously into the surrounding medium perhaps facilitates the removal of trapped or bound pathogens, toxicants and foreign matter as the gills, skin and certain environmental hazards. According to **Rajan and Banerjee (1991**), copious secretion of

mucous is perhaps a common response of the fish epidermis to a large number of irritants present in the environment. If the lamellae, unlike other external surface of fish such as the filaments of the gills and skin do not have a protective mucous coat, as suggested by the findings of **Handy and Eddy (1991**), these surface would be uniquely prone to waterborne pathogens and toxicants. The ability of glycoprotein to trap heavy metal ions is well illustrated **(Lock and Van Overbeeke 1981**).

The goblet cells of the respiratory organs of stress exposed fishes, after elaborating their secretary contents very often, show periodical fluctuations of increased, followed by decreased density, representing regenerative, secretary, and exhaustive stages. **Devi and Banjerjee (2006**) found that the density of the goblet cells increased simultaneously with increase in the protein contents of the gills of lead-exposed *Channa striata*. It is important to note that the same or different mucous cells of the differently exposed fishes secrete a mixture of neutral and acidic glycoproteins at the same or different stages of exposure (**Devi and Banerjee 2006). Zaccone et al. (1989**) also reported that the slime of stressed fish contains a mixture of neutral and acidic complex carbohydrates, the latter including 0-acetylated sialic acids. Increased secretion of acidic slime by the mucous cells of variously stressed fishes supports this finding.

Excessive secretion of mucous in thick layers over the respiratory epithelium might choke the respiratory surface, causing the failure of many biological processes and leading to asphyxiation and ultimate death of the fish (**Skidmore 1964). Laurent (1984**) also observed impaired bronchial gas exchange by the mucous by adding a nonconnective layer, which in turn affected ionic permeability of the gills. In the tongue sole, the structural characteristics of the mucous cells were similar to those described by **Perera (1993**) for the primary gill lamellae of the mackerel. It was also found that the mucous cells were very similar to those of the epidermis in the skin of other teleosts. Mucous cells secrete mucous through the expulsion of whole mucous globules (**Perera, 1993**) and not by fusion of mucous globules and posterior secretion of an amorphous mass.The histochemical results obtained would be consistent in as much as they could suggest a relationship with histochemistry with regard to the heterogeneity of the chemical composition of the mucous cells

Moreover, in some fresh-water fish, the epidermal mucous cells sulphated proteins predominate in the mucous composition, whereas in marine fish, GPs with sialic acid prevail (**Whitear and Mittal, 1984**). With regard to the histochemical compositions of mucous secretion, differences appear in the respiratory tract of the diverse species of mammals. Thus, sulphated GPs are abundant in rabbits, dogs and monkeys and are absent in hamsters (**Kennedy et al., 1978**). Differences have been also found in the composition of the mucosubstances of cells from the respiratory tract of different reptilian species (**Pastor et al., 1987**). In vertebrates mucous GPs have been demonstrated to be important in lubrication, control of infection and prevention of dehydration (**Mittal et al., 1994**). The mucous may act as an important diffusion barrier allowing ion absorption without direct contact of sea water with the epithelial cells. Moreover a lubricant role is provided by GPs containing O-sulphate esters. In addition, a protection activity against bacterial and virus invasion has been associated with GPs containing sialic acid residues (**Suprasert et al., 1987**). In fish epidermis too, GPs with oxidizable vicinal diols could control the acidity of the mucous secretion.

The results have shown in the skin and gills epithelium of *C.semifsciatus* numerous glandular mucus cells with different chemical composition. Infact, the mucus cells showed glycoproteins and acid proteoglycans with sulphate or carboxyl groups. This is in accordance with results reported in *Anguilla anguilla* (**Colombo, 1960**), in *Mugil cephalus*, and in *Anoptchthys jordani* **(Zaccone, 1989**).The mucus compounds differ in various teleost species according to their different living condition. The different chemical composition of the mucus in the skin can be correlated with the demersal life of *C.arel*. Further work is required to characterize the functional significance of the mucus cells with mucoprotein in the skin of *Cyanoglossus arel*.

5. Conclusion

From these results, it can be concluded that mucous cells from Skin and gills of *Cyanoglossus semifasciatus* have some characteristics that are similar to those of other fish skin and gills and to a lesser extent to those of epidermal location. Histochemical techniques suggest that neutral and acid mucopolysaccharides are the products of secretion. Accordingly this fact should indicate the achievement of different roles such as lubrication, control of infection, dehydration prevention, ion-regulation and ion diffusion. Further work is required

to characterize the functional significance of the mucous cells in gills of *Cyanoglossus arel*.

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